HIV & COMORBIDITY IN THE ERA OF MODERN ANTIRETROVIRAL THERAPY

Heritages of the past & challenges for the future

Berend van Welzen

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HIV & comorbidity in the era of modern antiretroviral therapy: heritages from the past & challenges for the future

Berend van Welzen

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HIV & COMORBIDITY IN THE ERA OF MODERN ANTIRETROVIRAL THERAPY

HERITAGES OF THE PAST & CHALLENGES FOR THE FUTURE

HIV en comorbiditeit in het tijdperk van moderne antiretrovirale therapie
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(met een samenvatting in het Nederlands)

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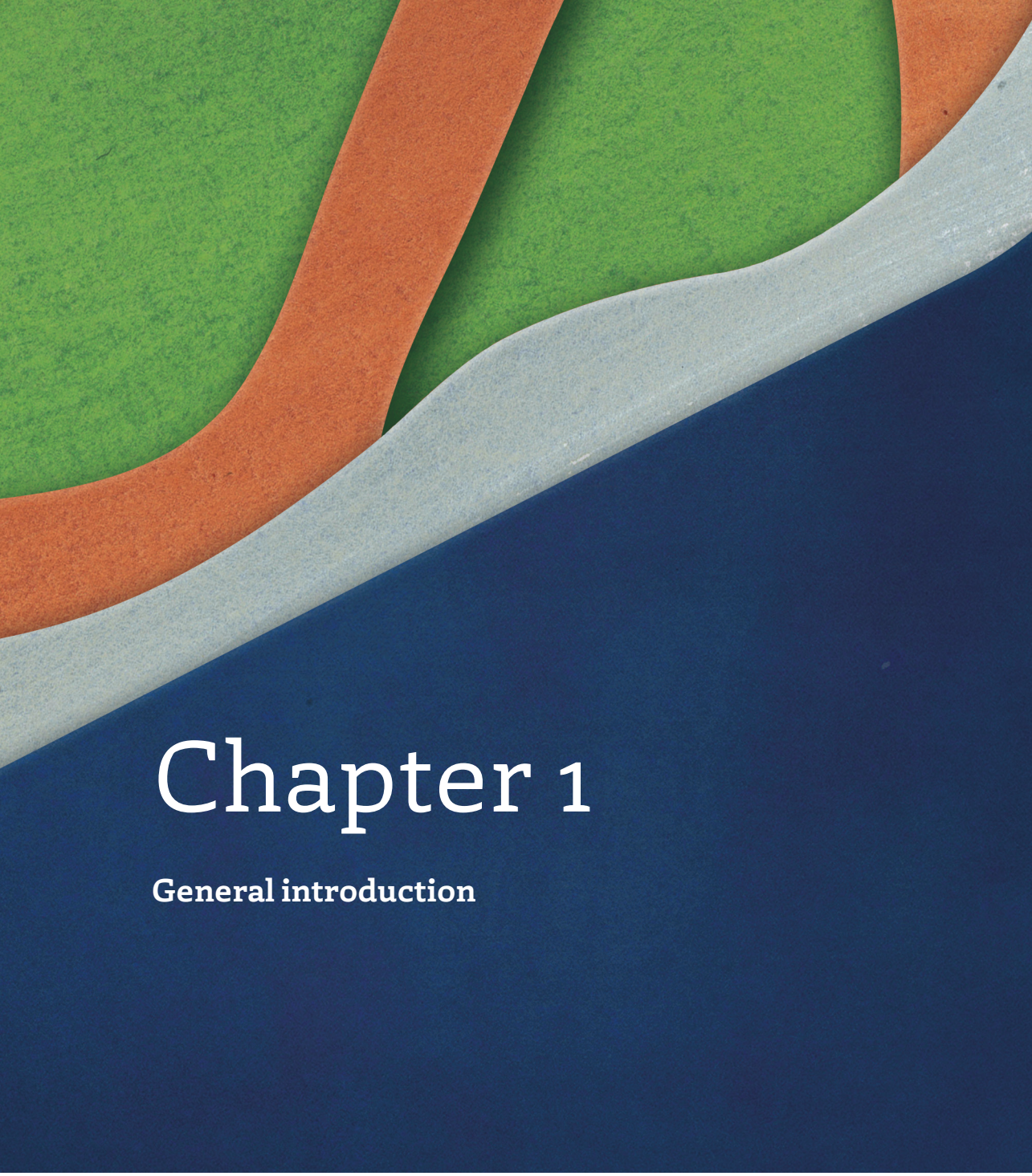


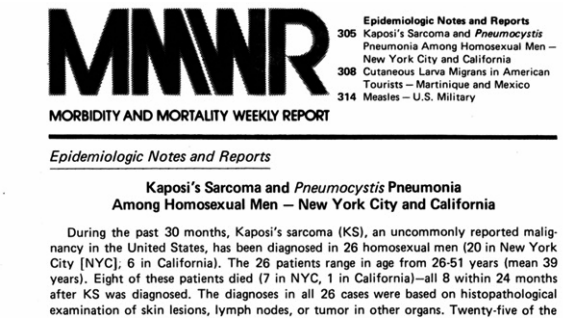
Chapter 1

General introduction

GENERAL INTRODUCTION

Human Immunodefiency Virus and Antiretroviral Therapy: A historical perspective

On June 5th 1981 the Centers of Disease Control (CDC) reported a cluster of *Pneumocystis Carinii Pneumonia* in five men who have sex with men (MSM) in Los Angeles, United States of America [1].Nowadays, this report is considered the starting point of the Human Immunodeficiency Virus (HIV) pandemic. At this moment, a total of 37.9 million people worldwide are living with HIV; it is estimated that HIV has already claimed the lives of the same number of people [2].In 1983, a group of French virologists identified a T-lymphotropic retrovirus – now known as HIV - as causative agent of the disease which was named Acquired Immunodeficiency Syndrome (AIDS) [3]. The virus mainly infects CD4-postive T-lymphocytes and macrophages and advanced disease is clinically characterized by opportunistic infections and malignancies due to progressive $CD4^+$ lymphocyte depletion [4].The virus appeared to be transmissible due to sexual or blood-blood contact.



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MORBIDITY AND MORTALITY WEEKLY REPORT

Epidemiologic Notes and Reports

Kaposi's Sarcoma and *Pneumocystis* Pneumonia Among Homosexual Men — New York City and California

During the past 30 months, Kaposi's sarcoma (KS), an uncommonly reported malignancy in the United States, has been diagnosed in 26 homosexual men (20 in New York City [NYC], 6 in California). The 26 patients range in age from 26-51 years (mean 39 years). Eight of these patients died (7 in NYC, 1 in California)—all 8 within 24 months after KS was diagnosed. The diagnoses in all 26 cases were based on histopathological examination of skin lesions, lymph nodes, or tumor in other organs. Twenty-five of the

Figure 1. The first report of a cluster Pneumocystis Carinii Pneumonia in 1981 – the starting point of the HIV pandemic. CDC Morbidity and Mortality Weekly Report, June 5, 1981.

The identification of HIV was followed by an intensive quest for effective antiretroviral drugs. Zidovudine (AZT) was the first therapeutic option available (1987) but showed only temporary effect as after a few months of its use viral resistance developed in the setting of monotherapy [5].In the early nineties other nucleoside reverse transcriptase inhibitors (NRTIs) were introduced – e.g. didanosine (ddI), stavudine (d4t) and zalcitabine (ddC) – but viral resistance also rapidly developed if used asmono- or dual therapy [6,7].It was till December 1995 that Saquinavir, the first protease inhibitor (PI) became available [8,9] – followed by other PIs and the non-nucleoside reverse transcriptase inhibitors (NNRTIs) [10,11]. The availability of these new drug classes caused a revolution in the treatment and prognosis of HIV. With the triple-

drug regimens, the virus was targeted at different stages of the replication cycle and thereby the risk for resistance development was greatly reduced. From this point on, a significant amount of the patients was able to reach prolonged virological suppression and immune reconstitution [12].The introduction of combination antiretroviral therapy (cART) led to a spectacular increase in life expectancy for HIV-positive individuals soon after its introduction [13–16]. But despite the success,therapy remained challenging due to high pill burden and serious, sometimes lethal side-effects [17,18].

Nowadays, a great proportion of the patients uses single-tablet regimens (STR) with an excellent safety profile. Life expectancy is almost equal to the general population as much less patients are threatened by opportunistic infections and AIDS-related malignancies[19]. Therefore, the current challenges in HIV-related care mainly concentrate on comorbidities and their prevention. As far as comorbidities are regarded, cardiovascular diseases have gained great attention and have been studied extensively [20,21]. Although less studied, the impact of HIV and its treatment on other organ systems is also significant. Liver-related mortality is the leading organ-specific cause of death in HIV-infected patients [14]. Reduced bone mineral density affects approximately half of the HIV-positive population, which is significantly more prevalent than most other comorbidities [22,23] and its importance grows with the aging of HIV patients. The high prevalence is associated with extensive diagnostic testing and therapeutic interventions and therefore with high costs. Finally, limited attention is paid to the long-term pulmonary outcomes in HIV despite the reduced quality of life reported by patients with chronic dyspnea [24]. Therefore, these three organ systems are the focus of this thesis.

Liver-related morbidity: drugs, viruses and fat

Liver-related morbidity among HIV-positive individuals is common [25]: hepatitis B virus (HBV) co-infection occurs in 5-15% of the HIV-positive population [26], hepatitis C virus (HCV) co-infection in approximately 6% [27] and non-alcoholic fatty liver disease (NAFLD) affects 30-40% of the HIV-positive individuals [28]. Even in the current era of cART, liver-related disease is the second leading cause of non-AIDS related mortality in HIV-infected individuals after malignancy [14]. When taking a closer look, there is a shift in the etiology of liver disease in HIV: In the pre-cART era, liver-related morbidity was mainly the result of opportunistic infections like Mycobacterium avium complex and Cryptosporidium [29], but soon after the introduction of cART the focus changed towards drug-induced hepatotoxicity.

The impact of drug-induced hepatotoxicity by antiretroviral drugs has been described extensively [30]:especially ddI, d4T and ddC – early generation NRTIs – were well known for their hepatotoxic potential. Mitochondrial toxicity is considered to be the main

responsible pathophysiological mechanism [31]. The clinical picture is characterized by liver failure, lactate acidosis and microvesicular steatosis [32,33]. The hepatotoxic potential of the modern NRTIs like tenofovir, lamivudine (3TC) and abacavir (ABC) is considered to be low [34]. In addition, the first generation NNRTIs (nevirapine and efavirenz) are also associated with hepatic injury and – in rare cases – the development of fulminant hepatic failure [35–38]. Most events occur in the first weeks after the initiation of NNRTI therapy, but data describing the toxic potential in long-term use are lacking.These data are of great importance, considering the need for life-long therapy and the fact that NNRTIs are still widely used as anchor in cART [39].

As antiretroviral therapy became safer over the years, there was increasing attention for the impact of certain types of viral hepatitis in HIV.Hepatitis A virus (HAV) outbreaks are increasingly observed among MSM [40]. The natural course is generally self-limiting also in the HIV-positive population [41]. As mentioned earlier, HBV and HCV are highly prevalent among HIV-positive patients as a result of the shared routes of transmission (blood-borne, sexual and vertical transmission). Last but not least, Hepatitis E virus (HEV) in increasingly recognized as a causative agent of unspecified hepatitis in the general population [42] – but currently there are no data that show a higher prevalence in the HIV-positive population.Considering the self-limiting course of HAV and the decreasing numbers of HIV/HCV co-infection in the era of effective anti-HCV drugs [43], we focused on HBV and HEV and their impact on HIV in this thesis.

Data from the eighties and nineties showed that patients with HIV/HBV coinfection display higher mortality rates than patients with either an HIV or HBV mono-infection [44,45].The introduction of 3TC brought a glimmer of hope as this drug has activity against both HIV and HBV [46]. Unfortunately, viral resistance emerged rapidly in HBV during 3TC treatment and the treatment of HIV/HBV co-infected patients remained challenging [47]. In 2003, tenofovir disoproxil fumarate (TDF) was introduced – also possessing potent dual antiviral efficacy, but with a high genetic barrier for HBV resistance compared to 3TC [48,49]. Therefore, current guidelines recommend the use of a tenofovir-containing regimen as preferential treatment in HIV/HBV co-infected patients [50,51].Although several studies described how the introduction of cART has led to a decrease in all-cause mortality in the general HIV-positive population [13–16] , data on the impact in HIV/HBV co-infected patients are lacking.

As mentioned above, there is growing attention for HEV infection.In general, HEV infection has a subclinical course and its seroprevalence is high in the general population [52]. Although HEV infection is considered to be a self-limiting infection, in 2008 the first report of chronic HEV was published on a patient that received a

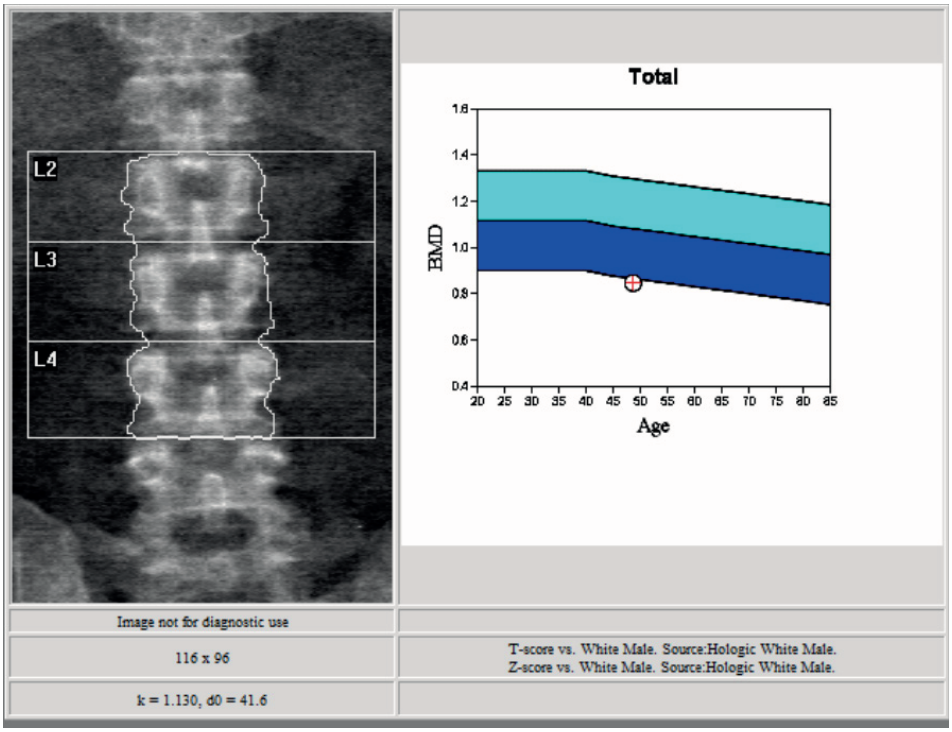
solid organ transplantation [53]. Small case series suggest that patients with chronic HEV infection display fast progression towards liver fibrosis and cirrhosis [54].These observations brought up the question whether HIV-infected patients with advanced immunodeficiency are also at risk for chronic HEV infection.Although there are no large scale reports on HEV chronicity in HIV, several cases have been reported in this population [55–57]. While HEV appeared to be the cause of unexplained liver enzyme abnormalities in the general population, it was unclear whether this was also significant in the HIV-positive population. Unexplained liver enzyme elevations are frequently encountered in this population and are commonly interpreted as drug-related [58] but a significant proportion might actually be the result of otherwise subclinical HEV infection.

Nowadays, while the impact of viral hepatitis is declining as a result of effective therapies and current cART displays limited hepatotoxic potential, NAFLD is the new kid on the block with respect to liver-related morbidity in HIV [25].In the general population, liver cirrhosis due to non-alcoholic steatohepatitis (NASH) has become the leading indication for liver transplantation [59]. After the successful introduction of cART, the HIV-positive population is increasingly adapting the common risk factor for NAFLD and NASH, namely obesity [60] – and soon NASH will replace viral hepatitis as most common liver-related disease.In a meta-analysis, the prevalence of NAFLD in the HIV-positive population was estimated around 35%, which is higher than the general population [28]. This high rate suggests that additional HIV-related factors play a role in the pathogenesis of NAFLD. Insulin resistance (IR) is considered to be the greatest risk factor for NAFLD and in the HIV-positive population IR is highly prevalent as it is driven by HIV-associated immune activation [61,62]. This link is supported by the high rates of steatosis in autopsy studies in the pre-ART era [63,64]. Moreover, certain antiretroviral drugs can also induce insulin resistance [65], hypertriglyceridemia and direct hepatic toxicity – this applies mainly to the early generation NRTIs [31,66]. The treatment of NAFLD in HIV should focus on diminishing immune activation by reaching full and sustained virological suppression and the use of antiretroviral drugs with a favorable metabolic profile. Furthermore, physicians should be aware of the increasing obesity rates in the HIV-infected population. At this moment, there are only a few effective medical therapies available for NALFD/NASH but some promising drugs are currently in the pipeline [67]. As HIV-positive patients live longer, the risk to develop NAFLD is increasing. In the setting of pre-existent liver disease – either virus-related or due to drug-induced toxicity – any additional damage to the liver should be avoided whenever possible.

Bone-related morbidity: Focus on pathophysiology and optimal screening

As mentioned earlier, the prevalence of reduced bone mineral density (BMD) is extremely high in the HIV-positive population and affects more than half of the patients [22] – outweighing the prevalence of cardiovascular disease [23]. A meta-analysis published in 2006 found a prevalence of osteoporosis (defined as a T-score ≤-2.5 measured by dual energy x-ray absorptiometry (DXA)) of 15%; osteopenia (defined as DXA T-score between -1 and -2.5) occurred in 52% of the population [68]. In this report, the risk for osteoporosis among HIV-infected patients is 3.6 fold elevated compared to matched, HIV-negative controls. These numbers are the result of a high prevalence of classical risk factors, but also due to specific HIV- and cART-related factors. The classical risk factors like low body mass index, hypogonadism, vitamin D deficiency and smoking are common in the HIV-positive population [69–71]. With respect to the HIV-specific factors, a prolonged period of immune activation and a low nadir $CD4^+$ cell count were implicated to be associated with reduced BMD [72] although other studies failed to establish this association [73].

The effects of cART on the occurrence of reduced BMD are better defined; especially the use of tenofovir disoproxil fumarate (TDF) was found to be associated with BMD loss when compared to other NRTIs, an effect that mainly occurs in the first 24 weeks of therapy [74].The pathophysiological mechanism of TDF-related BMD loss is subject of debate: a direct toxic effect on the bone, subclinical tubular dysfunction and parathyroid hormone (PTH)-driven increased bone resorption are the most accepted hypotheses [75].In 2016, tenofovir alafenamide (TAF) was introduced as an alternative with an improved renal and bone safety profile compared to TDF [76]. Several trials established that the use of TAF is associated with less BMD loss compared to TDF, although it should be noted that this difference is very small – approximately 3% at week 144 [77–80]. When patients receiving TDF-based cART were switched to TAF-based regimen a small but significant increase in BMD is observed [81]. It is implicated that the lower tenofovir levels in plasma in TAF are the reason for the favorable renal and bone safety profile [76], but the underlying pathophysiological mechanism of tenofovir-related bone mineral density loss remains unclear.In addition, the impact of decreased BMD on clinical relevant outcomes is subject of debate;a French case-control study showed no evidence for an excessive fracture risk in patients exposed to TDF [82].



Region	Area [cm^2]	BMC [(g)]	BMD [g/cm^2]	T-score	Z-score
L2	13.06	11.38	0.871	-2.0	-1.7
L3	14.64	11.90	0.813	-2.6	-2.3
L4	15.01	12.81	0.854	-2.1	-1.8
Total	**42.71**	**36.09**	**0.845**	**-2.5**	**-2.1**

Figure 2ab. The results of dual energy x-absorptiometry of a 50-year old male living with HIV, showing osteoporosis of the lumbar spine. Printed with the permission of the person concerned.

Not only the pathophysiological mechanism of reduced BMD in HIV is subject of ongoing research, there is also a need for optimal universal screening strategies to identify those patients at risk for osteoporosis. Considering the high prevalence especially in HIV-positive males aged ≥ 50 years and postmenopausal females, experts advocate standard DXA measurement in these patients [51,83].For patients aged 40-49 years without major risk factors for fractures – i.e. glucocorticoid use (≥ 5 mg x 3 months), a high risk of falls or the history of a fragility fracture – the use of Fracture Risk Assessment Tool (FRAX) is recommended to determine which patients should undergo BMD testing. FRAX is an algorithm including classical risk factors for osteoporotic fractures and was developed to estimate the 10-year probability of both major osteoporotic fractures (MOF) and hip fractures (HF) [84]. Despite the fact that FRAX is not originally meant as screening-tool

for osteoporosis, current HIV guidelines recommend that it is reasonable to assess BMD by DXA in HIV-infected patients aged 40-49 years if they have a 10-year probability of MOF exceeding 10% using FRAX [51,83].However, this approach in which a high FRAX score is used as surrogate outcome to identify patients at risk for osteoporosis has not been evaluated in this younger HIV-positive population. Studies in older patients demonstrated only moderate sensitivity of this policy [85–87]. Furthermore, studies investigating the absolute fracture risk among HIV-positive patients showed that this risk is low in the younger age group [88]. Therefore, the yield and cost-effectiveness of this screening is unclear.

Lung-related morbidity: Persistent effects after AIDS-related pulmonary infections?
In general, respiratory symptoms are among the most defining symptoms affecting quality of life. In the setting of chronic pulmonary disease, shortness of breath is considered as the most important determinant for reduced quality of life [24].Therefore, it is remarkable that long-term pulmonary outcomes in HIV-positive individuals are barely described in current literature.

In the Western world, PJP is currently still the most common AIDS-defining condition [89]. The clinical picture is characterized by a severe impairment of the pulmonary diffusion capacity due a massive influx of *Pneumocystis* and inflammatory cells in the pulmonary alveolus [90]. Besides antimicrobial therapy, there is an important role for corticosteroids in case of profound hypoxemia [91]. It is hypothesized that massive lysis of *Pneumocystis* after the initiation of antimicrobial therapy provokes a strong inflammatory response [92]. This response leads to progression of the alveolar infiltrates and worsening of the pre-existing hypoxemia. For some physicians the use of corticosteroids in this situation feels contra-intuitive as an already weakened immune system is further suppressed. Old data showed that the use of corticosteroids decreases the $CD4^+$ cell count on the short term [93,94]. However, there are no data whether the use of corticosteroids leads to suboptimal immunological recovery in HIV.

Despite the effectiveness of antimicrobial therapy,in the early cART era HIV-positive patients with PJP still had an in-hospital mortality risk of approximately 10% [95]. Nowadays, survival will be higher as the result of the current available antiretroviral therapy. Subsequently, the question was raised whether surviving PJP might affect long-term pulmonary outcomes. This could be of particular importance considering the increased incidence of pulmonary morbidity in HIV population. Earlier data showed that HIV-positive patients are at risk for obstructive pulmonary disease, but the exact prevalence of diffusion impairment is unknown [96]. In a recent meta-analysis, the overall prevalence of chronic obstructive pulmonary disease (COPD)

was 10.5%; when compared to HIV-negative controls the pooled odds ratio was 1.14 (95% confidence interval (CI) 1.05-1.25), after adjustment for tobacco consumption even higher [97]. The high prevalence of COPD in the general HIV-positive population emphasizes the need for more information regarding outcomes in patients with a history of PJP. After all, these patients did experience an additional hit on their pulmonary function due to PJP; older studies established that PJP is accompanied by the destruction of alveolar surfactant[98].This additional hit – which might transpose towards permanent diffusion impairment – could seriously jeopardize the pulmonary function in this specific group.

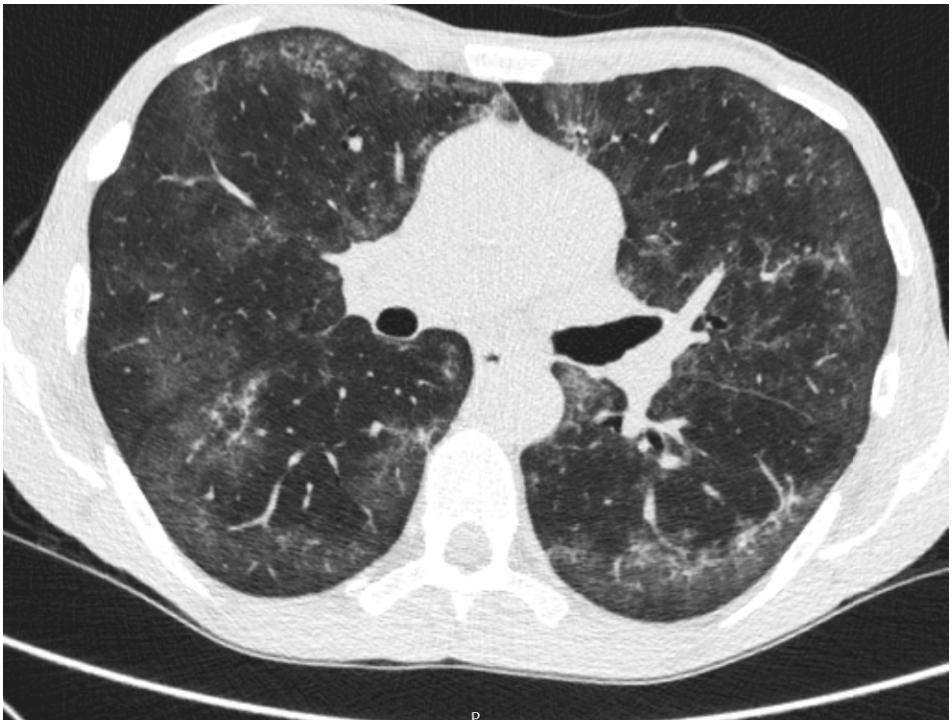


Figure 3. A high-resolution CT scan in a HIV-positive patient with Pneumocystis jirovecii pneumonia, showing diffuse ground glass opacities. Printed with the permission of the person concerned.

OUTLINE OF THIS THESIS

As previously described, there are several areas of uncertainty with regards to liver-, bone- and pulmonary-related disease in the HIV-positive population.While the life expectancy in HIV is approaching that of the general population, these co-morbidities are increasingly important. In this thesis we focus on the knowledge gaps which were addressed in the introduction.

In the first part, we evaluate whether patients who receive long-term NNRTI-containing cART are at increased risk for hepatotoxicity and describe the time course in which hepatotoxicity occurs **[chapter 2].**However, only a part of the unexplained liver enzyme elevations are caused by NNRTIs. In the following chapters we therefore address two other topics that might explain the observed liver test abnormalities: viral hepatitis and non-alcoholic fatty liver disease.In **chapter 3**, we investigate whether hepatitis E virus is a common causative agent for hepatitis in the HIV-positive population. Subsequently, in **chapter 4** we describe the impact of Hepatitis B virus in the HIV-positive population in the Netherlands and study the changes in the risk for all-cause and cause-specific mortality in the era of cART in these co-infected patients. Finally, in a narrative review we elaborate on the epidemiology, therapy and future perspective of non-alcoholic fatty liver diseases in HIV-positive individuals – which has become one of the leading causes of liver disease in this population **[chapter 5]**.

In the second part of the thesis, we focus on the bone-related morbidity in HIV-positive patients. We assess whether the switch of TDF to TAF results in lower parathyroid hormone levels – which would give us an insight on the mechanism in tenofovir-related bone mineral density loss **[chapter 6]** and evaluate whether current screening policy for osteoporosis is young HIV-infected patients is useful **[chapter 7]**.

In part III, we describe the outcomes in patients with a history of *Pneumocystis jirovecii* pneumonia (PJP) both short and long term.We study differences in immunological recovery between patients who received corticosteroids and those who did not **[chapter 8].** In **chapter 9** we present the results of a retrospective analysis assessing the prevalence of persistent pulmonary function abnormalities in patients with a history of PJP.

Finally, in **chapter 10** the main findings of this thesis are discussed and the future expectations and ideas for future research are presented.

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Part I

Liver-related morbidity in HIV-infected patients

Chapter 2

No increased risk of hepatotoxicity in long-term use of nonnucleoside reverse transcriptase inhibitors in HIV-infected patients

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ABSTRACT

Objective

The aim of this study was to assess the incidence of hepatotoxicity in patients who had used nonnucleoside reverse transcriptase inhibitors (NNRTIs) for at least 3 years.

Methods

The study group consisted of HIV-infected patients under follow-up at our clinic, who had continuously used an NNRTI-containing regimen (efavirenz or nevirapine) for at least 3 years. Patients who had used protease inhibitors (PIs) for the same time span constituted a control group. Hepatotoxicity was graded according to the modified AIDS Clinical Trial Group grading system, using alanine aminotransferase (ALT) as a marker.

Results

One hundred and twenty-two patients on an NNRTI regimen and 54 PI-using patients were included in the analysis. The mean follow-up time was nearly 6 years. Eighteen NNRTI-using patients (14.8%) developed a clinically relevant (≥grade II) event of hepatotoxicity during treatment; five of them (4.1%) developed severe hepatotoxicity (≥grade III). No significant difference in the hepatotoxicity rate was seen between NNRTI- and PI-using patients (14.8 *vs.* 18.5%, respectively; $P = 0.52$) or between patients using efavirenz and nevirapine (13.8% *vs.* 16.7%, respectively; $P = 0.51$). A hepatitis C virus (HCV) coinfection was associated with and increased risk of the development of hepatotoxicity during NNRTI therapy [odds ratio (OR) 1.83; 95% confidence interval (CI) 1.33–4.24; $P < 0.01$]. Finally, we observed that more hepatotoxic events occurred during the first year of NNRTI therapy compared with the entire period after 1 year (6.6 *vs.* 2.8 events, respectively, per 100 person-years of treatment; $P = 0.04$).

Conclusions

Long-term NNRTI use was not associated with a higher risk of clinically significant liver toxicity in patients who had been treated with NNRTI for at least 3 years.

