# Disentangling health risks for people ageing with - or whilst at risk of acquiring – HIV in the Netherlands

*Living with a legacy*

Verboeket, S.O.

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# LIVING WITH A LEGACY

Sebastiaan Olaf Verboeket

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# Disentangling Health Risks for People Ageing with - or whilst at Risk of Acquiring – HIV in the Netherlands

Living with a Legacy

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ter verkrijging van de graad van doctor aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus prof. dr. ir. K.I.J. Maex
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Aula der Universiteit
op woensdag 26 januari 2022, te 11.00 uur

door Sebastiaan Olaf Verboeket
geboren te BREDA

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Faculteit der Geneeskunde

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# 1

# General Introduction

# BACKGROUND

## HIV, AIDS and HAART

In 1996, at the 11th International AIDS Conference in Vancouver Canada, the first data were presented and later published to show that dual drug-class *combinations* of three antiretrovirals could effectively and sustainably reduce Human Immunodeficiency Virus (HIV)-RNA concentration in plasma to below the level of detection in people living with HIV (PLWH).[1,2] This Highly Active Antiretroviral Therapy (HAART), as it was named, was able to also induce a sustained recovery of the peripheral blood $CD4^+$ T-lymphocyte count and thereby proved highly effective in preventing Acquired ImmunoDeficiency Syndrome (AIDS)-defining comorbidities and AIDS-related mortality.[3] This scientific advancement signified a watershed moment in the history of HIV and AIDS, allowing to transform an inevitably fatal disease into a chronic condition with the prospect for longevity. Twenty-five years later, combination antiretroviral therapy (cART), as we call it today, remains the cornerstone of HIV care and has enabled increasing numbers of PLWH to reach an advanced age in relatively good health.[4] Naturally, this puts PLWH at increasing risk of developing non-AIDS-related *ageing-associated comorbidities* (AACs).

## Ageing-associated comorbidities in PLWH

Studies have reported AACs to occur *more frequently* and possibly *at younger ages* in PLWH compared to the general population.[5,6] Cardiovascular, pulmonary, renal and liver disease as well as neurocognitive decline, non-AIDS-defining malignancies, osteoporosis and frailty have all been reported to be more prevalent among older PLWH compared to similarly-aged people without HIV.[5,7,8] Determining the extent and drivers of these apparent health disparities between people with and without HIV is challenging; AACs are diverse in their pathophysiology and also commonly occur among ageing people without HIV. Furthermore, their occurrence is influenced by a multitude of risk factors that may be unevenly distributed between people with and without HIV, with the increasing age itself above all else remaining the most important risk factor for AAC development.[9]

AACs are typically slow-developing long-term degenerative processes which can take decades before becoming clinically apparent or symptomatic. These include for example slow-developing atherosclerotic plaques as precursors for acute arterial thrombotic events such as myocardial infarction and stroke, the pre-malignant stages of cancer or osteopenia/osteoporosis preceding osteoporotic bone fractures.[10,11] The development of such diseases is also typically influenced by long-term cumulative exposures to particular risk factors like cigarette smoking, overweight, dyslipidemia or hypertension.[12] To study the *causes* of AACs among PLWH it is therefore necessary to take into account

the *history* of these people. AACs experienced by older PLWH today may be driven by exposures which were accumulated over a lifetime: from before HIV-infection, during periods of uncontrolled HIV-replication, immune dysfunction and AIDS-related illness, and during the years after initiation of antiretroviral treatment with viral suppression.

## Commonalities in the exposome of people with and at risk for HIV

The *exposome* is defined as "every exposure to which an individual is subjected from conception to death", i.e. all lifetime non-genetic environmental exposures, including gestational influences and social interactions with others.[13,14] The exposome and the genome are together considered the two main interacting factors that determine the probability of disease in an individual.[15] The exposome can be divided into three domains of exposures: *general external* (social capital, education, financial status, mental stress, urban–rural environment, climate, etc.), *specific external* (radiation, infectious agents, chemical contaminants and environmental pollutants, diet, lifestyle factors (e.g. tobacco, alcohol), occupation, medical interventions, etc.), and *internal* (metabolism, endogenous hormones, body morphology, physical activity, gut microflora, inflammation, lipid peroxidation, oxidative stress, ageing, etc.).[14] Such exposures can be measured at the individual level (e.g. level of physical activity) or at the population level (e.g. levels of air pollution in a certain region).[15]

In this introduction I will attempt to illustrate which exposures the population of PLWH in the Netherlands are likely to have in common within their exposome (both HIV-related and -unrelated), while also evaluating the history of the people (having been) *at risk* for HIV-infection. As such, it provides the reader with a broad perspective and also a starting point from which to study the on average increased risk of AACs among PLWH (and potentially also in those at risk of acquiring HIV).

By definition PLWH share in their exposome an HIV infection and also, for the duration of the time spent living with HIV, likely share a common history of exposures to the evolving available therapeutic options for the infection, which have followed the scientific developments in HIV research since the start of the epidemic. Moreover, in higher income countries in particular, HIV infections typically occur among minority populations such as men who have sex with men (MSM), injecting drug users, sex workers or migrants.[16] For example, of the 20,612 people known to be in care with HIV in the Netherlands at the end of 2019, 12,985 (63%) were MSM and 8,435 (41%) had migrated to the Netherlands from other parts of the world.[4] People from such minority populations are also likely to have commonalities in their exposome, such as for example having experienced similar societal inequities or having been at increased risk of particular infectious diseases (including but not limited to HIV). When evaluating the multitude of potential exposures

leading to AACs among PLWH it is therefore important to not only take into account the effects of HIV infection and its treatments, but also the cultural and socio-demographic context of the people in whom the HIV-infection has occurred.

### A historical framework

The following paragraphs will provide an overview of key historical events regarding the scientific developments around HIV since the beginning of the epidemic, as well as events having affected the Dutch MSM, drug user and migrant communities over the last 65 years. This historical overview covers the lifespan of those PLWH who are currently 65 years of age, and as such provides a framework from which the commonalities in the exposomes of those ageing with HIV today can be envisioned. This framework can thereby be used to provide the reader with a more contextual interpretation of the findings described in this thesis.

Next, this introductory chapter will discuss the characteristics of the longitudinal prospective cohort studies that were used as the main sources of data for the studies presented in this thesis, followed by an outline of the remaining thesis chapters.

## SETTING THE SCENE: AN OVERVIEW OF HISTORICAL EVENTS AFFECTING THE HEALTH OF PEOPLE WITH - OR WHILST AT RISK FOR - HIV IN THE NETHERLANDS

*For a graphical representation of the timeline see Figure 1.4*

### Young and gay during the 1960s and 1970s

#### *LGBT activism*

In 1969, during the *Stonewall riots* in New York (USA) the local Lesbian Gay Bisexual and Transgender (LGBT) community for the first time took a united stand against belittling and the violence they had been subjected to by law enforcement officers.[17] Earlier that year in the Netherlands, protests had already been organized against the discriminating nature of criminal code article 248*bis* (Figure 1.1). The article stated sexual activity with a partner from the same sex to be a criminal offence if being 21 year of age or older and the partner being below the age of 21, while for heterosexual sexual contact this age limit was 16. After recommendations made by the Dutch Health Council the law was changed in 1971 making the age of consent 16, regardless of the partner's sex.[18] Furthermore, under the scrutiny of LGBT activists, homosexuality was removed as a psychiatric illness from the Diagnostic and Statistical Manual of Mental Disorders (DSM) by the


**FIGURE 1.1** | Demonstration against criminal code article 248bis

*Binnenhof, The Hague, the Netherlands. January 21, 1969. Source: Fotocollectie Anefo*

American Psychiatric Society in 1973, and as late as 1990, the World Health Organization followed by removing homosexuality from the International Classification of Diseases (ICD-10).[19,20]

These formal changes however certainly did not automatically lead to general *societal acceptance* of sexual diversity, including in the Netherlands. During the annual "Roze Zaterdag" demonstration (the Dutch equivalent of Christopher Street Day) in Amersfoort in 1982, riots erupted between LGBT-activists and local youth.[21] In contrast however, during the 1970s and 1980s the city of Amsterdam became renowned for providing an environment which was accepting of sexual diversity and which attracted people belonging to these communities from both the Netherlands and abroad.[18]

Societal acceptance of sexual diversity by the greater Dutch population has since continued to improve, resulting in the Netherlands becoming the first country to legalize same sex marriage in 2001. However, in a Dutch population study in 2018 21% of participants still indicated to be disgusted by the notion of men having sex with each other, and 29% to be offended by men kissing each other in public, while this was only 11% if a man and a woman were to kiss in public.[22] Moreover, even if currently living in a more accepting environment, people from sexual minority populations may still live with the *memories*

of traumatic experiences from times or places where acceptance of their sexual orientation was - or continues to be - poor. Especially memories from childhood or early adolescence related to social exclusion (e.g. bullying) or psychological or physical abuse may continue to affect the lives of older people belonging to these populations.[23] Such adverse social circumstances faced by people from minority communities have been termed *minority stress*, which is considered a potential contributing cause of health disparities, including mental health and substance use disorders.[24,25]

### *STI epidemics*

In the wake of the "S*exual Revolution*" in the 1960s and driven by the aforementioned liberation of sexual minorities, sexual behaviour changed during the 1970s and 1980s, which became apparent in a rising incidence of sexually transmitted infections (STIs).[26] While only 5 cases of syphilis and 450 cases of gonorrhoea had been treated at the Amsterdam municipal sexual health clinic in 1970, in 1979 this had increased to 533 and 3,918 cases, respectively (Figure 1.2).[27]

At this time, gonorrhoea was diagnosed two- and syphilis seven-fold more often in men compared to women, differences which were at the time thought to be partly driven by men who have sex with men (MSM) attending the clinic.[27] That MSM were at substantially increased risk of contracting STIs during those years was also illustrated by the high prevalence of exposure to hepatitis B virus (HBV) during a study conducted at the STI clinic among MSM in 1980-1981.[28] As many as 60.3% of participants had evidence of past or present HBV infection and 4.8% were positive for HBV surface antigen. The latter was significantly higher compared to the prevalence in blood donors from the general population (0.22%). In 1997, hepatitis B vaccination became available for MSM which since has gradually reduced the incidence of new infections in this population.[29,30] However, for those MSM who became infected prior to widespread vaccination, chronic HBV infection remains a potential health risk for developing liver fibrosis and hepatocellular carcinoma.

The appearance of AIDS in the early 1980s led to a drastic reduction in sexual risk-taking behaviour and subsequently in the rate of STIs.[31] However, this drop in STI incidence and sexual risk-taking behaviour was only temporary and driven by fear. After the introduction of cART in 1996, STI incidence rose steadily again, with the Amsterdam Municipal sexual health clinic in 2019 having diagnosed 3,034 cases of gonorrhoea, 621 of infectious syphilis, and 242 of *Lymphogranuloma venereum*, of which 84%, 96% and 100% respectively, were diagnosed in MSM.[32] Furthermore, in 1989 the hepatitis C virus (HCV) was identified as the cause of non-A non-B hepatitis.[33] Following its discovery, HCV infection was initially predominantly identified among people regularly receiving blood

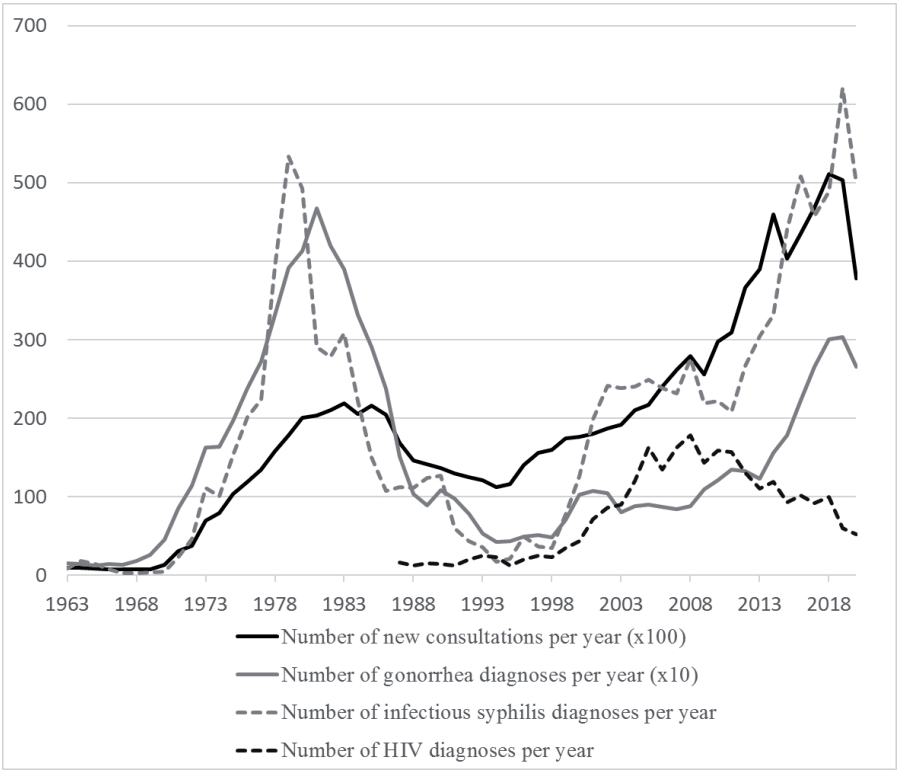



**FIGURE 1.2** | Annual number of new consultations and diagnoses of gonorrhea, infectious syphilis, and HIV at the Amsterdam Public Health Service's Sexual Health Clinic (APHSSHC) between 1963 and 2020

*Source: APHSSHC 2020 annual report. (https://www.ggd.amsterdam.nl/ggd/publicaties/jaarverslagen)*

transfusions and among injecting drug users who shared contaminated needles; the seroprevalence in the latter population in Amsterdam was estimated at 74% in 1990.[34] Currently however, the highest incidence of new HCV infection in the Netherlands is among HIV-positive MSM, with 94% of acute HCV infections between 1998 and 2019 in PLWH occurring among MSM, amongst whom the virus is most frequently transmitted through sexual contact.[4]

### *Substance use*

In the 1970s and 1980s the city of Amsterdam not only appealed to those seeking sexual freedom, but also to those seeking to use recreational drugs, especially among people from hippie communities.[35,36] In 1970, during the *Kralingen Music Festival* in Rotterdam ("*Woodstock* of the Netherlands") the Dutch police had experimented with a *laissez faire* approach regarding the sale and use of drugs, predominantly marijuana at the time.[37] The approach proved to be rather harmless. The experiment inspired the revolutionary 'tolerance policy' (i.e. *gedoogbeleid*) towards the sale (in small quantities) and use of

marijuana in the Netherlands, which was implemented in 1976.[35] Dutch policy makers believed criminal prosecution of people who use drugs should not be more harmful to the individual drug user or society than the drugs themselves.[35] However, when in 1972 heroin was introduced in Amsterdam among the hippie communities as 'just another drug', an epidemic of opioid addiction followed.[36] This also attracted foreign heroin-users towards Amsterdam's tolerant and free environment. In 1988 an estimated 15,000 to 20,000 people living in the Netherlands were addicted to heroin, 4,000 to 7,000 of whom were living in Amsterdam.[35] Among migrants from the former Dutch colonies Surinam and the Moluccas, risk of developing a heroin addiction was higher.[35] In the early 1980s this provided a population in which HIV could easily spread through the sharing of contaminated needles.[38]

Over the years new recreational drugs have steadily been introduced into the Netherlands, with gay men commonly being "early adopters" of such new trends. For example, in 1988 the party drug XTC (i.e. MDMA, 3,4-methylenedioxymethamphetamine) was introduced almost simultaneously with electronic dance music in the Netherlands. Initially, this scene centered around the Amsterdam dance club RoXY (1987-1999) which was famous for its sexually diverse visitors.[39]

MSM more frequently use recreational drugs compared to the general population, and often in different settings.[40] One such setting is that of *chemsex*, which has become more common among MSM in recent years.[41] During chemsex *drugs* (i.e. "*chem*icals") are combined with *sex*, often in settings with multiple sex partners and without the use of condoms, sometimes also involving injecting drug use.[42] Moreover, chemsex typically involves use of drugs with a relatively high risk of adverse health effects, such as crystal meth (N-methamphetamine), GHB (*gamma*-hydroxybutyric acid), 4-MMC (4-methylmethcathinone, mephedrone), and - most recently - 3-MMC (3-methylmeth-cathinone). In addition, Dutch MSM more often smoke tobacco compared to heterosexual men.[40]

## A novel deadly virus among gay men: 1981 – 1996

### *Gay Related Immunodeficiency*

In 1981 the first clusters of *Pneumocystis* pneumonia and Kaposi's sarcoma in US gay men were reported.[43] In July 1981 the New York Times headlined its first article about this new disease with "RARE CANCER SEEN IN 41 HOMOSEXUALS", which referred to Kaposi's sarcoma and was at the time related to a syndrome called "gay-related immunodeficiency" (GRID). The following year in the Netherlands a gay man was diagnosed with this syndrome at the Academic Medical Center in Amsterdam.[44]

The disease was renamed Acquired ImmunoDeficiency Syndrome (AIDS) when clusters of this syndrome appeared not only among gay men, but also among hemophiliacs and Haitians.[45,46] Although such clusters made an infectious agent highly likely, it took until 1983 for HIV (initially called LAV or HTLV-III) to be discovered as the causative agent.[47,48] Lack of treatment for the deadly disease caused significant fear, especially among gay men. Dutch public health campaigns encouraged gay men to stop having anal sex (see Figure 1.3), or at least to use a condom if they were to disregard this advice.