INTRODUCTION

Following the introduction of highly active antiretroviral therapy (HAART), the life expectancy of HIV-infected patients has increased dramatically. In view of the facts that HAART is a life-long therapy and a successful regimen is intended to be used for many years, the long-term side effects of these antiretroviral drugs are receiving increasing attention. The nonnucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz (EFV) and nevirapine (NVP) are frequently used as components of current antiretroviral regimens. However, NNRTIs are known for their potential to cause hepatotoxicity, which can lead to morbidity and therapy switches. Different studies have reported a cumulative incidence of severe hepatotoxicity varying from 1.4 to 15.6% in patients treated with NVP [1–5] and from 1.1 to 10% in patients treated with EFV [1–4]. However, the follow-up time in these studies was relatively short, up to 3 years. Data focusing on hepatotoxicity in long-term NNRTI use are scarce [6]. The aim of this retrospective cohort analysis was to evaluate whether the incidence of hepatotoxicity increases with increasing duration of a NNRTI regimen.

PATIENTS AND METHODS

Patient population

All HIV-infected patients under follow-up at our clinic until 1 November 2009, who had been receiving an NNRTI-containing HAART regimen for ≥3 years, were identified. Patients were included in the analysis if they had continuously used the same NNRTI for a minimum of three years and if at least one serum alanine transaminase (ALT) value per year was available throughout the treatment period. The control group consisted of patients who had exclusively received a protease inhibitor (PI)-based regimen for at least 3 years and for whom ALT data were available. Demographic, pharmacological and laboratory data at the start of therapy were retrieved from the clinical database and patient records. Patients were considered to have a hepatitis B virus (HBV) infection when HBV DNA and/or the HBV surface antigen (HBsAg) were found at baseline. Hepatitis C virus (HCV) infection was defined as the detection of HCV RNA by polymerase chain reaction. Patients for whom baseline ALT was unknown and those with acute viral hepatitis during NNRTI treatment were excluded from the analysis.

Hepatotoxicity

Hepatotoxicity was graded according to the modified toxicity scale of the AIDS Clinical Trials Group [1]. Serum ALT values were used rather than serum aspartate aminotransferase (AST) or cholestatic liver enzymes, as ALT is considered to be a more specific marker for liver damage [7]. To limit the possibility of the outcomes being biased as a result of pre-existent ALT values above the upper limit of normal (ULN), a distinction was made between patients with a normal serum ALT at baseline and patients with baseline values above the ULN. The ULN in our laboratory was changed on 30 November 2006; therefore, the ULN may differ between patients (50 U/L before this date and 35 U/L after this date). Liver enzyme elevations (LEEs) were graded as fold change compared with the ULN in patients with normal ALT at baseline, or compared with a baseline ALT (BL) in patients with elevated values at the start of therapy (grade 0: < 1.25 x ULN/BL; grade 1: 1.25–2.5 x ULN/BL; grade 2: 2.6–5.0 x ULN/BL; grade 3: 5.1–10 x ULN/BL; grade 4: > 10 x ULN/BL). LEEs of grade 2 or higher were considered to be clinically relevant; grade 2 was considered as moderate and grades 3 and 4 as severe hepatotoxicity. Every year of therapy in which LEEs occurred was considered as one event of hepatotoxicity. When multiple clinically relevant LEEs took place during one year, the highest elevation was used for the analysis.

Statistical analysis

To compare baseline characteristics, the χ^2 test was used for the analysis of categorical variables and the Mann–Whitney test for continuous variables. The incidence of liver toxicity was expressed as the number of episodes per 100 person-years for each treatment group (the ratio of the observed number of events to the total number of patient years of exposure). The χ^2test was used to calculate the statistical significance. All reported *P*-values are two-sided, with *P*-values of < 0.05 being considered statistically significant. The statistical analysis was performed using SPSS (version 15.0; SPSS, Chicago, IL).

RESULTS

We identified 146 patients under follow-up at our clinic who had been receiving an NNRTI-containing HAART regimen for at least 3 years without interruption. Twenty-one patients were excluded because ALT results were not available during treatment or at baseline. Three of these patients (14.2%) eventually developed moderate LEEs. Another three patients experienced an episode of acute viral hepatitis and were excluded. Therefore, 122 patients were included in this analysis. The median follow-up time after the start of the NNRTI-containing regimen was nearly 6 years (range 36–108 months). Eighty patients (65.6%) received an EFV-containing regimen and 42 patients (34.4%) an NVP-containing regimen. Fifty-four patients who received a PI-based regimen were used as the control group. Only 14 patients (26%) received a boosted-PI containing regimen, reflecting the fact that many patients in our cohort started a PI-based regimen before the introduction of PI boosting. During follow-up, there were many alterations in the HAART backbone – which generally consisted of two or more nucleot(s)ide reverse transcriptase inhibitors – in both groups. These are not described in detail. The baseline characteristics of the patients are displayed in Table 1.

Table 1. Pretreatment demographic and clinical characteristics.

	NNRTI-based regimen *(n=122)*	PI-based regimen *(n=54)*	P-value
Gender [n(%) male]	100 (82)	43 (80)	0.714
Age [mean (range)]	42.6 (19-71)	37.0 (12-71)	*0.002*
HCV coinfection [n(%)]	8 (6.6)*	10 (18.5)**	*0.016*
HBV coinfection [n(%)]	4 (3.3)	1 (1.9)	0.612
Baseline ALT (IU/L) [mean (range)]	32 (3-272)	34 (3-122)	0.384
Baseline CD4 count (cells/μL) [mean (range)]	452 (6-1528)	221 (4-766)	*<0.001*

ALT, alanine aminotransferase; HBV, hepatitis B virus. *Five patients were treated for their hepatitis C virus (HCV) infection during nonnucleoside reverse transcriptase inhibitor (NNRTI) therapy; two of them showed a sustained virological response (SVR). ** Three patients were treated for their HCV infection during protease inhibitor (PI) therapy; two of them showed an SVR.

Missing data were equally distributed in the two groups. Seventy-two patients (59.5%) in the NNRTI group and one patient (1.9%) in the PI group had undetectable viral load at baseline, defined as HIV RNA < 400 HIV-1 RNA copies/mL. Patients in the NNRTI group had a significantly higher CD4 count than those in the PI group (452 *vs.* 221 cells/mL, respectively; $P < 0.01$). These differences could be explained by the fact that many patients were switched from a PI-based regimen to an NNRTI-based regimen when these drugs became available. Regarding NVP users, 50% of female patients and 40% of male patients had CD4 counts < 250 and < 400 cells/mL, respectively, at the start of the

treatment. In 2006, the new therapeutic strategy was implemented which restricted the use of NVP to patients with CD4 cell counts below these cut-off values, because higher CD4 cell counts were shown to be associated with an increased risk of hepatotoxicity [8]. The results of viral hepatitis coinfection (both HBV and HCV) evaluations were available for 92.6% of all patients.

Liver enzyme elevations during treatment

During NNRTI therapy, 14.8% of the study population experienced a > 2.5-fold elevation in serum ALT (grade ≥ 2) (Fig. 1). A total of 21 events of moderate and five events of severe liver toxicity were observed during 691 person-years of therapy (PYT) with NNRTI (3.04 and 0.72 per 100 PYT, respectively). A sub-analysis showed an equal risk for the development of hepatotoxicity in patients using NVP and those using EFV (16.7% *vs.* 13.8%, respectively; $P = 0.51$). Regarding the incidence of severe hepatotoxicity, two events in the EFV group (0.47 per 100 PYT) and three events in the NVP group (1.1 per 100 PYT) were observed ($P = 0.37$). The baseline CD4 counts in these three NVP using patients with severe LEEs before the start of HAART were 508, 120 and 19 cells/mL, respectively. No significant difference in moderate hepatotoxicity between NVP and EFV was demonstrated (1.8 *vs.* 3.3 per 100 PYT, respectively; $P = 0.250$).

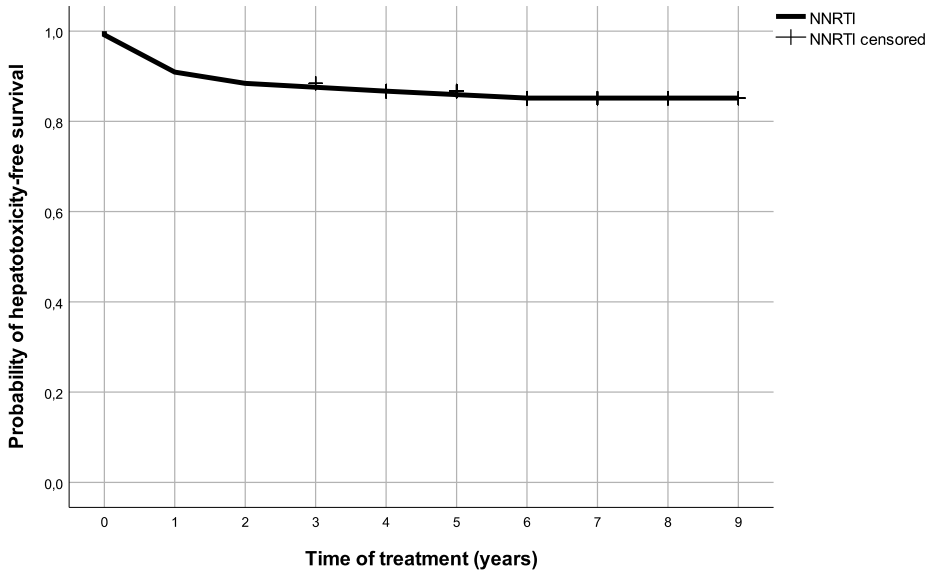

Figure 1. Kaplan-Meier curve illustrating the hepatotoxicity-free survival in patients using a NNRTI-based HAART regimen. NNRTI, nonnucleoside reverse transcriptase inhibitor

In the PI group, 10 patients (18.5%) showed at least grade 2 hepatotoxicity; 22 events of moderate and three events of severe hepatotoxicity were seen during the 468 PYT, with no significant difference in incidence between the NNRTI and PI groups (14.8% *vs.* 18.5%, respectively; $P = 0.52$). However, the two groups differed significantly in the baseline incidence of HCV coinfection, which is known to be associated with an increased risk of hepatotoxicity [1]. Excluding all HCV-positive patients from the analysis gave a cumulative incidence of 12.3% for NNRTI-using patients *vs.* 9.1% for those using PIs ($P = 0.57$). In the univariate analysis, only HCV coinfection was associated with the development of hepatotoxicity in the NNRTI group [odds ratio (OR) 1.83; 95% confidence interval (CI) 1.33-4.24; $P < 0.01$]. Hepatotoxicity was observed in 50% of coinfected patients compared with 12.3% in patients without HCV infection ($P < 0.01$). No association was detected between the development of LEEs and age, gender, HBV status, undetectable HIV RNA level at baseline, pretreatment ALT values or CD4 cell counts.

Short- vs. long-term incidences of hepatotoxicity

In the NNRTI group, eight events of hepatotoxicity in 122 PYT were observed in the first year of therapy (6.6%), while for the whole period beyond 1 year 16 episodes in 569 PYT were found (2.8%; $P = 0.04$). Thus, the risk of developing hepatotoxicity was significantly higher in the first year after NNRTI treatment initiation.

Clinical outcomes in the NNRTI group

All hepatotoxic events in our population occurred in 18 patients; four of them (22.2%) accounted for multiple LEEs over the years. All of these patients continued their NNRTI use despite these multiple events. Five patients (4.1%) accounted for the five events of severe hepatotoxicity; none of them discontinued therapy because of this severe event, as the LEE had either resolved spontaneously or was attributed to other medication which was adjusted or stopped. One hundred and four patients (85.2%) did not show any clinically relevant hepatotoxicity.

DISCUSSION

This retrospective cohort analysis shows that prolonged use of NNRTIs (≥3 years) is not accompanied by an increasing incidence of hepatotoxicity compared with the first year of NNRTI use. We did not find a difference in the risk for developing hepatotoxicity between patients using either EFV or NVP for ≥3 years. HCV coinfection was independently associated with the development of LEEs during NNRTI treatment. The incidence of hepatotoxicity did not differ significantly between the NNRTI and PI groups. To date, a few studies have reported on the liver safety of long-term use of NVP and EFV [6,9–11]. Most of these studies gave rates of discontinuation because of hepatotoxicity, but did not give the exact number of hepatotoxic events or describe the time course. The significantly higher risk of liver toxicity in patients with an HCV/HIV coinfection using NNRTI has been reported before [1,12]. The intriguing question is whether the occurrence of LEEs in these patients is indeed a marker of drug toxicity or the result of liver enzyme fluctuations in the context of chronic viral hepatitis infection [13]. It is remarkable that, although a higher proportion of patients in the PI group were HIV/HCV-coinfected, there was no difference with the NNRTI group in terms of the number of hepatotoxic events. We observed a distinct pattern in the incidence of hepatotoxic events over the years of therapy. The number of hepatotoxic events in the first year of NNRTI therapy was significantly higher than in the period that followed. It seems that the number of events declined over the years, even in patients who had already experienced moderate to severe hepatotoxicity in the first year. This observation suggests that it is safe to continue NNRTI-based HAART, even in case of (asymptomatic) hepatotoxicity in the first year of therapy. The debate regarding the pathogenesis of NNRTI-induced hepatotoxicity is ongoing. Hypersensitivity, mitochondrial toxicity, immune reconstitution and cumulative toxicity have been proposed as possible mechanisms [14,15], although a multifactorial cause is probably the most likely explanation. Our observation of a decline in the incidence of hepatotoxic events over the years suggests that a cumulative toxic effect is unlikely.

The representativeness of our study population for all NNRTI-using patients might be a point of discussion. After all, patients who develop (severe) toxicity in the first 3 years of therapy are less likely to continue using NNRTIs. However, our short-term outcomes did not differ significantly from those reported by Brück *et al.* [2] and Palmon *et al.* [3]. In those cohorts of all patients starting an NNRTI-based regimen, severe toxicity rates of 1.7% and 1.1%, respectively, were observed, compared with 1.4% in the first year in our cohort. Regarding moderate hepatotoxicity, the incidence in our group was actually slightly higher than the incidence reported by Brück *et al.* (4.9% *vs.* 3.1%, respectively). Because of the retrospective design of the study, we were not always able to obtain

complete biochemical data and information regarding the use of other potentially hepatotoxic medication (e.g. prescribed by the patient's general practitioner or over-the-counter drugs) and excessive alcohol use. We also cannot exclude the possibility that some of the LEEs were side effects of the nucleoside reverse transcriptase inhibitor (NRTI) backbone. The number of patients with an HBV or HCV coinfection was low; this raises the question of whether our results would have been the same if this group had been larger. In summary, long-term NNRTI use was not associated with a higher risk of clinically significant liver toxicity in our group of patients who had not discontinued their therapy within the first 3 years of treatment. There does not seem to be a long-term cumulative hepatotoxic effect of these antiretrovirals.

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Chapter 3

Hepatitis E virus as a causative agent of unexplained liver enzyme elevations in HIV-infected patients.

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ABSTRACT

Recent reports showed that hepatitis E virus (HEV) infection is a common causative agent for unexplained hepatitis and that HEV infection can become chronic in immunocompromised patients. The findings merit further evaluation in people living with HIV (PLWH). After all, unexplained liver enzyme elevations (LEEs) are frequently encountered during follow-up of PLWH and they are at risk for chronic hepatitis in case of advanced immunodeficiency. In this study we assessed the seroprevalence of HEV antibodies in 56 patients with otherwise unexplained LEEs and 50 patients without LEEs. A total of four patients (3.8%) tested positive for anti-HEV IgG antibodies, one of these patients was in the non-LEEs group. Only one patient with LEEs tested positive for both HEV IgM and IgG antibodies, suggestive for an acute HEV infection. We conclude that HEV infection is rarely a causative agent of unexplained LEEs in HIV – but one should remain aware of the sensitivity of serological tests in advanced immunodeficiency.

INTRODUCTION

Liver enzyme elevations (LEEs) in HIV-positive patients are mostly due to either viral hepatic coinfections or antiretroviral drug–induced hepatotoxicity, leaving a small number of patients with unexplained LEEs [1]. However, it was recently shown that a part of the unexplained LEEs in the general population is attributable to locally acquired hepatitis E virus (HEV) genotype 3 infections—probably as the result of zoonotic transmission [2,3]. Recent reports have shown that HIV-infected patients are at risk for persistent HEV carriage due to their immunocompromised state [4–6]. Since then, the prevalence of HEV in several cohorts of HIV-infected patients has been described [7–12].Renou *et al.* [9] observed a geographical gradient in HEV seroprevalence, being lower in HIV-infected patients in northern France compared with the south. However, data from northern Europe are scarce [13,14]. Considering the possibility of zoonotic transmission [15,16] and the high HEV seroprevalence among pigs and boars in these northern European countries [17,18], investigating the HEV epidemiology in susceptible populations in these countries is important. Therefore, we conducted a study in the Netherlands evaluating the prevalence of locally acquired HEV infection in a cohort of HIV-infected patients with and without unexplained LEEs under follow-up in our clinic.

METHODS

The laboratory results of all HIV-infected patients under follow-up from January 2007 through February 2011 in the University Medical Center Utrecht (n = 1117) were reviewed for the occurrence of elevations in serum alanine aminotransferase (ALT) above the upper limit of normal (45 U/L in our hospital). Because we focused on locally acquired infections with HEV genotype 3, immigrants from regions where non–genotype 3 HEV is highly prevalent were excluded. A number of selected serum samples of unexplained LEEs from the period - negative for hepatitis A, B, or C infection - were obtained for HEV serological testing. As controls, another group of white patients—matched for age and gender—without LEEs was selected and a random sample obtained. All samples were tested—together, in 3 batches—for the presence of both anti-HEV IgM and IgG antibodies with a commercial enzyme-linked immunosorbent assay kit (recomWell HEV; Mikrogen GmbH, Neuried, Germany); positive and borderline results were tested by line blot assay (recomLine HEV IgG/IgM; Mikrogen GmbH). All tests were performed according to the manufacturer's instructions. Patient characteristics at the time the serum sample was obtained were registered.

Continuous data were expressed as median value (with range) and analyzed using the Mann–Whitney U test, whereas categorical data are given as a percentage (with number) and analyzed using the Fisher exact test. Results are 2-sided, and $P \leq 0.05$ was considered to be statistically significant.

RESULTS

A total of 56 HIV-infected patients with unexplained LEEs were selected and matched for age and gender to a total of 50 HIV-infected patients without LEEs. Except for serum ALT values, there were no significant differences in patient characteristics between both groups (Table 1).

Table 1. Patient characteristics.

	Patients with LEEs (n=56)	Patients without LEEs (n=50)	P-value
Age (yr), median (range)	42.6 (22-63)	43.7 (23-74)	0.88
Gender (male), n (%)	51 (91)	47 (94)	0.57
Serum ALT (U/L), median (range)	129.7 (46-972)	25.6 (16-43)	*<0.001*
CD4+ count (cells/mm^3), median (range)	410.9 (25-1090)	494.9 (107-932)	0.10
HIV RNA n (%)			0.63
<400 copies/mL	27 (48.2)	26 (48)	
>400 copies/mL	28 (50.0)	24 (52)	
Missing	1 (1.8)	0 (0)	
Receiving cART (%)			0.27
No	22 (30.3)	25 (50.0)	
Yes	34 (60.7)	25 (50.0)	

Abbreviations: ALT – alanine aminotransferase; cART – combination antiretroviral therapy

In the total population (N = 106), 4 patients (3.8%) tested positive for anti-HEV IgG antibodies. Only 1 patient without LEEs tested HEV IgG positive. Within the group of patients with LEEs, 2 patients were HEV IgG positive, whereas 1 patient tested positive or both anti-HEV IgM and IgG antibodies indicating an acute HEV infection. However, a subsequently performed HEV polymerase chain reaction (PCR) on this serum sample was negative. No PCR was performed in the other patients who tested positive for IgG only because this indicated past exposure to HEV. No IgM was detected in any of the collected samples from the group without LEEs.

Regarding the 4 HEV-positive patients with detectable IgG antibodies, 3 (75%) were men, with 2 of them being men who have sex with men. Mean age was 32 years, and the mean baseline CD4+ count was 528 cells per cubic millimeter. None of these characteristics differed significantly from the IgG-negative population. Again, only the baseline mean ALT value was significantly higher in the anti-HEV IgG antibody–positive patients (288 vs. 76 IU/L, P=0.01).

DISCUSSION

This study shows that locally acquired HEV infection occurs among HIV-positive patients in the Netherlands—probably as the result of zoonotic transmission [20]. Furthermore, we observed an anti-HEV IgG seroprevalence rate of 3.8% in our whole cohort of HIV-infected patients, with a higher prevalence in those with LEEs (5.4% vs. 2%, P = 0.62). In the patients without LEEs, we did not detect any acute HEV infections. This observed seroprevalence is similar to the one found in the general Dutch population [2].In contrast, 2 recent studies [13,14] reported a slightly higher anti-HEV IgG seroprevalence of 4.9% (German cohort) and 9.4% (British cohort) in a predominantly northern European population. No acute infections were detected in these cohorts either. Differences in patient characteristics and different available assays [20,21] may have contributed to the observed differences inepidemiology between the study populations.

Because recent observations have suggested a delayed anti-HEV seroconversion in HIV patients with very low CD4 counts [4], questions have been raised about the suitability of these tests for the detection of HEV infections [7], and together with the varying diagnostic value between the available assays, they support the argument that serological screening alone may be insufficient to diagnose HEV and should be complemented with detection of HEV by PCR. The recent developments regarding the epidemiology of HEV and the altered natural course in immunocompromised patients led to the discussion of whether HIV-positive patients with unexplained LEEs should be tested for HEV infection [22]. With only 1 immunoblot- confirmed case of locally acquired acute HEV infection in the Dutch HIV-positive population, this might not be a cost-effective strategy.

In conclusion, although HEV infection should be considered as a causative agent of unexplained LEEs in HIV-infected patients in northern European countries, the number of acute infections is low.

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Chapter 4

Decreased all-cause and liver-related mortality risk in HIV/Hepatitis B virus coinfection coinciding with the introduction of tenofovir-containing combination antiretroviral therapy

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ABSTRACT

Background
The development of efficacious combination antiretroviral therapy (cART) has led to a dramatic decrease in mortality in HIV-positive patients. Specific data on the impact in HIV/Hepatitis B virus (HBV) co-infected patients are lacking. In this study, all-cause and cause-specific mortality risks stratified per era of diagnosis is investigated.

Methods
Data were analyzed from HIV/HBV co-infected patients enrolled in the ATHENA cohort between 1 January 1998 and 31 December 2017. Risk for (cause-specific) mortality was calculated using Cox proportional hazard regression analysis, comparing patients diagnosed before 2003 with those diagnosed ≥ 2003. Risk factors for all-cause and liver-related mortality were also assessed using Cox proportional hazard regression analysis.

Results
1301 HIV/HBV co-infected patients were included (14,882 person years of follow-up). One-hundred and ninety-eight patients (15%) died during follow-up. The adjusted hazard ratio (aHR) for all-cause mortality in patients diagnosed in or after 2003 was 0.50 (95% confidence interval (CI) 0.35-0.72)) relative to patients diagnosed before 2003. Similar risk reduction was observed for liver-related (aHR 0.29 (95% CI 0.11-0.75) and AIDS-related mortality (aHR (95% CI 0.44 (0.22-0.87)). Use of a tenofovir-containing regimen was independently associated with a reduced risk of all-cause and liver-related mortality. Prior exposure to didanosine/stavudine was strongly associated with liver-related mortality. Ten percent of the population used only lamivudine as treatment for HBV.

Conclusion
All-cause, liver- and AIDS-related mortality risk in HIV/HBV co-infected patients has markedly decreased over the years, coinciding with the introduction of tenofovir. Tenofovir-containing regimens, in absence of major contraindications, should be strongly encouraged in this population.

INTRODUCTION

Approximately 38 million people are currently living with HIV worldwide [1] with Hepatitis B virus (HBV) being a common coinfection in this population; an estimated 5-20% of HIV-positive patients are co-infected with HBV, but these estimates vary between risk groups and geographical regions – with the highest prevalence in Sub-Saharan Africa and Asia [2].In the Netherlands, 6 percent of all HIV-positive patients registered in the AIDS Therapy Evaluation in the Netherlands (ATHENA) observational cohort have ever tested positive for hepatitis B surface antigen (HBsAg) [3]. Data from the era during which effective anti-HBV therapy was not widely available show that patients with HIV/HBV coinfection display faster progression towards end-stage liver disease (ESLD) and have higher (liver-related) mortality rates compared to patients with either a HBV or HIV mono-infection [4].A more recent study demonstrated that in the current antiretroviral era, co-infected subjects had no increased risk for ESLD compared to HBV mono-infected patients, but their risk for all-cause and liver-related mortality was still significantly higher [5].

Lamivudine (3TC) became available in the mid-nineties and due to its dual activity against HIV and HBV, was an ideal therapeutic option for HIV/HBV coinfection [6]. Nevertheless, viral resistance rapidly emerged with the use of this agent [7].In 2003, tenofovir disoproxil fumarate (TDF) was introduced – also with potent dual efficacy, but with a higher genetic barrier to resistance [8]. Therefore, current guidelines recommend the use of a tenofovir plus either lamivudine or emtricitabine containing regimen as preferential treatment in HIV/HBV co-infected patients [9].

Earlier studies showed that the introduction of combination antiretroviral therapy (cART) has led to a dramatic decrease in all-cause mortality in the general HIV-positive population [10]. Data focusing on changes in mortality among HIV/HBV co-infected patients, particularly as more potent anti-HBV agents became available, are sparse. Considering the high prevalence and potential burden of liver-related disease, such data are of major interest.The main objective of this study was to describe mortality risk for HIV/HBV co-infected patients stratified by calendar periods of HIV-diagnosis, in relation to changes in HIV/HBV treatment including the introduction of tenofovir and the declining use of more toxic antiretroviral drugs.Furthermore, we aimed to identify risk factors for all-cause and liver-related mortality in this specific population.

METHODS

Study population

We performed a longitudinal analysis among HIV/HBV co-infected patients from the ATHENA observational cohort, which was initiated in 1998. Data are collected by the HIV Monitoring Foundation and cover 98% of all patients with a confirmed HIV-infection in care in the Netherlands. Medical history and data prior to 1998 were collected retrospectively. The structure of the cohort and procedures are described elsewhere [11].All patients aged ≥18 years with HIV/HBV coinfection in care between 1 January 1998 and 31 December 2017 were included in the analysis. HBV infection was defined by two consecutive HBsAg-positive and/or HBV DNA detectable results during a period of ≥6 months. Patients with evidence of hepatitis C virus (HCV) infection (i.e. a positive HCV RNA polymerase chain reaction (PCR)) were excluded from analysis.

Collected variables

Patients' demographic, clinical, and laboratory data were collected during follow-up. Laboratory data included HIV RNA viral load, HBV DNA viral load, $CD4^+$ cell count and alanine aminotransferase (ALT) levels. Laboratory data were retrieved time-updated per year If multiple results were available during the yearly interval, the last available measurement was used. If $CD4^+$ cell count and/or ALT were missing in a certain year, the last available observation was carried forward. Until April 2012, ALT levels were only collected if exceeding three times the upper limit of normal (ULN) and thus all missing ALT levels before this date were assumed to be ≤3x ULN.Due to varying levels of assay detection thresholds over the study period, undetectable HIV-RNA was defined at <400 copies per milliliter.

Treatment data and treatment periods

Treatment data included past and current use of antiretroviral agents, based on information provided by the patients' treating physicians in their medical record.We focused on the use of nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) with activity against both HIV and HBV: tenofovir (either disoproxil fumarate or alafenamide) (TDF/TAF) and 3TC.The use of the NRTIs stavudine (d4T) and didanosine (ddI) was also evaluated considering their hepatotoxic potential [12].

We defined two periods of HIV diagnosis calendar time based on both effectiveness of cART regimens and availability of potent anti-HBV treatment: diagnosis prior to 2003 (when cART was more readily available with only 3TC), and between 2003-2017 (with frequent use of more modern antiretroviral regimens and availability of tenofovir). In

subsequent analysis, we further stratified the period 2003-2017: between 2003-2007 - with less frequent use of TDF - and between 2008-2017 - with the advent of integrase strand inhibitors (INSTIs) as recommended first-line backbone therapy and widespread TDF/TAF use in the Netherlands[13,14].

Endpoints

The primary endpoint in this study was mortality. These data were obtained from the ATHENA cohort database, which used the Cause of Death (CoDe) protocol to classify causes of death [15]. Causes of death were categorized into liver-related, AIDS-related, non-AIDS malignancy, cardiovascular disease (CVD), non-natural, unknown or other. In addition, we assessed the occurrence of severe chronic liver disease (SCLD).In the ATHENA cohort, SCLD was categorized as either presumptive or definitive. In case of documented evidence of variceal bleeding, hepatic encephalopathy, hepatorenal syndrome *and/or* portal hypertension or cirrhosis by radiography or endoscopy, the patient was considered to have presumptive SCLD.If the abovementioned conditions were present in combination with histological evidence of severe chronic liver disease (histopathological Metavir score F3-F4) or a transient elastography ≥ 8 kPa, patients were considered to have definitive SCLD.

Statistical analyses

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).All reported P values were two-sided and $P < 0.05$ was considered to be statistically significant.

Follow-up began at the time patients first entered HIV care and consented to be enrolled into the ATHENA cohort [11].Since identification of HBV co-infection could be biased through failure to test for HBsAg, particularly in the earlier years of the ATHENA cohort, we decided to define the beginning of follow-up based on HIV-diagnosis.Patients diagnosed with HIV prior to the start of the ATHENA cohort were left-censored on 1 January 1998. Follow-up continued until the date of death, date last seen if lost to follow-up (withdrawn from care for >1 year), date of moving abroad or 31 December 2017, whichever occurred first.Since HBsAg-seroclearance was not systematically assessed across the entire study population, we decided not to censor after HBsAg-loss. The cumulative incidence rates of progression to all-cause mortality were modeled across calendar periods using Cox proportional hazards regression. Hazards ratios (HR) and their 95% confidence intervals (CI) were estimated with HIV diagnosis <2003 as the reference group. The cumulative incidence rates of progression to the different causes of death were also modeled across calendar periods with proportional hazards regression, while taking into account competing risk of other causes of death using

the method by Fine and Gray [16].To account for patient differences across periods, HRs were adjusted by age at inclusion, mode of HIV transmission and region of origin. Gender was not included in the adjustment due to its overlap with other demographic variables.In order to identify risk factors for all-cause and liver-related mortality, we used the proportional hazards models above to estimate differences in cumulative incidence between levels of risk factors. Variables with an associated P-value ≤0.2 in univariable analysis were included in the multivariable model without further selection. For this risk factor analysis, we included treatment variables that directly reflected certain calendar periods, hence these periods were not considered as independent variables.