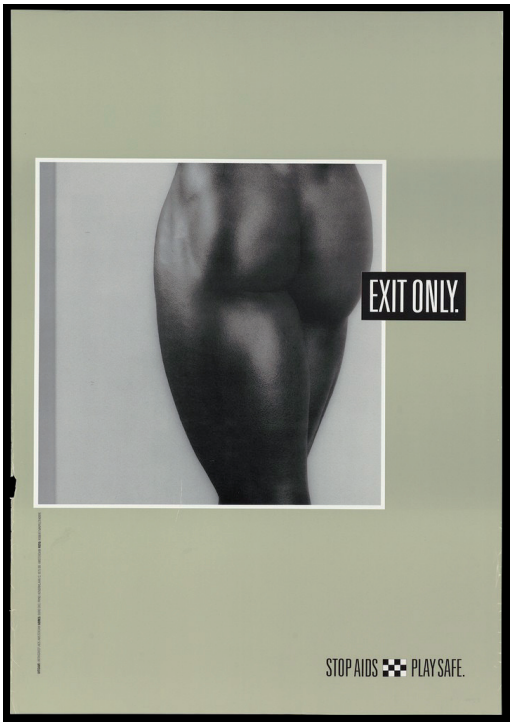



**FIGURE 1.3.** | The bare bottom of a black man with the words 'Exit only'; representing refusal of anal sex as a form of AIDS prevention

*Colour lithograph after Robert Mapplethorpe for the Werkgroep AIDS Amsterdam, 1986. Source: Wellcome Collection. Attribution-NonCommercial 4.0 International (CC BY-NC 4.0)*

That these campaigns were necessary became most apparent at the start of the prospective Amsterdam Cohort Studies (1984-present), which between October 1984 and April 1985 recruited 748 asymptomatic gay men from the city of Amsterdam and around.[49] This first large-scale Dutch HIV-seroprevalence study showed that already 31% of participants were HIV-seropositive at the time of enrolment, which increased to 39% in 1987. The 10-year cumulative incidence of AIDS in HIV-positive participants was later estimated at 60%, and the median time from AIDS to death at 20 months.[50] Tight-knit LGBT-communities had formed throughout the 1970s and 1980s which served as a safe haven for those expelled by family or friends because of their sexual preferences or transgender identities. Within these communities many became affected by HIV or

AIDS either directly by being diagnosed oneself or indirectly by friends or partners being diagnosed, even though the cumulative number of AIDS diagnoses in the Netherlands was only 3,172 by 1996 in a population of 15.5 million.[51] Phenomena like *multiple loss syndrome* (losing multiple close friends) and *survivors guilt* ("Why him and not me?") may resonate until today in the minds of members of the LGBT-communities who were confronted with AIDS between 1981 and 1996.[52]

### *AIDS and first treatments*

The increase in annual AIDS diagnoses in the Netherlands from 5 in 1982 to 533 in 1995 led to the establishment of dedicated wards for AIDS-patients in various hospitals.[51] Before 1990, over half of AIDS-related hospital admissions were for *Pneumocystis jirovecii (*then named *carinii)* pneumonia (PCP) and central nervous system toxoplasmosis.[53] From 1990 onwards the likelihood of admission for PCP and cerebral toxoplasmosis relatively decreased by the use of anti-microbial prophylaxis among those at increased risk of developing these infections ($CD4^+$ T-cell counts <200 cells/$mm^3$). A wide range of AIDS-related infections and malignancies nonetheless continued to affect the health of PLWH. Moreover, it was recognized that in some instances HIV itself could also directly affect organ function, such as in the case of *HIV-associated nephropathy (HIVAN)*, *AIDS-dementia* and *lymphocytic interstitial pneumonitis*, the latter mainly being observed in children.[54-56] Even if PLWH were able to temporarily survive the varied clinical manifestations of AIDS, organ dysfunction would sometimes persist, for example in the case of persistently reduced pulmonary function after resolution of a PCP.[57]

In 1987, the first antiretroviral drug zidovudine (AZT), a nucleoside-analogue reverse transcriptase inhibitor (NRTI) directly targeting virally-encoded reverse transcriptase, was shown to increase $CD4^+$ T-cell counts and provide clinical improvement.[58] The drug however improved the prognosis of PLWH only marginally, as viral resistance usually developed quickly resulting in loss of drug efficacy. In 1991 and 1992 two other NRTIs were introduced: didanosine (ddI) and zalcitabine (ddC). These were first also used as monotherapy, but proved to be slightly more effective when combined in dual-therapy regimens with AZT.[59,60] These dual-therapy regimens however in the vast majority of patients also ultimately resulted in the emergence of drug-resistant HIV, viral rebound, and disease progression.

## From a fatal disease to a chronic condition: 1996 – 2021

### *A Lazarus effect*

When introduced in 1996, the use of HAART, which next to a dual combination of NRTIs included either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease

inhibitor (PI), resulted in many people with AIDS remarkably quickly recovering and regaining their health. This effect was described by some as a *Lazarus* effect in reference to the biblical figure *Lazarus of Bethany* who was made to rise from the dead by Jesus Christ.[61] The ability to measure HIV-RNA by polymerase chain reaction (PCR), which was introduced more or less simultaneously with HAART, was key in allowing monitoring the course of patients' HIV plasma viral load as a marker of treatment effectiveness in clinical trials as well as in clinical settings. Different from what had been observed with mono- and dual therapy with just NRTIs, HAART for the first time was able to durably reduce plasma HIV-RNA to undetectable levels and increase $CD4^+$ T-cell counts.

***Side effects of antiretroviral therapy***

Although for all PLWH cART meant prolonged survival, for some this came at a high cost in the shape of severe drug toxicities. First generation NRTIs (which in the meantime also included stavudine [d4T] and lamivudine [3TC]) were discovered to be associated with mitochondrial toxicity which amongst others could result in peripheral neuropathy, fulminant liver failure, lactic acidosis and adipose tissue pathology. In 1999, *lipodystrophy* (i.e. peripheral fat wasting and central fat accumulation) was first reported as a characteristic HAART toxicity.[62] Lipoatrophy was subsequently found to be associated with the use of thymidine analogue NRTIs in particular, and shown to be poorly reversible after discontinuation or substitution of the culprit thymidine analogue. Facial lipoatrophy could change someone's appearance and result in significant psychological distress.

As a result of major drug development efforts during the next decades, currently more than twenty different antiretroviral agents are available which are combined into multiple cART regimens.[63] Increasingly novel drugs and drug combination have generally had more favourable toxicity and viral resistance profiles, and the more toxic first generation of antiretrovirals and regimens have become obsolete. Moreover, antiretroviral drugs have become less burdensome to take, with single-tablet once-daily combination regimens nowadays being the standard. Treatment toxicity nonetheless remains a subject of some concern. Each (new) drug may have its own toxicity profile and there may be variability between individuals in their susceptibility to such toxicity. The diversity in susceptibility to drug toxicity has proven to not always emerge from randomized clinical trials conducted by pharmaceutical companies seeking marketing authorization from drug licensing authorities. Trials conducted for the purpose of licensing may recruit selected populations of participants which are typically followed for no more than two or three years.[64]

*Changing guidelines*

With the emerging evidence for the potentially severe toxicity of cART the treatment became recommended only for PLWH with $CD4^+$ T-cell counts below 200 cells/$mm^3$ or AIDS. This made sure only those with the highest risk of disease progression and death would be subjected to the toxicities of the regimens available at the time. It also led to studies investigating whether cART could be temporarily interrupted after patients had shown a sufficient recovery of their $CD4^+$ counts on treatment. In 2006 the Strategies for Management of Antiretroviral Therapy (SMART) trial compared episodic vs. continuous use of cART in PLWH with >350 cells/$mm^3$. Episodic use meant deferral of cART until $CD4^+$ counts had decreased to <250 cells/$mm^3$, then use of cART only until $CD4^+$ counts had again increased to >350 cells/$mm^3$.[65] The trial showed continuous use of cART to be superior over episodic $CD4^+$-guided use in preventing opportunistic disease. Surprisingly, the trial also found that the occurrence of major cardiovascular, renal or hepatic disease was lower among the group using cART continuously. This unexpected finding for the first time indicated that uncontrolled HIV infection could adversely affect a range of organ systems and result in morbidities other than AIDS. Importantly, the SMART trial also showed that continuous use of cART was likely to be able to prevent these non-AIDS associated morbidities.

In subsequent years decreasing drug toxicity and increasing evidence regarding the benefits of cART have led to a continuously increasing $CD4^+$ count threshold for when to initiate treatment, i.e. earlier in the course of HIV infection. In 2015 the Strategic Timing of Antiretroviral Therapy (START) and TEMPRANO trials definitively demonstrated the clinical benefit of immediately starting cART in PLWH with more than 500 $CD4^+$ cells/$mm^3$ compared to deferring treatment.[66,67] In the START trial, immediate treatment was associated with hazard ratios for developing AIDS, and a composite endpoint of serious non–AIDS events of 0.28 (95% CI, 0.15 to 0.50; P<0.001) and 0.61 (95% CI, 0.38 to 0.97; P = 0.04), respectively.

In the wake of the START and TEMPRANO trials, HIV-treatment guidelines globally rapidly recommended immediate start of cART following HIV diagnosis, regardless of $CD4^+$ count.[68,69] Apart from the benefit of this strategy for the individual PLWH, a policy of immediate start of cART was also reinforced by the results from the HPTN 052 trial. This study demonstrated that heterosexual PLWH using cART, once their plasma HIV viral load had become undetectable, did not transmit HIV to their sexual partners.[70] The PARTNER and PARTNER2 studies provided the definitive evidence that this is the case for both heterosexual and male-male serodifferent couples, also when having condomless anal sex.[71,72]

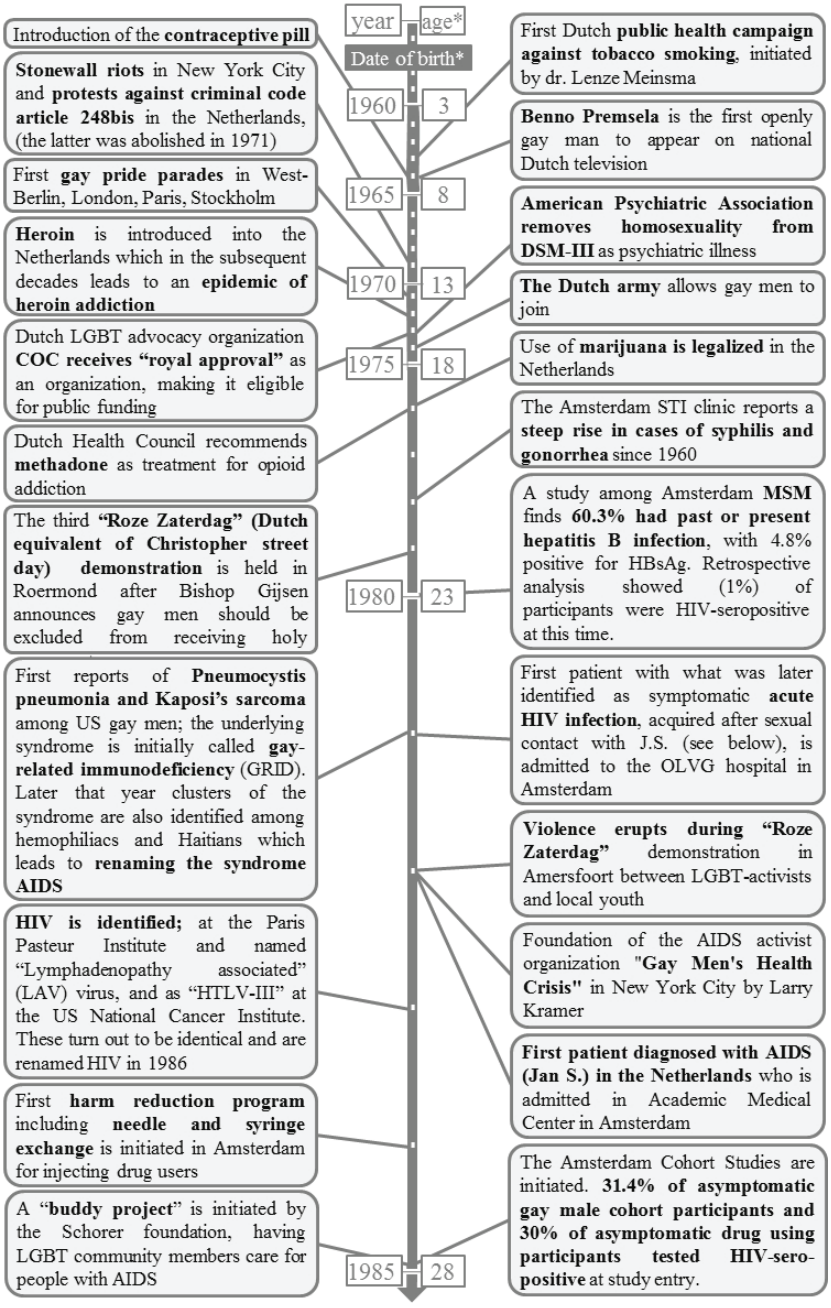
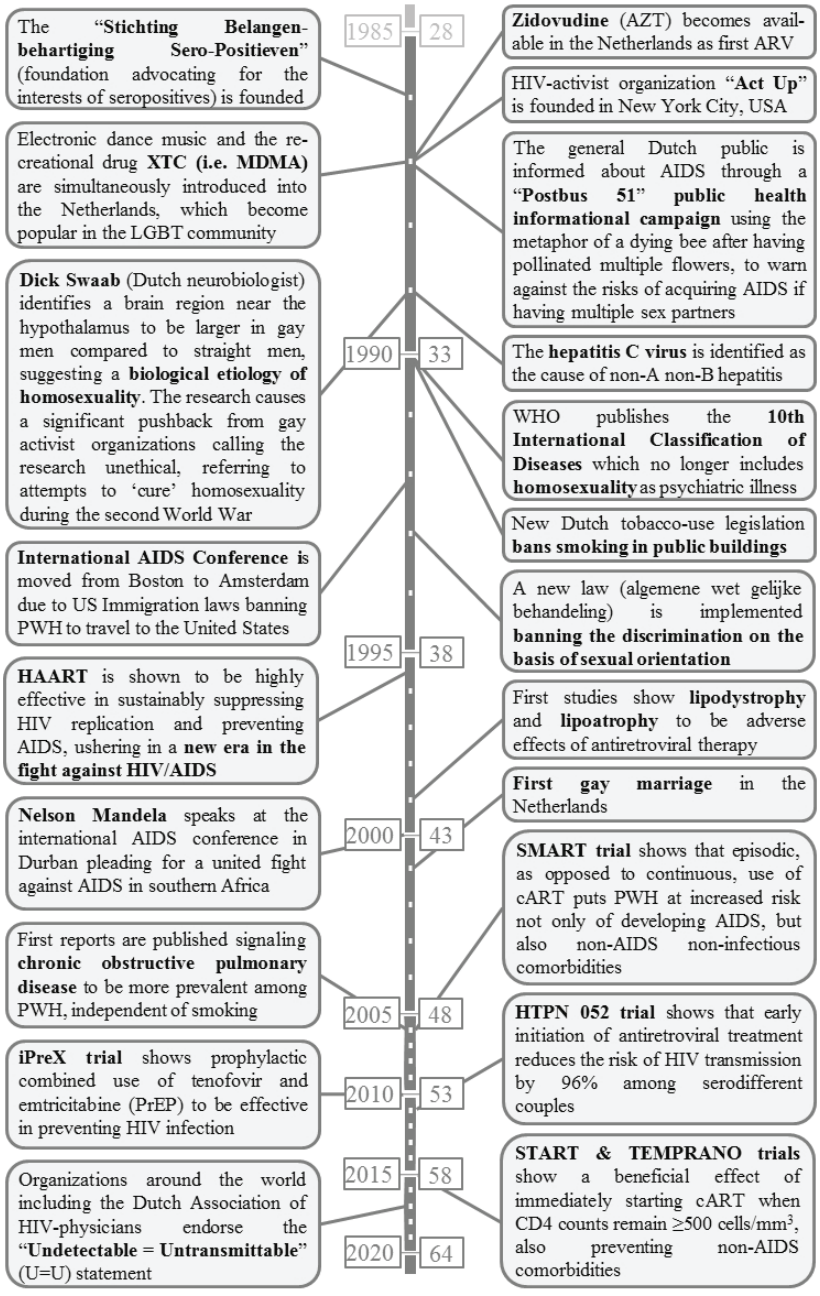