RESULTS

Study population characteristics

In the period ranging from 1 January 1998 until 31 December 2017, a total of 24,413 adult HIV-positive individuals had been in care and registered in the ATHENA cohort. Of them, 1398 patients met the definition for chronic HBV infection, after excluding 97 patients with an HCV coinfection we included 1301 individuals in our analysis. The vast majority were male with the most common HIV/HBV transmission risk group being men who have sex with men (MSM) [table 1].Description of the cohort at specific time points is also summarized in Table 1, showing that the cohort was ageing and that there was a shift in cART composition.

Median follow-up was 11 years (interquartile range (IQR) 6-17), totaling 14 882 person years of follow-up – including 12 577 years of follow-up on antiretroviral treatment.The majority of the study population entered the cohort during 1998-2005 with a remarkable decline in new cases thereafter [figure 1]. The large number of individuals entering in 1998 was mostly due to left-truncation. In 2017, no newly HBV-diagnosed patients entered the study cohort.Over the entire study period, eighty-six patients (7%) were lost to follow-up and eighty-five patients (7%) had moved abroad.

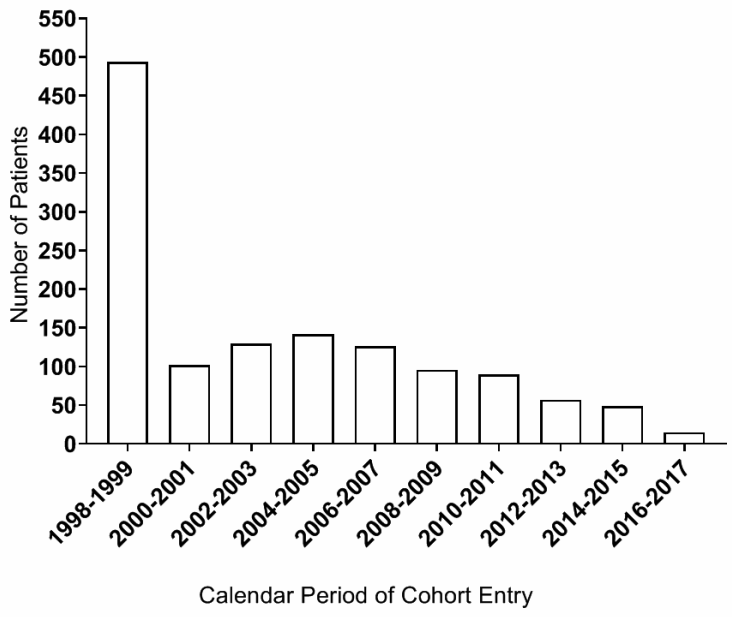

Figure 1.*Bar graph displaying the number of patients entering the cohort over the time of follow-up per two years.*

Table 1. Characteristics of study participants. Data are n (%) unless otherwise specified.

	Characteristics at cohort entry & death	
	Cohort entry	Death
Number of patients	1301 (100)	198 (100)
Men	1125 (86.5)	178 (89.9)
Age [Median (IQR)]	36.6 (30.8-43.7)	50.0 (43.1-58.3)
• <40	825 (63.4)	35 (17.7)
• 40-49	332 (25.6)	64 (32.3)
• 50-59	111 (8.5)	56 (28.3)
• ≥60	32 (2.5)	43 (21.7)
HIV transmission route		
• MSM	810 (62.2)	117 (59.1)
• Heterosexual	383 (29.4)	39 (19.7)
• IVD users	26 (2.0)	16 (8.1)
• Other	82 (6.3)	26 (13.1)
Region of origin		
• Europe	762 (58.6)	141 (71.2)
• Sub-Saharan Africa	315 (24.2)	30 (15.2)
• Caribbean	54 (4.2)	4 (2.0)
• Asian	63 (4.9)	6 (3.0)
• Other	106 (8.1)	17 (8.6)
HIV diagnosis era		
• <1998	401 (30.8)	118 (59.6)
• 1998 – 2002	261 (20.1)	37 (18.7)
• 2003 – 2007	332 (25.6)	29 (14.6)
• 2008 - 2017	307 (23.6)	14 (7.1)
$CD4^+$ cell count cells/mm^3 [Median (IQR)]	310 (150 – 506)	250 (110-490)
• <200 cells/mm^3	408 (31.6)	76 (38.3)
HIV Viral Load		
• Detectable (≥400 copies/ml)	973 (74.8)	69 (34.8)
• Undetectable (<400 copies/ml)	327 (25.2)	129 (65.2)
ALT level		
< 3.0x ULN*	1124 (86.4)	178 (89.9)
≥ 3.0x ULN	177 (13.6)	20 (10.1)
History of ddI/d4T exposure		88 (44.4)
• Median use in years [IQR]		3 (1-5)
• Cumulative use in years		320
Previous treatment with mono- or dual therapy		77 (38.9)
Ever TDF/TAF exposure		115 (57.5)
• Median use in years [IQR]		1 (0-4)
• Cumulative use in years		497
Time between HBV diagnosis and start TDF/ TAF in years [Median (IQR)]		6 (0.5 – 10)

	Characteristics at follow-up dates		
	1st of January 2003	1st of January 2008	31st of December 2017
	425 (100)	676 (100)	931 (100)
	370 (87.1)	583 (86.2)	812 (87.2)
	38.8 (33.7-44.0)	42.1 (36.5-47.5)	49.8 (43.0 – 55.4)
	242 (56.9)	271 (40.1)	171 (18.4)
	135 (31.8)	278 (41.1)	300 (32.2)
	42 (9.9)	104 (15.4)	323 (34.7)
	6 (1.4)	23 (3.4)	137 (14.7)
	294 (69.2)	453 (67.0)	623 (66.9)
	112 (26.4)	190 (28.1)	259 (27.8)
	5 (1.2)	7 (1.0)	8 (0.9)
	14 (3.3)	26 (3.8)	41 (4.4)
	274 (64.0)	409 (60.5)	568 (61.0)
	82 (19.3)	147 (21.7)	198 (21.3)
	21 (4.9)	35 (5.2)	43 (4.6)
	18 (4.2)	31 (4.6)	49 (5.3)
	32 (7.5)	54 (8.0)	73 (7.8)
	243 (57.2)	243 (35.9)	243 (26.1)
	182 (42.8)	182 (26.9)	182 (19.5)
	N/A	251 (37.1)	251 (27.0)
	N/A	N/A	255 (27.4)
	470 ((300-641)	480 (340-650)	630 (440-820)
	63 (14.8)	57 (8.4)	34 (3.6)
	162 (38.1)	207 (30.6)	48 (5.2)
	263 (61.9)	469 (69.4)	883 (94.8)
	393 (92.5)	631 (93.3)	912 (98.0)
	32 (7.5)	45 (6.7)	19 (2.0)
	159 (37.4)	172 (25.4)	177 (19.0)
	3 (2-5)	4 (2-6)	4 (2-6.5)
	515	744	820
	126 (29.6)	134 (19.8)	140 (15.0)
	63 (14.8)	425 (62.9)	866 (93.0)
	0 (0-0)	1 (0-4)	8 (5-11)
	81	1352	7250
	2 (0-6)	2.5 (0-7)	2 (0-6)

Table 1. Continued.

	Characteristics at cohort entry & death	
	Cohort entry	Death
Ever exposure to other drugs with anti-HBV activity		
• Lamivudine		145 (73.2)
• Emtricitabine		60 (30.3)
• Entecavir		4 (2.0)
• Telbivudine		2 (1.0)
Current ART use		
• None		73 (36.8)
• Mono- or dual therapy		10 (5.0)
• NNRTI based cART		44 (22.2)
• Protease inhibitor based cART		43 (21.7)
• Integrase strand inhibitor based cART		6 (3.0)
• Other		22 (11.1)

Abbreviations: ALT – Alanine Aminotransferase; ART – Antiretroviral Therapy;cART – Combination Antiretroviral Therapy;d4T – Stavudine;ddI – Didanosine; HBV – Hepatitis B virus;HIV – Human Immunodeficiency Virus; IQR – Interquartile Range; IVD – Intravenous Drugs;

Antiretroviral Therapy and Efficacy

Over time an increasing proportion of patients used antiretroviral therapy (ART), 76% on 1 January 2003 compared to almost everyone (98.8%) on 31 December 2017.Four-hundred and fifty-five patients (35%) did not start antiretroviral therapy in the first year they entered the cohort. Besides these patients, an additional number of two-hundred and six patients (16%) interrupted cART at some point during follow-up. In general, virological and immunological response was excellent at end of follow-up, with 96% of the patients having a HIV viral load <400 copies/ml and a median CD4 cell count of 630 cells/mm^3 (IQR 440-820).

Over time, 1095 patients (84%) were exposed to a TDF/TAF containing regimen, accounting for 8233 person years of tenofovir exposure.On 31 December 2017, 905 of the 931 patients (97%) remaining in follow-up were using drugs with activity against HBV (Figure 2).Most of them (n=766, 83%) were on a TDF/TAF containing regimen and sixteen (1%) patients were on entecavir. One-hundred and twenty-three patients (10%) were using only lamivudine as the HBV-active component of their ART regimen. Of these patients, sixty-two (50%) had been switched to dolutegravir/abacavir/lamivudine when this single-tablet regimen became available. Twenty-six patients did not use any anti-HBV therapy; twelve of whom displayed HBsAg seroclearance (i.e. loss of HBsAg, not necessarily with acquisition of anti-HBsAg antibodies) during follow-up.HBV DNA

Characteristics at follow-up dates		
1st of January 2003	1st of January 2008	31st of December 2017
313 (73.6)	456 (67.5)	536 (57.6)
1 (0.2)	111 (16.4)	782 (84.0)
24 (15.6)	33 (4.9)	47 (5.0)
2 (0.5)	2 (0.3)	4 (0.4)
104 (24.5)	144 (21.3)	11 (1.2)
16 (3.7)	8 (1.1)	25 (2.8)
169 (39.7)	299 (44.2)	367 (39.4)
102 (24.0)	180 (26.6)	173 (25.7)
N/A	N/A	285 (30.6)
34 (8.0)	45 (6.7)	70 (7.5)

MSM – Men who have Sex with Men; N/A – Not available; NNRTI – Non-nucleoside Reverse Transcriptase Inhibitors; TAF – Tenofovir Alafenamide Fumarate; TDF – Tenofovir Disoproxil Fumarate; ULN – upper limit of normal * (35 IU/L)

monitoring was infrequent in our cohort, with only 210 (16%) patients having an HBV DNA viral load measurement in their last year of follow-up.The lack of monitoring was not only observed among patients using highly effective agents, such as TDF/TAF or entecavir, but also among those using 3TC as single anti-HBV agent.Of the 123 patients with only 3TC for HBV treatment on 31 December 2017, 19 (15%) had an available HBV DNA viral load during the last year of follow-up. Of these 19 patients, twelve (63%) had a HBV DNA level < 40 copies/ml.

Trends in mortality risk

A total of 198 patients (15%) died during follow-up – with the most common causes of death being AIDS-related (24%), liver-related (19%) and non-AIDS-related malignancies (19%) (Table 1).As shown in Table 2, patients diagnosed after 2002 were significantly less likely to die from any cause compared to those diagnosed prior to 2003 (adjusted HR (aHR) 0.50 (95% CI 0.35-0.72), with similar effect sizes for the periods 2003-2007 (aHR 0.53 (95%CI 0.35-0.80) and 2008-2017 (aHR 0.46 (95%CI 0.26-0.81)). A similar reduced risk after 2002 was observed with respect to liver-related mortality (aHR 0.29 (95%CI 0.11-0.75)) and AIDS-related mortality (aHR 0.44 (95%CI 0.22-0.87)), but not for the other causes of death. We observed decreasing trends in mortality for the 2003-2007 and 2008-2017 subcategories with respect to liver-related, AIDS-related and non-AIDS malignancy related death, but these results did not reach statistical significance.

Table 2. Unadjusted and adjusted hazard ratios of underlying cause of death per time era of HIV diagnosis.

	<2003 *(reference)*
All-cause *(n=198)*	
• Non-adjusted	1.0
• Adjusted*	1.0
Liver-related *(n=38)*	
• Non-adjusted	1.0
• Adjusted*	1.0
AIDS-related *(n=48)*	
• Non-adjusted	1.0
• Adjusted*	1.0
Non-AIDS defining malignancy *(n=38)*	
• Non-adjusted	1.0
• Adjusted*	1.0
Cardiovascular disease *(n=16)*	
• Non-adjusted	1.0
• Adjusted*	1.0
Non-natural *(n=10)*	
• Non-adjusted	1.0
• Adjusted*	1.0
Unknown *(n=18)*	
• Non-adjusted	1.0
• Adjusted*	1.0
Other *(n=30)*	
• Non-adjusted	1.0
• Adjusted*	1.0

*Adjusted for demographical factors (baseline age, HIV transmission route and region of origin)
†Estimates could not be calculated.

Two-period analysis	Three-period analysis	
≥2003	2003 - 2007	2008 -2018
0.55 (0.38-0.78)	0.53 (0.35-0.79)	0.60 (0.34-1.05)
0.50 (0.35-0.72)	0.53 (0.35 - 0.80)	0.46 (0.26 - 0.81)
0.30 (0.13-0.78)	0.33 (0.11-0.94)	0.21 (0.03-1.61)
0.29 (0.11-0.75)	0.34 (0.12-0.97)	0.17 (0.02 - 1.33)
0.48 (0.24-0.95)	0.52 (0.24-1.12)	0.39 (0.12 - 1.30)
0.44 (0.22-0.87)	0.49 (0.22 - 1.06)	0.33 (0.10-1.12)
0.95 (0.45-2.00)	0.90 (0.40-2.07)	1.09 (0.31 - 3.86)
0.78 (0.36-1.68)	0.85 (0.37-1.95)	0.61 (0.17-2.24)
0.44 (0.12-1.62)	0.67 (0.18-2.48)	†
0.35 (0.09-1.32)	0.66 (0.18-2.47)	†
0.84 (0.20-3.49)	0.40 (0.05-3.37)	2.27 (0.57-13.86)
0.90 (0.21-3.74)	0.44 (0.05-3.72)	2.19 (0.36-13.40)
0.40 (0.11-1.42)	0.38 (0.08-1.70)	0.43 (0.05-3.51)
0.33 (0.09-1.21)	0.34 (0.08-1.56)	0.30 (0.04-2.52)
0.65 (0.24-1.71)	0.32 (0.07-1.43)	1.41 (0.43 - 4.60)
0.72 (0.27-1.94)	0.39 (0.09 - 1.76)	1.34 (0.40 - 4.55)

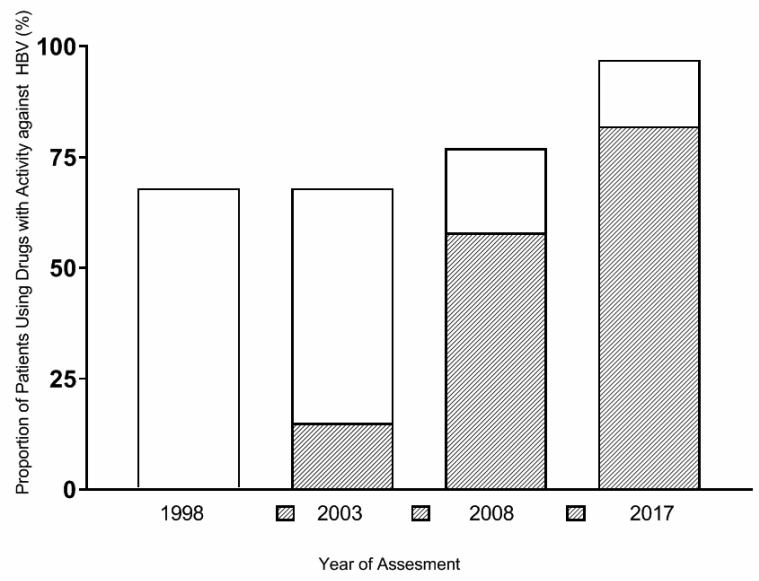

Figure 2. Graph bar displaying the proportion of HIV/HBV co-infected patients using drugs with activity against HBV (e.g. tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), lamivudine, entecavir, telbivudine, adefovir). The shaded area represents the proportion of patients using either TDF or TAF.The date of assessment was 1 January 1998, 1 January 2003, 1 January 2008 and 31 December 2017.

Risk factors for mortality

Lower age at baseline, being of non-European origin, deferral or interruption of antiretroviral therapy, having ALT levels <3.0x ULN and higher time-updated $CD4^+$ cell counts, as well as time-updated use of a TDF/TAF containing regimen were independently associated with a lower risk of all-cause mortality (Table 3). Patients using TDF/TAF had a significantly lower risk for all-cause mortality, with an aHR of 0.47 (95%CI 0.34-0.64) when compared to those who did not receive TDF/TAF treatment. Factors associated with all-cause mortality also applied for liver-related mortality, with the exception of non-European origin and deferralor interruption of antiretroviral therapy. Cumulative exposure to d4T and/or ddI was strongly associated with liver-related mortality aHR per additional year of exposure 1.15 (95%CI 1.02 – 1.29). The all-time risk for liver-related mortality among patients being exposed to ddI/d4T was 7.4% versus 1.6% in patients who never used these agents ($p < 0.001$).

Table 3. Adjusted hazard ratios for the composite endpoint of all-cause mortality and liver-related mortality

	All-cause mortality		Liver-related mortality	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Age at baseline (per 5 year increase)	1.35 (1.26-1.45)	<0.001	1.25 (1.05-1.50)	<0.001
Transmission route				
• MSM	1.0		1.0	
• Other (male)	1.41 (0.99-1.98)	0.051	0.91 (0.38-2.18)	0.950
• Other (female)	1.00 (0.57-1.73)	0.982	0.97 (0.27-3.40)	0.941
Region of origin				
• European	1.0		1.0	
• Other	0.62 (0.43-0.91)	0.013	0.52 (0.21-1.30)	0.162
ALT (category)*				
• <3x ULN	1.0		1.0	
• ≥3x ULN	2.43 (1.52-3.91)	<0.001	4.03 (1.63-9.94)	0.003
CD4+ count square root (per unit increase)*	0.87 (0.85-0.89)	<0.001	0.87 (0.82-0.91)	<0.001
Use of TAF/TDF*				
• No	1.0		1.0	
• Yes	0.43 (0.32 – 0.58)	<0.001	0.43 (0.21 – 0.86)	0.017
Cumulative ddI/d4T use (per year increase)*	1.01 (0.95-1.07)	0.749	1.14 (1.01-1.27)	0.028

Adjusted for baseline age, transmission route, region of origin, time-updated CD4+ cell count,time-updated ALT levels, time-updated use of TDF/TAF, time-updated cumulative ddI/d4T use. * Time-updated variables. Abbreviations: ALT – Alanine Aminotransferase; CI – Confidence Interval; d4T – Stavudine; ddI – Didanosine; MSM – Men who have Sex with Men; TAF – Tenofovir Alafenamide; TDF – Tenofovir Disoproxil Fumarate; ULN – Upper Limit of Normal (35 IU/L)

Liver-related morbidity

Of the 1301 patients included in the cohort, 325 (25%) were classified as having SCLD, 61 (5%) with a definitive and 264 (20%) with a presumptive diagnosis. The majority of the cases of definitive SCLD were established after 2003 (79% of the total) with the highest number of incident definitive SCLD in 2015 (n=10). Of the 61 patients with definitive SCLD, 41 (67%) were still alive at the end of the study period.During follow-up, there were 17 cases (1%) of hepatocellular carcinoma – with the first case diagnosed in 2003 and the last in 2013. One patient in our cohort underwent a liver transplantation as a result of ESLD.

DISCUSSION

This is one of the first studies evaluating trends in risk of mortality in HIV-HBV co-infected individuals during the cART era. We build on previous studies in the general HIV-positive population by assessing exclusively an HIV/HBV co-infected population and with extensive follow-up of up to twenty years.In this study we found a marked decrease in risk of all-cause, AIDS-related and liver-related mortality among patients diagnosed after 2002 compared to those diagnosed in earlier years. These findings are likely the result of a shift from moderately effective and potentially toxic antiviral therapy with limited anti-HBV activity towards highly potent and much less toxic antiretroviral drugs including agents with potent activity against HBV.

Several large cohort studies have established declining mortality rates in patients living or diagnosed with HIV during the modern cART era compared to earlier calendar periods. For example, the D:A:D Study Group showed a steep decline in mortality rates over the past decade for almost all underlying causes of death, with the exception of non-AIDS malignancy [10].Although 11% of almost 50 000 HIV-positive individuals included in this study had HIV-HBV coinfection, no analysis of mortality rates in this subgroup of patients was reported. In another study conducted in HIV-HBV co-infected patients, Klein *et al* [17] failed to demonstrate a significant decline in ESLD adjusted incidence rate ratios in the 'late cART era' (2006-2010) compared to the 'early cART era' (1996-2000). This may have been the result of a relatively short median follow-up time of 2.9 years and low uptake of anti-HBV treatment in the late cART era (only 65% of the HIV/HBV co-infected patients received tenofovir-containing cART).With a much longer follow-up and increased uptake of TDF/TAF-containing regimens in the ATHENA cohort, we were able to establish that the use of tenofovir was one of the strongest factors associated with a decrease in both all-cause and liver-related mortality.It was remarkable that the mortality risk for the separate calendar periods 2003-2007 and 2008-2017 was not significantly reduced compared to patients diagnosed in the pre-tenofovir era; this was probably the result of a lack of power leading to wide confidence intervals. Our findings are in line with numerous reports demonstrating that the use of tenofovir diminishes the risk for hepatocellular carcinoma [18] and all-cause and liver-related mortality [19].Taken together, tenofovir-containing regimens, in the absence of major contraindications, should be strongly encouraged in HIV/HBV coinfection.

In addition to the declining risk of mortality in this cohort, we observed that the influx of new HIV/HBV co-infected patients in our cohort decreased drastically from 2005, with no such patients entering the cohort in the last year of study period.The declining rate of new (acute) HBV infections matches trends observed in the general

European population [20]. In the ATHENA cohort, the overall prevalence of chronic HBV coinfection among HIV-positive individuals has decreased from 9.8% in 1998 to 5.8% in 2018[14].These trends are likely the result ofvaccination campaigns carried out by the Dutch Community Health Services in high-risk populations and awareness among HIV-treating physicians to offer HBV vaccination services to non-immune patients [21].Furthermore, there is increasing evidence for the prophylactic effects of TDF/TAF against HBV acquisition [22]. The extensive uptake of tenofovir-containing regimens provided a prophylactic benefit for HIV mono-infected patients and virological suppression leading to reduced onwards transmission for HBsAg-positive patients, both of which probably contributed to fewer new cases.

We observed a strong association between the cumulative usage of ddI/d4T and the risk for liver-related mortality.The hepatotoxic potential of these drugs was already recognized in the 1990s after several case reports described patients developing fulminant hepatitis with microvesicular steatosis by histological examination [23]. However, later reports identified the use of these thymidine - and deoxyadenosine analogues – to be also associated with the development of liver fibrosis and cirrhosis [12].Both d4T and ddI are strong inhibitors of the mitochondrial polymerase-γ, which is essential for mitochondrial DNA (mtDNA) replication. Inhibition of polymerase-γ leads to a loss of functional mitochondria and subsequently hepatic lipid accumulation and steatohepatitis [24]. The close interplay between these agents and mitochondrial toxicity could explain the increased risk of liver-related mortality with their use. Although d4T or ddI should no longer be used, clinicians should remain aware that patients ever exposed to these drugs may be at continued increased risk of liver-related disease.

Our data show that current treatment is highly successful, but challenges remain.A remarkable finding was that a significant part of the patients in our cohort did not receive any HBV-active agents or only lamivudine. Potential explanation may include patients having documented HBsAg clearance or controlled HBV infection with only lamivudine.Nonetheless, we found that several patients switched to a single-tablet regimen with possibly ineffective HBV-active agents. The introduction of tenofovir as part of ART mayhave reduced clinicians' concern about HBV coinfection, including the need for regular HBV-DNA monitoring, given thevirtual zero risk of selecting HBV-resistant mutants on tenofovir [25].Data from France have however reported approximately 15% of patients on TDF-containing cART to display persistent HBV viremia even after years of treatment [26]. Such patients are less likely to achieve HBsAg and HBeAg loss, but the impact on clinical endpoints is unknown.In addition, a recent study showed that the adherence for HCC screening in patients with HIV/HBV

coinfection with advanced fibrosis/cirrhosis is strikingly low [27]; in this cohort only 5-18% of the patients underwent bi-annual HCC screening in accordance to guidelines. Although treatment of HBV has become simpler in the tenofovir era, physicians need to remain vigilant on HBV management.Our HIV/HBV cohort is aging with currently nearly half of the patients being ≥ 50 years – placing this population at risk for several age-related diseases. Currently, non-alcoholic fatty liver disease (NAFLD) is one of the most pervasive liver-related co-morbidities in HIV-positive populations [28]. Even in the setting of optimal HIV/HBV treatment, a notable proportion of the co-infected patients display significant fibrosis [29] – also in the ATHENA cohort [30]. Therefore, the extra hit due to NAFLD could potentially lead to increased progression towards ESLD.

Our study has some limitations.Given the many changes in immunological recovery, viral suppression of both HIV and HBV, and improvement in antiretroviral medication occurring simultaneously over calendar periods, it is difficult to state which of these had a specific effect on liver-related mortality. Furthermore, the ATHENA cohort is a real-life cohort based on data that are gathered on different treatment sites during routine care. For this reason, other data related to liver-related or cause-specific mortality, such as time-updated alcohol use, liver-specific laboratory results and HBV serological markers, are not collected in a standardized manner and not all could be taken in account in the analyses.

In conclusion, we demonstrate that HIV/HBV co-infected patients diagnosed after 2002 were far more likely to survive than patients diagnosed in the early cART era, coinciding with the introduction of safe and highly efficacious antiretroviral medications against HIV and HBV. Importantly, our data demonstrate a need for continued awareness by physicians to maintain optimal HBV suppression. Future research should focus on how the aging HIV/HBV co-infected population is affected by co-morbidities like NAFLD, and how the decline in mortality risk compares to populations with HIV mono-infection.

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Chapter 5

A Review of Non-Alcoholic Fatty Liver Disease in HIV-infected Patients: The Next Big Thing?

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ABSTRACT

The burden of liver-related morbidity remains high among HIV-infected patients, despite advances in the treatment of HIV and viral hepatitis. Especially the impact of non-alcoholic fatty liver disease (NAFLD) is significant with a prevalence of up to 50%. The pathogenesis of NAFLD and the reasons for progression to non-alcoholic steatohepatitis (NASH) are still not fully elucidated, but insulin resistance, mitochondrial dysfunction and dyslipidemia seem to be the main drivers. Both HIV-infection itself and combination antiretroviral therapy (cART) can contribute to the development of NAFLD/NASH in various ways. As ongoing HIV-related immune activation is associated with insulin resistance, early initiation of cART is needed to limit its duration. In addition, the use of early generation nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) is also associated with the development of NAFLD/NASH. Patients at risk should therefore receive antiretroviral drugs with a more favorable metabolic profile. Only weight reduction is considered to be an effective therapy for all patients with NAFLD/NASH, although certain drugs are available for specific subgroups. Since patients with NASH are at risk of developing liver cirrhosis and hepatocellular carcinoma, several non-antifibrotic and antifibrotic drugs are under investigation in clinical trials to broaden the therapeutic options.The epidemiology and etiology of NAFLD/NASH in HIV-positive patients is likely to change in the near future. Current guidelines recommend early initiation of cART that is less likely to induce insulin resistance, mitochondrial dysfunction and dyslipidemia. In contrast, as a result of increasing life expectancy in good health, this population will adopt the more traditional risk factors for NAFLD/NASH. HIV-treating physicians should be aware of the etiology, pathogenesis and treatment of NAFLD/NASH in order to identify and treat the patients at risk.

INTRODUCTION

In the current era of combination antiretroviral therapy (cART), the all-cause mortality in Human Immunodeficiency Virus (HIV)-infected patients is low [1].However, liver-related complications remain one of the major causes of mortality in this population [2].Although viral hepatitis and excessive alcohol consumption are traditionally considered the most important causes of liver fibrosis and cirrhosis in HIV-infected patients, metabolic liver disease – mostly non-alcoholic fatty liver disease (NAFLD) - is increasingly recognized as an aetiological factor in the development of liver disease [2–5]. In fact, due to the introduction of effective therapies against viral hepatitis, it is likely that fatty liver disease will become the leading cause of liver cirrhosis in HIV-infected patients, as is already happening in the general population [6].It should not be forgotten that NAFLD can coexist with other liver diseases, which will lead to faster progression of fibrosis towards end-stage liver disease. The term NAFLD encompasses a wide spectrum of entities ranging from 'simple' steatosis to non-alcoholic steatohepatitis (NASH) [7]. Since the major risk factors for NAFLD –insulin resistance [8,9], mitochondrial dysfunction[10] and concurrent viral infections [11] - are highly prevalent in HIV-infected patients, this population is at risk for liver-related morbidity.

In this review, we describe the epidemiology and pathogenesis of NAFLD in the HIV-infected patients. We also discuss the future with respect to novel (antiretroviral) medication and anti-NAFLD interventions. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

EPIDEMIOLOGY

The worldwide prevalence of NAFLD varies greatly among different geographical areas, with the highest numbers in Northern America and the lowest in Africa; overall prevalence is estimated to be around 25% [12]. In addition to many epidemiological studies in the general population, several studies describe the prevalence in a general HIV-infected population, with a prevalence varying from 28 – 48% [13–18]. In the meta-analysis by Maurice et al. the prevalence of imaging-based diagnosis of NAFLD was 35%, which is higher than the general population [19]. In patient populations with persistent liver enzyme elevations, the prevalence is even higher with approximately three-quarter of the patients having NAFLD [20–24] [Table 1].However, Price et al. performed a matched-control study which surprisingly showed a higher prevalence of NAFLD in the HIV-negative control group compared to their HIV-positive counterparts (19% versus 13% P=0.02) [25]. This prevalence will be an underestimation of the 'real-life' prevalence due to exclusion of patients with a history of cardiovascular surgery and those with severe excess body weight. In another report, the imaging-based prevalence of NAFLD in a small cohort of HIV-positive patients was compared with HIV-negative controls [26]. The prevalence of steatosis tended to be higher among HIV-infected men compared to HIV-negative men (41% versus 33% - not statistically significant), but was lower in HIV-positive versus HIV-negative women (17% versus 33%). Both studies showed that classic risk factors – such as obesity-related insulin resistance - were the most important determinants for the development of NAFLD; HIV-related factors certainly contributed to this risk but its impact appeared to be limited. Therefore, it remains difficult to draw conclusions from these small studies because of methodological issues and small sample sizes; larger cross-sectional studies are thus needed.

Table 1. Overview of epidemiological studies describing the prevalence of NAFLD in HIV-infected patients.

Author, year and country	Population characteristics	Diagnostic test	NAFLD prevalence
Studies describing the prevalence of NAFLD in a general HIV-positive population			
Guaraldi et al.[15] (2008). Italy	N=225. Mean BMI: 23.8 Consecutive patients evaluated in metabolic clinic	CT-scan	36.9%
Crum-Cianflone et al.[14] (2009). USA	N=216. Mean BMI: 26.0 Consecutive patients in American military clinic.	Ultrasound	31.0%
Macias et al.[13] (2014). Spain	N=505. Median BMI: 23.2 Consecutive patients under follow-up in 5 different clinics	CAP	40.0%
Nishijima et al.[17] (2014). Japan	N=435. Mean BMI: 22.8 All HIV-infected patients that underwent ultrasound between 2004-2013. HBV&HCV co-infection excluded.	Ultrasound	31.0%
Lui et al.[16] (2016). China	N=80. Mean BMI: 23.6 Consecutive patients under follow-up in ID clinic.	MRS	28.8%
Vuille-Lessard et al.[18] (2016). Canada	N=300. Mean BMI: 26.6 Consecutive patients under follow-up in ID clinic.	CAP	48.0%
Studies describing the prevalence of NAFLD in HIV-patients with persistent liver enzyme elevations			
Lemoine et al.[23] (2006). France	N=14. Mean BMI: 23.0 HIV-mono infection with ALT levels ≥ 2x ULN over 3 months	Liver biopsy	57.1%
Ingiliz et al.[20] (2009). France	N=60. Mean BMI: 23.0 ALT or AST >2x ULN on two occasions in previous 6 months	Liver biopsy	60.0%
Sterling et al.[22] (2013). USA	N=14. Mean BMI: 29.9 AST or ALT 1.25-5x ULN over ≥ 6 months.	Liver biopsy	64.3%
Morse et al.[21] (2015). USA	N=62. Mean BMI: 28.0 ALT or AST > ULN on three occasions in previous 6 months.	Liver biopsy	72.6%
Lombardi et al.[24] (2017). UK	N=66. Mean BMI: Not available Retrospective cohort analysis of patients with ALT or AST > ULN on two occasions in six months.	Ultrasound	71.0%

Abbreviations: ALT-Alanine Aminotransferase; AST- Aspartate Aminotransferase; BMI - Body Mass Index; CAP – Controlled Attenuation Parameter; ID – Infectious Diseases; HBV – Hepatitis B Virus; HCV – Hepatitis C Virus; MRS – Magnetic Resonance Spectroscopy; NAFLD – Non-Alcoholic Fatty Liver Disease; UK – United Kingdom; ULN- Upper limit of Normal; USA – United States of America

DIAGNOSIS

The gold standard in diagnosing NAFLD is liver biopsy by which discrimination between steatosis and NASH is possible [27].Given its invasive nature with risks such as haemorrhagic complications and pain, liver biopsy is not useful as an epidemiological screening tool [28]. As a result, current cross-sectional epidemiological data are mainly based on surrogate markers such as imaging, rather than on histopathological data. Considering the higher sensitivity, lower costs and wide availability, ultrasound is preferred over CT-scanning for diagnosing NAFLD [29]. However, its sensitivity is limited in the setting of mild NAFLD and in morbidly obese patients [30,31]. Magnetic resonance spectroscopy proton density fat fraction (MRS-PDFF) is a very sensitive imaging modality, with a sensitivity up to 100% [31]. Due to its wider availability and low risk for sampling error, magnetic resonance imaging proton density fat fraction (MRI-PDFF) is increasingly used in research. In a recent review by Caussy et al, the differences and impact of MRS-PDFF and MRI-PDFF were discussed extensively. Considering the high costs and limited availability, MRI-based imaging is currently still not a useful screening tool and its use remains limited to research [32]. In 2010, a new non-invasive tool for the detection of steatosis called controlled attenuation parameter (CAP) was introduced [33]. It is an addition to the Fibroscan (Echosens, Paris, France) and measures steatosis simultaneously with fibrosis. CAP determines the total ultrasonic attenuation at a frequency of 3.5 MHz and is reported in decibel/meter (dB/m). In a meta-analysis by *Wang et al.* CAP provided good sensitivity and specificity [34].For example, the sensitivity for the detection of ≥ S1 steatosis – ≥ 5% of the hepatocytes affected with lipid accumulation – was 0.78 with an area under the curve (AUC) of 0.86 (95% CI 0.82 – 0.88). For stage S3 - > 66% of the hepatocytes affected – the sensitivity was 0.86 with an AUC of 0.94 (95% CI 0.91 – 0.96).It should be emphasized that none of the above-mentioned imaging modalities can assess the degree of NAFLD/NASH as assessed by the histological Brunt classification [35].Differentiating between NAFLD and NASHis important since 25-35% of patients with NASH eventually progress to liver fibrosis or even cirrhosis [36,37]. Using serum alanine aminotransferase (ALT) levels in addition to imaging to discriminate between steatosis and NASH turns out to be disappointing. Several studies showed that a significant proportion of the patients with biopsy-proven NASH had normal ALT values, although some studies suggested that the common laboratory cut-off value for ALT was too high [38–40]. There are several risk scores – such as the NAFLD Fibrosis score - available to evaluate which patients with NAFLD are at risk for (advanced) fibrosis, but its utility still needs to be evaluated in the HIV-positive population [41]. There are currently no guidelines that recommend universal screening for NAFLD in the general population or in

specific subpopulations. In contrast, the European AIDS Clinical Society (EACS) guideline recommends screening for NAFLD in HIV-infected patients with metabolic syndrome using ultrasound [42]. Since the prevalence of NAFLD among HIV-infected patients with persistent liver enzyme elevations is high, ultrasound screening should also be considered in this population.

PATHOGENESIS

NAFLD is a clinical-histological diagnosis characterized by the presence of fat accumulation in hepatocytes resulting in necro-inflammation and hepatocyte ballooning in absence of excessive alcohol use [43]. According to the American Association for the Study of Liver Diseases (AASLD), the threshold of 'significant alcohol consumption' is > 21 standard drinks per week on average for men and > 14 standard drinks on average in woman when evaluating patients with suspected NAFLD [44]. In general, NAFLD is considered to be the hepatic manifestation of the metabolic syndrome. The study of Mellinger et al. showed an association between presence of NAFLD and coronary artery calcium (OR 1.20 (1.10 – 1.30) P < 0.001) [45]. This finding was confirmed in the HIV-positive population in the study of Crum-Cianflone et al. showing that high coronary artery calcification values are associated with the presence of steatosis [46].Although the natural history of steatosis is usually benign, approximately 10% of patients with 'simple' steatosis progress to NASH. Eventually, 25-35% of the patients with NASH progress to liver fibrosis or even cirrhosis. Eventually, 10% of patients with cirrhosis develop hepatocellular carcinoma (HCC) over a period of several years [36,37] [Figure 1].

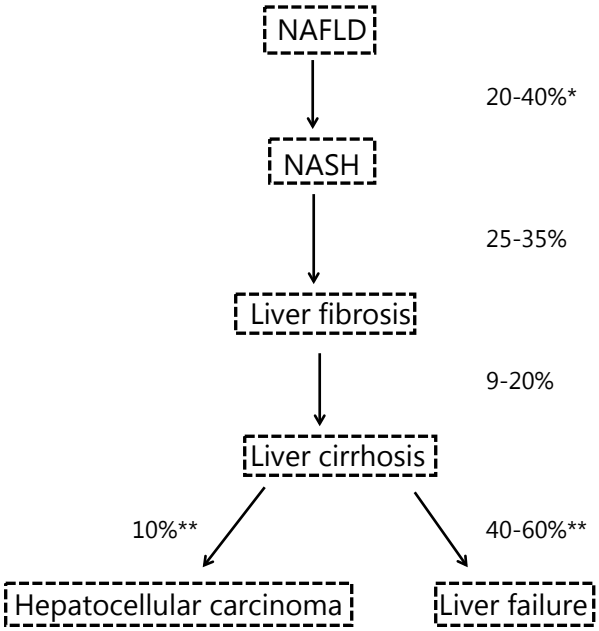

Figure 1. Schematic representation of the natural history of non-alcoholic fatty liver disease (NAFLD). NASH nonalcoholic steatohepatitis. *In 3–6 years of follow-up **In 5–7 years of follow-up

Insulin resistance (IR) is considered to be the key mechanism in the development of steatosis [47]. IR contributes to hepatic triglyceride accumulation in two ways. First, insulin normally supresses the activity of hormone-sensitive lipase (HSL).HSL is present in all adipocytes and is able to hydrolyse stored triglycerides into free fatty acids (FFAs) [48].In case of IR, the suppression of HSL is diminished, resulting in an increased hydrolysis in peripheral adipose tissue and therefore an increase in delivery of FFAs to the liver [49]. In the liver, the FFAs undergo esterification into triglycerides contributing to the process of steatosis. Second, the synthesis of lipids in the liver is increased in the setting of NAFLD. Donnelly et al. showed that hepatic *de novo* lipogenesis (DNL) attributes for 26.1%of the hepatic triglyceride formation in NAFLD-patients [50]. In contrast, the contribution of DNL in healthy individuals is less than 5% [51] [Figure 2].Several studies in animals and humans showed that both hyperinsulinemia and hyperglycaemia – as markers of IR – stimulate DNL by various pathways, leading to the development of steatosis [52]. The reason why steatosis progresses to NASH is still not fully elucidated. Traditionally, the two-hit theory was widely adopted [53]. In this theory, the 'first hit' is the hepatic accumulation of triglycerides – as a result of an increase in circulating free fatty acids (FFAs). The accumulation increases the susceptibility of the liver for additional hepatotoxic hits.Such an additional factor – 'second hit' - eventually leads to local inflammation and NASH. Suggested contributing factors (second hit) include mitochondrial dysfunction, adipose tissue dysfunction and genetic factors. However, some authors state that the 'two-hit' hypothesis is obsolete, as it is inadequate to explain the several molecular and metabolic changes that take place in NAFLD [47]. Therefore, current literature tends to speak of a 'multiple hit theory' that states that the pathogenesis of NASH is very complex and is the result of multiple hits and not limited to one additional hit besides the presence of steatosis.A third theory suggests that accumulation of triglycerides itself provokesoxidative stress and eventually leads to inflammation and NASH [54].

The human microbiome is increasingly recognized as an important factor in the development of NAFLD/NASH. Suggested mechanisms, like changes in intestinal permeability and production of microbe-derived metabolites, are more extensively addressed in the excellent recent reviews of *Leung et al.* and *Chu et al.* [55,56]. There are no specific studies linking microbiome dysfunction to development of NASH in HIV-infected patients. Furthermore, there is strong evidence on the impact of hereditary component with polymorphisms for hepatic lipid regulation and insulin signalling pathways, both in the HIV-infected and general population [57,58].

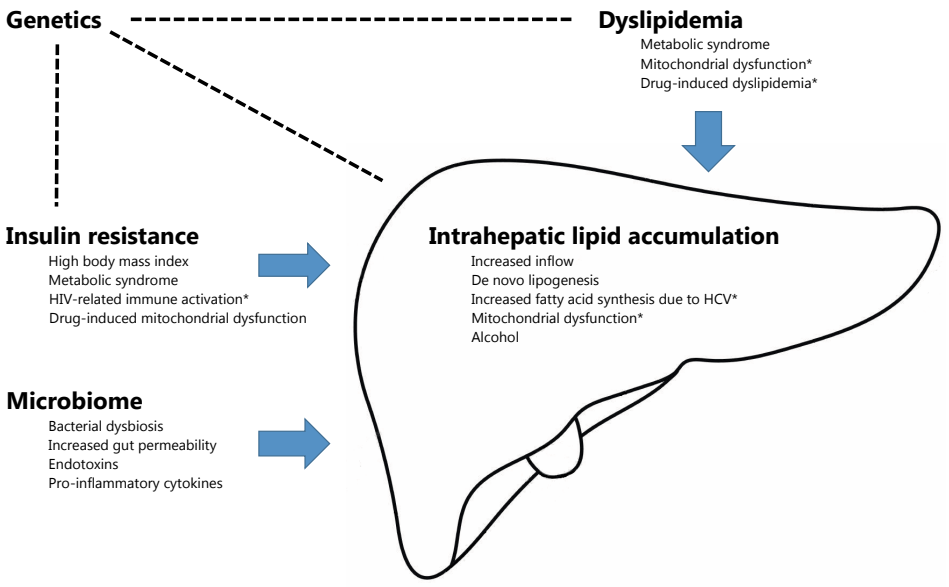

Figure 2. Schematic representation of the pathogenesis of NAFLD. As shown, there are four major hallmarks in the pathogenesis of NALFD—insulin resistance, dyslipidemia, hepatic accumulation and the microbiome—with a certain overlap between these factors. As mentioned, genetics play an important role in the overall pathogenesis influencing most of these factors. The arrows represent a direct impact of a certain hallmark on the development of NAFLD. The contributing factors are mentioned below the hallmarks. Risk factors more common in HIV-infected population are marked with an asterisk

NAFLD IN HIV-INFECTED PATIENTS

HIV as risk factor for NAFLD

Although NAFLD is common in HIV-infected patients as a result of traditional risk factors, it is suggested that its exact aetiology may differ from the general population [59]. Interestingly, histopathological studies from the pre-cART era report high numbers of steatosis in treatment-naïve HIV-infected patients – suggestive for a direct steatotic effect of HIV itself [60,61]. Furthermore, HIV-infected patients with NAFLD tend to have a lower BMI than HIV-negative controls with NAFLD [62]. These data suggest that HIV-related factors are associated with NAFLD, even in the absence of traditional risk factors such as obesity-related IR. Insulin resistance is highly prevalent – up to 35% - in HIV-infected patients, as a result of ongoing immune activation [63,64]. This phenomena has also been described in the setting of rheumatoid arthritis in which high levels of pro-inflammatory markers – e.g. TNF alpha and IL-6 – were associated with IR [65]. These markers have also been shown to be elevated in chronic HIV-infection, but it is unclear to what extend other immune activation markers like sCD14 and the expansion of CD8+/HLA-DR+ subtype T cells contribute to IR in HIV [66].Most studies on IR in this population describe cART-treated patients but only one study evaluated the occurrence of IR in treatment-naïve HIV-positive patients [67]. In this heterogeneous prospective cohort there was a clear association between advanced HIV-infection – defined as low CD4 counts and detectable viral load – and IR, measured by fasting lipids, glucoses and insulin levels. This and other observations strongly suggest a link between chronic HIV-induced immune activation and IR with subsequent steatosis development [68–70].

In contrast to the limited number of studies on the glucose haemostasis in HIV-positive patients, several studies were performed on the occurrence of dyslipidemia–also associated with NAFLD [50]. A study in the early nineties established that untreated HIV-infected patients with advanced immunodeficiency have higher triglycerides and FFA levels compared to healthy controls [71]. In addition to hypertriglyceridemia, untreated HIV-infected patients have decreased high-density lipoprotein (HDL) cholesterol and total cholesterol levels. These observations were confirmed in several other studies, although the mechanism of HIV-related dyslipidemia is poorly understood [72,73]. The study by El-Sadr et al. even established an association between high HIV RNA levels and hypertriglyceridemia [67].Based on these observations, it seems reasonable to assume that HIV-infection itself plays a role in the development of NAFLD.

Antiretroviral therapy as a risk factor for NAFLD

As the vast majority of HIV-infected patients treated with cART will reach virological suppression and (near) inhibition of immune activation, the impact of hypertriglyceridemia and IR caused by the virus itself will diminish. In contrast, certain antiretroviral therapy can contribute to the development of NAFLD [74]. cART seems to influence the development of NAFLD in two different ways.First, several antiretroviral drugs cause unfavorable metabolic changes such as dyslipidemia and insulin resistance [75]. Furthermore, the use of certain antiretroviral drugs is associated with mitochondrial dysfunction.

Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs)

Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) are still an essential part of current cART regimens. Especially early generation NRTIs are associated with insulin resistance and dyslipidemia [76–78]. The main driver for both metabolic disturbances is mitochondrial toxicity.The first reports of mitochondrial dysfunction in NRTIs were published in the early nineties, but this adverse event gained increasing attention after the introduction of combination therapy [79–81]. NRTI-related metabolic side-effects are caused by the inhibition of the replication of mitochondrial DNA (mtDNA) by binding to intra-mitochondrial polymerase gamma. This leads to impairment of the oxidative phosphorylation and promotes the formation of reactive oxygen species. These reactive oxygen species eventually damage the mtDNA even further, resulting in mitochondrial dysfunction [82].

NRTI-related mitochondrial dysfunction contributes to the development of NAFLD in several ways. First, both IR and dyslipidemia can be the result of mitochondrial dysfunction in the peripheral fat tissue [83].Even though the mechanism is not exactly clear, mitochondrial dysfunction seems to induce adipocyte apoptosis, leading to peripheral lipodystrophy. The clinical syndrome is characterized by the degeneration of peripheral fat tissue combined with metabolic changes such as IR and dyslipidemia [81,84].In addition to peripheral effects, mitochondrial dysfunction also occurs in the liver. Hepatic mitochondria play an essential role in the oxidation of FFAs. In the setting of mitochondrial dysfunction, mitochondria are unable to process this oxidation, leading to a local accumulation of triglycerides which is the hallmark of NAFLD [85]. Early generation NRTIs are most commonly associated with mitochondrial dysfunction; especially stavudine, didanosine, zalcitabine and - to a lesser extent – zidovudine [86]. Modern NRTIs – e.g. tenofovir, abacavir, lamivudine and emtricitabine – are rarely implicated in clinically significant mitochondrial dysfunction and are less likely to contribute to NAFLD development in this way [87].

Protease inhibitors (PIs)
Introduction of protease inhibitors (PIs) in 1995 broadened the possibilities in the treatment of HIV-infection. However, PIs display an unfavorable metabolic profile with an increased risk for insulin resistance and dyslipidemia.

Especially the early generation PI – e.g. indinavir and therapeutic-dosed ritonavir – were known for their ability to induce IR [88]. Two animal studies showed that these PIs act as potent isoform-specific inhibitors of the transport function of the GLUT4-receptor, resulting in hyperglycemia and hyperinsulinemia [89,90].Additional data suggested the ability to directly inhibit insulin secretion from the beta cells [91,92].In contrast to early generation PIs, current PIs like atazanavir and darunavir display a far more favorable profile with regard to IR [93]. Although the newer generation PIs seem to have little impact on the lipid levels in monotherapy, when combined with ritonavir or cobicistat as a pharmacological booster, these drugs still have an unfavorable lipid profile compared to most other classes of antiretroviral drugs [94–97]. The exact mechanism of PI-induced dyslipidaemia remains subject of debate. Data suggest that PIs attribute to an increase of ApolipoproteinB which transports LDL- and VLDL-cholesterol and triglycerides in the circulation [98]. Secondly, experimental research in mice showed that ritonavir inhibits the clearance of triglycerides from the circulation [99].

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
The amount of data describing the metabolic profile of the non-nucleoside reverse transcriptase inhibitors (NNRTIs) is limited compared to the number of studies for PI-based treatment. Rilpivirine and nevirapine have a more favorable profile compared to efavirenz [100,101]. Etravirine does not seem to influence lipid levels when compared with placebo.[102]Recently, the new NNRTI Doravirine was approved by the FDA; in the DRIVE-AHEAD and DRIVE-FORWARD Trials this novel drug showed lower triglyceride and LDL levels when compared to Efavirenz and Darunavir [103,104].

Integrase Strand Transfer Inhibitors (INSTI)
In 2008, the first integrase strand transfer inhibitor (INSTI) Raltegravir was introduced. This new drug class is generally considered advantageous in respect with the metabolic profile [105,106]. The lipid profile of the recently approved Bictegravir is comparable to Dolutegravir [107,108].

Other antiretroviral classes
Data on entry-inhibitors (e.g. maraviroc and enfuviritide) are limited, but no negative effects have been reported so far [109,110].None of these agents are implicated in the development of NAFLD.

Non-HIV or –cART-related risk factors for NAFLD

In addition to the risk factors that are directly linked to HIV-infection and cART, HIV-infected patients are exposed to other risk factors for the development of NAFLD.First of all, a significant proportion of the population is suffering from a hepatitis C virus (HCV)/HIV co-infection [111].HCV genotype 3 was identified as an independent risk factor for the development of NAFLD [112].Current literature suggests that these specific genotype 3 antigens induces upregulation of hepatic fatty acid synthesis [113,114]. When HCV genotype 3 infected patients achieve sustained viral response (i.e. cure) after treatment, the degree of steatosis diminishes [115]. In contrast to HCV genotype 3 infection, current literature suggest that Hepatitis B Virus (HBV) is associated with a lower risk for NAFLD [116]. However, the most important non-HIV/non-cART risk factors are probably the previously mentioned 'traditional' risk factors such as obesity-related IR and diabetes mellitus.Nowadays, HIV-patients grow old enough to be exposed to these traditional risk factors. Recent publications describe increases in median body mass index (BMI) and the incidence of diabetes mellitus in the HIV-infected population, adding more risks for this population [14,117,118]. The impact of traditional risk factors was emphasized by the results of the meta-analysis of Maurice et al., identifying high BMI, waist circumference, type 2 diabetes, hypertension and high triglycerides as the significant risk factors for NAFLD [19].

TREATMENT

Lifestyle modification

Despite extensive research over the past years, treatment of NAFLD remains challenging. Weight loss is still the most important intervention in all patients [119,120]. A sustained weight loss of approximately 10 percent is needed to improve the majority of the histopathological features of NASH [121]. Furthermore, excessive use of alcohol is discommended as alcohol itself can cause significant liver disease. Although the AASLD guideline recommends that patients with NAFLD should not consume heavy amounts of alcohol – 4 standard drinks a day or >14 per week for men and 3 standard drinks per day or 7 per week for women – it states that there are insufficient data to make recommendations with regard to non-heavy alcohol consumption [44]. In the setting of NASH, alcohol consumption is the most significant factor associated with the risk of HCC [122]. For this population, total abstinence is mandatory according to the European Association for Study of the Liver (EASL) [123].

Medical treatment

Next to behavioural interventions, several medical treatments have been described to be effective [44]. The most recent AASLD guideline supports the use of pioglitazone and vitamin E in biopsy-proven NASH. These recommendations are mainly based on the study of *Sanyal et al*, describing a trial comparing three groups - pioglitazone (30 mg daily) or vitamin E (800 IE daily) or placebo - in patients with biopsy-proven NASH [124]. Patients were randomized and treated for 96 weeks; all patients underwent follow-up liver biopsy. The primary outcome was an improvement in histological features of NASH as a composite endpoint of standardized scores of steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis. Vitamin E therapy versus placebo showed a significantly higher rate of improvement in biopsy proven NASH (43% vs. 19% P=0.001). However, the use of vitamin E is not strongly advocated since the SELECT trial from 2011 suggested an increased risk for prostate cancer in patients receiving 400 mg vitamin E daily with a hazard ratio of 1.16 (99% confidence interval 1.004 – 1.36. P=0.008) compared to placebo. In the trial of *Sanyal et al*., the use of pioglitazone also seems to improve the histological features of NASH although statistical significance was not reached – 34% of the pioglitazone group versus 19% of placebo showed improvement (p=0.04). A major side-effect of pioglitazone was weight gain [125]. Furthermore, its use seems to be associated with the development of bladder cancer although data are conflicting [126,127].The EASL guideline recommends that an insulin-sensitizer can be used, in particular in patients with Type II Diabetes Mellitus (T2DM) [123]. The AASLD suggests its use in biopsy-proven NASH regardless of T2DM status [44]. Both the EASL and

AASLD suggest considering vitamin E only in non-diabetic non-cirrhotic adults with biopsy proven NASH.Other medical interventions, such as metformin, glucagon-like peptide-1 (GLP-1) agonists and ursodeoxycholic acid are suggested as alternatives, but have not been proven effective and are therefore not recommended in current guidelines.

Bariatric surgery
Bariatric surgery is another treatment option that has increasingly been recognized as a potential intervention for NAFLD [128]. Several studies established high rates of histological improvement in NASH in patients with significant weight loss after bariatric surgery [120,129]. The AASLD guideline recommends that bariatric surgery can be considered in otherwise eligible obese individuals with NAFLD, but it is premature to consider surgery to specifically treat NAFLD [44].

With respect to HIV-infected patients, interventions should be guided on the specific pathophysiological aspects regarding HIV- and cART-related NAFLD in addition to standard care. First of all, these interventions should focus on reducing the degree of immune activation in HIV-infection by early introduction of effective antiretroviral therapy, aiming to reach full virological suppression and optimal immunological recovery. After publication of the START Study, current HIV treatment guidelines recommend the start of antiretroviral therapy regardless of $CD4^+$ cell count [130,131]. As a result of this early initiation of cART, the time of immune activation is limited and the degree of IR will decrease – as one of the most important factors of HIV-related NAFLD. Several studies showed that even in the setting of virological suppression, there is some residual immune activation [132–134]. However, the impact of the low-grade immune activation on the development of insulin resistance and NAFLD is currently unclear. Second, if patients are using a cART regimen with an unfavourable metabolic profile – like protease inhibitors or early generation NRTIs – changes in cART should be considered. Although current guidelines prefer the use of INSTI-based over PI-based regimens and modern PIs display a more favourable metabolic profile, some patients are still at risk for NAFLD as a result of cART-related dyslipidaemia.

FUTURE EXPECTATIONS

Epidemiology

In the upcoming years, the epidemiology and management of NAFLD is expected to change. As a result of an increased life expectancy and the number of years living in good health, risk factors for the general population are increasingly applicable for the HIV-infected population. In their 2010 paper, Crum-Cianflone *et al.* demonstrated significant increases in weight in HIV-infected patients over the years [135]. Among those diagnosed in the cART era, nearly two-thirds of the population were overweight or obese at last visit. This percentage is similar to the United States of America general population, confirming the observation that HIV-infected patients increasingly resemble the general population in respect to risk factors for NAFLD. Therefore, the upcoming years will be characterised by the transition from the HIV- and cART-related risk factors towards the 'classical' risk factors for the development of NAFLD. During this transitional period, HIV-treating physicians should be aware of the high prevalence of NAFLD in their patient population, especially for those that were diagnosed in the pre- and early-cART eras, those with low nadir CD_4 counts and those that received early generation antiretroviral therapy. Furthermore, it should not be forgotten that NAFLD can be the first utterance of underlying cardiovascular disease. Its finding may require other diagnostic or therapeutic interventions.

Treatment

In addition to changes in epidemiology and diagnostics, several drugs are currently under investigation which can be divided in non-antifibrotic and antifibrotic drugs [136]. Current phase 3 studies for the non-antifibrotic drugs focus on regulation of triglycerides and diminishing IR. These include farnesoid X receptor (FXR) and peroxisome proliferator activated alpha/delta (PPAR α/δ) receptor agonists. For example, PPAR α/δ receptors are present in adipose tissue, muscles and the liver among other tissues and stimulation promotes mitochondrial beta oxidation and diminishes IR by various pathways [137]. Elafibranor is a PPAR α/δ agonist which is currently tested in a phase 3 clinical trial. An earlier study using follow-up liver biopsies suggested significantly more resolution of NASH without fibrosis worsening in patients receiving Elafibranor versus placebo (19% vs. 12%. Odds ratio = 2.31 (95% confidence interval: 1.02 – 5.24 ($p=0.045$)) [138]. With respect to the antifibrotic drugs, selonsertib is currently investigated in a phase 3 clinical trial [139].Selonsertib is an inhibitor of apoptosis signal-regulating kinase 1 (ASK-1), a serine/threonine signalling kinase that induces hepatic inflammation, apoptosis and fibrosis in the setting of oxidative stress [140]. In an animal model, selonsertib seemed effective in diminishing hepatic inflammation and fibrosis [141]. A phase 2 study evaluated the efficacy of selonsertib;

patients were randomized in five groups: selonsertib monotherapy (18 mg or 6 mg) *versus* selonsertib (18 mg or 6 mg) plus simtuzumab (another antifibrotic drug) *versus* simtuzumab monotherapy in patients with biopsy-proven NASH [142]. As another study in the meantime established that simtuzumab was ineffective, the selonsertib groups with and without simtuzumab were pooled per dosage [143]. Reduction of one or more stages in biopsy-proven fibrosis was observed in 43% of patients treated with 18 mg selonsertib, 30% among those treated with 6 mg selonsertib and 20% in those treated with simtuzumab monotherapy – suggesting beneficial effects of selonsertib. Further studies will follow in the near future.