**FIGURE 1.4** | Overview of lifetime historical events relevant to the average individual ageing with, or whilst at risk of acquiring HIV, in the Netherlands




**Based on the birth year of the average HIV-positive participant and aged 53 at the time of enrolment into the AGE$_n$IV cohort study in 2010-12. Abbreviations: LGBT, Lesbian Gay Bisexual and Transgender; COC, Cultuur en Ontspanningscentrum; US, United States; AIDS, Acquired Immunodeficiency Syndrome; DSM, Diagnostic and Statistical Manual of Mental Disorders; STI, Sexually Transmitted Infection; HBsAg, Hepatitis B surface Antigen; MDMA, 3,4-methylenedioxymethamphetamine; PLWH, people with HIV; PrEP, Pre-exposure prophylaxis; WHO, World Health Organization.*

### *A pandemic*

Although in high-income countries the large majority of new HIV infections over the years continues to occur among relatively small specific key populations, this has to a lesser extent been the case in low- and middle-income countries (LMIC). Especially in sub-Saharan Africa HIV has spread among the general population, with for example South-Africa's HIV-seroprevalence having risen from 0.7% in 1990 to 19% in 2019 within the general adult (15-49 year old) population.[16] In this country alone in 2019 7.7 million people were living with HIV, making it the largest national HIV-epidemic globally. In 2000 it was estimated half of South Africa's young people would die of AIDS, "robbing schools of both students and teachers" as was stated by Nelson Mandela at the International AIDS conference in Durban that year.[73,74] Since then international collaborations have mounted an unprecedented global HIV-response. International funding and the waiver of antiretroviral drug-patents in LMIC has allowed for global access to affordable cART. At the end of 2019, 38.0 million people were estimated to be living with HIV globally of whom 67% were receiving cART.[16] As a result, an increasingly large number of PLWH are now ageing with HIV globally.

Through migration the HIV-*pandemic* has also influenced the nature of the epidemic in the Netherlands. In 2019, 41% of all PLWH in the Netherlands were born outside the Netherlands; 33% of these migrants were born in Africa, 13% in Eastern Europa and 10% in South America.[4] Although they may not have experienced the same specific adversities MSM have been subjected to in the Netherlands over the last 65 years as described earlier, they are likewise part of minority populations. A minority not only based on their migration status or ethnicity, but also based on their HIV status. In many societies being HIV-positive is highly stigmatized which can result in social exclusion and thereby have adverse effects on health. As such, individuals from minority populations with HIV may experience minority stress or social inequity on the basis of more than one characteristic (e.g. ethnicity *and* gender identity *and* sexual preference *and* HIV status), a phenomenon described as *intersectionality*.[75]

## 2021: AGEING WITH HIV

### An ageing population

The average age of PLWH in the Netherlands is increasing. While in 1996 only 11% of PLWH in care were 50 years or older, in 2019 this had increased to 52%, with 21% already being 60 years or older.[4] This is mainly a result of cART improving the life expectancy of PLWH, but also because a substantial proportion of newly diagnosed people are over 50 (23% in 2019).[4] It is estimated that 73% of PLWH in the Netherlands will be 50 years or

older in 2030.[76] Even if PLWH would be at similar risk of AACs compared to the general population, AACs will over the coming years increasingly demand the attention from those caring for PLWH.

### Aetiology of the higher prevalence of age-associated comorbidities in PLWH

As previously stated, AACs appear to occur more often and possibly at a younger age in PLWH compared to people without HIV. However, it remains unclear *to what extent* this is the case and whether the underlying *pathophysiology is* different in PLWH. As I have aimed to portray in the preceding paragraphs, there is a multitude of factors that may – at some point in time over the last 65 years – have contributed to organ damage now becoming apparent in this older cohort of PLWH in the form of AACs. Such factors may be related to HIV infection and its treatment, but may also be related to belonging to a population *at risk* of HIV. Moreover, these risk factors may not only have occurred in the past, but could also continue to cause organ damage in spite of HIV infection being suppressed by treatment. Gaining insight into the multifactorial processes contributing to AACs in PLWH may guide our approach on how to best mitigate them, including any AAC treatment strategies tailored toward PLWH specifically, and thereby improve the quality of life and longevity of PLWH.

## THE AGE$_H$IV, POPPY AND COBRA COHORT STUDIES

### The AGE$_h$IV cohort study

To study the incidence and underlying pathogenesis of AACs among PLWH the prospective AGE$_h$IV cohort study was initiated in 2010. Between 1 October 2010 and 30 September 2012, 598 HIV-positive participants were recruited and enrolled from the Amsterdam University Medical Centers (location AMC) HIV outpatient clinic, and 550 HIV-uninfected control participants from the sexual health clinic and the Amsterdam Cohort Studies at the Amsterdam Public Health Service. All participants were 45 years of age or older and for controls HIV-negative status was confirmed by a 4$^{th}$ generation HIV-antigen/-antibody test. Recruitment of control participants at a sexual health clinic resulted in controls having a similar sociodemographic and behavioural background compared to HIV-positive participants; 441 (69%) HIV-positive and 370 (67%) HIV-negative participants were MSM (i.e. >1 lifetime male-male sexual contact). Ten (2%) participants who were HIV-negative at enrolment (all MSM) had seroconverted during study follow-up by the end of 2020.

Participants are screened for AACs and associated risk factors every two years during on-site study visits. Assessments include multiple biometric measurements, i.e.: blood pressure, height, weight, waist- and hip circumference, frailty, vascular elasticity, pulmonary function, electrocardiogram, and bone density. Blood, urine and faecal samples are obtained for laboratory testing and cryopreservation. Participants are asked to report if new AACs have been diagnosed by health care professionals since their last study visit. These diagnoses are confirmed using the AMC electronic medical records in the case of HIV-positive participants and general practitioners' records in the case of HIV-negative participants. Participants are asked to complete a questionnaire regarding demographics, family medical history, use of medications (both prescribed and over-the-counter), participation in population screening programs, substance use, quality of life, depression, sexual orientation/behaviour/dysfunction, cognitive complaints, calcium/vitamin D intake, physical exercise, social behaviour, and work participation/income. Detailed information concerning HIV and ART history of HIV-positive participants is obtained from the Dutch HIV Monitoring Foundation.

## The POPPY cohort studies

The Pharmacokinetic and clinical Observations in PeoPle over fiftY (POPPY) Study is a multicenter prospective cohort study and was initiated in 2013.[77] The cohort includes 699 PLWH ≥50 years of age, 374 PLWH <50 years of age and 304 people without HIV ≥50 years of age. HIV-positive participants were recruited from 8 different clinical sites in England and Ireland. Only HIV-positive participants that had acquired HIV through sexual contact (both male-male and male-female) were included. Where possible, HIV-negative control participants were recruited from similar underlying populations; for MSM this was done in sexual health clinics and for people of black African origin in local community settings. Amongst the included participants 550 (79%), 269 (72%) and 114 (47%) were MSM in the older HIV-positive, younger HIV-positive and HIV-negative subgroups, respectively.

During annual study visits information is collected on demographics, socioeconomic status, anthropometrics and lifestyle factors. A clinical history is taken, and information is collected on medications and health care resources that have been used over the past year. Biennially, cognitive function (cortical and sub-cortical function), bone health (including DEXA scan) and pain are assessed, and blood samples are stored. Data linkage is performed with two HIV cohort studies (UK CHIC and Dublin ID cohort) to provide access to historical longitudinal HIV data.

### The COBRA cohort study

134 HIV-positive and 79 HIV-negative participants from the $AGE_hIV$ and POPPY studies were included in the nested COmorBidity in Relation to AIDS (COBRA) cohort study.[78] This cohort study consisted of a baseline study visit between January 2013 and October 2014 and a follow-up study visit after two years. COBRA participants underwent one or two MRI brain scans, lumbar punctures and detailed cognitive function assessments, in addition to the assessments performed in the context of the $AGE_hIV$/POPPY studies. Stored blood, urine and spinal fluid samples were used to perform in-depth biomarker assessments evaluating potential pathophysiological mechanisms underlying the development of AACs, including biomarkers of biological ageing, in HIV-positive and HIV-negative participants.

## THESIS OUTLINE

The overall aim of this thesis is to study the multifactorial risk profile related to the development of AACs in PLWH and those *at risk* of HIV. A specific focus will be put on studying the extent, characteristics and drivers of *chronic pulmonary disease* in ageing PLWH, an issue that has received relatively little attention in the field of "HIV and ageing" research. Furthermore, I will aim to gain insight into several other pathophysiological processes potentially driving the more frequent and possibly premature development of AACs in PLWH, including cART toxicity, multi-morbidity and chronic inflammation.

**Chapters 2 and 3** focus on the pulmonary function of PLWH using the longitudinally collected spirometry measurement data in the $AGE_hIV$ cohort study. **Chapter 4** is an analysis into the alleged effects of Integrase Strand Transfer Inhibitors (INSTIs) on increases in body weight, an important risk factor for several AACs. In **chapter 5** a data-driven approach is used to study patterns of co-occurring comorbidities in PLWH in both the POPPY and $AGE_hIV$ cohorts, in order to explore whether co-occurring co-morbidities may have common underlying pathogenic mechanisms. **Chapter 6** focusses on the relationship between sexual behaviour and $CD4^+$ and $CD8^+$ T-cell counts and the $CD4^+/CD8^+$ T-cell ratio in *HIV-negative* $AGE_hIV$ participants, a marker which in PLWH has been suggested to be predictive of AAC development. The findings from these chapters are discussed and integrated for a broader perspective in the general discussion (**Chapter 7**).

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# 2

# Reduced Forced Vital Capacity Among Human Immunodeficiency Virus-Infected Middle-Aged Individuals

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## ABSTRACT

**Introduction** Pulmonary function impairments are more common among people living with HIV (PLWH), as are contributing risk behaviours. To understand the effects of HIV infection independent of risk behaviours, pulmonary function was evaluated in lifestyle comparable HIV-positive and -negative $AGE_h$IV-cohort participants.

**Methods** Prevalence of obstructive lung disease in 544 HIV-positive and 529 HIV-negative participants was determined using spirometry. Logistic regression was used to assess HIV as a determinant of obstructive lung disease. Additional explanatory models were constructed to explain observed differences.

**Results** The unadjusted obstructive lung disease prevalence was similar in HIV-positive (23.0%) and -negative (23.4%) participants. Multivariable logistic regression analysis showed an effect modification whereby obstructive lung disease prevalence among persons with limited smoking experience was notably lower among HIV-positive compared to HIV-negative participants. This resulted from a lower Forced Vital Capacity (FVC) in HIV-positive participants, but similar 1-second Forced Expiratory Volume ($FEV_1$), especially in those with limited smoking experience.

**Discussion** The lower FVC in HIV-positive participants could indicate HIV-related restrictive or fibrotic pulmonary changes. Factors that decrease the FVC could obscure emphysematous changes in the lungs of PLWH when using the $FEV_1$/FVC ratio as single diagnostic measure.

## INTRODUCTION

As a result of effective combination antiretroviral therapy (cART), people living with HIV (PLWH) are notably ageing and consequently prone to develop age-related comorbidities. Chronic obstructive pulmonary disease (COPD), similar to other age-related comorbidities, is reported to occur earlier and more frequently in PLWH compared to the general population.[1-4] In the developed world, including the Netherlands, HIV-infection occurs predominantly among identifiable key populations, such as men who have sex with men (MSM).[5] These key populations often exhibit a lifestyle that is associated with greater exposure to environmental and behavioural risk factors such as active and passive smoking and illicit drug use.[6-8] Thus, lifestyle-comparable control groups are essential when evaluating incidence, prevalence, and HIV-related causes of morbidity in PLWH. This is especially relevant for conditions highly correlated with risk behaviours, as is the case with COPD development and smoking exposure.

The aim of this study was to evaluate the prevalence of obstructive lung disease in a group of middle-aged, mostly virologically suppressed, PLWH compared to an appropriately selected HIV-negative control group with similar lifestyles.

## METHODS

### Study participants and procedure

The $AGE_hIV$ Cohort Study is an ongoing prospective cohort study evaluating the occurrence of age-related comorbidities in 598 HIV-1-infected participants compared to 550 HIV-negative participants. HIV-positive participants were recruited from the HIV outpatient clinic of the Academic Medical Center (AMC), Amsterdam, The Netherlands. HIV-negative participants were recruited from the sexual health clinic at the Amsterdam Public Health Service and from the Amsterdam Cohort Studies on HIV/AIDS.[9] The recruitment method of the control group allowed for inclusion of a majority of MSM, comparable to the index group in geographical and socio-demographical background, and at risk for HIV-infection. The inclusion criteria were being at least 45 years of age and controls had a documented negative HIV antibody test at inclusion. Study enrolment was conducted between 2010 and 2012. Biennially, participants undergo a standardized screening program to diagnose age-related comorbidities, which includes an extensive questionnaire, multiple biometric measurements, and laboratory testing. More details about the study protocol have previously been published.[10] Detailed information on recent and historical HIV characteristics prior to study entry was obtained from the Dutch HIV Monitoring Foundation registry. Written informed consent was obtained from

all participants and the study was approved by the ethical review board of the AMC in Amsterdam and registered at ClinicalTrials.gov (identifier NCT01466582).

Information on demographics and risk behaviour was obtained via questionnaires, including a detailed history of current and past smoking behaviour. At each study visit, a spirometry measurement without bronchodilation was performed by trained nurses according to ERS/ATS-criteria (European Respiratory Society / American Thoracic Society)[11] using a SpiroUSB™ (Carefusion) spirometer. Forced Expiratory Volume in 1 second ($FEV_1$) and Forced Vital Capacity (FVC) were determined in a minimum of 3 up to a maximum of 6 efforts. Afterwards, an (unforced) Expiratory Vital Capacity (EVC) was measured in 3 to 6 separate efforts.

## Data quality

Highest $FEV_1$ and FVC values were selected only from ATS qualifying efforts, of which there needed to be at least one. If the spirometry measurement did not meet quality control criteria, indices from a follow-up visit were used when available (n=56, Supplementary Figure 2.1). Missing covariate data were supplemented with data from follow-up visits (max. 42 cases per variable) or imputed using a mean or median value of the overall cohort (max. 35 cases per variable). Participants without any data on smoking behaviour were excluded from the analysis (n=48).

## Definitions

The threshold for Obstructive Lung Disease was set at an $FEV_1$/FVC ratio <70%, following Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria[12] to allow for comparability with previous studies. An alternative threshold for obstructive lung disease was set at a z-score of the $FEV_1$/FVC ratio <-1.64 (i.e. lower limit of normal) using the Global Lung Initiative's (GLI) spirometry reference calculations and criteria.[13] These reference calculations were also used to calculate the percentage of the per individual predicted $FEV_1$ and FVC values, based on participants' gender, age, height and ethnicity. Severity of airway obstruction ($FEV_1$/FVC <70%) was classified using the GOLD guidelines into GOLD 1 to 4; $FEV_1$ % predicted being ≥80%, <80%, <50% and <30% respectively. Cumulative smoking pack-years was defined as the product of the duration of smoking career in years and the average number of cigarettes smoked daily (20 cigarettes = 1 pack).

## Laboratory measurements

Levels of a panel of inflammatory markers were determined centrally at the Amsterdam UMC (location AMC) research laboratories; high sensitivity C-reactive protein (hsCRP) and D-dimer were determined in fresh plasma samples using immunoturbidimetry.