With respect to HIV-infected patients, not many specific trials are performed. To the best of our knowledge, only two studies focussing on NAFLD in HIV-infected patients are currently active. Recently, the ARRIVE Trial was completed with the results pending. In this study, HIV-infected patients with documented NAFLD were randomized to Aramachol versus placebo. The effect on steatosis will be evaluated using MRI-imaging [144].Aramchol is a synthetic fatty-Acid/bile-acid conjugate that inhibits the synthesis of fatty acids, which appeared to be safe and effective in an earlier trial [145].A second trial is currently evaluating the effects of Tesamorelin – a growth-hormone-releasing hormone (GHRH) analogue – on the degree of steatosis, with the endpoint measured by MRS-PDFF [146]. Two earlier studies in HIV-infected patients suggested beneficial effects when comparing Tesamorelin versus placebo [147,148]. One study determined that the amount of liver fat diminished –assessed by MRS -as result of GHRH analogue treatment, while the other report found that the use of Tesamorelin significantly reduced liver enzyme values compared to placebo. It is not completely clear how GHRH augmentation alters the hepatic fat storage, but it is suggested that it inhibits de novo lipogenesis in the liver [149].

In conclusion, although life expectancy of HIV-infected patients has increased dramatically after the introduction of cART, liver related-morbidity continues to have a great burden in this population.In some patients residual inflammation persists, certain antiretroviral drugs continue to influence the metabolic profile and HIV-infected patients will grow old enough to face the traditional risk factors for NAFLD. These developments should make us aware of the high risk of NAFLD in this population and warrant further research on modification of – both the traditional and HIV-related -risk factors and therapeutic interventions.

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Part II

Bone-related morbidity in HIV-infected patients

Chapter 6

Switching tenofovir disoproxil fumarate to tenofovir alafenamide results in a significant decline in parathyroid hormone levels: uncovering the mechanism of tenofovir disoproxil fumarate-related bone loss?

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ABSTRACT

An increasing number of patients have been switched from tenofovir disoproxil fumarate (TDF) to tenofovir alafenamide because of its improved bone safety profile, although the pathophysiological mechanism is not fully understood. We show that serum parathyroid hormone levels drop significantly after the switch from TDF to tenofovir alafenamide. This observation supports the theories that TDF-related bone loss is parathyroid hormone-driven and that this effect is dose-dependent.

INTRODUCTION

Reduced bone mineral density (BMD) among HIV-infected patients is common, with a prevalence up to 50% depending on the study population [1]. The pathogenesis is multifactorial including both traditional risk factors – e.g. vitamin D deficiency and low body mass index – and HIV-related factors like the use of antiretroviral therapy [2–4]. Especially the use of tenofovir disoproxil fumarate (TDF) is associated with accelerated BMD loss, although the mechanism is not fully understood [5–7]. One possible explanation is TDF-induced hyperparathyroidism [8].The prevalence of hyperparathyroidism in HIV-infected patients is significant with numbers up to 17.5% and several reports identified TDF as independent risk factor for elevated parathyroid hormone (PTH) levels [9,10].Prolonged exposure to high PTH levels is associated with cortical bone loss and higher fracture rates [11,12]. A recent study suggested that this increase is the result of a direct and dose-dependent inhibitory effect of TDF on the calcium sensing receptor (CaSR) in the parathyroid glands [13]. The authors raised the question whether Tenofovir Alafenamide (TAF) – the new drug formulation of tenofovir (TFV) – has less effect on the CaSR because of the lower plasma TFV levels associated with its use [14].

In this study, we assessed the dynamics of PTH and alkaline phosphatase (ALP) as a bone turnover marker in patients switching from TDF to TAF.We hypothesized that this reduction in plasma TFV exposure will be accompanied by a decline in serum PTH and eventually lead to lower bone turnover marker levels.

METHODS

We performed a single-centre, retrospective observational study in a cohort of HIV-infected patients under follow-up in the University Medical Centre Utrecht, Utrecht, the Netherlands.All patients who were switched from TDF- to a TAF-containing combination antiretroviral therapy (cART), in the period ranging from the 1st of June 2016 – when TAF became available in the Netherlands – and 1st of March 2018, were identified. Patients were included in the analysis if at least two PTH measurements were available – one during the use of TDF and the other while using TAF. As a matter of routine care in our centre, the serum PTH and 25-hydroxy vitamin D levels are measured yearly.

The following biochemical data were recovered from the hospital records for the two periods: PTH (reference range (rr): 1.0 – 6.9 pmol/L), 25-hydroxy vitamin D (rr: ≥ 50 nmol/L), ALP (rr: 0-120 U/L), ionized calcium (iCa^{2+}) (rr: 1.15-1.32 mmol/L) and the estimated glomerular filtration rate (eGFR). Patients with primary hyperparathyroidism and an eGFR < 60 ml/min were excluded from the analysis considering the altered PTH metabolism in these patients.In case PTH was measured multiple times during follow-up, we chose to use the measurement most closely to the point of switching.To compare variables, χ^2 was used for categorical variables and the Mann-Whitney U test for continuous variables. All reported P-values are two-sided, with a value < 0.05 being considered statistically significant. The statistical analysis was performed using SPSS (version 25.0; SPSS, Chicago, IL).

RESULTS

We identified 132 patients who switched from TDF to TAF and of whom a sufficient number of PTH measurements were available. After excluding 8 patients with an eGFR <60 ml/min, the cohort consisted of 124 patients [table 1]. The majority of the patients was male (n=94 (75.8%)) and had a median age of 49 years (interquartile range (IQR) 42.0-57.0). Most patients were from European descent (n=92 (74.2%), sixteen patients (12.9%)) originated from Sub-Saharan Africa.

Table 1. Baseline characteristics at the moment of the first parathyroid hormone measurement.

Patient characteristics	Total population (n=124)
Gender [n(%)]	
• Male	95 (76.6)
Region of origin [n(%)]	
• Europe	93 (75.0)
• Sub-Saharan Africa	16 (12.9)
• Other	15 (12.1)
Age [Median(IQR)]	49.5(42.3-57.8)
BMI [Median (IQR)]	24.6 (22.9 – 26.5)
Nadir CD4 count [Median(IQR)]	279 (113-375)
Duration of TDF therapy in months [Median (IQR)]	80.5 (48.3 – 108.5)
Time living with HIV in years [Median(IQR)]	11 (5 – 16.75)
Anchor changes when switching to TAF [n(%)]:	
• PI > PI	41 (33.1)
• NNRTI > NNRTI	36 (29.0)
• INSTI > INSTI	20 (16.1)
• PI > INSTI	11 (8.9)
• NNRTI > INSTI	8 (6.5)
• Other	8 (6.5)
Time between PTH measurements in months [Median(IQR)]	12(11-13)

Abbreviations: BMI – Body Mass Index; INSTI – Integrase Strand Inhibitors; IQR – Interquartile range; NNRTI – Non-nucleoside reverse transcriptase inhibitor; PI – Protease Inhibitor; PTH – Parathyroid hormone; TAF – Tenofovir Alafenamide; TDF – Tenofovir disoproxil fumarate

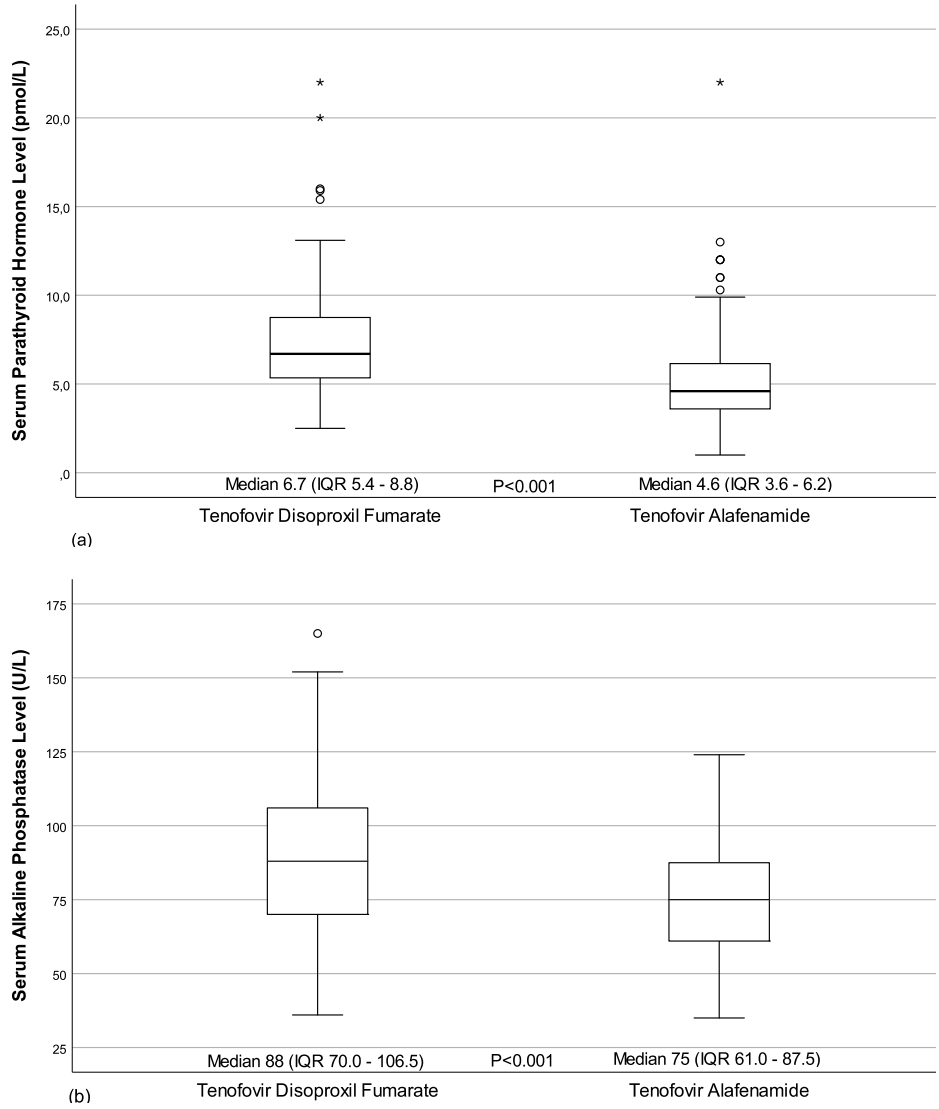

Fig. 1ab. Boxplots showing serum levels of parathyroid hormone (a) and alkaline phosphatase (b) in our study cohort while patients were either using tenofovir disoproxil fumarate or tenofovir alafenamide. The box represents the interquartile range with the thicker black line being the median. The whiskers represent the highest and lowest value within 1.5x interquartile range of either the upper or lower quartile. The dots and asterisks are the outliers

The median PTH level during TDF therapy was 6.7 pmol/L (IQR 5.4-8.8). After the switch to TAF, PTH levels had significantly decreased to a median of 4.6 pmol/L (IQR 3.6-6.2) ($p < 0.001$) [Figure 1a]; we observed a decline in PTH levels in 93 patients (75.0%). While using TDF, approximately half of the patients (n=59) had a PTH level >6.9 pmol/L; PTH levels were in the normal range in 105 patients (84.7%), after switching. With respect to ALP levels, data were available in 104 of the patients while being on TDF and in 119 patients on TAF. The median ALP level significantly dropped from 88 U/L (IQR 70.0-106.5) to 75 U/L (IQR 61.0-87.5) ($p < 0.001$) [figure 1b]. During TDF therapy, 14 patients (13.3%) had ALP levels above the upper limit of normal; after the switch to TAF, the ALP level was normal in 118 patients (99.2%) ($p < 0.001$). There was no difference in median iCa^{2+} (1.19 mmol/L for TDF versus 1.20 mmol/L for TAF ($p=0.372$)), nor in vitamin D levels (66.0 nmol/L during TDF use versus 64.0 nnmol/L while using TAF ($p=0.611$)).

DISCUSSION

This study shows that switching from a TDF- to TAF-containing cART, results in a significant decline in the serum PTH and ALP levels. Several trials demonstrated that patients on TAF show less decrease in BMD with a favorable bone turnover marker profile, compared to patients on TDF [15–19]. The pathophysiological mechanism of TDF-related bone loss is not fully understood: a direct toxic effect on the bone, subclinical tubular dysfunction and PTH-driven increased bone resorption, are the most accepted theories [7]. An earlier study established that PTH levels increase shortly after the initiation of TDF [8] – probably the result of a direct and dose-dependent inhibitory effect of TDF on the CaSR [13].From this perspective, it is reasonable to assume that in the use of TAF – characterized by very low plasma TFV levels – this inhibitory effect is weaker and the rise of PTH will be limited. Our finding that serum PTH sharply declines after the switch to TAF supports this hypothesis and suggests that TDF-related bone loss is PTH-driven. Due to the retrospective design of the study, we did not assess BMD alterations, but our data could help to understand the physiology behind the improved bone safety profile of TAF.Additional research is needed to compare the PTH dynamics in TAF-treated patients, versus those receiving a non-TFV containing regimen.

In conclusion, this is the first study describing PTH dynamics in patients switching from TDF to TAF. The significant decline in serum PTH levels is remarkable and might explain the favorable bone safety of TAF.

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Chapter 7

Low Sensitivity of the Fracture Risk Assessment Tool in Young HIV-infected Patients: Time to Revise Our Screening Strategy.

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ABSTRACT

Objectives
The burden of reduced bone mineral density (BMD) is high among HIV-infected patients. As screening strategy, current guidelines recommend calculating a Fracture Risk Assessment Tool (FRAX) score in patients aged 40-49 years. Patients with a 10-year risk for a major osteoporotic fracture ≥10% should undergo dual energy x-ray absorptiometry (DXA) to assess bone mineral density (BMD). The aim of this study was to establish the sensitivity of this threshold to identify patients with risk of osteoporosis in this age category – as surrogate marker for high fracture risk.

Methods
The study group consisted of patients aged 50-59 years and living with HIV for at least ten years who recently underwent dual energy x-ray absorptiometry (DXA). A clinical risk factor based FRAX score was calculated using patient characteristics from ten years earlier. In this way, we assessed which patients would have undergone DXA while they were 40-49 years old.

Results
The cohort consisted of 126 patients; 23 patients (18.3%) had osteoporosis. Ten years prior to the DXA, none of them met the guideline threshold of a 10-year major osteoporotic fracture probability of ≥10%, resulting in a sensitivity of 0% in this cohort. There was no difference between the median FRAX score between patients who developed osteoporosis and those who did not (3.3% versus 3.4%. P=0.55).

Conclusions
FRAX lacks sensitivity to determine which HIV-infected patients aged 40-49 years should undergo BMD testing to identify reduced bone mineral density. Its role should be limited to treatment decisions.

INTRODUCTION

The use of combination antiretroviral therapy (cART) has led to a dramatic increase in life expectancy of HIV-infected patients [1,2]. As most patients are no longer threatened by opportunistic infections, the focus in current HIV-related care lies on management of long-term effects of HIV-infection and cART [3].For example, reduced bone mineral density (BMD) can be found in approximately half of the patients [4]. This high burden is probably the result of the high prevalence of traditional risk factors such as low body weight and vitamin D deficiency [4,5]. The use ofcertain antiretroviral drugs like tenofovir disoproxil fumarate is also associated with a decrease in BMD [6].Considering an osteoporosis prevalence of approximately 15% - which is higher than in the general population - it is recommended to routinely screen all HIV-infected patients aged ≥ 50 years with dual energy x-ray absorptiometry (DXA) scan [7,8].

For patients aged 40-49 years without a major risk factor for fractures, i.e. history of fragility fracture, glucocorticoid use (≥ 5 mg x 3 months) or high risk of falls - the current guidelines recommend calculating Fracture Risk Assessment Tool (FRAX) score to determine which patients should undergo BMD testing [7,8].FRAX is an algorithm including classical risk factors for osteoporotic fractures and was developed to estimate the 10-year probability of both major osteoporotic fractures (MOF) and hip fractures (HF)[9].The FRAX score can be calculated based on clinical risk factors (CRFs) alone or combined with femoral neck BMD as determined by DXA, to help in treatment decision. The use of FRAX as case-finding tool or as screening for osteoporosis is less common[10,11], although experts state that it is reasonable to assess BMD in HIV-infected patients aged 40-49 years if they have a 10-year probability of MOF ≥ 10% calculated by CRF-based FRAX [8]; the European AIDS Clinical Society (EACS) guideline advocates a threshold of > 20% [7].

This approach is evaluated in a few studies that describe the accuracy of FRAX to identify patients at risk for osteoporosis - as a surrogate outcome - but none specifically addresses this younger population[12–14]. Therefore, the aim of our study was to evaluate the sensitivity of FRAX for the diagnosis of (future) osteoporosis in HIV-infected patients aged 40-49 years by calculating the FRAX score in a retrospective manner. Based on clinical observations, we hypothesized that the current FRAX thresholds underestimate the risk on this outcome.

METHODS

Study Population & Design

We performed a cross-sectional analysis in a cohort of patients with HIV infection under follow-up in the University Medical Center Utrecht, the Netherlands, a tertiary hospital with 1800 HIV-infected patients in care.As matter of routine care, all patients aged ≥ 50 years are offered DXA scanning. We identified all patients aged 50-59 years and living with HIV for at least ten years who recently underwent a DXA. For these patients, a CRF-based FRAX was calculated in a retrospective manner, using the data from ten years earlier.

By retrospectively applying the current practice for HIV-infected patients aged 40-49 years, we could establish how many patients would have undergone BMD measurement according to the current guidelines, and thus the sensitivity of the current strategy for osteoporosis as outcome. In addition, we compared median fracture probability scores between patients who eventually had osteoporosis and those who did not.In this analysis, we considered the date of the FRAX calculation as baseline. Patients receiving corticosteroids with a dose ≥ 5.0 mg for over three months were excluded as they already have an indication for DXA scan regardless the FRAX score. Patients from Sub-Saharan African were excluded as well since there are no FRAX databases available for these populations.

The study was conducted in accordance with local ethical guidelines.

FRAX calculation

FRAX scores were computed on the website of the University of Sheffield (https://www.sheffield.ac.uk/FRAX/) to assess 10-year risks for both MOF and HF for all patients. CRFs were filled in with the patient's characteristics ten years prior to the DXA scan - retrieved from the medical records.For all patients the box for 'Secondary osteoporosis' was checked with 'Yes' according to the guidelines.Considering the possibility that the 'Parent Fractured Hip' (PFH) was underreported, the FRAX was calculated with the box checked with 'Yes' in case it was not mentioned in the medical records.When calculating the FRAX score, the country-specific algorithm was used – with no algorithm available, a country with comparable population characteristics was used as surrogate. As mentioned above, there are no FRAX algorithms available for sub-Saharan Africa.

DXA

Bone mineral density of the femoral neck and lumbar spine were measured by DXA (Discovery A (S/N80675) Hologic Inc.®).In accordance to the World Health Organization (WHO) criteria, all patients with a T-score <-1.0 at either the lumbar spine and/or femoral neck were considered to have reduced BMD; osteoporosis was defined as a T-score <-2.5.

Statistical Analysis

Dichotomous variables of baseline characteristics were analyzed using χ^2 testing. FRAX scores were compared between groups using a 2-sided, non-parametric Mann-Whitney U test. All reported P-values are two-sided, with P-values of <0.05 being considered statistically significant. The statistical analysis was performed using SPSS (version 25.0; SPSS, Chicago, IL).

RESULTS

Patient's characteristics

We identified 143 HIV-infected patients who met the inclusion criteria. After the exclusion of 16 patients of sub-Saharan African descent and one patient using corticosteroids, 126 patients were eligible for analysis.Most patients were male (n=112 (89%)) and of Caucasian descent (n=112 (88.9%)); the median BMI was 23.6 kg/m^2 (interquartile range (IQR) 21.5 – 25.4) [table 1]. At total number of 23 patients (18.3%) were diagnosed with osteoporosis and 47 (37.3%) with osteopenia. At baseline, patients who turned out to have osteoporosis had a lower BMI (22.1 versus 23.9 kg/m^2. p=0.02) and lower nadir CD4$^+$ cell counts (111 versus 194 cells/mm^3 p=0.02) compared to the patients without osteoporosis.

Table 1. Baseline table showing patient characteristics at the moment the CRF-based FRAX score was calculated – 10 years prior to DXA.

Patient characteristics	Total population (n=126)	Osteoporosis (n=23)	Non-osteoporosis (n=103)	P-value
Gender				
• Male [n(%)]	112 (88.9)	21 (91.3)	91 (88.3)	0.684
Region of origin				
• Caucasian [n(%)]	112 (88.9)	18 (78.3)	94 (91.3)	0.073
Age	44 (42-46)	42 (42-46)	44 (42 -46.5)	0.638
BMI	23.6 (22.5 – 25.4)	22.1 (20.6 – 23.9)	23.9 (21.6 – 25.5)	0.016
Nadir CD4 count	185 (70 – 275)	111 (22-184)	194 (81.5 -275)	0.02
Years living with HIV	7 (3 – 13)	5 (1-13)	7 (3-12.5)	0.707
Excessive alcohol use [n(%)]	18 (14.3)	15 (14.6)	3 (13.0)	0.851
Previous fracture [n(%)]	1 (0.8)	0 (0)	1 (4.3)	0.034
Current smoking [n(%)]	51 (40.5)	10 (43.5)	41 (39.8)	0.746
Parental Hip Fracture [n(%)]				0.785
• Yes	2 (1.6)	0 (0)	2 (8.7)	
• No	12 (9.5)	2 (8.7)	0 (0)	
• Missing	112 (88.9)	21 (91.3)	21 (91.3)	
Rheumatoid arthritis [n(%)]	0 (0)	0 (0)	0 (0)	-
Use of TDF [n(%)]	48 (38.1)	11 (47.8)	37 (35.9)	0.288

All values are reported as median (interquartile range) unless noted otherwise. Abbreviations: BMI – Body Mass Index; HIV – Human Immunodeficiency Virus; TDF – Tenofovir Disoproxil Fumarate.

FRAX scores

We calculated FRAX scores for MOF and HF for all patients as these were ten years prior to the DXA scan. At that point, none of the 126 patients met the MOF risk threshold of 10.0% indicating referral for early DXA scanning – resulting in a sensitivity of 0% for the detection of future osteoporosis.The calculated FRAX scores in these patients ranged between 0.9 and 5.1%. If missing PFH variables were checked as 'No', the score ranged from 0.5% – 2.6%. Furthermore, the median FRAX score for a major osteoporotic fracture did not differ between patients with or without the future osteoporosis (3.3% vs 3.4% p=0.55) [Table 2]. The median FRAX score for HF was also not able to predict which patients would be at risk to have osteoporosis ten years later [Table 2].

Table 2. Ten-year probability for major osteoporotic fracture and osteoporotic hip fracture according to CRF-based calculated FRAX comparing patients having osteoporosis versus those who have not.

	Osteoporosis (n=23)	Non-osteoporosis (n=103)	P-value
Risk of MOF with PFH marked 'Yes' [Median (IQR)]	3.3 (3.0 – 3.7)	3.4 (3.1 – 3.8)	0.552
Risk of MOF with PFH marked 'No' [Median (IQR)]	1.7 (1.5 – 2.0)	1.7 (1.5 – 1.9)	0.684
Risk of OHF with PFH marked 'Yes' [Median (IQR)]	0.2 (0.1 – 0.4)	0.2 (0.1 – 0.3)	0.286
Risk of OHF with PFH marked 'No' [Median (IQR)]	0.1 (0.1 – 0.3)	0.1 (0.1-0.2)	0.684

Abbreviations: IQR – Interquartile range; MOF - Major Osteoporotic Fracture; PHF – Parenteral Hip Fracture.

The FRAX score was also assessed at the moment of DXA scan: The median MOF probability risk was 4.6% in patients having osteoporosis versus 4.7% of the patients that did not have osteoporosis (p=0.86) with a range of 0.9 - 8.9% for the entire population.

Fragility fractures

After ten years of follow-up after the initial FRAX calculation, three patients (2.4%) had developed a fragility fracture [metatarsal, olecranon and (subclinical) vertebral fracture], but this does not qualify as MOF. Only the patient with metatarsal fracture had a T-score <-2.5.

DISCUSSION

HIV-infected patients are at high risk for reduced BMD; in this study 18.3% was diagnosed with osteoporosis and 37.3% with osteopenia. Our analyses demonstrate that FRAX lacks sensitivity to assess which patients aged 40-49 years are at risk for the development of osteoporosis, as none of them met the threshold of 10% for early BMD assessment. In addition, the median FRAX score 10 years before the DXA did not differ between patients that eventually turned out to have osteoporosis versus those who did not.

Only a few studies evaluated the accuracy of FRAX in HIV-infected patients for predicting the occurrence of osteoporosis [12–14]. The reported sensitivity in these three reports ranged from 22 - 38.5%. In two of the studies median FRAX scores were calculated and these did not differ between patients that had osteoporosis and those who had not.Our study differs from these earlier reports by being the first to describe probability scores exclusively in in the 40-49 years age category to which the current guidelines apply.

The use of FRAX in the HIV guidelines is not keeping in with the original design and aim of the tool, which is to establish the 10-year MOF-risk for purposes of therapy, using intervention thresholds [9]. The HIV guidelines apply a more case-finding approach using assessment thresholds. These are – as our data show - mistakenly considered to be of predictive value for the occurrence of osteoporosis. In the general population, there is indeed a shift towards a policy in which the tool is used in case-finding [15], but this approach is adopted and validated in a limited number of countries [10,11]. In addition, for the general population intervention and assessment thresholds vary greatly between countries due to country-specific cost-effectiveness analyses and DXA availability [11]. These geographical differences make it difficult to apply the fixed thresholds formulated for HIV-infected patients, as they can deviate significantly from the local approach. Secondly, we observed no major osteoporotic fractures in our cohort. This finding is supported by another study that established that the absolute number of MOF is limited in young HIV-infected patients[16], despite the high burden of reduced BMD in this population. These findings once again confirm that reduced BMD does not necessarily lead to fractures. In fact, earlier studies validated FRAX to predict the occurrence of MOF, although some suggest an underestimation in HIV-infected patients[17,18]. The addition of specific HIV-related factors associated with reduced BMD like low nadir CD4 cell counts could improve the performance of FRAX [4].

FRAX lacks sensitivity to identify patients at risk for osteoporosis, its use varies greatly between countries and there is no database for a significant proportion of patients – e.g. patients from sub-Saharan Africa [19]. These observations make that the usage of FRAX as a kind of case finding strategy need to be reconsidered. However, we believe that FRAX could have a role in the current guidelines, but only for intervention assessment in general rather than case finding in patients aged 40-49 years. The policy of identifying patients with so-called major risk factors - e.g. who have a high risk of falling, a history of a fragility fracture or who were exposed to corticosteroids - might be sufficient to identify those who benefit from early BMD measurement in this age category. However, this hypothesis needs to be confirmed in large cohorts.

Some limitations should be taken into account in the interpretation of our findings. For example, we are aware that FRAX scores in the Netherlands tend to be lower than in other European countries as a lower incidence of osteoporotic fractures is reported [20].But as mentioned earlier, the finding of low sensitivity rates is not limited to our cohort [10-12].

In conclusion, the sensitivity of the FRAX tool in our cohort of young HIV-infected patients turned out to be extremely low for predicting the risk of osteoporosis and therefore its role in case finding strategy need to be reconsidered.

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Part III

Lung-related morbidity in HIV-infected patients

Chapter 8

The use of corticosteroids does not influence CD4+ lymphocyte recovery in HIV-infected patients with advanced immune-deficiency.

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ABSTRACT

Corticosteroids inhibit HIV-related immune activation and seem to have a mild favorable effect on immunological recovery in patients with $CD4^+$ counts ≥200 cells/mm^3. Data in patients with advanced immunodeficiency are lacking, despite the fact that these patients are likely to receive corticosteroids and are vulnerable for incomplete immunological recovery after the initiation of combination antiretroviral therapy (cART). We analyzed whether corticosteroids negatively influence the short term $CD4^+$ lymphocyte recovery in patients with $CD4^+$ cell counts <200 cells/mm^3 started on cART.

We performed a retrospective cohort analysis including all HIV-infected patients under follow-up in our hospital with a documented episode of Pneumocystis jirovecii Pneumonia (PJP) in the cART era. $CD4^+$ lymphocyte recovery was assessed at three months after the episode of PJP and subsequent start of cART, comparing patients that received adjunctive corticosteroids (AC) versus patients that did not receive corticosteroids (standard care (SC)).

In total, 66 patients with an episode of PJP were identified with 38 patients in the AC-group versus 28 patients in the SC-group. Almost all baseline characteristics were similar, including mean $CD4^+$ lymphocyte counts.After three months, the mean $CD4^+$ cell count did not differ; 222 cells/mm^3 for the SC-group versus 259 cells/mm^3 for the AC-group ($p=0.29$). Neither there were differences in mean $CD4^+$ cell count after twelve months or the occurrence of new opportunistic infections in the first year.

The use of corticosteroids does not alter $CD4^+$ lymphocyte recovery in HIV-infected patients with advanced immunodeficiency in the first months of antiretroviral therapy.

INTRODUCTION

Corticosteroids are frequently used in modern medicine, also in the setting of HIV-related care [1]. Immune reconstitution inflammatory syndrome (IRIS) and Pneumocystis jirovecii pneumonia (PJP) with profound hypoxemia are the most common indications for corticosteroids in HIV-infected patients [2–4]. Besides side effects like diabetes mellitus, corticosteroids are potent immunosuppressants – leading to concerns of new (opportunistic) infections [5–7].

Several studies described a mildly favorable effect of corticosteroids on $CD4^+$ cell recovery in HIV-infected patients with $CD4^+$ cell counts >200 cells/mm^3 [8–11]. In contrast, data on the impact of corticosteroids in patients with advanced immunodeficiency – e.g. $CD4^+$ cell counts ≤200 cells/mm^3 – are limited [12]. This lack of data is remarkable, considering that especially these patients are likely to receive corticosteroids for the indications mentioned above. As studies in healthy volunteers have shown that corticosteroids induce lymphopenia - in particular in the $CD4^+$ compartment - [13,14], the use of corticosteroids in patients with severe $CD4^+$ lymphopenia might lead to incomplete immune recovery and other opportunistic infections [15]. These observations emphasize the need for a better understanding of the dynamics of $CD4^+$ cell recovery in patients with advanced immunodeficiency using corticosteroids.

In this study we retrospectively analyzed whether the use of corticosteroids leads to incomplete immunological recovery in HIV-infected patients with $CD4^+$ lymphocyte counts ≤200 cells/mm^3 in the first months after the initiation of combination antiretroviral therapy (cART).