Interleukin-6, soluble CD14 and soluble CD163 were determined by enzyme-linked immunosorbent assay using stored plasma samples from the baseline visit.[14] Regular HIV-treatment related laboratory markers (e.g. lymphocyte phenotyping) were determined directly in samples collected during the study visits or, only with HIV-positive participants, <3 months prior to the study visits during routine clinic visits.

## Statistical analysis

Statistical analyses were performed using Stata software (version 12; StataCorp LP, College Station, Texas, USA). All tests were two sided and p-values <0.05 were considered statistically significant. Baseline characteristics were compared using student's t-tests, $\chi^2$ tests, Mann-Whitney U tests or nonparametric tests for trend, where appropriate. Multivariable logistic regression was used to evaluate the association between obstructive lung disease and HIV, while adjusting for confounding factors. Possible confounding variables were selected via univariable logistic regression analyses. Variables associated with obstructive lung disease at a p-value <0.2 in univariable analyses and considered a biologically plausible confounder were included in the multivariable models. In case continuous covariates were associated with obstructive lung disease, this association was evaluated for linearity by categorizing the variable into quintiles and evaluating the association with obstructive lung disease per quintile. When the association was determined to be non-linear these variables were appropriately transformed, categorized or dichotomized. A manual stepwise backwards variable selection process was used to generate the most parsimonious multivariable models. All biologically plausible interaction effects were evaluated. To clarify the results of the primary analysis, exploratory analyses were performed using the $FEV_1$, FVC and EVC spirometry indices as separate continuous outcome variables in multiple linear regression models, to evaluate their associations with HIV infection, and also their relation to smoking history. Where possible, these indices were transformed to z-scores using the GLI reference calculations to reduce the number of confounding variables in the models and to allow for a comparison with the general population. Associations between the spirometry indices and historical HIV characteristics and chronic inflammation markers were also explored.

## Sensitivity analyses

To verify the robustness of our results several sensitivity analyses were performed. These included an analysis of spirometry measurements restricted to those with at least three ATS qualifying efforts (72% of spirometry measurements), and also analyses using the lower limit of normal (LLN; Z-score <-1.64) of the $FEV_1$/FVC ratio and the GOLD class 2 ($FEV_1$/FVC <70% & $FEV_1$ <80%-predicted) as alternative outcome measures.

# RESULTS

## Characteristics of study participants

Spirometry measurements from 544 HIV-positive and 529 HIV-negative controls were available for analysis, representing 91% and 95% of the $AGE_hIV$ participants in the two study arms, respectively (Supplementary Figure 2.1). HIV-positive participants were more often male (89% vs. 85%), MSM (77% vs. 70%), of non-white ethnicity (12% vs. 6%), unfit for work (23% vs. 7%) and had a significantly lower average BMI (24.6 kg/$m^2$ vs. 25.2 kg/$m^2$). (Table 2.1) HIV-negative participants more often had a higher educational level (56% vs. 42%) and were more frequently employed or self-employed (71% vs. 53%). Smoking behaviour also significantly differed between groups, with HIV-positive

**TABLE 2.1** | Characteristics of $AGE_hIV$ study participants by HIV status

| | HIV – positive N = 544 | HIV – negative N = 529 | *P* |
|---|---|---|---|
| Age in years | 53 (48-59) | 52 (48-58) | 0.2[a] |
| Male gender at birth | 486 (89) | 447 (85) | **0.019**[b] |
| MSM | 411 (77) | 366 (70) | **0.008**[b] |
| Non-white ethnicity | 64 (12) | 30 (6) | **<0.001**[b] |
| Height, cm [mean, (SD)] | 178 (9.4) | 178 (9.2) | 0.3[c] |
| BMI, kg/$m^2$ [mean, (SD)] | 24.6 (3.5) | 25.2 (3.6) | **0.006**[c] |
| High educational level [e] | 214 (42) | 286 (56) | **<0.001**[b] |
| Employed or self-employed | 273 (53) | 370 (71) | **<0.001**[b] |
| Wholly or partly unfit for work | 120 (23) | 36 (7) | **<0.001**[b] |
| Smoking status | | | |
| Never | 172 (32) | 192 (37) | **0.008**[d] |
| Former | 186 (34) | 202 (38) | |
| Current | 186 (34) | 135 (25) | |
| Number of pack-years [f] | 18 (8-35) | 15 (5-28) | **<0.001**[a] |
| Years since cessation [g] | 10 (4-20) | 14 (6-27) | **0.004**[a] |
| Former injection drug use | 24 (4) | 6 (1) | **0.001**[b] |
| Alcohol consumption in last 6 months | | | |
| Daily or nearly daily ≥1 IU | 172 (32) | 198 (37) | 0.05[b] |
| Heavy daily drinking [h] | 23 (4) | 36 (7) | 0.06[b] |
| Binge drinking [i] | 107 (21) | 159 (31) | **<0.001**[b] |
| Use of recreational drugs during last 6 months | 152 (29) | 140 (27) | 0.5[b] |

**TABLE 2.1 *(continued)*** | Characteristics of AGE$_h$IV study participants by HIV status

| | HIV – positive N = 544 | HIV – negative N = 529 | *P* |
|---|---|---|---|
| Daily, weekly or monthly use of | | | |
| Marijuana | 52 (10) | 39 (7) | 0.2[b] |
| MDMA | 22 (4) | 45 (9) | **0.003**[b] |
| Cocaine | 19 (4) | 13 (2) | 0.3[b] |
| Poppers | 48 (9) | 34 (7) | 0.1[b] |
| Daily marijuana use | 16 (3) | 12 (2) | 0.5[b] |
| Number of sexual partners in 6 months preceding study visit | 1 (0-4) | 3 (1-10) | **<0.001**[a] |
| Estimated number of lifetime sexual partners | 53 (15-250) | 100 (25-500) | **<0.001**[a] |
| Use of inhaler 24 hours prior to spirometry | 12 (2) | 14 (3) | **0.6**[b] |
| Self-reported history of obstructive pulmonary disease | 86 (16) | 61 (12) | **0.03**[b] |
| Years since diagnosis of HIV-1 infection | 12.1 (6.3 - 7.1) | - - | - |
| Mean $CD4^+$ cell count over the 12 months prior to enrolment, cells/$mm^3$ | 565 (434 - 742) | - - | - |
| $CD4^+$ nadir cell count, cells/$mm^3$ | 180 (73 - 260) | - - | - |
| Receiving cART at time of enrolment | 521 (95.8%) | - - | - |
| Viral load < 200 copies/mL in the year prior to enrolment | 498 (92.1%) | - - | - |
| Duration of suppressed viremia (<200 c/mL), years | 9.2 (4.0-12.8) | - - | - |
| Prior CDC class-C AIDS defining illness | 166 (30.5%) | - - | - |
| Prior pulmonary CDC class-C AIDS-defining illness[j] | 70 (12.8%) | - - | - |
| Prior non-pulmonary CDC class C AIDS-defining illness | 129 (24%) | - - | - |
| Prior PCP | 51 (9.4%) | - - | - |
| Prior bacterial pneumonia | 54 (10%) | - - | - |
| Prior pulmonary tuberculosis | 9 (1.7%) | - - | - |

*All values are n (%) or median (IQR) unless otherwise stated. Participants with missing or invalid spirometry measurements (n=27) or data on smoking behaviour (n=48) were excluded. a. Wilcoxon rank-sum test b. $\chi^2$ test c. Two sample t-test d. non-parametric test for trend e. Having completed an education at higher vocational level or university level. f. 1 Pack-year = 20 cigarettes/day during 1 year, former/current smokers only g. Number of years since having stopped smoking, former smokers only. h. Near daily >4 International units (IU) (females) or >5 IU (males) of alcohol. i. >6 IU / day at least once per month in last 6 months. j. any pulmonary mycobacterial infections, Pneumocystis jirovecii pneumonia, recurrent bacterial pneumonia or candidiasis in trachea, bronchi or lungs. Abbreviations: MSM=men having sex with men, BMI=Body mass Index, SD= Standard deviation, IQR=Interquartile range, IDU=injection drug use, MDMA=3,4-Methylenedioxymethamphetamine. cART=combination Antiretroviral Therapy. PCP=Pneumocystis jirovecii pneumonia. IU=International Units.*

participants more often being current smokers (34% vs. 25%), having on average more pack-years (18 vs. 15) and had more recently quit smoking when cessation was achieved (10 vs. 14 years since cessation). HIV-positive participants however had lower levels of alcohol use; being less often a daily drinker (32% vs 37%), a heavy daily drinker (4% vs 7%) or a binge drinker (21% vs 31%). Other recreational drug use only differed regarding former injection drug use (4% HIV-positive vs. 1% HIV-negative) and less daily to monthly MDMA (3,4-Methylenedioxymethamphetamine) use (4% HIV-positive vs. 9% HIV-negative). HIV-positive participants also had less sexual risk behaviour with a lower median number of partners in their lifetime (53 vs. 100) and in the 6 months before study-enrolment (1 vs. 3).

The majority of HIV-positive participants were on cART at the time of the spirometry measurement (95.8%) with 92.1% being virologically suppressed and having high $CD4^+$ cell counts (median 565 cells/$mm^3$). The HIV-positive participants were known to be HIV-positive for a median of 12 years, with a median $CD4^+$ nadir cell count of 180 cells/$mm^3$, 31% having previously been diagnosed with a CDC category C AIDS-defining illness and 9% with a history of *Pneumocystis jirovecii* pneumonia (PCP); prior pulmonary tuberculosis was rare (1.7%).

## Prevalence of obstructive lung disease

Using GOLD criteria, prevalence of obstructive lung disease was 23.0% in the HIV-positive group and 23.4% in the HIV negative group (Figure 2.1). Using GLI criteria, prevalence was 14.0% and 15.6%, respectively. Neither difference was statistically significant (p-values = 0.9 and 0.5, respectively). Subdivision based on disease severity using the GOLD

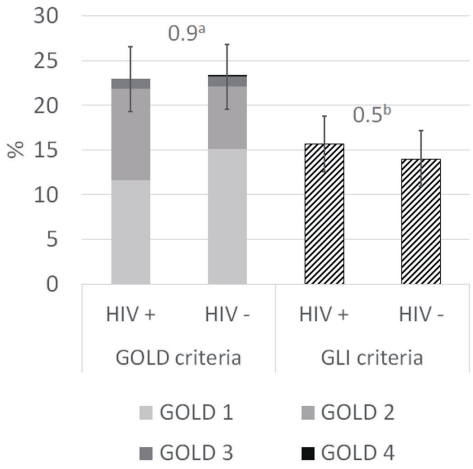


**FIGURE 2.1** | Prevalence of obstructive lung disease by HIV status

*a. p-value of non-parametric test for trend b. p-value of $\chi^2$ test. Obstructive lung disease was defined as $FEV_1/FVC$ ratio <70% (GOLD-criteria) and $FEV_1/FVC$ ratio z-score < -1.64 (GLI-criteria). GOLD disease severity criteria: $FEV_1/FVC$ <70% and $FEV_1$ >80% (GOLD 1), $FEV_1$ 50-80% (GOLD 2), $FEV_1$ 30-50% (GOLD 3) or $FEV_1$ <30% (GOLD 4)*

disease-severity classifications [12] also failed to show a significant difference (p-value = 0.9 for non-parametric test for trend, Figure 2.1). There was however a trend towards more GOLD class ≥2 spirometry patterns in the HIV-positive group (p-value 0.09, chi-square test).

## Adjustment for potential confounders

Table 2.2 shows multivariable models including adjustment for demographic and behavioural factors in the probability of having obstructive lung disease ($FEV_1$/FVC <70%). HIV-status and number of pack-years strongly interacted with each other (*P* <0.001): for those reporting zero pack-years, HIV was significantly associated with a lower probability of obstructive lung disease (OR 0.35, 95% CI 0.21-0.58, *P* <0.001). This negative association between HIV and obstructive lung disease diminished with an increasing number of pack-years, and was (non-significantly) reversed from around 25 pack-years (Figure 2.2, $P_{interaction}$ 0.006). Age, height and BMI were significantly associated with obstructive lung disease, but did not modify the effect of HIV (Table 2.2, model 2). The interaction term of smoking cessation years with pack-years was significant and showed

**TABLE 2.2** | Multivariable logistic regression models evaluating factors independently associated with obstructive lung disease, with (model 2) and without adjustment for smoking and recreational drug use (model 1).

| | Model 1 | | | Model 2 | | |
|---|---|---|---|---|---|---|
| | **OR** | **95% CI** | ***P*** | **OR** | **95% CI** | ***P*** |
| **HIV infection** | 0.88 | 0.65-1.18 | 0.4 | 0.35 | 0.21-0.58 | <0.001 |
| Interaction: HIV infection x $\sqrt{pack\text{-}years}$ [a] | x | x | x | 1.23 | 1.09-1.38 | <0.001 |
| **Demographics** | | | | | | |
| Age per 10 year increase | 1.92 | 1.58-2.34 | <0.001 | 2.08 | 1.67-2.59 | <0.001 |
| Height >170 cm (dichotomized) [a] | 2.32 | 1.49-3.62 | <0.001 | 2.26 | 1.43-3.60 | 0.001 |
| BMI <23 kg/$m^2$ (dichotomized) [a] | 1.86 | 1.36-2.52 | <0.001 | 1.72 | 1.24-2.38 | 0.001 |
| **Smoking & recreational drug use** | | | | | | |
| Pack-years per $\sqrt{pack\text{-}years}$ increase [a] | x | x | x | 1.13 | 1.04-1.23 | 0.006 |
| Smoke cessation years per 10 years longer ago | x | x | x | 1.10 | 0.88-1.37 | 0.4 |
| Interaction: smoke cessation years x $\sqrt{pack\text{-}years}$ [a] | x | x | x | 0.91 | 0.85-0.98 | 0.009 |
| Daily marijuana use | x | x | x | 2.06 | 0.92-4.64 | 0.08 |
| Former IV drug use | x | x | x | 1.87 | 0.84-4.19 | 0.1 |

*Model 1 includes the variables HIV-status, age, height and BMI. Model 2 includes all variables and interaction terms listed in the table. a. Variables were transformed or categorized appropriately to fit the linearity assumption of the logistic regression model. Variables that were excluded from the model in the backwards selection process were: ethnicity, sexual orientation, employment status, smoking status, daily and heavy daily alcohol use. Diagnostics Model 2: Likelihood ratio test: $\chi^2$ 149 (p-value <0.001). Hosmer-Lemeshow $\chi^2$ test: 5.02 (p-value 0.76). Maximal leverage 0.15. Maximal Pregibon's dbeta 0.19.*

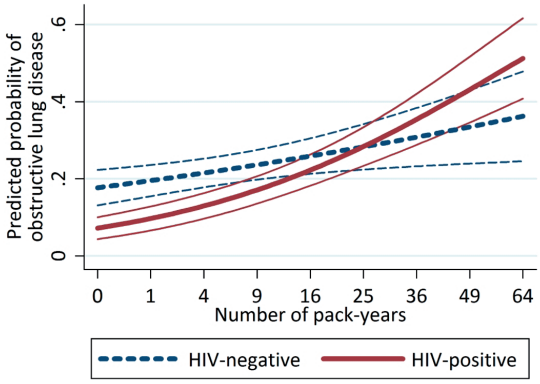


**FIGURE 2.2** | Predicted probability with 95% confidence interval of obstructive lung disease by number of pack-years smoking, stratified by HIV status

*Predicted probability was calculated using model 2 in table 2.2. Other covariates in the multivariable model were kept constant at their mean values in the overall study population. Number of pack-years were back calculated from the transformed* $\sqrt{pack\text{-}years}$.

an attenuation of the smoking effect when having quit smoking for longer periods. Sensitivity analyses using the obstructive lung disease definition by GLI or using a GOLD ≥2 definition as outcome measures yielded similar results. (data not shown) Also in an analysis restricted to participants with at least three ATS qualifying efforts the lower probability of obstructive lung disease in those HIV-positive participants with limited smoking experience remained significant.

## Lower vital capacity in HIV-positive participants

Table 2.3 shows a two times higher prevalence of obstructive lung disease (21% vs 9%, *P* 0.001) in the never-smoking HIV-negative participants compared to HIV-positive never-smoking participants, and a 5 times higher prevalence of obstructive lung disease (15% vs 3%, *P* <0.001) when using GLI criteria. Within this subgroup, FVC and EVC were significantly lower in the HIV-positive participants (resp. *P* 0.02 and <0.001), while $FEV_1$ was not significantly different. The percentage of the predicted FVC was also not significantly lower in the HIV-positive never-smoking subgroup compared to the never-smoking HIV-negative subgroup. Figure 2.3 shows the predicted z-scores of $FEV_1$, FVC, the $FEV_1$/FVC ratio and the absolute EVC by HIV-status and pack-years of smoking, using multivariable linear regression to adjust for confounding variables. $FEV_1$ z-score levels and their declines with increasing pack-years were similar in HIV-positive and –negative participants. The interaction effect of pack-years smoking with HIV on the $FEV_1$/FVC ratio z-score appeared therefore primarily driven by differences in FVC. The HIV-positive participants had an on average lower FVC z-score at 0 pack-years (-0.169, 95% CI -0.321 to -0.018, *P* 0.028), which remained relatively unaffected by an increasing numbers of pack-years smoking, in contrast to the decline in FVC observed with increasing numbers of pack-years in the HIV-negative participants. The interaction term HIV*pack-years was however non-significant (+0.005, 95% CI -0.001 to 0.11, *P* 0.14). Similarly the EVC,

**TABLE 2.3** | Pulmonary function and characteristics of never-smoking participants

| | HIV-positive N=172 | HIV-negative N=192 | *P* |
|---|---|---|---|
| Obstructive lung disease, GOLD criteria | **15 (8.7)** | **41 (21.4)** | **0.001**[a] |
| Obstructive lung disease, GLI criteria | **6 (3.5)** | **28 (14.6)** | **<0.001**[a] |
| $FEV_1$/FVC | **0.78 (0.1)** | **0.75 (0.1)** | **0.003**[b] |
| $FEV_1$/FVC % predicted[c] | **98.7 (8.7)** | **96.0 (8.8)** | **0.003**[b] |
| $FEV_1$ (liter) | 3.38 (0.8) | 3.51 (0.8) | 0.2[b] |
| $FEV_1$ % predicted[c] | 94.8 (14.0) | 93.7 (14.5) | 0.5 |
| FVC (liter) | **4.39 (1.1)** | **4.66 (1.1)** | **0.02**[b] |
| FVC % predicted[c] | 96.2 (15.9) | 97.3 (13.7) | 0.5 |
| EVC (liter) | **4.35 (1.2)** | **4.79 (1.1)** | **<0.001**[b] |
| Self-reported history of obstructive lung disease | 21 (12.4) | 18 (9.4) | 0.36[a] |
| Male at birth | 146 (84.9) | 166 (86.5) | 0.7[a] |
| MSM | 121 (70.3) | 140 (73.0) | 0.6[a] |
| Height, cm | **176 (10.4)** | **179 (8.9)** | **0.03**[b] |
| BMI, $kg/m^2$ | 25 (3.3) | 25 (3.6) | 0.7[b] |
| Non-white ethnicity | **29 (16.9)** | **14 (7.3)** | **0.005**[a] |
| Use of recreational drugs during last 6 months | 27 (15.8) | 40 (20.8) | 0.2 |
| Binge drinking[d] | **19 (11.7)** | **46 (24.3)** | **0.002** |
| Prior pulmonary CDC class-C AIDS-defining illness [e] | 49 (28.5) | - | - |
| Prior PCP infection | 18 (10.5) | - | - |

*All values are n (%) or mean (standard deviation). a. $\chi^2$ test b. Two sample t-test. c. using Global Lung Initiative reference calculations. d. >6 IU/day at least once per month in last 6 months. e. any pulmonary mycobacterial infections, Pneumocystis jirovecii pneumonia, recurrent bacterial pneumonia or candidiasis in trachea, bronchi or lungs. Abbreviations: GOLD=Global initiative for Chronic Obstructive Lung Disease, GLI=Global Lung Initiative. $FEV_1$=1-second Forced Expiratory Volume, FVC=Forced Vital Capacity, EVC=Expiratory Vital Capacity. MSM=men who have sex with men. PCP= Pneumocystis jirovecii pneumonia.*

which also measures the vital capacity, was lower among never-smoking HIV-positive participants compared to never-smoking HIV-negative participants (-228 ml, 95% CI -330 ml to -127 ml, *P* <0.001). This difference also appeared to be relatively unaffected by an increasing number of pack-years smoking in the HIV-positive participants (interaction term HIV-status*pack-years: +0.003 95% CI -0.001 to +0.007, *P* 0.22). Considering the interaction terms of HIV with both FVC and with EVC were not statistically significant in these models, it appears the FVC was on average lower in the HIV-positive compared to the HIV-negative participants regardless of their smoking history.

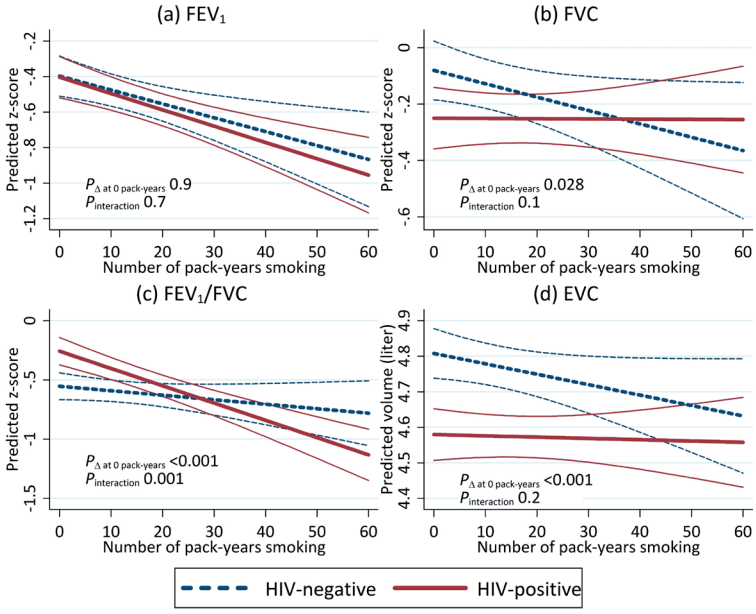


**FIGURE 2.3** | Predicted mean $FEV_1$, FVC, $FEV_1$/FVC z-scores and predicted mean absolute EVC by pack-years of smoking, stratified by HIV status

*For each covariate in table 2.1 univariable analyses were performed to assess their association with all of the four outcome variables (supplementary table 2.2). Variables associated with a p-value <0.2 were subsequently included in multivariable models to generate the adjusted mean values shown in this figure. A stepwise backward variable selection process was used to generate the most parsimonious models. The mean outcome values were adjusted for the following covariates in the final models, which were fixed at their mean values in the overall study-population: Mean z-score $FEV_1$: ethnicity, education level, current smoking status, alcohol use. Mean z-score FVC: age, ethnicity, BMI, education level, daily marijuana use. Mean z-score $FEV_1$/FVC: age, gender, ethnicity, smoking status. Mean EVC: age, gender, BMI, sexual orientation, ethnicity, height, education-level. Abbreviations: $FEV_1$=1-second Forced Expiratory Volume, FVC=Forced Vital Capacity, EVC=Expiratory Vital Capacity.*

## HIV-related characteristics, markers of chronic inflammation and lung function

In further exploratory analyses, we evaluated associations of HIV-related characteristics and chronic inflammation with the z-scores of $FEV_1$/FVC, $FEV_1$ and FVC (Table 2.4). A prior AIDS-defining diagnosis (pulmonary and/or non-pulmonary) was associated with having both a lower $FEV_1$ and FVC, and specifically in the case of prior pulmonary AIDS, resulted in a lower $FEV_1$/FVC ratio. Prior didanosine use was associated with a lower $FEV_1$/FVC ratio, primarily mediated by being associated with a higher FVC. Of the immunological markers in the HIV-positive participants, the strongest association was observed between higher levels of IL-6 with a lower FVC z-score.

**TABLE 2.4** | Association of HIV-related characteristics and markers of chronic inflammation with the $FEV_1$/FVC, $FEV_1$ and FVC z-scores within HIV-positive and –negative subgroups using multiple linear regression.

| | $FEV_1$/FVC z-score[a] | | $FEV_1$ z-score[b] | | FVC z-score[c] | |
|---|---|---|---|---|---|---|
| | **Coeff.** | ***P*** | **Coeff.** | ***P*** | **Coeff.** | ***P*** |
| **HIV characteristics (HIV-positive subgroup only)** | | | | | | |
| CD4-nadir (per 50 cells/$mm^3$ increase) | +0.02 | 0.3 | +0.03 | 0.06 | +0.03 | 0.09 |
| Current CD4 (per 50 cells/$mm^3$ increase) | -0.00 | 0.9 | -0.00 | 0.8 | -0.00 | 1.0 |
| Years since HIV-diagnosis | -0.00 | 0.5 | -0.00 | 0.9 | +0.00 | 0.6 |
| Prior AIDS diagnosis [d] | | | | | | |
| No AIDS diagnosis | ref | ref | ref | ref | ref | ref |
| Prior pulmonary AIDS[e] | **-0.36** | **0.046** | **-0.34** | **0.048** | -0.13 | 0.5 |
| Prior non-pulmonary AIDS | -0.04 | 0.7 | **-0.24** | **0.041** | -0.23 | 0.058 |
| Both prior pulmonary [e] & non-pulmonary AIDS | -0.14 | 0.4 | **-0.60** | **0.001** | **-0.55** | **0.004** |
| Prior PCP diagnosis | -0.29 | 0.06 | **-0.40** | **0.009** | **-0.38** | **0.017** |
| Prior pulmonary TB | -0.25 | 0.5 | -0.37 | 0.3 | -0.49 | 0.2 |
| Prior bacterial pneumonia | -0.25 | 0.1 | -0.24 | 0.1 | -0.13 | 0.4 |
| Prior D4T use | -0.10 | 0.3 | -0.07 | 0.5 | -0.06 | 0.5 |
| Prior DDI use | **-0.25** | **0.012** | +0.12 | 0.2 | **+0.29** | **0.004** |
| **Markers of chronic inflammation (by HIV-positive and HIV-negative subgroups)** | | | | | | |
| IL-6 (pg/ml) | | | | | | |
| HIV-positive | | | | | | |
| 2-10 vs. < 2 | **+0.22** | **0.03** | -0.00 | 1.0 | **-0.22** | **0.04** |
| >10 vs. < 2 | +0.24 | 0.1 | -0.20 | 0.2 | **-0.44** | **0.008** |
| HIV-negative | | | | | | |
| 2-10 vs. < 2 | +0.07 | 0.4 | -0.11 | 0.3 | -0.15 | 0.1 |
| >10 vs. < 2 | +0.09 | 0.6 | -0.02 | 0.9 | -0.07 | 0.6 |
| Soluble CD14 (per log increase) | | | | | | |
| HIV-positive | -0.09 | 0.5 | -0.12 | 0.3 | +0.22 | 0.08 |
| HIV-negative | -0.08 | 0.5 | -0.04 | 0.8 | +0.6 | 0.6 |
| Soluble CD163 (per log increase) | | | | | | |
| HIV-positive | +0.02 | 0.8 | **+0.17** | **0.049** | +0.16 | 0.07 |
| HIV-negative | -0.07 | 0.5 | -0.03 | 0.8 | -0.00 | 0.7 |
| hsCRP (per quartile increase) | | | | | | |
| HIV-positive | -0.02 | 0.7 | -0.04 | 0.3 | -0.04 | 0.3 |
| HIV-negative | -0.00 | 1.0 | **-0.12** | **0.004** | **-0.13** | **0.001** |

**TABLE 2.4 *(continued)*** | Association of HIV-related characteristics and markers of chronic inflammation with the $FEV_1$/FVC, $FEV_1$ and FVC z-scores within HIV-positive and –negative subgroups using multiple linear regression.

| | $FEV_1$/FVC z-score[a] | | $FEV_1$ z-score[b] | | FVC z-score[c] | |
|---|---|---|---|---|---|---|
| | **Coeff.** | ***P*** | **Coeff.** | ***P*** | **Coeff.** | ***P*** |
| D-dimer (per quartile increase) | | | | | | |
| HIV-positive | **-0.09** | **0.03** | +0.04 | 0.3 | +0.07 | 0.07 |
| HIV-negative | -0.03 | 0.6 | -0.03 | 0.5 | -0.00 | 0.9 |
| CD4-CD8 ratio (per log increase) | | | | | | |
| HIV-positive | -0.02 | 0.9 | +0.08 | 0.4 | +0.11 | 0.2 |
| HIV-negative | -0.02 | 0.8 | +0.06 | 0.5 | +0.07 | 0.4 |

*Each variable was included in a separate multivariable model. [a] adjusted for gender, ethnicity, current smoking-status, pack-years smoking, former IV drug use, daily marijuana use, BMI. [b] adjusted for ethnicity, education-level, current smoking status, daily alcohol use. [c] adjusted for age, ethnicity, education-level, daily marijuana use, BMI. [d] Prior CDC category-C AIDS defining illness. [e] Any pulmonary mycobacterial infections, Pneumocystis jirovecii pneumonia, recurrent bacterial pneumonia or Candidiasis in trachea, bronchi or lungs. Abbreviations: Coeff=coefficient, PCP=Pneumocystis jirovecii pneumonia, TB=Tuberculosis, D4T=Stavudine, DDI=Didanosine, IL-6=Interleukine-6, hsCRP= high-sensitivity C-reactive protein.*

## DISCUSSION

We show a strong interaction between HIV-status and pack-years smoking concerning the probability of having spirometry-defined obstructive lung disease, with a 2-5 times lower prevalence of spirometry-defined obstructive lung disease among HIV-positive participants compared to HIV-negative participants with limited smoking exposure. It is highly unlikely for this to be explained by an actual beneficial effect of HIV, since multiple studies have shown a remarkably high prevalence of chronic pulmonary disease in PLWH, also among never-smokers.[15] For example, in a subgroup analysis of never-smokers in a study using CT-scan based criteria to diagnose obstructive lung disease in PLWH, 15.5% had emphysematous changes. [16] PLWH also report higher levels of respiratory symptoms compared to HIV-negative people.[17,18] Furthermore, several HIV characteristics such as a low current CD4 count[19-21], low CD4 nadir[22], high viral load[20,23], prior pulmonary infections[24,25] and parameters of chronic inflammation[22,26] have each been associated with a higher occurrence of obstructive lung disease. Moreover, the higher $FEV_1$/FVC ratio was not related to a higher $FEV_1$ – which would truly support an advantageous effect – but with a lower FVC in the HIV-positive participants.

### Lower vital capacity in PLWH

The lower FVC and EVC with a rather preserved $FEV_1$ in HIV-positive participants may suggest the presence of restrictive lung disease. Unfortunately, total lung capacity (TLC), the gold standard for restrictive lung disease, was not measured. Several studies have

reported similar results. HIV-positive African (never-smoking) children had a lower FVC compared to HIV-negative children, unrelated to their higher frequency of pulmonary co-infections including tuberculosis.[27] Results from studies using CT-scans as diagnostic tool report not only emphysematous but also fibrotic changes, where emphysema was correlated with smoking history, but fibrosis was associated with HIV-indices such as viral load.[28,29] Furthermore, long-term HIV-infection has been strongly associated with a lower diffusion capacity (DCLO).[30-32] One study found a low DCLO to be associated with a lower FVC in never-smokers, but with a lower $FEV_1$ in smokers.[32] Within a multi-cohort study, spirometry-defined restrictive lung disease prevalence was reported to be as high as 10% among PLWH, however these results were also not verified by TLC measurements.[24]

In our study, prior pulmonary and non-pulmonary AIDS diagnoses were associated with both a lower $FEV_1$ and FVC. Didanosine-use was associated with a higher FVC; this unexpected finding could be the result of a survival bias or a chance finding in the context of multiple testing. In summary, these findings all suggest HIV-specific effects on pulmonary function measured by spirometry.

Morris et al[25] similarly showed long-lasting declines in pulmonary function following HIV-associated pulmonary infections. $FEV_1$, FVC and the $FEV_1$/FVC ratio all declined and did not recover, most significantly after episodes of PCP. This study also included TLC-measurements, the gold standard for diagnosing restrictive lung disease. TLC did not decline after PCP - disputing the theory of permanent fibrotic changes and leaving an enhanced residual volume as explanation for the decrease in VC probably as an expression of small airways disease. The main difference with our study is that the $FEV_1$ remains constant which is highly unlikely in small airways disease. This illustrates the need for caution when using spirometry to detect restrictive changes. FVC and EVC are biased in many ways including the effort and strength of the test subject, which results in a <60% positive predictive value for spirometry to diagnose restrictive lung disease.[33] HIV infection has been associated with the early development of sarcopenia[34,35], which might also affect the strength and function of respiratory muscles, thus contributing to a lower average measured FVC and higher $FEV_1$/FVC ratio. Since we did not inquire about respiratory symptoms or use other tools to measure pulmonary function, determining both the clinical significance of these findings as well as their long-term consequences requires further studies.