METHODS

Patients with HIV infection treated for a PJP in the University Medical Center Utrecht (the Netherlands) between January 1st 1996 (start of the cART era) and July 1st 2017 were identified. We chose to include only patients that were diagnosed with PJP in our analysis for two reasons. First of all, the use of corticosteroids as adjunctive treatment in the setting of PJP is standardized with a fixed dosage for three weeks [16]. This is in contrast to the treatment of IRIS that does not have a predefined dosage and duration of therapy, which makes the interpretation of results difficult.PJP patients without profound hypoxemia and therefore no indication for corticosteroids created a control group.

All patients with a minimal follow-up time of three months after the episode of PJP were considered for the analysis. The diagnosis of PJP was based on clinical history with either microbiological or cytological evidence. HIV-positive patients with $CD4^+$ counts ≤200 cells/mm^3 presenting with a clinical picture highly suspicious for PJP - severe hypoxemia and ground glass opacities on the imaging studies - and with clinical recovery while given anti-PJP therapy were also considered eligible for this analysis. Patients were excluded in the following situations: 1) not naïve for antiretroviral therapy at the time of PJP; 2) no initiation of cART within 8 weeks after PJP diagnosis; 3) corticosteroids for a period longer than 21 days. Baseline demographical, clinical and biochemical characteristics were retrieved from patient's charts, as were the $CD4^+$ cell counts in the year after the PJP diagnosis. The primary outcome of this analysis was the difference in the mean $CD4^+$ cell count at three months after the diagnosis of PJP between patients receiving adjunctive corticosteroids (AC) – prednisone dosage according to the NEJM consensus statement - next to antimicrobial treatment compared to those who only received antimicrobial therapy (standard care (SC)) for the treatment of PJP.Furthermore, we evaluated the occurrence of other AIDS defining conditions – e.g. toxoplasmosis, *Mycobacterium avium complex* infection, candidiasis and Kaposi sarcoma - in the first year. We used χ^2 test to compare dichotomous variables and Student's T-test to compare means – also for mean $CD4^+$ counts during follow-up. A 2-sided $p < 0.05$ was regarded as significant. For calculations SPSS (version 22.0, Chicago, IL) was used, the figure was created with GraphPad Prism 7.

RESULTS

We identified 89 patients with PJP of whom 23 were excluded – flow-chart is shown in supplement. Of the remaining 66 patients, 38 patients received AC and 28 patients received SC. All patients were alive and in follow-up after 12 months.At baseline, patients did not differ between groups (AC versus SC) regarding age, gender and HIV transmission route [table 1]. The mean $CD4^+$ cell count at baseline was 47 cells/mm^3 in the AC group versus 59 cells/mm^3 in the SC-group (p=0.35). As expected, more patients from the AC group were admitted at the Intensive Care Unit (ICU) during the episode of PJP compared to patients receiving SC (26.3% vs. 3.6% P=0.02), but there was no difference in mean baseline CD4 cell count between these patients (ICU versus non-ICU: 42.9 vs 54.2 cells/mm3 (p=0.46)). All patients received initially trimethoprim/sulfamethoxazole (TMP/SMX), but a significant part (24.4%) was switched to pentamidine because of TMP/SMX-associated toxicity.

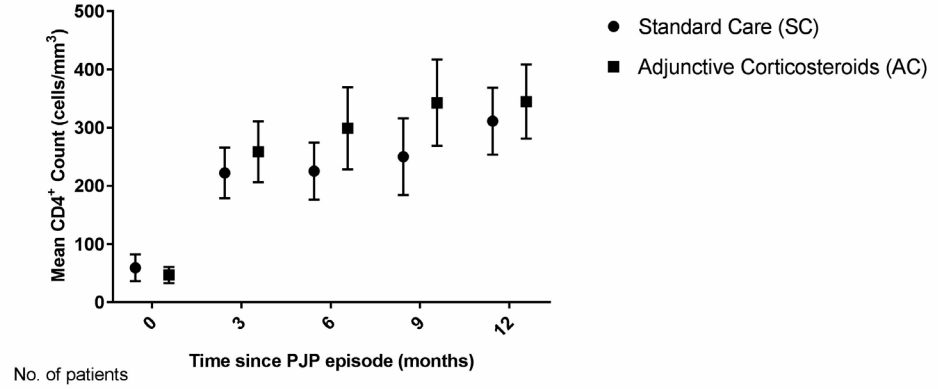
At three months after the PJP diagnosis, the mean $CD4^+$ count between both groups did not differ; 222 cells/mm^3 for the SC group versus 259 cells/mm^3 for the AC group (p=0.29). Analysis at twelve months after PJP diagnosis did not reveal a significant difference either: 311 cells/mm^3 (SC) versus 345 cells/mm^3 (AC) (p=0.43) [Figure 1]. Separate sub-analyses for time eras (1996-2002; 2003 – 2007 and 2008 -2017) or cART backbone did not reveal significant differences between both groups (data not displayed).

The percentage of patients reaching a CD4+ count of >200 cells/mm3 within the first year was 84.2% in the AC-group versus 71.4% for the patients from the SC-group (p=0.24). All but one patient (1.5%) had a viral load <400 copies/ml after one year. There was no difference in the percentage of patients that suffered from another AIDS-defining condition; 21.4% for patients receiving SC versus 13.2% of those from the AC-group (p=0.50) in the first year.

Table 1. Baseline characteristics.

	Adjunctive Corticosteroids (n=38)	Standard Care (n=28)	P-value
Gender [n (%) male]	31 (81.6)	24 (85.7)	0.75
Route of HIV transmission [n (%)]			0.45
• MSM	17 (44.7)	16 (57.1)	
• Heterosexual	15 (18.4)	7 (7.6)	
• Other	2 (5.3)	1 (3.6)	
ICU admission [n (%)]	10 (26.3)	1 (3.6)	*0.02*
Concurrent AIDS-defining condition [n (%)]	9 (23.7)	5 (17.9)	0.76
$CD4^+$ cells/mm^3 [Mean (SD)]	47.0 (42.8)	59.5 (59.2)	0.35
Age [Mean (SD) years]	44.1 (11.7)	42.5 (8.6)	0.51
Antimicrobial therapy for PJP [n (%)]			0.38
• Only TMP/SMX	29 (76.3)	19 (67.9)	
• TMP/SMX followed by Pentamidine	8(21.5)	8 (28.5)	
• Other	1(2.6)	1 (3.6)	
Time of HIV diagnosis [n (%)]			0.75
• Prior to PJP diagnosis	2 (5.3)	2 (7.4)	
• At the time of PJP diagnosis	36 (94.7)	26 (92.6)	
cART anchor in first year [n (%)]			0.09
• Protease inhibitor	16 (42.1)	20 (71.4)	
• NNRTI	20 (52.6)	6 (21.4)	
• Integrase Strand Inhibitor	1 (2.6)	1 (3.6)	
• Other	1 (2.6)	1 (3.6)	
Time period of PJP episode[n (%)]			0.74
• 1996 – 2002	4 (10.5)	5 (17.9)	
• 2003 – 2007	12 (31.6)	6 (21.4)	
• 2008 – 2017	22 (57.9)	17 (60.7)	

cART - combination antiretroviral therapy; ICU – Intensive Care Unit; MSM - Men who have sex with men; NNRTI – Non-nucleoside reverse transcriptase inhibitor; SD – Standard deviation; PJP – Pneumocystis Jirovecii Pneumonia; TMP/SMX – Trimethoprim/Sulfamethoxazol



No. of patients					
SC	28	27	27	18	28
AC	38	37	35	31	38

Figure 1. Mean $CD4^+$ cell counts over the time after initation of antiretroviral therapy.

DISCUSSION

In this retrospective cohort study in HIV-infected patients with advanced immunodeficiency, the use of corticosteroids had no impact on $CD4^+$ cell recovery in the first months after the initiation of cART.

In the past, several randomized controlled trials studied the effects of corticosteroids in HIV-infected patients in respect to clinical and immunological outcomes. Most of these reports describe dynamics in $CD4^+$ counts and immune activation in the setting of HIV-infection with $CD4^+$ counts >200 cells/mm^3[8–11].

For example, the largest cohort by *Kasang et al.* with a total number of 326 untreated HIV-infected patients with a $CD4^+$ count ≥300 cells/mm^3,were randomized to 5 mg prednisolone per day or placebo for 2 years [17]. After two years, the rise in $CD4^+$ count compared to baseline in patients receiving corticosteroids was significantly higher than the control group – an increase 77.4 cells/mm^3 versus a decline of 37.4 cells/mm^3 ($p < 0.01$).Furthermore, several markers for immune activation in the prednisone-group were significantly lower compared to placebo. It was hypothesized that corticosteroids inhibit HIV-related immune activation - slowing down $CD4^+$ destruction.

However, information on the effects of corticosteroid use in HIV-infected patients with advanced immunodeficiency are lacking despite that these data in this vulnerable population are needed. After all, two major indications for corticosteroids in HIV-infected patients – treatment of IRIS and adjunctive therapy in PJP with profound hypoxemia – mainly occur in patients with advanced immunodeficiency and this population is at risk for new opportunistic infections. To our best knowledge, only *McComsey et al.* describes the short-term dynamics of $CD4^+$ cell recovery exclusively in patients with advanced immunodeficiency [12]. In this placebo-controlled trial, 41 HIV-infected patients with a mean baseline $CD4^+$ count of 129 cells/mm^3 were randomized to 8 weeks 0.5 mg/kg body weight prednisone daily or placebo. Almost all patients were on cART for at least two months before randomization, with a median of 207 days for the prednisone group.After 8 weeks, there was no significant difference between both groups; the mean $CD4^+$ cell count in the placebo group remained stable and there was a slight decrease of 3 cells/mm^3 in patients receiving corticosteroids.

There were no data available on $CD4^+$ cell dynamics after eight weeks. We built on the data by *McComsey et al.* by describing the impact of corticosteroids on $CD4^+$ cell recovery in the setting of subsequent start of cART. As the largest increase in $CD4^+$ cells tend to happen in the first three months, it is likely that possible negative effects of the

corticosteroids will be visualized during this period [18]. Furthermore, the observation period in our study was extended up to twelve months after the course of corticosteroids. A long follow-up time is needed, considering that the immunosuppressive effects of corticosteroids can persist up till three months after discontinuing prednisone – especially when the cumulative dose of 700 mg is exceeded [5].

A limitation of our study is the small sample size, which results in reduced statistical power. Considering the trend towards better $CD4^+$ cell recovery in patients treated with corticosteroids it seems unlikely that a negative effect is missed as result of a lack of power.In addition, the described study period is long – this poses a risk for bias due to changes in (antiretroviral) therapy over the years. However, sub-analyses per time era or cART backbone did not reveal any differences.

In conclusion, although corticosteroids have several potential side-effects, its use does not alter $CD4^+$ cell recovery in HIV-infected patients with advanced immunodeficiency in the first months of antiretroviral therapy.

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Chapter 9

High rates of diffusion capacity impairment after Pneumocystis jirovecii pneumonia in HIV: The result of a multiple hit model?

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In preparation

ABSTRACT

Introduction

Pneumocystis jirovecii pneumonia(PJP) became one of the leading causes of death in HIV-positive patients. In the acute setting, the clinical picture is characterized by a profound hypoxemia as a result of diminished alveolar diffusion capacity. However, it is unknown whether the diffusion capacity will return to normal or whether there is residual pulmonary damage after successful treatment.

Methods

We performed a retrospective observational analysis in HIV-positive patients with a history of PJP under follow-up in het University Medical Center Utrecht (UMCU) who underwent pulmonary function testing at least one year after the episode of PJP. Pulmonary function tests were performed in a standardized matter and included spirometry and CO diffusion measurement. The pulmonary function tests were reviewed by a pulmonologist for the presence of restriction, obstruction and/or diffusion capacity impairment.

Results

A total of 54 HIV-positive patients with a history of PJP underwent a pulmonary function test; the median time since PJP was ten years and only a few patients reported respiratory symptoms. The occurrence of restrictive (6%) and obstructive pulmonary (9%) disease was limited. In contrast, a significant percentage of the patients displayed diffusion impairment (39%), although mostly mild. Multivariate analysis did not identify one specific risk factor explaining this high number.

Conclusion

A significant number of patients with a history of PJP displays some degree diffusion impairment in the years after PJP. Further research is needed to elucidate whether this is related to PJP or to HIV infection itself.

INTRODUCTION

In the eighties, the report of five cases of young men with Pneumocystis jirovecii pneumonia (PJP) marked the beginning of the HIV-pandemic [1] with PJP becoming one of the common causes of death in HIV-positive patients [2]. Pneumonia with Pneumocystis jirovecii, a yeast-like fungus, is characterized by respiratory insufficiency as the pulmonary alveolus is filled with a foamy mix of pathogens and inflammatory cells which limits the alveolar gas exchange [3].Therefore, the assessment of carbon oxide (CO) diffusion capacity was historically used as non-invasive diagnostic test for PJP [4].

Standardization of antimicrobial treatment and the use of adjunctive corticosteroids in severe hypoxemia has resulted in a decrease in mortality rates and the use of antimicrobial prophylaxis led to fewer new cases [5]. Moreover, the introduction of combination antiretroviral therapy (cART) with subsequent immunological recovery had a big impact on the long-term survival of HIV-infected patients, including those who suffering from AIDS-defining conditions such as PJP [6].As a result of an increased life expectancy, the focus in HIV-related care has shifted from opportunistic infections towards the long-term effects of both HIV-infection and antiretroviral therapy [7]. However, the possible long-term sequelae of previous AIDS-defining conditions are less frequently studied, such as the outcomes after PJP which in the active phase is characterized by significant lung damage [8]. A Danish study in the pre-cART era demonstrated that diffusion capacity impairment persisted in the majority of patients nine months after the episode of PJP [9]. However, there is a lack of data with longer follow-up times but this information would be of interest since persistent pulmonary function abnormalities can pose patients at risk for a more severe course of respiratory infections or faster development of smoking-related lung disease [10]. Studies are needed as lung disease is frequent in the HIV-positive population due to high prevalence of smoking, respiratory tract infections and immune activation [11].We hypothesize that PJP in combination with HIV-related immune activation causes significant lung damage resulting in pulmonary function abnormalities over years.

Therefore, we retrospectively analyzed the prevalence of diffusion capacity impairment, restrictive and obstructive pulmonary diseases in our clinical cohort of HIV-positive patients with a history of PJP who underwent pulmonary function testing as part of routine care. Furthermore, we analyzed the associated clinical factors with these outcomes.

METHODS

Design and patient selection

We performed a retrospective observational analysis in HIV-positive patients with a history of PJP who are under follow-up in the University Medical Center Utrecht (UMCU) (Utrecht, the Netherlands) – a tertiary hospital with approximately 1500 patients in care. After the identification of all patients ever in care in the UMCU, their medical records were reviewed for the occurrence of an episode of PJP in their medical history at least one year prior to April 1st 2017. Those with a history of PJP at some point during the course of the HIV-infection and who have undergone pulmonary function testing as a matter of routine care were considered to be eligible for analysis.Of these patients several demographical, clinical and biochemical data were retrieved from the medical records.Furthermore, the records were reviewed for notifications on respiratory symptoms.

Pulmonary function testing and definitions

All pulmonary function testing were performed in a standardized manner; it included both spirometry and CO diffusion capacity assessment. Smokers were asked not to smoke in the four hours prior to the pulmonary function test.All pulmonary function tests were reviewed by a pulmonologist

Pulmonary obstruction was defined as a FEV_1/FVC-ratio < 0.70 [12]. Pulmonary restriction was diagnosed if the percentage of predicted value for total lung capacity (TLC) fell within the lowest 5th percentile, corresponding to a Z-score < -1.64 [12].The Combined European Respiratory Society (ERS)/American Thoracic Society (ATS) document guideline was used for the standardized measurement and interpretation for the carbon oxide diffusion capacity [13]. Impaired diffusion capacity was defined as a percentage of predicted value for transfer factor for carbon monoxide (TLco) within the lowest 5th percentile, thus a Z-score < -1.64 [12,14]. In case impaired diffusion capacity was established, a diffusion capacity > 60% of predicted was considered as mild, 40-60% as moderate and < 40% as severe.All outcomes were dichotomized to either present or absent.

Statistical analysis

The primary outcome of this analysis was the presence of pulmonary function abnormalities – obstruction, restriction or diffusion impairment. Furthermore, we analyzed the associated factors for these outcomes. The statistical analysis was performed using IBM SPSS Statistics 25.0. Associations between patients' characteristics and outcomes were assessed using a Chi Square test for dichotomous variables and Mann-Whitney-U test for continuous variables. Only variables displaying a p-value ≤ 0.2 in univariate analysis were included in the multivariate regression analysis for further evaluation. P-values < 0.05 were considered to be statistically significant.

RESULTS

Patients' characteristics

A total of 213 patients who had a documented episode of PJP longer than one year ago till 1 April 2017 were identified. Fifty-eight of those patients (27%) had undergone pulmonary function testing at some point in follow-up. Of four pulmonary function tests, interpretation was not possible because of insufficient quality – leaving 54 patients for final analysis. Baseline characteristics from the moment of the pulmonary function test are displayed in table 1. Forty-seven (87%) patients were male with a median age of 54 years [interquartile range (IQR) 9] and nine patients (17%) were current smokers. In the cohort, men having sex with men (MSM) represented the largest risk group for HIV acquisition. The median time since the episode of PJP was ten years (IQR 12); this episode was accompanied by severe hypoxemia in 33 patients (61%) – reflected by the need for adjunctive corticosteroids beside standard antimicrobial therapy. In general, there was good immunological recovery with a median CD4 count of 478 cells/mm^3 (IQR 224) at the time of pulmonary function testing.

Obstructive and restrictive pulmonary disease

First, we set out to investigate the prevalence of general pulmonary abnormalities focusing on restrictive and obstructive pulmonary diseases [table 2]. The number of patients with restrictive pulmonary disease was limited (n=3 (6%)), with only one patient experiencing shortness of breath. None of these patients had a history of an illness associated with restrictive lung disease, like pulmonary fibrosis, interstitial pneumonia or neuromuscular disease.

Obstructive pulmonary disease was found in 5 patients (9%) with three of these patients previously being diagnosed with obstructive pulmonary disease – either asthma or chronic obstructive pulmonary disease (COPD). Since there was no standard use of bronchodilation agents prior or during the procedure, we could not establish whether observed abnormalities were the result of either asthma or COPD. Three patients had mild obstruction, defined as FEV1 of ≥ 80% with only one patient reporting respiratory symptoms. Remarkably, the two patients with moderate obstruction did not report any respiratory complaints. Although the prevalence of obstructive pulmonary disease was higher among (former) smokers [n=4 (19%)] versus never smokers [n=1 (3%)], this difference did not reach statistical significance (p=0.08). None of the other baseline characteristics was associated with the development of obstructive pulmonary disease.

Table 1. Patients' characteristics at the moment of pulmonary function (n=54) All values are reported as n (%) unless noted otherwise.

Age in years (median (IQR))	54 (9)
Gender	
• Male	47 (87)
Smoking	
• Never smoked	31 (57)
• Former smoker	14 (26)
• Current smoker	9 (17)
Mode of transmission	
• MSM	27 (50)
• Heterosexual	8 (15)
• Other	19 (35)
Current cART regimen	
• PI-based	24 (44)
• INSTI-based	7 (13)
• NNRTI-based	15 (28)
• Other	8 (15)
HIV viral load at moment of pulmonary function test	
• Undetectable	51 (94)
Admission at ICU during PJP episode	
• Yes	9 (17)
History of pneumothorax	
• Yes	5 (10)
Diagnosis of COPD/Asthma established by any physician	6 (11)
Time since HIV diagnosis (median (IQR))	10 (11)
Time since PJP diagnosis (median (IQR))	10 (12)
Adjunctive steroids during PJP episode	33 (61)
$CD4^+$ cell count during pulmonary function test (median (IQR))	478 (224)
$CD4^+$ cell count during PJP episode (median (IQR))	29 (60)
Peripheral oxygen saturation in percent (median (IQR))	98 (0)

Abbreviations: COPD – Chronic Obstructive Pulmonary Disease; HIV – Human Immunodeficiency Virus; ICU – Intensive Care Unit; INSTI – Integrase Strand Transfer Inhibitor; IQR – Interquartile Range; MSM – Men who have sex with men; NNRTI – Non-Nucleoside Reverse Transcriptase Inhibitor; PI – Protease Inhibitor; PJP -Pneumocystis Jirovecii Pneumonia.

Table 2. Main outcomes of the pulmonary function tests.

Restrictive pulmonary disease	
• No	51 (94)
• Yes	3 (6)
Obstructive pulmonary disease	
• No	49 (91)
• Mild	3 (6)
• Moderate	2 (4)
• Severe	0 (0)
Carbon oxide diffusion impairment	
• No	33 (61)
• Mild	16 (30)
• Moderate	5 (9)
• Severe	0 (0)

All values are reported as n (%) unless noted otherwise

Impaired diffusion capacity

Next, we evaluated the occurrence of impaired CO diffusion capacity in our cohort. A total of 21 (39%) patients displayed some degree of diffusion impairment [table 2]. Five patients (9%) classified as having moderate and sixteen patients (30%) as having mild diffusion impairment. There were no patients with a diffusion capacity below 40% of predicted. Only five of the patients with impaired diffusion (24%) reported respiratory symptoms in daily life, two of them with moderate and three with mild disease. Two patients qualified as moderate impaired diffusion and three as mild. Two patients with impaired diffusion had also restrictive pulmonary function and three had concurrent obstructive pulmonary disease.In univariate analysis age, a history of smoking, history of pneumothorax during the episode of PJP, time since PJP and being MSM as risk factor for HIV transmission were associated with the occurrence of impaired diffusion capacity [table 3]. However, in multivariate analysis none of these factors reached statistical significance.

DISCUSSION

This retrospective cohort analysis among HIV-positive patients with a history of PJP demonstrated that nearly 40% of patients have some degree of diffusion impairment, while the prevalence of obstructive and restrictive pulmonary disease was relatively low. In our cohort, we could not identify specific risk factors associated with these outcomes.

The first proof of long term effects of PJP in HIV-infected patients was demonstrated in the study by Nelsing et al. – which was published in the pre-cART era (1995) [9]. They described the follow-up of pulmonary function abnormalities exclusively in HIV-positive patients with recent PJP [9]. In this study, nineteen patients underwent pulmonary function test during the initial episode of PJP – with all patients having reduced diffusion impairment with a median value of 43% of predicted (normal range defined as ≥ 80%). Serial follow-up pulmonary function tests were performed up to nine months after the initial episode of PJP. At nine months, data were available in thirteen patients with only three of them (23%) showing normalization of the diffusion capacity; the median diffusion capacity was 64% of predicted. The use of adjunctive corticosteroids did not influence the outcomes, nor did a history of smoking.

Although this study gives an insight in the dynamics of diffusion impairment after PJP, it only describes a relatively short follow-up time in a limited number of patients. Moreover, no efficient antiretroviral therapy was started after PJP. When compared to our data, we observed a lower prevalence of impaired diffusion, leaving the possibility that pulmonary function could further improve and sometimes even normalizes after one year of the episode of PJP. Such a protracted course is also observed in other pulmonary conditions in which diffusion impairment is also one of the main characteristics like in acute respiratory distress syndrome (ARDS) [15]. Long-term follow-up data in patients with ARDS showed that improvement can occur up to five years after the initial episode of ARDS [16]. However, it should be noted that also in this setting in a significant number of patients the diffusion capacity remained impaired.

The mechanism of impaired diffusion capacity in the acute setting of PJP is presumed to be the result of the presence of a high inoculum of Pneumocystis species and the subsequent influx of inflammatory cells into the alveolus during infection [3]. During this inflammatory reaction several alterations in the composition of alveolar surfactant take place and these changes attribute to the impaired diffusion in both the short- as the long-term [17–19]. Several mouse models and clinical studies have shown that there is severe reduction in the amount of large surfactant aggregations in PJP, resulting in

diminished surface tension in the alveolus and subsequently a risk for alveolar collapse [17,18]. In the study of Schmidt et al. this effect was most pronounced in patients with severe PJP – e.g. those needing mechanical ventilation – when compared to patients with moderate PJP or a bacterial pneumonia [19].

Our analysis did not include a matched control group of HIV-positive patients without a history of PJP and therefore the high prevalence of impaired diffusion could not be attributed solely on PJP. In fact, the background prevalence of impaired diffusion among HIV-positive patients is high with numbers reported up to 30% [20]. Although the numbers are not compared in a direct manner, the prevalence in our cohort seems to exceed this number and therefore merits further consideration.

The most important study on diffusion impairment in the general HIV population was published by Crothers et al [20]. They assessed pulmonary function in a cross-sectional manner, comparing HIV-positive patients (n=300) with HIV-negative controls (n=289). HIV-positive patients were more likely to have moderately to severely reduced CO diffusion capacity compared to the controls (30% versus 18%. $P<0.001$). Multivariate analysis revealed that HIV-infection itself was independently associated with impaired diffusion next to more traditional risk factors like smoking. There was no association between impaired diffusion and a history of PJP, but this effect might have been outweighed by the high prevalence of (former) smokers in this cohort (70%).The finding that HIV itself is independently associated with impaired diffusion, suggests that HIV-related immune activation directly contributes to the development of this outcome. Moreover, in this study the effect of HIV itself was most pronounced in patients with advanced immunodeficiency – e.g. $CD4^+$ cell count <200 cells/mm^3. Since a history of respiratory tract infection was not associated with impaired diffusion, this finding supports the hypothesis that longstanding HIV-related immune activation plays an important role. Moreover, there are several studies that support this relation in a more pathophysiological manner: patients with advanced immunodeficiency are at risk for a $CD8^+$ associated lymphocytic alveolitis [21] which alters the diffusion capacity of the alveolus. Other studies established that there is an upregulation of matrix metalloproteinases expression in the alveolar macrophages of HIV-positive individuals not receiving antiretroviral therapy [22]. These enzymes are important mediators in the degradation of the alveolar wall extracellular matrix [23]. Inflammatory processes like this are directly linked to the development of emphysema and thus diffusion impairment [24].

Therefore, we hypothesize that the high prevalence of impaired diffusion in our population is the result of multiple hits. At first, the pulmonary alveolus is affected by longstanding HIV-related immune activation - reflected by low nadir $CD4^+$ cell counts

– by the pathophysiological mechanisms described above. Subsequently, due to this advanced immunodeficiency patients eventually develop PJP; this additional hit leads to even more extensive alveolar damage with a slow tendency to recover and persistent diffusion impairment as a result. Lastly, more traditional risk factors such as smoking should also be taken in consideration as additional hit. Depending on the severity of these multiple hits, alveolar recovery over time varies from return to normal (with normal lung function testing) to impaired (with affected lung function testing).

There are some limitations that need to be taken in account in the interpretation of these data. Besides the small sample size and its retrospective design, the most important one was already discussed above; the high background prevalence of impaired diffusion in the general HIV population makes it difficult to assess the exact impact of PJP on this specific outcome. The inclusion of an HIV-positive control group without a history of PJP – matched for age, gender, nadir $CD4^+$ cell counts (as surrogate for time of HIV-infection) and smoking habits – would help in the interpretation of our results. Furthermore, the time between the episode of PJP and the pulmonary function assessment was highly variable in our cohort. While some patients underwent pulmonary function testing only a few years after the episode of PJP, in other patients the test was performed nearly twenty years after the initial episode. Ideally, the serial pulmonary function tests as in the study of Nelsing et al. [9] should be performed for a longer period of time and while patients use effective antiretroviral medication to determine at what point no further improvement in the diffusion capacity can be expected – like in the large ARDS trials [16].However, in the era in which the number of cases of PJP is decreasing in the Western world, it would be challenging to perform such a study [2].

Although this is only a small retrospective study, these data give some guidance on the expected long-term pulmonary function outcomes after an episode of PJP. With HIV itself being a risk factor for diffusion impairment, an episode of PJP is an additional hit that further compromises the pulmonary function in HIV-positive patients. These findings should make physicians aware of the fact that the patients with a history of PJP have a high a priori probability for significant lung damage, which possibly puts them at risk for a more severe course of respiratory infection or the earlier development of respiratory disease in the setting of cigarette smoking.

In conclusion, the prevalence of impaired diffusion capacity in HIV-positive patients with a history of PJP was high in this retrospective analysis. However, there is a need for an HIV-positive control group without a history of PJP to determine the impact of PJP itself.

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Part IV

General discussion & summary

Chapter 10

Discussion

GENERAL DISCUSSION

The first reports of acquired immunodeficiency syndrome (AIDS) and the subsequent identification of the human immunodeficiency virus (HIV) were published in the early eighties[1,2].After fifteen years of limited therapeutic options, combination antiretroviral therapy (cART) was introduced in the mid-nineties and led to a dramatic increase in life expectancy for HIV-positive individuals [3,4]. Nowadays, the life expectancy for HIV-positive individuals approximates that of the general population and most patients use single-tablet regimens with few side-effects and a high genetic barrier for resistance [5].Subsequently, the focus in HIV-related research has shifted from AIDS-defining illnesses towards comorbidities and the long-term effects of HIV and cART in this population.

In this thesis I focus on liver-, bone- and lung-related comorbidities in the HIV-positive population. Although cardiovascular disease and non-AIDS malignancies draw most attention, the impact of these other domains might be underestimated in current research. As a matter of fact, liver disease is the leading organ-specific cause of death in the HIV-positive population [6]. Moreover, reduced bone mineral density (BMD) occurs in more than half of patients, exceeding the burden of cardiovascular disease in HIV [7]. Finally, data on morbidity of the respiratory tract in HIV are scarce although pulmonary infections are common – from pneumococcal pneumonia in early stages of HIV [8,9] to Pneumocystis jirovecii pneumonia (PJP) as prevalent AIDS-defining condition [10]. In addition to infectious comorbidity, lung cancer is of increasing importance in the HIV-positive population [11].

Below, I discuss the results of the analyses performed for this thesis, the future perspectives and make recommendations regarding new research projects.

Part I: Liver-related morbidity: drugs, viruses and fat

Liver-related diseases in HIV have evolved in conjunction with the developments of the course of HIV infection over the years: initially, opportunistic infections were the main threat for the liver [12], but over the years drug-induced hepatotoxicity [13] and viral hepatitis became the leading causes of liver disease [14]. Nowadays, non-alcohol fatty liver disease is one of the most important liver-related morbidities in the HIV-positive population [15].

The hepatotoxic potential of some antiretroviral drugs is widely recognized; especially the early generation nucleoside reverse transcriptase inhibitors (NRTIs) like didanosine (ddI) and stavudine (d4T) can cause severe liver damage. In general, these drugs are not used anymore but some of the currently used antiretroviral drugs

also display hepatotoxicity. For example, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz (EFV) and nevirapine (NVP) are known for their potential to induce liver injury – with a cumulative incidence of severe hepatotoxicity in up to 10-15% of the patients [16–18].Earlier studies reported on the occurrence of hepatotoxicity in the first three years of therapy, but there was a lack of studies with a longer follow-up time. In **chapter 2** we report on the finding that clinically relevant hepatotoxicity occurs in approximately 15% of patients receiving NNRTI-therapy for at least three years, but that severe hepatotoxicity was rare. Moreover, most of these hepatotoxic events occurred in the first year of NNRTI-treatment. This observation gave an insight on the pathophysiological mechanisms and natural course of NNRTI-induced hepatotoxicity: first of all, a cumulative toxic effect is unlikely and data suggest that prolonged NNRTI-treatment is safe if no severe hepatotoxicity occurred in the first years of therapy. In addition, these findings emphasize that physicians should consider alternative etiologies if liver enzyme elevations occur after years of NNRTI treatment, like viral hepatitis or steatohepatitis. Our study was published in 2012, but remains relevant in current practice.In 2018 more than 3,000 patients in the Netherlands were still using either EFV or NVP [19] and until recently EFV-based cART was the first line therapy according to the World Health Organization [20]. In my experience, many patients do not want to change their ART combination if it is successful and well tolerated, and therefore the use of NNRTI-based therapy will remain significant. In addition, recent data show that patients are open to switch to generic medication like nevirapine and hereby contributing to a significant cost reduction [21]. The safety data are reassuring and support prolonged NNRTI-based therapy with efavirenz and nevirapine.

The second pillar in liver-related morbidity in HIV-positive patients is viral hepatitis. In this thesis I focused on Hepatitis E (HEV) and Hepatitis B Virus (HBV).After the first reports on the potential of hepatitis E infection to become a chronic disease in immunocompromised hosts, several studies were performed to assess the impact of these findings in the HIV-positive population [22,23]. In **chapter 3** we studied the seroprevalence of HEV in a HIV-positive population with and without otherwise unexplained liver enzyme elevations in the calendar period 2007-2011.In this cohort, a low overall HEV IgG seroprevalence (3.8%) and one patient having detectable IgM antibodies were found, suggestive of an acute HEV infection as a cause of the elevated ALT. These numbers are in line with the low seroprevalence in the general Dutch population at that moment [24] as well as in other cohorts of European HIV-infected patients [25,26]. However, since 2011 there has been increasing attention for HEV in the general population;in a study among young healthy blood donors in the Netherlands a rise in seroprevalence from 4.3% in 2000 to 12.7% in 2011 [27] was

observed and additional data showed that HEV viremia is present in 1 of 762 blood donations [28]. More recently, a sexual mode of transmission was suggested for HEV[29]. In this study, a population attending a sexual transmitted infections (STI) clinic who were diagnosed with chlamydia and/or gonorrhea displayed a slightly higher IgG HEV seroprevalence than those who tested negative for these STIs (odds ratio 1.60 (95% confidence interval 1.02 – 2.49)). These findings need to be confirmed in other studies, as this might change our perspective for HEV testing in case of unexplained liver enzyme elevations in HIV-positive patients with advanced immunodeficiency with high risk sexual behavior. Screening all patients would not be an efficient strategy considering the self-limiting course of HEV infection in immunocompetent hosts.

The direct acting antivirals (DAAs) in the treatment for Hepatitis C virus (HCV) have been major game changers in the field of infectious diseases in the last years [30]. The extremely high cure rates have resulted in a six-fold reduction of the prevalence of HIV/HCV co-infection in the ATHENA cohort [19]. Because of this, hepatitis B virus (HBV) might become the sole chronic co-infection left in the HIV-positive population. Traditionally, the all-cause and liver-related mortality was considered to be higher in HIV/HBV co-infected patients compared to patients with either an HIV or HBV mono-infection [31,32].More recent data from our group suggested that in the current era of cART the risk for end-stage liver disease (ESLD) is comparable to patients with a HBV mono-infection [33]. However, there is a lack of robust data on the mortality risk in the era of tenofovir – a nucleoside reverse transcriptase inhibitor (NRTI) displaying an excellent efficacy against both HIV and HBV [34]. Therefore, it is currently the treatment of choice in co-infected patients [35]. In **chapter 4** we compared the risk for all-cause and cause-specific mortality over time in 1,301 HIV/HBV co-infected patients from the ATHENA cohort, accounting for 14,882 person years of follow-up. In this report, patients diagnosed in the tenofovir era – starting in 2003 – had an adjusted hazard ratio of 0.50 for all-cause mortality when compared to patients diagnosed prior to 2003; the adjusted hazard ratio for liver-related mortality was 0.29. These major improvements are the result of the fact that patients diagnosed after 2002 could fully benefit from safe and highly efficacious therapy against both HIV and HBV (tenofovir) and were less likely to be exposed to toxic antiretroviral drugs (didanosine and/or stavudine) or suboptimal HBV treatment (lamivudine, adefovir, telbuvidine). Despite these major improvements in the care for HIV/HBV co-infected patients, we should not rest on our laurels. Even in the era of potent anti-HBV drugs, a significant minority of the patients received no or suboptimal anti-HBV therapy and HBV DNA monitoring was infrequent in this population. It sometimes seems that there is a mindset that one should not worry about HBV anymore in the tenofovir era, with high rates of virological suppression and nil risk for resistance. In addition, the infrequent

monitoring of HBV DNA and low adherence to hepatocellular carcinoma screening policy are examples that the focus is drifting away from HBV. These observations raise the question whether HBV treatment in the setting of HIV-infection is sometimes too inattentively handled.

In the upcoming decade, the most important challenge which lays ahead is to bring drugs into clinical practice that achieve higher rates of 'functional cure' – defined as loss of hepatitis B surface antigen (HBsAg) - compared to the current nucleoside analogues (NAs) tenofovir and entecavir. In current practice, long-term virological suppression of HBV replication represents the main endpoint of current HBV therapy, while HBsAg clearance is considered an optimal endpoint [36]. Although the NAs are highly successful in achieving the main endpoint, the acquisition of immunological control and thereby HBsAg loss is rare: 12% after 5-8 years of therapy in HBeAg positive patients and 2% after 8 years in HBeAg negative patients [37]. It is suggested that ongoing low grade replication despite NA therapy is a mechanism for long-term viral persistence [38]. In addition, NAs do not target the viral nuclear reservoir of episomal covalently closed circular DNA (cccDNA), so the production side and reservoir for HBV virions is not tackled. The inability of the NAs to interfere with the cccDNA also explains why complete cure - eradication of the integrated HBV DNA from the body – could not be achieved. Currently, several HBV targeting drugs are under investigation which hopefully will lead to higher rates of functional cure. The main classes of these new drugs are the entry inhibitors, cccDNA targeting agents, the capsid modulators, nucleic acid polymers (NAPs) and small-interfering RNAs (siRNA) [39]. Although several drugs show great potential to achieve virological suppression in early stage clinical trials, there are currently no data giving us an insight whether as to these drugs could lead to higher functional cure rates. However, with the current drug development pipeline in mind, possible combinations are endless and therefore strategies to find the optimal ones are challenging.

In the upcoming ten years, the adage will remain that virological suppression is the main endpoint and HBsAg clearance is the optimal endpoint. However, we should strive to take these endpoints to a next level, namely that the functional cure will become the main endpoint and complete cure the optimal endpoint.To reach these endpoints, we will also need further progress in immunomodulatory approaches in order to target the infected hepatocytes; proposed treatments include the use of checkpoint inhibitors, chimeric antigen receptor (CAR) T cells, immunomodulatory vaccinations or gene therapy [40]. Future HBV therapy is therefore likely to consist of at least two drugs – a direct acting antiviral like the NAs and an immunomodulatory agent to interact with the already infected hepatocytes. Although such a regimen might lead to more side effects, this could eventually result in complete cure.

While new antiretroviral drugs show less hepatotoxic potential and the treatment of viral hepatitis has been improving over the years, non-alcohol fatty liver disease (NAFLD) might be the next big thing regarding liver disease in HIV. **Chapter 5** is a narrative review on the epidemiology, pathogenesis, diagnosis, treatment and future expectations of NAFLD in HIV.We describe the high prevalence of NAFLD among HIV-positive patients and the similarities and differences in pathogenesis compared to the general population. Weight loss is currently the only effective intervention in all patients with NAFLD, but several drugs are in the pipeline [41]. Unfortunately, patients with HIV are excluded in most of these current trials.After the completion of our review, three clinical trials on the treatment of NAFLD in HIV were published [42–44]. At this moment, tesamorelin is the most promising therapeutic option in reducing the hepatic fat fraction. Tesamorelin is a growth-hormone releasing hormone (GHRH) analogue that was originally introduced as treatment for HIV-associated lipodystrophy and needs to be administrated subcutaneously once daily and is only available in the United States of America [45]. The drug is thought to reduce fat by stimulating lipolysis and diminishing *de novo* lipogenesis through increasing endogenous growth hormone, but it can induce insulin resistance leading to type 2 diabetes mellitus [46]. In the trial reported by Stanley et al., 62 HIV-positive patients with NAFLD were randomized to receive either tesamorelin or placebo and underwent MRI spectroscopy and liver biopsy during follow-up [42]. After twelve months, there was a significant reduction in the hepatic fat fraction assessed by MRI in patients receiving tesamorelin compared to placebo. In addition, patients without liver fibrosis who used tesamorelin were less likely to progress to fibrosis than patients receiving placebo. There was no effect in patients who already had fibrosis at baseline. Although these data are promising for early stage NAFLD, some issues should be kept in mind. For example, there are no long-term safety data available for tesamorelin – the follow-up time in all reports is limited to twelve months [47]. Moreover, the subcutaneous route of administration could be a threshold for some patients; in the trial of Stanley et al. there were 28 reports of injection site complaints among the patients using tesamorelin versus 13 reports among those using placebo. More recently, a Canadian group published data on the effects of vitamin E therapy in HIV-positive patients with presumed NASH [43], which is also an option in the current guidelines for the general population [48]. In this single-arm trial that included 27 patients, the authors observed a decline in ALT levels, controlled attenuation parameter scores and markers for hepatocyte apoptosis (cytokeratin-18) levels after 24 weeks of therapy. Although it is interesting to see that vitamin E administration had effect on NAFLD in HIV-positive patients in this study, it has important limitations. The non-invasive markers used for both diagnosis and outcome display only moderate sensitivity and specificity for NASH, and the lack of a control group make the interpretation of these data difficult [49]. Furthermore, vitamin E therapy was linked with prostate cancer

and should therefore be used with caution [50].A third trial on the efficacy of Aramchol (a stearoyl-coenzyme-A-desaturate-1 inhibitor), failed to identify a significant effect on the hepatic fat fraction when compared to placebo [44].

An interesting option – in particular in the HIV-positive population – is the CCR2/CCR5-receptor antagonist cenicriviroc (CVC). While originally developed as a new entry blocker in the treatment of HIV, CVC is increasingly recognized as an option for the treatment of NASH and liver fibrosis [51]. CVC inhibits the upregulation of pro-fibrotic pathways by the blockage of both CCR2 and CCR5 on the hepatic stellate cells, which are considered to be the key players in the development of liver fibrosis [52]. The effect of CVC on liver fibrosis was evaluated in a small cohort of HIV-positive patients who were either using CVC or EFV with a backbone of tenofovir disoproxil fumarate and emtricitabine [53]. The degree of liver fibrosis was evaluated using the Enhanced Liver Fibrosis (ELF) index as a non-invasive marker; a higher ELF index is correlated with more significant fibrosis [54]. After 48 weeks, patients in the CVC arm showed significant improvement, while the ELF index remained stable in patients receiving TDF/FTC/EFV (-9% versus +2%. $p < 0.01$).

Data from an HIV-negative population with biopsy-proven NASH suggested beneficial effects with respect to improving the degree of liver fibrosis when compared to placebo [55]. Considering its activity against HIV, CVC would be an ideal option for HIV-positive patients with NAFLD. In 2016 a rather positive phase 2b trial for CVC as third drug in cART was published [56], but ever since no new trials have been initiated to study CVC in HIV treatment according to clinicaltrials.gov. Therefore, CVC should only be considered as an anti-fibrotic drug and not as a third drug option in cART. In contrast, there are some data that support the use of Maraviroc (MVC) – which targets the CCR5 receptor – as an anti-fibrotic drug [57,58]. Although MVC does not target the CCR2 receptor, its clinical utility is far more established than CVC and this might be an option for HIV-positive patients with liver fibrosis.

In the upcoming years, I expect more trials to include HIV-positive patients because the burden of NAFLD is increasingly recognized in this population. But given the current data, I would not expect major pharmacological changes in the short term. Weight loss will remain the cornerstone for NAFLD treatment, but this is hard to achieve, especially in the setting of the ongoing debate on cART-related (excessive) weight gain [59]. Although data on tesamorelin are promising for HIV-positive patients without liver fibrosis, its position in the treatment needs to be defined in the upcoming years. The main challenge will be to identify those patients who could benefit the most from this renewed drug. Patients who already have liver fibrosis and should therefore benefit the most, are the least likely

to respond to treatment in means of clinical endpoints. In contrast, patients with NAFLD without concurrent fibrosis could benefit the most from this treatment – but it will be challenging to motivate these asymptomatic patients to administer a subcutaneous drug once daily for a rather abstract endpoint. Furthermore, uncertainty exists whether as to they would ever show progression to severe liver disease like cirrhosis. Not to mention the potential financial burden to treat a disease that affects one out of three HIV-positive patients. I would suggest focusing on anti-fibrotic drugs like CVC. These drugs target especially the patients who are at risk for progression to end-stage liver disease and it can be easilyincorporated into the current HIV treatment protocols.

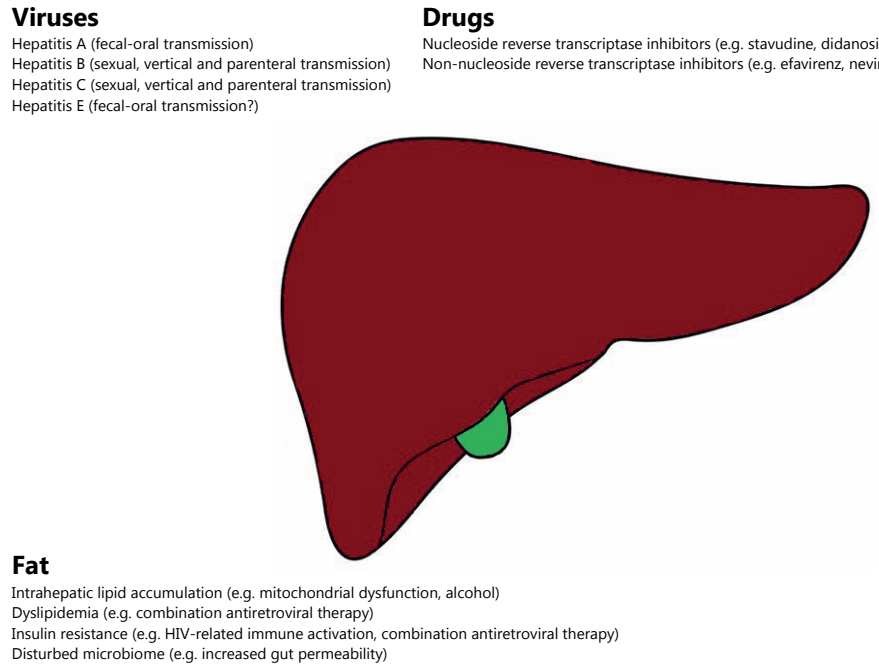

Figure 1. *A schematic representation of the factors influencing liver health in people living with HIV.*

Part II:Bone-related morbidity: Safety profile in modern antiretroviral regimens and a more pragmatic screening policy

The high prevalence of reduced bone mineral density (BMD) in the HIV-positive population could lead to increased health care consumption and associated costs. Screening with dual energy x-absorptiometry (DXA) in a cohort in the University Medical Centre Utrecht revealed that nearly 60% of the HIV-positive patients aged ≥ 50 years had reduced BMD [60], which is in line with other reports [7].

Tenofovir disoproxil fumarate (TDF) is currently among the most used antiretroviral drugs in the HIV-positive population, but is associated with the development of reduced BMD [61]. While the exact pathophysiological mechanisms of TDF-related bone loss are subject of debate, our study presented in **chapter 6** strongly suggests that this effect is driven by the parathyroid hormone (PTH) and is dependent on tenofovir plasma levels. Switching from TDF to tenofovir alafenamide (TAF) resulted in lower PTH levels and subsequently lower bone turnover markers; this explains the favorable bone profile of TAF. However, the actual impact of this biochemical improvement needs to be evaluated in more robust clinical trials. Several registration studies showed that on the long term the difference in bone mineral density is only 3% between patients using TDF or TAF [62–65] and there is a lack of data on whether the observed difference actually leads to less osteoporotic fractures. This question is even more intriguing since there is no robust evidence that links the use of TDF with an increased fracture risk [66]. Some suggest that the unfavorable bone and renal safety profile in TDF only applies when it is combined with a pharmacological booster like cobicistat or ritonavir [67]. Furthermore, it is unknown how the bone safety profile of TAF relates to non-TDF/TAF containing antiretroviral regimens. What is the impact of TAF-based regimens on PTH, bone turnover markers and DXA outcomes when compared to the long-acting injectable antiretroviral drugs or the novel dual-therapy agents like dolutegravir/lamivudine (DTG/3TC)?The registration trials with injectables did not include bone safety as an endpoint. The TANGO study in cART experienced patients who switched to either DTG/3TC or a TAF-based triple drug regimen found that DTG/3TC had a more favorable profile with respect to bone turnover markers, but data on more robust clinical outcomes like changes in BMD – assessed by DXA – or (calculated) fracture risks are also needed [68]. Studies on this subject need to be prioritized, as DTG/3TC is currently among the first line options in HIV-treatment guidelines [69].

Following the observation that reduced BMD is prevalent among HIV-positive patients, there is the quest for optimal screening strategy to identify patients with reduced BMD who would benefit from early treatment. Current guidelines recommend universal screening for all males aged ≥50 years and postmenopausal females and suggest the use of Fracture Risk Assessment (FRAX®) Tool in the remaining patients [70,71]. However, in **chapter 7** we showed that the latter screening policy lacks sensitivity for identifying those at risk for osteoporosis. Therefore we are advising to abandon FRAX as a screening tool.For the young population, I propose to follow a more pragmatic policy in which only patients with a high risk of falling, a history of a fragility fracture or prolonged corticosteroid usage qualify for early bone mineral density assessment. Universal screening of young HIV-positive patients is probably not a cost-effective policy, considering the low absolute fracture risk in these patients [72]. Indeed, we

should keep in mind that in the end it is all about the prevention of fragility fractures and not just the diagnosis of reduced BMD. From this perspective, I do see a role for FRAX in treatment decision rather than as a screening strategy. Although FRAX might underestimate the fracture risk in HIV-positive patients [73,74], I believe that FRAX has the potential to prevent overtreatment. In my opinion, the upcoming years need to be used to identify specific HIV-related risk factors for fragility fractures so these parameters can be applied in a more sensitive FRAX-like model for HIV-positive patients. In that way, physicians could also evaluate which older HIV-positive patient could benefit from BMD measurement rather than the current 'one size fits all' approach.

Part III:Lung-related morbidity: Sequelae after AIDS-related pulmonary infections and new threats on the horizon

In the field of comorbidities in the HIV-positive population, respiratory tract diseases tend to be overlooked. This is remarkable considering the fact that the lungs are a frequently encountered location of various diseases through all stages of the HIV-infection. Pneumococcal pneumonia is often one of the first presentations of an –unrecognized –impaired immune system [8] while *Pneumocystis jirovecii* pneumonia (PJP) – previously known as *Pneumocystis carinii* pneumonia (PCP) -is one of the most common AIDS-defining conditions [10]. Furthermore, symptoms associated with pulmonary disease like dyspnea are associated with major reduction in quality of life [75]. In this thesis I addressed two topics associated with PJP.

In **chapter 8** we studied the influence of corticosteroids on $CD4^+$ cell recovery in HIV-positive patients with Pneumocystis jirovecii pneumonia (PJP). In this small cohort we found that a short course of corticosteroids does not alter the degree of immunological recovery in the first year after the initiation of cART. Although the sample size is small and patients undergo a relatively short course of corticosteroids, these data give some guidance what to expect regarding immunological recovery in patients with severe immunodeficiency with a need for corticosteroid treatment. Not only the immunological recovery in patients with a history of PJP is of interest, there is also a need for data on functional outcomes in these patients. From one small study in the pre-cART era, it is known that CO diffusion impairment can persist at least nine months after the episode of PJP, but data on the long-term consequences are lacking. In **chapter 9** it was shown that the prevalence of impaired diffusion capacity is as high as 40% among patients that suffered from PJP at least one year ago.However, these findings should be interpreted with caution; in the general HIV-positive cohorts a prevalence up to 30% is reported and a history of PJP was not associated with this outcome.Some state that these high rates in the general HIV population are the result of HIV-related immune activation, in which CD8+ cell predominant alveolitis and upregulation of alveolar macrophage matrix

metalloproteinases lead to emphysema [76]. A new study is needed to assess whether PJP is indeed associated with persistent diffusion impairment or that the observations in our study are there result of high background prevalence of impaired diffusion in HIV-positive patients.To perform such a study, the control group should consist of at least an equal number of HIV-positive patients without a history of PJP (n=54) that are matched for age, gender, nadir CD4+ cell count, time living with HIV and smoking history with the study group from our previous analysis. This limits the number of confounders and would therefore be the best control group to answer the question whether a history of PJP is indeed associated with persistent diffusion impairment.

Nowadays, PJP is not the biggest threat among the respiratory diseases in HIV-positive patients anymore. It is the high rate of lung cancer and its presentation at a relative young age in HIV-positive patients that is gaining increasing attention [77]. Although pulmonary cancers are not part of this thesis, I believe that this remains the most significant pulmonary comorbidity in the HIV-positive population in the upcoming years and merits therefore some consideration in this discussion.

Currently lung cancer is the leading malignancy-related cause of death in HIV-positive patients [78]. As the prevalence of smoking among HIV-positive patients remains high [79], it is expected that the impact of lung cancer will remain significant in this population. Smoking is the most important risk factor for lung cancer, but HIV infection itself is an independent risk factor for the development of lung cancer [80]. In that respect, I believe it would be interesting to review lung carcinoma development in HIV-positive patients on a more basic level, for example to evaluate the histological characteristics of the resected or biopsied tumors in relation to the tumors in HIV-negative patients.It is well known for example that non-Hodgkin lymphomas tend to have more aggressive characteristics in HIV-positive patients compared to those without HIV [81]; it would be interesting to see whether there are similar differences with respect to pulmonary carcinoma.Furthermore, the efficacy, safety and tolerance of the standard and new treatment regimens (e.g. immunotherapy) in patients with HIV are worth to studying [82].

TO SUMMARIZE:

In the discussion of this thesis, the clinical perspectives in the upcoming ten years were addressed and recommendations regarding future research on several topics were made.

- The introduction of tenofovir containing cART has led to an impressive decrease in mortality risk in HIV/HBV co-infected patients.
- As functional cure of HBV will remain achievable in only a limited number of patients, we need to keep in mind that the HIV/HBV co-infected patients should receive optimal treatment for both infections.
- There are several interesting options in the HBV treatment pipeline, but finding the ultimate therapy combination will be challenging.
- Non-alcoholic fatty liver disease will become the most important liver disease in HIV-positive patients and its pathophysiological mechanism is multifactorial.
- Tesamorelin could be an important option in the treatment of NAFLD in HIV-positive patients, but its indication field needs to be explored further.
- Short-term research should focus on the development and clinical introduction of anti-fibrotic drugs, targeting the patients most at need for intervention.
- The prevalence of reduced BMD among HIV-patients is high, but uncertainty exists as to which patients are at increased risk for fragility fractures.
- There is a need for a more directed screening policy in order to prevent fragility fractures in HIV-positive individuals.
- Clinical trials with new antiretroviral drugs should use clinical relevant outcomes like T-scores rather than bone turnover markers alone as outcome for bone safety.
- The prevalence of diffusion impairment among patients with a history of PJP is high, but it unclear whether this exceeds the background prevalence in the general HIV-positive population.
- In addition to infectious diseases, the burden of lung cancer among HIV-positive patients is and will remain significant.
- More basal studies are needed to determine how lung cancer in HIV-patients differs from lung cancer in the general population and efficient screening programs should be developed.

In conclusion, this thesis describes the clinical perspective of HIV-related morbidity in three different organ systems. In a few decades, HIV has changed from a death sentence towards a manageable chronic condition which can be treated with one pill a day. Such a paradigm shift was never seen before in medicine and we would not have talked about long-term morbidity without the tremendous efforts by all people involved in HIV-

related care and science. In the nineties, media showed images of cachectic HIV-positive patients who needed to take a great amount of pills to stay alive. Nowadays I discuss healthy aging with my HIV-positive patients and tell them about the new therapeutic options displaying fewer side effects and give them more autonomy. Considering the developments in immunology, hopefully I will be discussing the ultimate goal for HIV during consultations:

CURE

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SUMMARY

Background
The first reports of acquired immunodeficiency syndrome (AIDS) and the identification of the human immunodeficiency virus (HIV) were published in the beginning of the eighties. After fifteen years of limited therapeutic options, combination antiretroviral therapy (cART) was introduced. For that reason, the clinical perspective of HIV changed from a lethal disease to a chronic manageable condition that can be treated with safe and highly efficacious drugs. Subsequently, the focus in scientific research shifted towards comorbidities, related to either HIV or cART. In this thesis I present several studies on liver-, bone- and lung-related comorbidities in the HIV-positive population.

Part I: Liver-related morbidity: drugs, viruses and fat
In the first part of this thesis I address several aspects of liver-related comorbidity in HIV. Nowadays, liver-related diseases are still among the leading causes of death in the HIV-positive population, but its clinical perspective has changed over the years.In the early years of the HIV pandemic, most liver diseases were the result of opportunistic infections but over the years cART-related hepatotoxicity and coinfections with viral hepatitis became the most important reasons for liver-related morbidity.

In **chapter 2** we studied the occurrence of clinical significant hepatotoxicity in a retrospective cohort of patients that received the non-nucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz or nevirapine for at least three years, as long-term follow-up data regarding hepatic safety of NNRTIs were lacking. We found that the prevalence of clinically significant hepatotoxic events (15%) in this cohort was comparable to the data in the cohorts with short follow-up time. Furthermore, we observed that most hepatotoxic events occurred in the first year of NNRTI therapy (6.6 events in the first year versus 2.8 per 100 person-years of treatment in the following years), which makes a cumulative toxic effect highly unlikely. These findings are reassuring for both physicians and patients who worry about the long-term hepatotoxic potential of NNRTIs.

In the following chapters, two studies were presented addressing the subject of viral hepatitis in HIV-positive individuals. **Chapter 3** is the report of a study that evaluated the occurrence of hepatitis E virus (HEV) as causative agent for unexplained liver enzyme elevations in autochthonous HIV-positive patients (n=56) and the background prevalence of a past HEV infection in patients without liver enzyme elevations (n=50). These data are of interest considering the potential of HEV to become chronic in severely immunocompromised patients. In this study we found that in only one patient – 2% of the total population – the elevated alanine-aminotransferase could be attributed

to an acute HEV infection. In addition, the prevalence of a past HEV infection – defined as the presence of IgG HEV antibodies – was also low with a seroprevalence of 3.8% in the entire cohort.In **chapter 4** we present a study how the risk for all-cause and cause-specific mortality in HIV/HBV coinfected patients have developed over the years, coinciding with the improvements in care for this specific population. This analysis included all patients (n=1301) from the ATHENA cohort in care in the period 1998-2018, accounting for 14,882 person years of follow-up.Over the years, the incidence of newly diagnosed HIV/HBV coinfections declined with no new cases entering the cohort in 2017. We found that the adjusted hazard ratio (aHR) for all-cause mortality in patients diagnosed in or after 2003 was 0.50 (95% confidence interval (CI) (0.35-0.75) relative to patients diagnosed before 2003. This risk reduction was more pronounced for liver-related mortality (aHR 0.29 (95% CI 0.11 – 0.75). The use of a tenofovir-containing antiretroviral regimen was independently associated with a reduced risk for all-cause and liver-related mortality. Prior exposure to didanosine and/or stavudine was strongly associated with liver-related mortality. Furthermore, it was remarkable that a significant part of the patients under follow-up in the modern cART era did not receive optimal HBV therapy. This study illustrates the success of the current therapeutic approach in HIV/HBV coinfection, but also emphasizes that we should not rest on our laurels regarding HBV management.

The final chapter of the first part of this thesis (**chapter 5**) is a narrative review on what might be the next big thing in liver-related morbidity in HIV: non-alcoholic fatty liver disease (NAFLD). In this review, we discuss the prevalence, pathophysiology and treatment of NAFLD in the HIV-positive population. In the final sections of this chapter, we address the future expectations regarding epidemiology and therapy, with special interest for the anti-NAFLD and anti-fibrotic drugs that are currently studied in HIV-positive populations.

Part II: Bone-related morbidity: Focus on pathophysiology and optimal screening

In the second part of this thesis, I focus on bone-related morbidity in HIV and in particular on osteoporosis. The prevalence of osteoporosis is extremely high in the HIV-positive population, with nearly 20 percent of the patients aged >50 years being affected. The high prevalence of reduced bone mineral density in this population is the result of multiple factors, including both HIV- and cART-related factors.