## Living with HIV and being a heavy smoker

The effect of HIV on the $FEV_1$/FVC-ratio was attenuated with increasing pack-years of smoking; there was a lower $FEV_1$/FVC ratio in the heavy smoking HIV-positive partici-

pants compared to HIV-negative participants with similar smoking exposure. However, this difference did not result in a higher prevalence of obstructive lung disease and seemed to be resulting from a preserved FVC instead of a lower $FEV_1$. Follow-up of these heavy smoking participants over the coming years may further clarify these results.

## Strengths and limitations

Our results were strongly influenced by the very high prevalence of obstructive lung disease (21%) in the HIV-negative never-smoking control group, being more than 3 times higher compared to similarly aged never-smokers in a Dutch general population study [36] and also demonstrable by the negative mean $FEV_1$ and $FEV_1$/FVC z-scores. Tobacco smoking (active or passive) is the primary risk factor for COPD in the Netherlands, with generally limited occupational hazards or exposure to other indoor toxic fumes. In the recruitment of our control group, we successfully included a majority of MSM comparable to our HIV-positive participants and reflective of the HIV epidemic in the Netherlands. These mostly urban citizens without children are not comparable to the general population, which was the reason for their selection. Their lifestyle may have involved more going out in clubs or bars, reflected in their common alcohol and recreational drug use and the high number of sexual partners. This would likely have resulted in a significant amount of passive smoking exposure, as smoking was ubiquitous in bars and clubs in the Netherlands until 2008, when new regulations were put in place. The strength of our cohort is its ability to account for bias from difficult to measure confounders such as second hand smoking using lifestyle-comparable control subjects. However - since we did not measure these exposures - we cannot exclude the possibility of sampling bias and recruitment of HIV negative MSM with heavy second hand smoke exposure, especially since recent alcohol use and sexual risk behaviour was higher in the HIV-negative study group. A further limitation of this study was the possibility of measurement bias due to the spirometry being performed at two study-sites for the two respective study groups. However, equipment was identical and study nurse training was repeatedly synchronized to ensure comparable measurements across study sites. Furthermore, we were unable to distinguish between COPD and asthma as post-bronchodilator spirometry was not performed. Since these two conditions have a very different underlying pathogenesis they could also be differently affected by HIV. Although very few participants reported ever having been diagnosed with a form of obstructive pulmonary disease, we cannot exclude the possibility of pre-existent asthma or any form of HIV-associated asthma to be influencing our results.

## CONCLUSION

Chronic pulmonary disease in PLWH in the context of adequate antiretroviral treatment is likely to be diverse and multifactorial in origin. PLWH, similar to their lifestyle-comparable peers, are at high risk of obstructive lung disease due to their behavioural and social background, extending beyond measurable traditional risk factors like personal smoking histories. This study shows that HIV might influence pulmonary function differently compared to traditional risk factors such as smoking. It could shed light on a repeatedly reported discrepancy between higher levels of respiratory symptoms in studies comparing people with and without HIV-infection, but normal spirometry findings.[17,18,37] HIV might inflict a more fibrotic or restrictive rather than obstructive pulmonary dysfunction pattern, which could be masked by the effects of heavy smoking. Longitudinal analyses will have to evaluate if these changes should be considered as stabilized scars from prior uncontrolled HIV or associated pulmonary infections, or as part of an ongoing process during suppressed HIV infection, in the context of chronic inflammation. Clinicians should therefore be cautious in using spirometry as a sole diagnostic tool, and consider measuring total lung and diffusion capacity and/or CT-imaging to appropriately diagnose a potential mixed pattern of pulmonary conditions in PLWH.

## AUTHOR CONTRIBUTIONS

SOV, FW, GDK, MBD, RvS, MFSvdL and PR contributed to the conceptualization and design of the study. SOV performed data curation and contributed to data collection and coordination of the study. SOV, FWW, MBD, GDK, designed the methodology of the study and SOV conducted the formal analysis. PR obtained funding and supervised the conduct of the study. SOV provided the original draft of the manuscript including figures and tables. All authors critically revised and approved the final version for publication.

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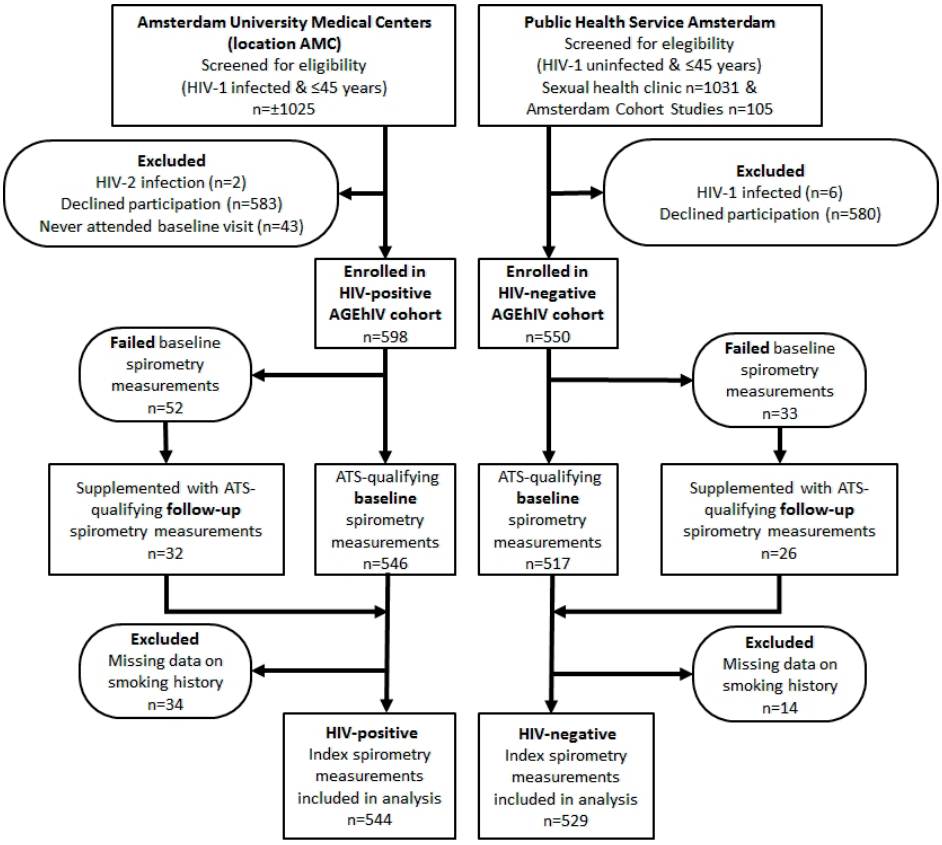
## SUPPLEMENTARY MATERIAL




**SUPPLEMENTARY FIGURE 2.1** | CONSORT flow-diagram of included participants and spirometry measurements from the AGE$_h$IV cohort

*Abbreviations: ATS=American Thoracic Society*

**SUPPLEMENTARY TABLE 2.1** | Univariable logistic regression analyses evaluating associations of demographics and behavioural factors with obstructive lung disease (FEV/FVC <70%) within the whole AGEhIV cohort

| | OR[a] | 95% CI | P-value |
|---|---|---|---|
| HIV infection | 0.97 | 0.73-1.29 | 0.9 |
| Male at birth | 2.39 | 1.41-4.06 | 0.001 |
| Age per 10 years increase | 1.83 | 1.51-2.21 | <0.001 |
| Height (dichotomized: > 170 cm) [b] | 2.23 | 1.45-3.44 | <0.001 |
| BMI (dichotomized at < 23 kg/m$^2$) [b] | 1.70 | 1.27-2.28 | <0.001 |
| Non-white ethnicity | 0.51 | 0.28-0.92 | 0.03 |
| MSM | 1.36 | 0.97-1.90 | 0.08 |
| Having employment | 0.64 | 0.48-0.86 | 0.003 |
| Smoking status | | | |
| Never | *ref* | | |
| Former | 1.66 | 1.15-2.40 | 0.007 |
| Current | 2.60 | 1.80-3.76 | <0.001 |
| Packyears per $\sqrt{pack\text{-}years}$ [b] increase (without interaction) | 1.21 | 1.15-1.28 | <0.001 |
| Heavy daily drinking [c] | 1.49 | 0.84-2.65 | 0.2 |
| Daily alcohol use | 1.35 | 1.01-1.80 | 0.05 |
| Daily Marijuana use | 2.56 | 1.19-5.48 | 0.02 |
| Former intravenous drug use | 2.31 | 1.10-4.86 | 0.03 |

*Variables excluded from this table for having a p-value >0.2 were: daily-monthly use of marijuana or poppers, being unfit for work, binge drinking, current use of any drug, education level, years since having quitted smoking (without the interaction with pack-years). [a] Odds ratio for OLD [b] variables were transformed or categorized appropriately to adhere to the linearity assumption of the logistic regression model. [c] (Nearly) daily > 4 International units (IU) (women) or >5 IU (men) of alcohol. Abbreviations: OR=Odds ratio, CI=Confidence Interval, BMI=Body mass index, MSM=Men who have sex with men.*

**SUPPLEMENTARY TABLE 2.2** | Univariable linear regression analyses evaluating associations of demographics and behavioural factors with the $FEV_1$, FVC, $FEV_1$/FVC z-scores and the absolute EVC

| | $FEV_1$ (z-score) | | FVC (z-score) | | $FEV_1$/FVC (z-score) | | EVC (Liter) | |
|---|---|---|---|---|---|---|---|---|
| | **coeff** | **p-value** | **coeff** | **p-value** | **coeff** | **p-value** | **coeff** | **p-value** |
| **HIV infection** | **-0.14** | **0.03** | **-0.13** | **0.03** | **+0.017** | **0.8** | **-0.26** | **<0.001** |
| Age (per 5 years increase) | +0.04 | 0.08 | +0.07 | 0.001 | -0.05 | 0.06 | -0.14 | <0.001 |
| BMI (per $kg/m^2$) | -0.00 | 0.8 | -0.04 | <0.001 | +0.06 | <0.001 | -0.06 | <0.001 |
| Male gender at birth | -0.11 | 0.3 | +0.01 | 0.9 | -0.18 | 0.08 | +1.60 | <0.001 |
| MSM | +0.00 | 0.3 | +0.15 | 0.04 | -0.08 | 0.3 | +0.99 | <0.001 |
| Non-white ethnicity | -0.23 | 0.04 | -0.40 | <0.001 | +0.31 | 0.01 | -1.46 | <0.001 |
| Height (per 5 cm increase) | -0.01 | 0.4 | -0.01 | 0.7 | -0.03 | 0.08 | +0.42 | <0.001 |
| High education level | +0.21 | 0.001 | +0.25 | <0.001 | -0.06 | 0.4 | +0.52 | <0.001 |
| Current employment | +0.14 | 0.04 | +0.07 | 0.3 | +0.09 | 0.2 | +0.48 | <0.001 |
| Smoking status | | | | | | | | |
| Never | *ref* | *ref* | *ref* | *ref* | *ref* | *Ref* | *ref* | *ref* |
| Former + > 10 cessation years | +0.12 | 0.2 | +0.24 | 0.005 | -0.22 | 0.02 | +0.23 | 0.01 |
| Former + < 10 cessation years | -0.32 | 0.001 | -0.05 | 0.6 | -0.43 | <0.001 | +0.32 | 0.002 |
| Current | -0.35 | <0.001 | -0.007 | 0.9 | -0.56 | <0.001 | -0.00 | 0.9 |
| Number of pack-years (per 5 years increase) | -0.05 | <0.001 | -0.01 | 0.3 | -0.06 | <0.001 | -0.00 | 0.7 |
| Former IV drug use | -0.38 | 0.049 | +0.11 | 0.6 | -0.80 | <0.001 | -0.15 | 0.4 |
| Alcohol consumption last 6 months | | | | | | | | |
| (Nearly) daily ≥ 1 IU | +0.12 | 0.067 | +0.24 | <0.001 | -0.18 | 0.01 | +0.32 | <0.001 |
| Heavy daily drinking | -0.13 | 0.4 | +0.14 | 0.3 | -0.49 | 0.001 | +0.06 | 0.7 |
| Binge drinking | -0.01 | 0.9 | +0.07 | 0.3 | -0.15 | 0.06 | +0.39 | <0.001 |
| Daily-monthly use of recreational drugs during last 6 months | | | | | | | | |
| Any drug | +0.00 | 1.0 | +0.08 | 0.3 | -0.10 | 0.2 | +0.33 | <0.001 |
| Marijuana | -0.08 | 0.5 | +0.87 | 0.4 | -0.24 | 0.047 | +0.16 | 0.2 |
| MDMA | +0.04 | 0.8 | +0.10 | 0.4 | -0.09 | 0.5 | +0.29 | 0.03 |
| Cocaine | +0.22 | 0.3 | +0.09 | 0.6 | +0.23 | 0.2 | +0.31 | 0.1 |
| Daily Marijuana use | -0.19 | 0.4 | +0.33 | 0.1 | -0.73 | 0.001 | -0.04 | 0.9 |

*Abbreviations: coeff = coefficient, MSM=men who have sex with men, MDMA=3,4-methylenedioxymethamfetamine.*

# 3

# Changes in lung function among treated HIV-positive and HIV-negative individuals: analysis of the prospective $AGE_hIV$ cohort study

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## ABSTRACT

**Background** The $AGE_hIV$ cohort study is a prospective cohort study evaluating the occurrence of age-related comorbidities in people living with and without HIV. We previously reported a lower forced vital capacity (FVC) in HIV-positive compared with HIV-negative participants in those without heavy smoking exposure at time of enrolment in the $AGE_hIV$ cohort study. In this study we evaluate longitudinal changes in spirometry indices in the same $AGE_hIV$ cohort accounting for smoking behaviour and other risk factors.

**Methods** We obtained pre-bronchodilator spirometry measurements in $AGE_hIV$ cohort participants during biennial visits over a median of 5.9 years (IQR 5.7–6.0). Adjusted declines in forced expiratory volume in 1 s ($FEV_1$), FVC, and $FEV_1$/FVC ratio were modelled using linear mixed-effects models and compared by HIV status and smoking status. To evaluate whether changes in spirometry measurements could be driven by increased levels of chronic inflammation, we assessed associations between rates of $FEV_1$ and FVC decline and CD4 and CD8 T-cell counts, and plasma concentrations of C-reactive protein (CRP), interleukin 6, soluble CD14, soluble CD163, and intestinal fatty-acid-binding protein in separate models. The study is registered at ClinicalTrials.gov, NCT01466582.

**Findings** 500 HIV-positive and 481 HIV-negative participants were included with spirometry data from Oct 29, 2010, to Aug 14, 2018. HIV-positive participants were virally suppressed (<40 copies per mL) during 1627 (95%) study visits, and 159 (32%) HIV-positive and 183 (38%) HIV-negative participants had never smoked. Adjusted declines in $FEV_1$ were 10.0 mL per year faster in HIV-positive non-smokers (95% CI 4.2 to 15.7, p=0.00066) compared with HIV-negative non-smokers, and 11.1 mL per year faster in HIV-positive smokers (95% CI 0.7 to 21.4, p=0.036) compared with HIV-negative smokers. In comparison, smoking was associated with a 16.4 mL per year steeper decline in $FEV_1$ among HIV-positive participants (95% CI 8.0 to 24.7, p=0.00012), and 15.3 mL per year steeper decline among HIV-negative participants (95% CI 6.7–24.0, p=0.00052) compared with not smoking. Adjusted yearly declines in $FEV_1$ and FVC, but not $FEV_1$/FVC, were significantly greater in HIV-positive than HIV-negative participants overall (additional decline in HIV-positive participants, $FEV_1$ 10.5 mL per year [95% CI 4.7 to 16.3], p=0.00040; FVC 11.5 mL per year [2.8 to 20.3], p=0.0096; $FEV_1$/FVC 0.07% per year [−0.05 to 0.19], p=0.26), with a similar observation for never-smokers ($FEV_1$ 6.0 mL per year [−1.8 to 13.7], p=0.13; FVC 9.1 mL per year [−3.0 to 21.1], p=0.14; $FEV_1$/FVC ratio 0.00% per year [−0.18 to −0.18], p=0.97). Higher CRP concentrations during follow-up were associated with accelerated declines in $FEV_1$ and FVC among HIV-positive participants but not among HIV-negative participants.

**Interpretation** Treated HIV infection was associated with faster declines in both $FEV_1$ and FVC, but not in the $FEV_1$/FVC ratio. These changes were independent of smoking and might have been driven by ongoing interstitial or small airway damage, potentially related to increased inflammation.

## INTRODUCTION

Chronic obstructive pulmonary disease is more frequently diagnosed among people living with HIV compared with the general population, independent of risk behaviours such as tobacco smoking.[1,2] HIV infection appears to have distinct consequences for pulmonary health, including in people living with HIV who have suppressed viraemia using combination antiretroviral therapy (cART).[3] These changes have been most evidently characterised by a lower pulmonary diffusion capacity in people living with HIV.[4] Studies evaluating airway obstruction or emphysema have been less consistent in showing differences between people living with HIV on cART and uninfected controls.[5,6,7]

Disentangling the ongoing effects of suppressed HIV infection on pulmonary health from those effects related to previously untreated HIV infection and historical risk behaviours remains challenging, mainly because most studies evaluating such differences have been cross-sectional in design. In a previous cross-sectional analysis of baseline data from the $AGE_hIV$ cohort, we showed that HIV-positive participants reporting less than 25 pack-years of cumulative smoking had a lower forced vital capacity (FVC) but a similar forced expiratory volume in 1 s ($FEV_1$) compared with HIV-negative participants with similar smoking exposure. This finding resulted in a seemingly lower prevalence of obstructive lung disease ($FEV_1$/FVC ratio <70%) in the HIV-positive participants with little smoking exposure.[8]

Evaluating the effects of HIV on pulmonary health during adequate viral suppression requires longitudinal studies that include HIV-negative controls, while adequately controlling for confounding risk behaviours. To date, the only adequately powered study evaluating such longitudinal changes in spirometry indices is the AIDS Linked to the Intravenous Experience (ALIVE) study, which included HIV-positive and HIV-negative people who inject drugs. Compared with HIV-negative controls, a faster decline in $FEV_1$ was reported among HIV-positive participants with a viral load greater than 75 000 copies per mL,[9] and among HIV-positive virally-suppressed participants younger than 50 years of age, although not among those older than 50 years.[10] However, most of these participants were smokers, which might have influenced or obscured any HIV-specific effects.

We therefore aimed to evaluate the effects of HIV infection on longitudinal spirometry indices in middle-aged, predominantly virally-suppressed, HIV-positive and HIV-negative $AGE_hIV$ cohort participants, a substantial number of whom were never or former smokers. Moreover, we evaluated whether such changes in spirometry indices could

be driven by increased levels of chronic inflammation, which can accelerate functional decline of other organ systems in ageing people living with HIV on cART.

## RESEARCH IN CONTEXT

### Evidence before this study

We searched Pubmed on October 1, 2020 using the search terms "spirometry", "respiratory function tests", "pulmonary function test", "chronic obstructive pulmonary disease", "COPD", "obstructive lung disease", "restrictive pulmonary disease", or "pulmonary fibrosis", and "HIV Infections" or "HIV" (both free text and MeSH terms). We restricted our search to publications from 2006 onwards, corresponding to the year at which a higher prevalence of obstructive lung disease was first described in people living with HIV despite the use of antiretroviral therapy. We also searched conference abstracts of major HIV-conferences to include more recent findings. Although cross-sectional analyses evaluating the pulmonary health of people with treated HIV have been numerous, studies reporting longitudinal measurements while including HIV-negative controls are scarce. Only one adequately powered cohort has assessed longitudinal follow-up with spirometry measurements during suppressed HIV-infection, while including an HIV-negative comparison group. This cohort (SHIELD cohort) includes people who inject drugs, the majority of whom smoked during follow-up, potentially obscuring HIV-specific changes in spirometry indices. In that study, accelerated declines 1-second Forced Expiratory Volume were reported in HIV-positive participants with a viral load >75,000 copies, and in HIV-positive participants under 50 years of age with a suppressed viral load, compared to HIV-negative controls.

### Added value of this study

To our knowledge, we are the first to report long-term longitudinal spirometry measurements in people living with HIV on antiretroviral therapy compared to HIV-negative individuals, while including a sufficient number of never- and former-smokers to appropriately evaluate any smoking-independent effect. We show that HIV-infection, independent of smoking, is associated with accelerated declines in spirometry indices despite adequate viral suppression and that these changes are phenotypically different from those associated with smoking (i.e. declines in both FEV1 and FVC, but not the FEV1/FVC ratio). Although the precise aetiology of these HIV-associated changes cannot be determined in the context of our study, several markers of chronic systemic inflammation were associated with accelerated declines in spirometry indices among HIV-positive participants only.

### Implications of all the available evidence

Clinicians should be aware that HIV may have an independent effect on pulmonary function, which also arises in the context of adequate viral suppression. These effects may not be evident when using typical cut-offs for obstructive lung disease (FEV1/FVC below 70% or below the lower limit of normal). In both clinical practice as well as future research, pulmonary diagnostic modalities other than spirometry should be employed to determine the aetiology of HIV-associated respiratory impairments presenting in people living with HIV on antiretroviral therapy.

# METHODS

## Study design and participants

The AGE$_h$IV Cohort Study is an ongoing prospective cohort study of 1148 participants evaluating the occurrence of age-related comorbidities in HIV-1-infected and HIV-negative participants. HIV-positive participants were recruited from the HIV outpatient clinic of the Amsterdam University Medical Centers, location AMC, Amsterdam, Netherlands. HIV-negative participants were recruited from the sexual health clinic and the Amsterdam Cohorts Studies at the Amsterdam Public Health Clinic.[11] Recruiting the control group from a sexual health clinic allowed for a comparable group with respect to sexual orientation, geographical region, and sociodemographic background. The inclusion criteria were age 45 years or older and, for controls, a documented HIV-negative antibody test at enrolment. Study enrolment was open between Oct 29, 2010, and Oct 9, 2012. Once every 2 years (ie, biennially), participants underwent a standardised screening programme to diagnose age-related comorbidities, details of which are included in Supplementary Text 3.1. Detailed information on current and past HIV characteristics were obtained from the Dutch HIV Monitoring Foundation.[12] Written informed consent was obtained from all participants and the study was approved by the ethical review board of the Academic Medical Center, University of Amsterdam.

## Procedures

Information on demographics and risk behaviours was prospectively collected via questionnaires, including a detailed record of ongoing and past smoking behaviour. At each study visit, a spirometry measurement without bronchodilation was obtained by trained nurses according to European Respiratory Society/American Thoracic Society (ERS/ATS) criteria[13] using a SpiroUSB spirometer (Carefusion; Hoechberg, Germany). A maximum of six forced expiratory efforts were performed. Three or more efforts meeting the ERS/ATS criteria were needed to determine $FEV_1$ and FVC. If fewer than three efforts met ERS/ATS criteria, the highest $FEV_1$ and FVC were selected from the qualifying values. If no ERS/ATS-qualifying effort was recorded, the spirometry measurement was excluded. All participants with more than one successful spirometry measurement were included in the analysis.

At each study visit, high-sensitivity C-reactive protein (CRP) concentrations were assessed by immunoturbidimetry (E702 Roche Diagnostics; Indianapolis, IN, USA) and absolute CD4 and CD8 T-cell counts by flow cytometry (BD FACSCantoTM II, BD Biosciences; San Jose, CA, USA). Interleukin 6 (IL-6), soluble CD14, soluble CD163, and intestinal fatty-acid-binding protein (I-FABP) concentrations were assessed by ELISA (DuoSet ELISA; R&D systems, Minneapolis, MN, USA) with use of stored plasma samples from

the enrolment visit. HIV-negative participants received a fourth-generation HIV antibody test at each study visit to confirm HIV status.

## Outcomes

Outcome measures were mean yearly changes in $FEV_1$, FVC, and the $FEV_1$/FVC ratio.

## Statistical analysis

Baseline was defined as the first visit with a successful spirometry measurement and follow-up continued until the fourth scheduled study visit, loss to follow-up, or death. HIV-negative participants who seroconverted during follow-up (n=5) continued follow-up in the HIV-positive group. Baseline characteristics of included versus excluded participants and of HIV-positive versus HIV-negative included participants were compared with Wilcoxon rank-sum, Pearson's $\chi^2$, or Student's t tests as appropriate.

We modelled mean yearly changes in $FEV_1$, FVC, and $FEV_1$/FVC ratio over study follow-up using mixed-effect linear regression. We included a random intercept to account for baseline variability between participants. The following covariates were included in the multivariable models: baseline age; sex at birth; ethnicity (based on region of origin); (time-updated during follow-up) weight; (time-updated) height; baseline number of pack-years smoking (ie, number of years smoking multiplied by packs of 20 cigarettes smoked daily); baseline number of years since cessation of smoking (for former smokers); the interaction between baseline pack-years and years since smoking cessation; (time-updated) daily marijuana use; former injecting drug use; and (time-updated) bronchodilator use during the 24 h before spirometry measurements. These covariates were selected a priori on the basis of findings from the baseline analysis of $AGE_hIV$ cohort spirometry data[8] or known associations with variation in spirometry indices.[14] Moreover, the variables of HIV status, time-updated recent (during past month) smoking behaviour (ie, smoking [current-smoking] vs non-smoking [former-smoking or never-smoking]), and follow-up time were included, as well as all two-way and three-way interactions of these three variables. Differences in the yearly decline in spirometry indices between HIV-positive and HIV-negative groups were assessed, while stratifying by time-updated smoking status. This test was done with the contrast command in Stata. From this model, we also calculated mean yearly changes in spirometry indices by HIV and smoking status using the lincom command. To evaluate whether previous severe immunodeficiency or AIDS-related conditions might influence differences observed between HIV-positive participants and controls, we assessed whether nadir CD4 count, previous Pneumocystis jirovecii pneumonia, or pulmonary tuberculosis were associated with rates of decline in $FEV_1$ and FVC in HIV-positive participants. In sensitivity analyses, we restricted the analysis to (1) high-quality spirometry measurements (ie, ≥3 ERS/ATS-qualifying efforts),

(2) measurements from HIV-positive participants for which HIV viral load was undetectable (<40 copies per mL) and all measurements from HIV-negative participants, and (3) never-smokers. We also did a sensitivity analysis to account for covariate imbalances between HIV-positive participants and HIV-negative controls at baseline by estimating pulmonary function declines using linear mixed effects models weighted by the inverse probability of belonging to the HIV-positive participant group (Supplementary Table 3.5). Furthermore, we explored the influence of all other covariates included in the multivariable model (eg, age, sex, pack-years smoking) on the main associations between HIV status and change in $FEV_1$ and FVC (Supplementary Table 3.4 and Supplementary Text 3.2), and whether HIV status was associated with a more rapid (ie, highest quintile) decline in $FEV_1$ and FVC (Supplementary Table 3.5).

We evaluated the association between individual markers of inflammation and the rates of both $FEV_1$ and FVC decline. All marker concentrations were natural log-transformed and were considered time-fixed (for markers measured at baseline only) or time-updated (for markers measured at baseline and during follow-up). We used mixed-effects linear regression while including the same covariates as in the primary analysis and a random intercept to account for participant variability at baseline. We included an interaction term between continuous inflammatory marker concentrations and follow-up time, from which we could test whether changes in spirometry indices were faster or slower with increases in marker concentrations. These analyses were stratified by HIV status. Sensitivity analyses were restricted to participants who had never smoked. Statistical significance was defined as p less than 0.05.

Statistical analyses were done with Stata software (version 12.0). The study is registered at ClinicalTrials.gov, NCT01466582.

### Role of the funding source

None of the study funders had a role in the design or conduct of the study, the analysis and interpretation of the results, the writing of the report, or the decision to publish.

## RESULTS

We included 1700 spirometry measurements from 500 HIV-positive participants in the analysis, of which 1264 (74.4%) had at least three ERS/ATS-qualifying efforts, and 1732 spirometry measurements from 481 HIV-negative participants, of which 1396 (80.6%) had at least three qualifying efforts. Included data in the study were collected between Oct 29, 2010, and Aug 14, 2018. 99 (17%) HIV-positive and 68 (12%) HIV-negative

participants were excluded for having one or no spirometry measurements. Excluded participants were more likely to be HIV-positive, female, younger, and of black ethnicity than included participants (Supplementary Table 3.1). At baseline, spirometry results from excluded participants with a single qualifying spirometry measurement (141 [84%] of excluded participants) did not seem to differ from those of participants included in the analysis (Supplementary Table 3.1).

Among individuals included in our analysis, HIV-positive participants were more likely to be of black ethnicity, had a slightly lower body-mass index, were more likely to be smokers and had more pack-years of smoking compared with HIV-negative participants (Table 3.1). Most HIV-positive participants were virally suppressed (<40 copies per mL) and had

**TABLE 3.1** | Baseline demographic, behavioural and clinical characteristics of included participants

| | HIV-positive N = 500 | | HIV-negative N = 481 | | *P* |
|---|---|---|---|---|---|
| | **N** | **n (%) or median (IQR)** | **N** | **n (%) or median (IQR)** | |
| **Demographics** | | | | | |
| Male sex at birth | 500 | 447 (89%) | 481 | 411 (85%) | 0.062 |
| Age in years | 500 | 53.2 (48.3, 59.6) | 481 | 52.5 (48.4, 58.7) | 0.21 |
| Ethnic descent | 500 | | 481 | | |
| White | | 445 (89%) | | 454 (95%) | 0.0025 |
| Black | | 46 (9%) | | 18 (4%) | |
| Asian | | 9 (2%) | | 9 (2%) | |
| MSM | 477 | 371 (74%) | 474 | 344 (72%) | 0.063 |
| BMI ($kg/m^2$) | 500 | 24.2 (22.5, 26.7) | 481 | 24.5 (22.9, 27.0) | 0.027 |
| **Risk behaviours** | | | | | |
| Smoke-status | 474 | | 470 | | |
| Never | | 159 (32%) | | 183 (38%) | 0.017 |
| Former | | 164 (33%) | | 176 (37%) | |
| Current | | 151 (30%) | | 111 (23%) | |
| Pack-years smoking, Former/current smokers only | 308 | 22 (8, 37) | 284 | 14 (4, 28) | <0.0001 |
| Marijuana use | 466 | | 466 | | |
| No or less than weekly use | | 412 (82%) | | 426 (89%) | 0.29 |
| Weekly use | | 29 (6%) | | 23 (5%) | |
| Daily use | | 25 (5%) | | 17 (4%) | |

**TABLE 3.1 *(continued)*** | Baseline demographic, behavioural and clinical characteristics of included participants

| | HIV-positive N = 500 | | HIV-negative N = 481 | | *P* |
|---|---|---|---|---|---|
| | N | n (%) or median (IQR) | N | n (%) or median (IQR) | |
| Prior intravenous drug use | 464 | 16 (3%) | 468 | 5 (1%) | 0.014 |
| Hepatitis C RNA positive | 500 | 13 (3%) | 480 | 5 (1%) | 0.069 |
| **HIV-related characteristics** | | | | | |
| Years since HIV-diagnosis | 498 | 12 (7, 17) | N/A | N/A | N/A |
| Last HIV-1 viral load measurement <40 c/mL | 497 | 456 (91%) | N/A | N/A | N/A |
| Last CD4 count (cells/mm$^3$) | 496 | 570 (450, 760) | N/A | N/A | N/A |
| Nadir CD4 count (cells/mm$^3$) | 500 | 180 (80, 260) | N/A | N/A | N/A |
| Prior CDC class-C AIDS-defining illness | 500 | 146 (29%) | N/A | N/A | N/A |
| Prior *Pneumocystis jirovecii* pneumonia | 500 | 48 (10%) | N/A | N/A | N/A |
| Prior pulmonary tuberculosis | 500 | 8 (2%) | N/A | N/A | N/A |
| **Baseline spirometry indices** | | | | | |
| Absolute $FEV_1$ (mean litre, SD) | 500 | 3.37 (0.77) | 481 | 3.51 (0.80) | 0.0068 [1] |
| $FEV_1$ (mean % predicted [2], SD) | 500 | 91.4 (15.0) | 481 | 93.6 (14.7) | 0.017 [1] |
| $FEV_1$ < z-score -1.64 [2] | 500 | 70 (14%) | 481 | 48 (10%) | 0.053 |
| Absolute FVC (mean litre, SD) | 500 | 4.56 (1.00) | 481 | 4.72 (1.01) | 0.012 [1] |
| FVC (mean % predicted [2], SD) | 500 | 96.5 (14.8) | 481 | 98.4 (13.3) | 0.034 [1] |
| FVC < z-score -1.64 [2] | 500 | 32 (6%) | 481 | 25 (5%) | 0.42 |
| $FEV_1$/FVC (mean %, SD) | 500 | 74.5 (8.7) | 481 | 74.3 (7.7) | 0.82 [1] |
| $FEV_1$/FVC < 70% | 500 | 117 (23%) | 481 | 110 (23%) | 0.84 |
| $FEV_1$/FVC < z-score -1.64 [2] | 500 | 78 (16%) | 481 | 63 (13%) | 0.26 |
| **Baseline inflammatory marker levels** | | | | | |
| High sensitivity C-reactive protein (mg/L) | 491 | 1.5 (0.7, 3.1) | 479 | 1.0 (0.6, 2.0) | <0.0001 |
| Interleukin-6 (pg/mL) | 499 | 1.5 (1.0, 2.8) | 480 | 1.9 (1.2, 3.1) | 0.0001 |
| Soluble CD14 (ng/mL) | 499 | 1565 (1310, 1975) | 480 | 1358 (1081, 1734) | <0.0001 |
| Soluble CD163 (ng/mL) | 499 | 284 (206, 417) | 478 | 249 (182, 343) | <0.0001 |
| Intestinal fatty acid binding protein (ng/mL) | 499 | 2.2 (1.5, 3.7) | 480 | 1.1 (0.7, 1.6) | <0.0001 |