Especially the use of tenofovir disoproxil fumarate (TDF) is associated with the development of reduced bone mineral density. However, the pathophysiological mechanisms of TDF-related bone loss are largely unknown. Recently was hypothesized that tenofovir directly interacts with the calcium sensing receptor in the parathyroid

glands and that tenofovir induces hyperparathyroidism in a dose-dependent manner. Considering this hypothesis, we performed a study on the effects of switching TDF to tenofovir alafenamide (TAF), a tenofovir pro-drug with lower blood levels and therefore less systemic effects – on bone turnover markers and parathyroid hormone (PTH) levels. The result of this study was presented in **chapter 6** and showed a significant decline in median serum PTH levels when patients were switched from TDF to TAF: From 6.7 (interquartile range (IQR) 5.4 – 8.8) to 4.6 (IQR 3.6-6.2). Subsequently, we found a decline in the levels of the alkaline phosphatase as a bone turnover marker. This finding gives us an important insight on the mechanism of TDF-related bone loss and helps to understand the favorable bone safety profile of TAF.

In **chapter 7** the current policy regarding osteoporosis screening in young HIV-positive patients was evaluated. Current guidelines recommend the use of the Fracture Risk Assessment Tool (FRAX®) as screening tool to determine which patients should undergo bone mineral density testing (BMD). In case patients have a >10% probability for an osteoporotic fracture in ten years, they are eligible for dual energy x-absorptiometry (DXA). In our study we found that this policy lacks sensitivity as a case-finding strategy for osteoporosis, as none of the patients who developed osteoporosis in our cohort were identified by screening ten years prior to the diagnosis. Furthermore, there was no difference between FRAX scores between patients that eventually develop osteoporosis and those who do not. Based on our findings, we strongly advice to revise our current screening strategies for osteoporosis in young HIV-positive patients.

Part III: Lung-related morbidity: Persistent effects after AIDS-related pulmonary infections?

In the field of comorbidities in the HIV-positive population, respiratory tract diseases tend to be overlooked. Considering that Pneumocystis jirovecii pneumonia (PJP) still being the most common AIDS-defining condition, data on the long-term outcomes in patients with PJP are of interest. In this thesis, the focus was on immunological recovery and the long-term sequelae in pulmonary function after PJP.

Chapter 8 describes the immunological recovery in HIV-positive patients with advanced immunodeficiency who receive corticosteroids. Corticosteroids are frequently used in HIV-positive patients with $CD4^+$ cell counts <200 cells/mm^3, especially to treat immune reconstitution inflammatory syndrome (IRIS) and PJP with profound hypoxemia. However, as corticosteroids can induce significant lymphopenia, some physicians fear that corticosteroids lead to suboptimal immunological recovery after the initiation of cART. We found that a short-course of corticosteroids during an episode of PJP did not lead to worse immunological recovery compared to patients that did not receive

corticosteroids as adjunctive therapy for PJP: after three and twelve months, there was no difference between mean $CD4^+$-cell count between these groups. Furthermore, there was no increase in the incidence of additional opportunistic infections in the first year after the initiation of cART in patient that received corticosteroid treatment.

In **chapter 9** the data on the long-term pulmonary function outcomes of PJP are presented. In this retrospective analysis, the pulmonary function tests of 58 patients with a history of PJP were evaluated for the presence of pulmonary function abnormalities. In this cohort, we found that nearly 40% of the patients had an impaired diffusion capacity. The question that needs to be answered is whether this is the result of the past PJP – which is characterized by diffusion impairment in the acute setting – or due to a high background prevalence of diffusion impairment among HIV-positive patients. Despite the limitations of this analysis, this finding merits further evaluation.

Finally, in **chapter 10** I discuss the main findings of this thesis and what these findings add to current knowledge in the field of HIV-related comorbidity in the era of modern combination antiretroviral therapy. Furthermore, I address expectations for the future and make recommendations for new research projects.

Appendices

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Appendices

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Achtergrond

De onderzoeken die in dit proefschrift besproken worden hebben betrekking op comorbiditeit bij patiënten met het humaan immunodeficiëntie virus (HIV), het virus dat uiteindelijk tot AIDS (Acquired Immunodeficiency Syndrome) kan leiden.

HIV is een virus dat in begin jaren 80 van de twintigste eeuw werd ontdekt toen een aantal jonge mannen zonder medische voorgeschiedenis werden gediagnostiseerd met een ernstige longontsteking (*Pneumocystis carinii* pneumonie) die normaal gesproken alleen bij mensen met een ernstig verzwakt immuunsysteem voorkomt. HIV bleek tot verzwakking van het immuunsysteem te leiden doordat het virus rechtsreeks de $CD4^+$-cellen aanvalt en vernietigt. Omdat deze cellen een essentiële rol spelen in de afweer tegen ziekteverwekkers, zijn HIV-positieve patiënten vatbaar voor allerhande ernstige infectieziekten en vormen van kanker die bij gezonde mensen niet voorkomen. De transmissie van HIV verloopt via seksueel contact, door bloed-bloed contact of door de overdracht van moeder op kind. Besmetting met HIV via deze routes kan ook gepaard gaan met de overdracht van andere virussen die op eenzelfde wijze worden overgebracht zoals hepatitis B, een virus dat leverontsteking veroorzaakt.

Het duurde enkele jaren tot er medicijnen waren ontwikkeld die HIV enigszins konden afremmen, de zogeheten antiretrovirale therapie. In 1985 werd de eerste HIV-remmer geïntroduceerd– Zidovudine (AZT) – dat in staat bleek om het ziektebeloop van HIV af te remmen. Dit betrof echter maar een kortdurend effect, omdat het virus na enkele maanden resistent werd tegen het medicijn. Hetzelfde fenomeen werd gezien bij alle andere antiretrovirale medicatie die in de jaren 80 en begin van de jaren 90 op de markt werd gebracht. Bovendien ging het gebruik van deze middelen gepaard met veel en soms ernstige bijwerkingen. Veel (jonge) mensen overleden in deze periode aan de gevolgen van AIDS en het aantal HIV infecties bleef groeien.

Deze sombere situatie veranderde radicaal toen in 1996 een nieuwe groep HIV-remmers werd geïntroduceerd, de protease inhibitors. Uit onderzoeken bleek dat als deze middelen gecombineerd werden met de oudere medicijnen het virus effectief en langdurig onderdrukt kon worden. Door de combinatie van medicatie werd de kans op resistentie-ontwikkeling aanzienlijk kleiner dan eerder. De therapie voorkwam nu niet alleen dat de afweer nog slechter werd, bij een groot deel van de patiënten werd zelfs een (gedeeltelijk) herstel van de afweer gezien. De behandeling was echter zwaar door een grote hoeveelheid pillen die geslikt moesten worden en door de bijwerkingen. Om deze redenen moesten sommige patiënten de medicatie (tijdelijk) staken.

De grote maatschappelijke aandacht en de inspanningen van vele wetenschappers en artsen hebben er toe geleid dat sinds 1996 de behandelmethoden voor HIV sterk zijn verbeterd. De middelen die anno 2020 worden gebruikt zijn zeer effectief en de bijwerkingen zijn relatief beperkt. Het gevolg hiervan is dat de levensverwachting van de HIV-positieve patiënt nu vrijwel gelijk is aan die van de algemene, HIV-negatieve populatie. In de spreekkamer gaat het tegenwoordig ook niet meer over de ernstige infecties die op korte termijn kunnen optreden en het risico op overlijden, maar juist over de langetermijngevolgen van een HIV-infectie en het gebruik van de antiretrovirale therapie. We zien dat verschillende ouderdomsziekten bij HIV-patiënten op jongere leeftijd optreden omdat zij hebben blootgestaan aan langdurige activatie van het immuunsysteem en het gebruik van toxische medicatie, wat beiden op de lange termijn tot verschillende aandoeningen kan leiden. Dergelijke aandoeningen worden met name gezien aan de bloedvaten, lever, botten, longen en hersenen.

In dit proefschrift worden verschillende onderzoeken beschreven die betrekking hebben op co-morbiditeit bij HIV-patiënten op het gebied van de lever, botten en de longen.

Deel I: Lever-gerelateerde ziekten bij HIV: medicijnen, virussen en vet
Leverziekten komen veelvuldig voor bij patiënten met HIV. Lever-gerelateerde aandoeningen zijn na AIDS-gerelateerde ziekten ook de belangrijkste doodsoorzaak bij deze patiënten. De leverziekten die bij HIV worden gezien zijn over de tijd sterk veranderd – in het begin van de HIV pandemie waren het met name infectieuze problemen, maar na de introductie van effectieve medicatie verschoof de focus naar levertoxiciteit van deze medicatie en virale leverziekten. Echter, in het tijdperk van de moderne antiretrovirale therapie lijkt leververvetting de belangrijkste oorzaak van leverschade bij HIV-patiënten te worden.

In **hoofdstuk 2** beschrijven we het optreden van levertoxiciteit bij patiënten die gedurende langere periode (≥ 3 jaar) de non-nucleoside reverse transcriptase remmers (NNRTI's) efavirenz of nevirapine gebruiken. Het is bekend dat ongeveer 10-15% van de patiënten die deze middelen gebruiken ergens gedurende hun behandeling levertoxiciteit ontwikkelen. Echter, de studies die dit fenomeen beschrijven hebben allemaal maar een relatief korte follow-up periode tot hooguit drie jaar terwijl patiënten deze middelen soms jarenlang gebruiken. In onze studie stelden we vast dat het optreden van levertoxiciteit in deze groep patiënten vergelijkbaar is met de incidentie in de studies met een korte follow-up periode (< 3 jaar). Daarnaast zagen we dat de meeste episodes van toxiciteit zich in het eerste jaar van therapie voordeden, nadien neemt het

aantal episodes van toxiciteit duidelijk af. Deze uitkomsten zijn geruststellend omdat ze ondersteunen dat het langdurige gebruik van efavirenz of nevirapine op de lange termijn veilig lijkt te zijn als het gaat om effecten op de lever.

Zoals gezegd is een andere bekende oorzaak van leverproblemen bij HIV-patiënten een bijkomende infectie met een virale hepatitis. Er zijn verschillende hepatitis virussen die ziekte kunnen veroorzaken en in dit proefschrift heb ik mij gericht op hepatitis E virus (HEV) en hepatitis B virus (HBV).**Hoofdstuk 3** beschrijft een studie waarin wordt gekeken hoe vaak HEV de oorzaak vormt van leverenzymstoornissen bij HIV-patiënten waarbij andere redenen hiervoor zijn uitgesloten. Dit onderzoek is ingegeven door het feit dat in de algemene populatie ongeveer 10% van de onverklaarde leverenzymstoornissen veroorzaakt blijkt te worden door een infectie met HEV. Bij HIV-patiënten is deze bevinding extra belangrijk gezien het feit dat bij mensen met een ernstig verstoorde afweer het hepatitis E virus ook een chronische infectie kan veroorzaken hetgeen op de lange termijn leidt tot levercirrose. In onze studie bleek het aantal gevallen van een acute HEV infectie beperkt en maar 2% van de onverklaarde leverenzymstoornissen te verklaren. Daarmee leek HEV op dat moment geen relevante oorzaak voor verstoorde leverbiochemie bij HIV-positieve patiënten te vormen.

In **hoofdstuk 4** onderzoeken we hoe het overlijdensrisico bij patiënten met een infectie met zowel HIV als HBV over de jaren is veranderd. Vanwege de gemeenschappelijk transmissie route komt een HIV/HBV coinfectie regelmatig voor en patiënten met een dergelijke coinfectie hebben een verhoogd risico op een vroegtijdig overlijden. In deze studie bekijken we in een cohort met alle HIV/HBVpatiënten die sedert 1998 in Nederland in zorg zijn geweest hoe het overlijdensrisico zich heeft ontwikkeld en welke factoren geassocieerd zijn met sterfte. In de resultaten van deze studie komt naar voren dat het risico op vroegtijdig overlijden bij patiënten die na 2003 zijn gediagnostiseerd met ongeveer 50% is afgenomen ten opzichte van patiënten die voor 2003 de HIV-diagnose hebben gekregen. Die reductie is zelfs 70% als er wordt gekeken naar het risico om te overlijden aan lever-gerelateerde aandoeningen. Het gebruik van de nucleotide reverse transcriptase inhibitor tenofovir – dat zowel actief is tegen de HIV als de HBV -blijkt de belangrijkste beschermende factor tegen overlijden te zijn. Verder zagen we dat patiënten die in het verleden behandeld zijn met de ouderwetse middelen didanosine of stavudine juist een sterk verhoogd risico hebben om te overlijden aan lever-gerelateerde oorzaken. Naast de afname van de sterfte zien we dat door succesvolle vaccinatie strategieën van de GGD's het aantal nieuwe gevallen van hepatitis B sterk afneemt. Ondanks deze successen moet er voldoende aandacht blijven voor de optimale behandeling van HBV bij HIV-patiënten. Uit ons cohort blijkt namelijk dat >10% van de patiënten geen optimale HBV therapie krijgt.

Zoals duidelijk wordt zijn er veel successen geboekt in de behandeling van lever-gerelateerde co-morbiditeit bij HIV-patiënten: de medicatie die we tegenwoordig gebruiken wordt steeds veiliger en het aantal nieuwe gevallen van een bijkomende HBV infectie wordt minder. De nieuwste uitdaging op het gebied van leverziekten bij de HIV-patiënt is een ziektebeeld dat ook in de algemene populatie in toenemende mate wordt gezien: non-alcoholic fatty liver disease (NAFLD) – in de volksmond ook wel leververvetting geheten. In **hoofdstuk 5** wordt op basis van de bestaande literatuur een overzicht gegeven van de epidemiologie, pathofysiologie, diagnostiek en behandeling van NAFLD bij HIV-patiënten. Daarnaast wordt er in deze review ingegaan op de verwachtingen met betrekking tot nieuwe therapieën in de behandeling van NAFLD in het algemeen en die behandeling bij HIV-patiënten in het bijzonder.

Deel II: Bot-gerelateerde ziekten bij HIV: Onderliggende mechanismen en screening
De prevalentie van verminderde botdichtheid bij HIV-patiënten is zeer hoog: in het UMC Utrecht heeft meer dan 50% van de patiënten ouder dan 50 jaar een verminderde botdichtheid, in totaal voldoet bijna 20% van de patiënten aan de diagnose osteoporose ('botontkalking'). De reden hiervan is multifactorieel en wordt bepaald door traditionele risicofactoren (vitamine D tekort, ondergewicht), HIV-gerelateerde factoren (slechte afweer) en door medicatie. In dit proefschrift wordt een hypothese getoetst die betrekking heeft op het onderliggende mechanisme van medicatie-gerelateerd botverlies en wordt de vraag gesteld of de huidige screeningsstrategie voor osteoporose bij jonge HIV-patiënten zinnig is.

Een belangrijke speler in medicatie-gerelateerd botverlies is tenofovir disoproxil fumarate (TDF). Dit middel werd aan het begin van de 20e eeuw op de markt gebracht en heeft vanwege zijn effectiviteit en relatief gunstige bijwerkingenprofiel een centrale plaats verworven in de behandeling van HIV. Het gebruik van TDF kan echter leiden tot botontkalking, maar het onderliggende mechanisme hiervan is onduidelijk. Een van de theorieën hieromtrent is dat de werkzame stof tenofovir zich aan de bijschildklieren bindt waardoor deze minder goed aangestuurd kunnen worden en een overmaat van het bijschildklierhormoon PTH gaan produceren. Het PTH zorgt er vervolgens voor dat er botafbraak plaatsvindt, hetgeen uiteindelijk resulteert in verminderde botdichtheid. In eerder onderzoeken werd gesuggereerd dat dit effect afhankelijk is van de hoeveelheid tenofovir waaraan de bijschildklieren worden blootgesteld. In 2016 kwam een alternatief voor TDF op de markt: tenofovir alafenamide (TAF).

Door een alternatieve formulering is de tenofovir spiegel in het bloed lager bij TAF dan bij TDF, maar juist een stuk hoger in de cellen waar tenofovir zijn werk moet doen. Om die reden is TAF net zo effectief als TDF, maar door de lagere bloedspiegels zijn de effecten op de rest van het lichaam inclusief de botten minder. Om de hypothese te toetsen dat het effect van tenofovir op de bijschildklier dosis-afhankelijk is, werd een onderzoek verricht waarvan de resultaten in **hoofdstuk 6** worden gepresenteerd. Hierbij werden de waarden van het PTH en van de botafbraakmarker alkalisch fosfatase (AF) bekeken bij patiënten die van TDF naar TAF switchten. In deze studie zagen we het PTH met gemiddeld 32% zakken, naast een daling van 16% van het AF – hetgeen de hypothese ondersteunt. Hoewel deze uitkomsten niet direct te vertalen zijn naar relevante uitkomsten voor patiënten, geven deze data ons wel een inzicht in het mechanisme waarom TAF gunstiger voor de botten is dan TDF en hiermee begrijpen we ook beter waarom er bij het gebruik van TDF een verhoogd risico op botontkalking is.

Zoals gesteld komt osteoporose veel voor in de populatie HIV-patiënten boven de 50 jaar en derhalve wordt in de richtlijnen gesteld dat alle patiënten in deze leeftijdscategorie een zogeheten dual energy x-absorptiometry (DXA) scan, oftewel een botdichtheidsmeting moeten ondergaan. Voor patiënten tussen de 40-50 jaar wordt gebruik gemaakt van een risico score (Fracture Risk Asessment Tool (FRAX®) waarbij patiënten die hierbij een hoge score (>10% risico op een osteoporotische fractuur in de aankomende 10 jaar) hebben, geadviseerd wordt om een DXA scan te ondergaan.

In **hoofdstuk 7** evalueren we hoe gevoelig deze test is om de patiënten met osteoporose te identificeren. In deze studie bekijken we alle patiënten die tussen hun 50^{e} en 59^{e} een DXA scan hebben ondergaan en rekenen met de gegevens van tien jaar eerder uit of zij op dat moment al in aanmerking zouden zijn gekomen voor een DXA. Vervolgens bekijken we of er een verschil zit tussen de mensen die uiteindelijk osteoporose blijken te hebben en degenen die dat niet hebben. In onze studie zien we dat geen enkele patiënt in aanmerking zou zijn gekomen voor een vroegere DXA scan omdat niemand de geformuleerde afkapwaarde van 10% voor het 10-jaars risico voor een fractuur haalt. Nog belangrijker is dat er geen verschil in de FRAX score is tussen de mensen die uiteindelijk osteoporose hebben (ongeveer 20% van alle patiënten) en degenen die dat niet hebben. Met andere woorden, het gebruik van de FRAX score helpt niet bij de vroegere identificatie van mensen die uiteindelijk osteoporose hebben. Daarnaast lijkt het ook niet zinnig om de afkapwaarde aan te passen omdat er uiteindelijk geen enkel verschil in de FRAX score zit tussen de patiënten met en zonder osteoporose. Wij concluderen in dit hoofdstuk dan ook dat we op zoek moeten naar een andere manier van osteoporosescreening voor deze jongere HIV-patiënten.

Deel III: Long-gerelateerde ziekten bij HIV: Lange termijn effecten na infecties
In dit laatste gedeelte van het proefschrift ga ik in op de effecten die een eerdere HIV-gerelateerde longziekte – in deze een longontsteking veroorzaakt door een schimmel (Pneumocystis jirovecii pneumonie (PJP)) - en haar behandeling kan hebben op de lange termijn.

In **hoofdstuk 8** ga ik in op de vraag of het gebruik van ontstekingsremmers (corticosteroïden) een negatief effect heeft op het herstel van het immuunsysteem bij HIV-patiënten die een ernstig gestoorde afweer hebben. In de behandeling van PJP worden in het geval van een ernstig zuurstoftekort corticosteroïden zoals prednison gebruikt omdat afweerreactie van het lichaam de patiënt soms juist nog zieker kan maken. Uit oude literatuur weten we echter dat corticosteroïden voor een daling van het aantal $CD4^+$-cellenkan zorgen en kan zorgen voor andere infecties doordat de afweer verder wordt onderdrukt, juist het tegenovergestelde van wat wordt beoogd bij de HIV behandeling. In deze studie hebben we gekeken wat er met het aantal $CD4^+$-cellengebeurt bij patiënten die starten met HIV-remmers en tegelijkertijd ook corticosteroïden krijgen en degenen die dat niet krijgen. Er zit geen verschil in de mate van stijging van het aantal $CD4^+$-cellentussen beide groepen na 3 of 12 maanden na de behandeling met corticosteroïden. Ook zagen we niet meer ernstige infecties optreden bij de mensen die corticosteroïden krijgen. Dat betekent dan ook dat artsen die te maken krijgen met deze HIV-patiënten met een ernstig gestoorde afweer zich geen zorgen hoeven maken over negatieve effecten op het immuunsysteem bij patiënten die om wat voor reden dan ook gedurende korte tijd behandeld worden met prednison.

Als laatste beschrijf ik in **hoofdstuk 9** in hoeverre patiënten die in het verleden een PJP hebben gehad hier restafwijkingen in de longfunctie aan overhouden. In deze studie hebben we achteraf gekeken hoeveel patiënten een verstoring van de gaswisseling in de longen houden in de jaren nadat ze een PJP hebben doorgemaakt. Tijdens de episode van de PJP is de gaswisseling namelijk in alle patiënten ernstig verstoord, maar het is onduidelijk in hoeverre dit herstelt. Bij deze retrospectieve analyse waarbij de longfuncties van alle patiënten die in het verleden PJP hebben gehad zijn bekeken vonden we dat 40% van deze patiënten in enige mate afwijkingen in de gaswisseling blijft houden. Omdat er geen controle groep bij deze studie zat is er niet goed uit te maken hoe groot het effect van de doorgemaakte PJP was, zeker omdat bekend is dat een aanzienlijk percentage van de HIV-patiënten door de HIV-infectie zelf al een verstoorde gaswisseling heeft. Het zou overigens goed kunnen dat een groot gedeelte van de patiënten al longschade heeft door de langdurige HIV-infectie maar dat dit nog eens versterkt wordt door het doormaken van PJP naast eventueel andere risicofactoren voor longschade zoals roken.

In **hoofdstuk 10** volgt tenslotte de discussie van dit proefschrift. Hierbij ga ik in op de betekenis van mijn bevindingen en wat voor implicaties dit heeft voor patiëntenzorg en toekomstig onderzoek. In de aanbevelingen richt ik mij met name op het behoud van adequate behandeling van hepatitis B bij HIV-patiënten en hoe we in de toekomst genezing van de HBV-infectie kunnen bereiken. Daarnaast roep ik op om in de grote onderzoeken naar de behandeling van NAFLD ook HIV-patiënten te betrekken en om op zoek te gaan naar een meer doelgerichte screeningsstrategie om vast te stellen welke HIV-patiënten gescreend moeten worden voor osteoporose.

Appendices

List of publications

LIST OF PUBLICATIONS

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Appendices

Dankwoord

DANKWOORD

Als laatste het hoofdstuk wat uiteindelijk door de meeste mensen gelezen zal worden, het dankwoord.

Het sluitstuk van een proefschrift waarvan de oorsprong ruim tien jaar geleden lag tijdens de geneeskunde-opleiding en dat zich in wisselend tempo over de jaren tot het huidige boekje heeft ontwikkeld. Dat zou uiteraard niet mogelijk zijn geweest zonder de steun en hulp van een aantal mensen die ik hier wil bedanken.

Geachte professor Hoepelman, beste Andy. Ik wil je bedanken voor het vertrouwen wat je me de afgelopen jaren hebt geschonken om dit promotie-traject met succes af te ronden en hoe je me hebt gevormd tot de internist-infectioloog die ik nu ben.Zowel als opleider als promotor weet je de perfecte balans te vinden wanneer je moet prikkelen en wanneer je iemand vrij moet laten. Je hebt op meerdere momenten je nek voor me uitgestoken, wat heeft geresulteerd in mijn boekje en mijn huidige aanstelling. Ik waardeer je zeer, zowel als clinicus als onderzoeker. Enerzijds benadrukte je meer dan eens dat we terug moesten naar de basis, welke vraag wilde ik beantwoorden? Anderzijds keek je ook telkens weer vooruit, welke lijn moesten we volgen – wat vertelden de tot dan toe gegenereerde resultaten ons en wat konden we daar verder mee. De geschiedenis van het PTH stuk illustreert hoe je klinische observatie met wetenschap verbindt.

Geachte dr. Mudrikova. Tania, ik heb je leren kennen toen ik als 4^{e} jaars geneeskundestudent een wetenschappelijke keuzestage deed waarbij we keken naar de dynamiek in HBV DNA bij HIV-patiënten die behandeld werden met het toen relatief nieuwe middel tenofovir. Dat onderzoeksproject heeft qua wetenschap niet veel opgeleverd, maar heeft de basis gelegd voor een verdere samenwerking. In eerste instantie als student, later als mijn poli supervisor en uiteindelijk als collega internist-infectioloog. In al die jaren heb ik je leren kennen als iemand met een schat aan ervaring en kennis, maar bovenal als een prettig persoon. Je staat altijd open voor nieuwe onderzoeks ideeën, je toetst mijn ideeën aan de klinische praktijk en je bent altijd bereid om met een kop koffie om 'het wereldje' te becommentariëren. Daarnaast waardeer ik je oprechte interesse en je betrokkenheid.

Geachte dr. Arends. Beste Joop, ook jou heb ik al vroeg tijdens mijn 'onderzoektijd' leren kennen. In eerste instantie als meelezer/meeschrijver van het NNRTI-stuk en later als mijn begeleider voor het hepatitis E stuk. Een aantal maanden geleden heb ik de speech terug gevonden die jij in 2011 hebt geschreven voor mijn afstuderen met een

beschouwing of ik een echte internist ben. Hierin sprak je al uit dat je verwachtte me nog wel eens tegen te komen. Dat heeft uiteindelijk tot 2015 geduurd toen ik mijn opleiding tot internist vervolgde in het UMC Utrecht – wat ook een soort herstart (of echte start?) van mijn promotietraject is geweest. In die tijd ben ik onder de indruk geraakt van hoe je van alle markten thuis bleek te zijn. Je was niet alleen een goede, pragmatische internist, maar ook een ondernemer en goede wetenschapper. Je commentaar op mijn stukken was er altijd snel, je zette de lijnen uit en kwam met nieuwe hypotheses waar ik op kon voort borduren. Je wist me telkens toch weer te motiveren om de zoveelste versie van het NAFLD stuk te herschrijven. Daarnaast ben ik je enorm dankbaar voor de kansen en het vertrouwen die je me hebt geschonken tijdens het promotietraject en om je te vervangen tijdens je sabbatical en later na je definitieve vertrek. Ik vind het enorm jammer dat we afscheid van je hebben moeten nemen maar ook ik weet zeker dat ik je in de toekomst weer tegen ga komen.

De leden van de beoordelingscommissie en opponenten, prof. dr. Reiss, prof. dr. Vleggaar, prof. dr. Emmelot-Vonk, prof. dr. Heijerman en prof. dr. van der Ven, graag wil ik u bedanken voor de bereidheid mijn proefschrift te beoordelen.

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Het grootste gedeelte van mijn proefschrift heb ik geschreven tijdens de periode dat ik werkzaam was als AIOS in het Diakonessenhuis en UMC Utrecht en later als internist-infectioloog in het UMCU. Gedurende deze periode heb ik mogen samenwerken met veel mensen, die in meer of mindere mate het voor mij mogelijk hebben gemaakt om dit proefschrift af te ronden. Sommige motiveerden mij wetenschappelijk, anderen waren van belang voor mijn werkplezier in het algemeen. Hiervan zijn er een aantal mensen die ik in het bijzonder wil bedanken.

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En dan mijn paranimfen, CJ-dokters van het eerste uur. Dokter Vincent! Wij zijn tegen het einde van de studie bevriend geraakt. Even leek je voor de interne geneeskunde te kiezen, maar uiteindelijk bleek je toch liever een hartcardioloog te worden. Die wens bleek zo sterk te zijn dat je hiervoor naar de andere kant van het land bent verhuisd. Hoewel ik het jammer vind dat je hier niet meer in de buurt woont, vind ik de keuze om te verhuizen bewonderingswaardig. Niet alleen kon je doen wat je het allerliefste wilde, je woonplaats levert in ieder geval genoeg stof voor grappen. Het was ook fijn om te merken dat ondanks de afstand ons contact goed blijft, zodat we ons kunnen blijven verbazen over van alles. Dank dat je vandaag hier wil staan als paranimf. Fleur, bedankt dat je mijn paranimf wil zijn. Zoals je weet heb ik je uitgekozen vanwege je uitgesproken academische profiel. Ik ken je het langste van het groepje en het is mooi hoe we na al die jaren nog steeds bevriend zijn. Onze levens zijn over die 15 jaar natuurlijk heel erg veranderd, maar nog steeds vinden we elkaar in onze humor, onze niet-altijd-even-genuanceerde meningen over eigenlijk alles en andere gemeenschappelijke interesses. Dank voor onze discussies, borrels en rants. En zoals beloofd: +1.

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Appendices

Curriculum Vitae

CURRICULUM VITAE

Berend Joost van Welzen was born on the 3th of 1986 in Leiden, the Netherlands.In 2005, he completed secondary school at the Visser 't Hooft lyceum in Leiden. Subsequently, he started studying medicine at the University of Utrecht in 2005 and obtained his medical degree in 2011. During medical training he performed several research projects at the Department of Infectious Diseases at the University Medical Centre Utrecht.

Upon graduation, he started working as a medical doctor at the Internal Medicine department of the Diakonessenhuis in Utrecht. In April 2013 he started his residency in Internal Medicine in the same hospital under supervision of dr. A.F. Muller.

After nearly three years he continued his residency at the University Medical Centre Utrecht (UMCU) (supervisor prof. dr. H.A.H. Kaasjager) and participated in several HIV-related research projects. These projects were supervised by Prof. dr. I.M. Hoepelman, Dr. J.E. Arends and Dr. T. Mudrikova and formed the basis of this thesis. In 2016 he started a fellowship in Infectious Diseases, which was finished in July 2019. Afterwards he began working as an internist-infectiologist in the UMCU till now. He is living in Utrecht with Mariëlle Gidding.