*N represents number of individuals with available data. Wilcoxon rank-sum and Pearson's $\chi^2$ tests were used for statistical comparisons, unless stated otherwise. 1.Student's t-test, 2. Based on Global Lung Initiative reference calculations (i.e. predicted based on age, sex, ethnicity and height). Abbreviations: MSM, men who have sex with men; BMI, body mass index; $FEV_1$, Forced 1-second expiratory volume; FVC, forced vital capacity; N/A, not available.*

high CD4 counts at baseline, but had low nadir CD4 counts (Table 3.1). Median follow-up was 6.0 years (IQR 4.0–6.1) in HIV-positive and 6.0 years (5.8–6.0) in HIV-negative participants. 129 (26%) HIV-positive and 71 (15%) HIV-negative participants were censored before the fourth follow-up visit due to poor spirometry measurement quality (16 [3%] HIV-positive and 19 [4%] HIV-negative), discontinuation of study participation (32 [6%] and seven [1%]), loss to follow-up (71 [14%] and 41 [9%]), or death (ten [2%] and four [1%]). During follow-up, HIV-positive participants were virally suppressed (<40 copies per mL) at 1627 (95.5%) of spirometry measurements. 159 (32%) HIV-positive and 183 (38%) HIV-negative participants had never smoked. Smoking during the month before spirometry was reported at 522 (31%) measurements in HIV-positive participants and 392 (23%) measurements in HIV-negative participants. Recent inhaler use was reported at 56 (3.3%) measurements in HIV-positive participants and 66 (3.8%) measurements in HIV-negative participants.

Baseline spirometry measurements of the $AGE_hIV$ cohort have previously been reported in detail.[8] In the current selection of participants, baseline mean $FEV_1$ was 3.37 L for HIV-positive participants versus 3.51 L for HIV-negative participants (p=0.0068), and mean FVC was 4.56 L versus 4.72 L (p=0.034), respectively. 78 (16%) HIV-positive and 63 (13%) HIV-negative participants had an $FEV_1$/FVC ratio below the lower limit of normal cut-off values (Z score less than −1.64; p=0.26).[14]

Unadjusted mean declines in $FEV_1$, FVC, and $FEV_1$/FVC ratio were 46.5 mL per year, 29.4 mL per year, and 0.55% per year in HIV-positive participants, and 35.9 mL per year, 16.7 mL per year, and 0.49% per year in HIV-negative participants, respectively. Significant differences in declines in $FEV_1$ (p<0.0001) and FVC (p=0.00070), but not in the $FEV_1$/FVC ratio (p=0.29), were observed between HIV-positive and HIV-negative participants. The distribution of the rates of change in spirometry indices by HIV status is shown in Supplementary Figure 3.1.

Adjusted mean declines in spirometry indices by HIV status and smoking behaviour are shown in Figure 3.1 and Supplementary Table 3.5. In adjusted models, HIV-status was associated with faster declines in $FEV_1$ (10.5 mL/year [95% CI 4.7 to 16.3], p=0.00040), FVC (11.5 mL/year [2.8 to 20.3], p=0.0096), but not in the $FEV_1$/FVC ratio (0.07 %/year [−0.05 to 0.19], p=0.26). $FEV_1$ declined 10.0 mL per year faster in HIV-positive non-smokers (95% CI 4.2–15.7, p=0.00066) compared with HIV-negative non-smokers, and 11.1 mL per year faster in HIV-positive smokers (95% CI 0.7–21.4, p=0.036) compared with HIV-negative smokers. In comparison, smoking was associated with a 16.4 mL per year steeper decline in $FEV_1$ among HIV-positive participants (95% CI 8.0–24.7, p=0.00012), and 15.3 mL

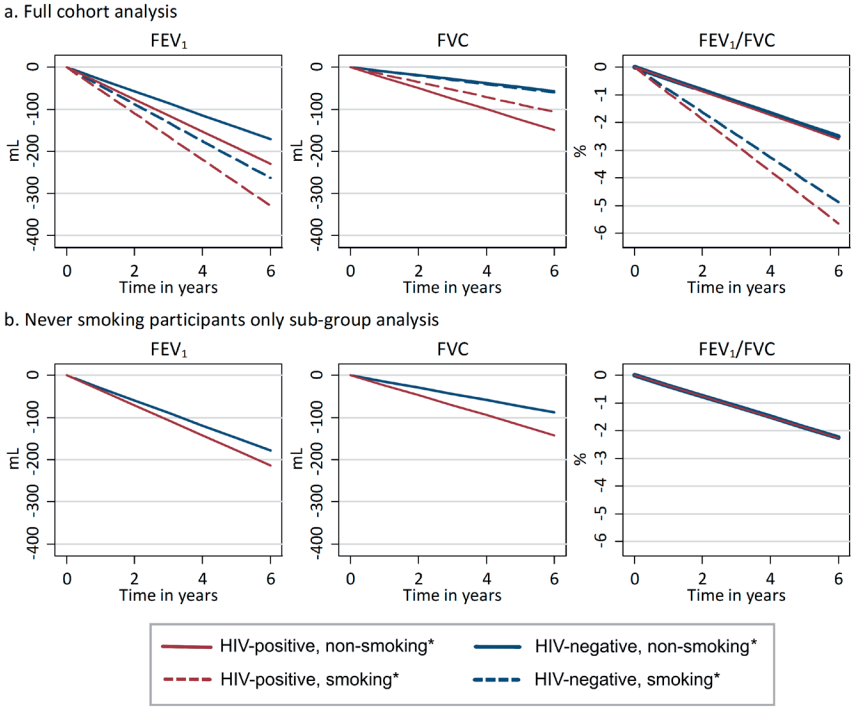


**FIGURE 3.1** | Adjusted predicted mean yearly declines in $FEV_1$, FVC and $FEV_1$/FVC from baseline by HIV-status and smoking behaviour during follow-up

*Figures are a visual representation of the mean predicted yearly declines in spirometry indices provided in supplementary table 3.2. *Smoking or non-smoking during the month prior to spirometry measurements, i.e. time-updated smoking-behaviour.*

per year steeper decline among HIV-negative participants (95% CI 6.7–24.0, p=0.00052) compared with not smoking.

FVC declined significantly faster at 15.4 mL per year (95% CI 6.8–24.0, p=0.00046) in non-smoking HIV-positive versus non-smoking HIV-negative participants, and non-significantly faster at 7.7 mL per year (95% CI −7.9 to 23.2, p=0.33) in HIV-positive smokers versus HIV-negative smokers. There were no significant differences between smokers and non-smokers in FVC decline (HIV-positive p=0.26, HIV-negative p=0.93).

Decline in the $FEV_1$/FVC ratio did not differ between HIV-positive versus HIV-negative participants: 0.01% per year (95% CI −0.14 to 0.11, p=0.81) among non-smoking and 0.13% per year (−0.35 to 0.09, p=0.26) among smoking participants. However, smoking was associated with a 0.51% per year steeper decline in $FEV_1$/FVC ratio in HIV-positive

participants (95% CI 0.33–0.68, p<0.0001) and a 0.40% per year steeper decline in HIV-negative participants (0.21–0.58, p<0.0001).

Nadir CD4 count, previous P jirovecii pneumonia, or pulmonary tuberculosis were not associated with faster $FEV_1$ or FVC decline in HIV-positive participants (Table 3.2).

**TABLE 3.2** | HIV-related predictors of $FEV_1$ and FVC decline over time among HIV-positive participants

| | $FEV_1$ (mL) | | FVC (mL) | |
|---|---|---|---|---|
| | ***mL/y additional decline* (95% CI)** | ***P*** | ***mL/y additional decline* (95% CI)** | ***P*** |
| CD4 nadir count (cells/μL) | | | | |
| <50 | 0.7 (-10.0, 11.4) | 0.90 | -3.8 (-19.9, 12.2) | 0.64 |
| 50 - <200 | -0.7 (-9.4, 8.0) | 0.87 | -3.0 (-16.1, 10.1) | 0.66 |
| 200 - <500 | *Ref* | *Ref* | *Ref* | *Ref* |
| ≥500 | 11.3 (-8.9, 31.6) | 0.27 | -3.9 (-34.2, 26.4) | 0.80 |
| Prior PCP | 2.5 (-10.9, 15.9) | 0.71 | -15.1 (-35.2, 4.9) | 0.14 |
| Prior pulmonary TB diagnosis | -1.9 (-31.8, 28.0) | 0.90 | -15.5 (-60.2, 29.2) | 0.50 |

*Estimations were derived from an interaction term of the indicated variables with time in a mixed effects model, including only HIV-positive participants while adjusting for the same covariates as reported in the main adjusted models. Abbreviations: CDC, Centres for Disease Control and Prevention; PCP, Pneumocystis jirovecii pneumonia; TB, Tuberculosis.*

Sensitivity analyses showed that when restricting analyses to high-quality spirometry measurements (n=2707), the $FEV_1$/FVC ratio declined significantly faster among HIV-positive versus HIV-negative participants overall (0.17% per year, p=0.014) but not within the smoking or non-smoking subgroups. Results from the main analysis did not change when restricting analyses to only virally-suppressed HIV-positive participants (and HIV-negative participants; Supplementary Table 3.2). When restricting analyses to never-smokers, the declines in $FEV_1$ and FVC were faster in HIV-positive, compared with HIV-negative, participants, but the difference did not reach statistical significance (additional decline in HIV-positive individuals 6.0 mL per year, 95% CI −1.8 to 13.7, p=0.13 for FEV1; 9.1 mL per year, 95% CI −3.0 to 21.8, p=0.14 for FVC; Figure 3.1, Supplementary Table 3.2). Results from the inverse probability-weighted model were similar to results from the main analysis (overall additional decline in HIV-positive participants: $FEV_1$ 11.9 mL per year [p=0.0014], FVC 12.7 mL per year [p=0.010], and $FEV_1$/FVC ratio 0.07% per year [p=0.34]; Supplementary Table 3.3). Further analyses in Supplementary Table 3.4 and Supplementary Text 3.2 show that any imbalances in other covariates (eg, age, sex, and pack-years of smoking) between HIV-positive and HIV-negative participants were unlikely to have changed the associations between HIV-status and declines in $FEV_1$ and

FVC. Positive HIV status was also independently associated with a higher probability of rapid $FEV_1$ and FVC decline (highest quintile of change, Supplementary Table 3.5).

Figure 3.2 shows the associations between concentrations of inflammatory markers and declines in $FEV_1$ and FVC. Higher baseline and time-updated high-sensitivity CRP concentrations were associated with significantly steeper declines in $FEV_1$ (baseline p=0.036, time-updated p=0.00029) and FVC (p=0.044 and p=0.0012) among HIV-positive participants, but not among HIV-negative participants ($FEV_1$ p=0.63 and p=0.97, FVC p=0.56 and p=0.15, respectively). In contrast, in both HIV-positive and HIV-negative participants higher concentrations of IL-6 at baseline were associated with less steep declines in $FEV_1$ (HIV-positive p=0.025, HIV-negative p=0.10) and FVC (HIV-positive p=0.036, HIV-negative p=0.039). Higher concentrations of baseline soluble CD14 were associated with a steeper decline in FVC in HIV-positive participants (p=0.0018), while higher baseline concentrations of soluble CD163 and lower baseline concentrations of I-FABP were associated with a steeper decline in FVC in HIV-negative participants (p=0.0015 and p=0.0077, respectively). Analyses limited to participants who never smoked are shown in Supplementary Figure 3.2. Among never-smoking HIV-positive participants, high-

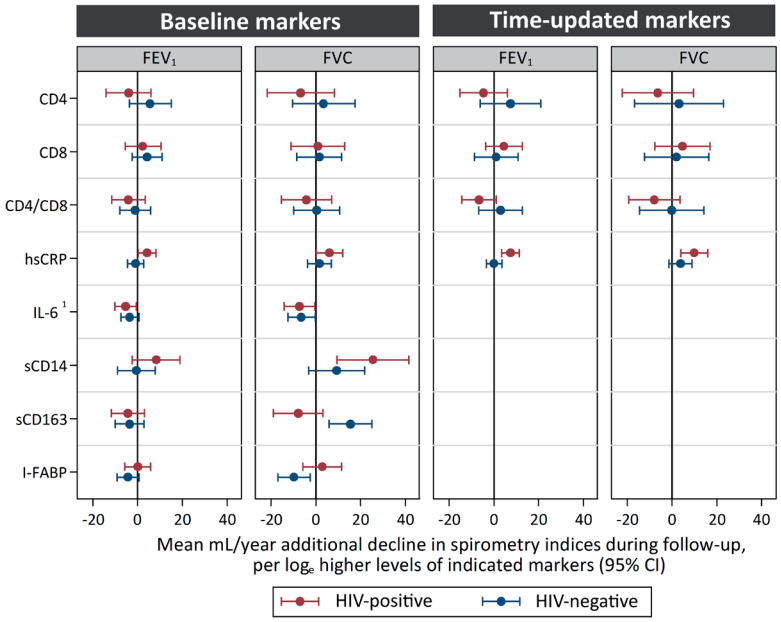


**FIGURE 3.2** | Associations between markers of chronic inflammation and rate of yearly decline in $FEV_1$ and FVC

*1. Log-log-transformation. Abbreviations: hsCRP, highly sensitive C-reactive protein; IL-6, Interleukin-6, sCD14, soluble CD14; sCD163, soluble CD163; I-FABP, Intestinal fatty acid binding protein.*

sensitivity CRP concentrations at baseline and during follow-up and IL-6 and soluble CD14 concentrations at baseline were not statistically associated with differences in the rates of $FEV_1$ or FVC decline.

## DISCUSSION

During 6 years of follow-up we found an accelerated decline in $FEV_1$ and FVC, but not in the $FEV_1$/FVC ratio, in predominantly virally-suppressed HIV-positive $AGE_h$IV cohort participants compared with HIV-negative controls, independent of smoking. HIV status was also independently associated with a higher probability of rapid decline in $FEV_1$ and FVC. Tobacco smoking during follow-up was associated with accelerated $FEV_1$ and $FEV_1$/FVC decline, but not an accelerated decline in FVC. These results suggest that declines in spirometry-measured HIV-related pulmonary function are phenotypically different from declines related to smoking and could continue even during suppressive antiretroviral therapy.

Simultaneous declines in $FEV_1$ and FVC might indicate development of isolated interstitial restrictive pulmonary disease or restrictive pulmonary disease combined with obstructive disease.[16] Both pathophysiological processes have been associated with HIV in previous studies. In a cross-sectional analysis of baseline data from the Danish COCOMO cohort,[6] HIV-positive compared with HIV-negative participants had lower $FEV_1$ and FVC, but slightly higher $FEV_1$/FVC ratios. In that cohort, CT imaging did not indicate more frequent emphysema, but did show more interstitial pulmonary abnormalities among HIV-positive participants.[5,17] In a subgroup of never-smoking participants, small airway dysfunction was also identified to be more frequent among HIV-positive participants with use of a lung clearance index (ie, multiple breath nitrogen washout) compared with HIV-negative participants.[18] Several studies have reported higher lung densities on CT imaging in people living with HIV versus controls, suggesting interstitial pulmonary pathology.[7,19] However, CT imaging studies evaluating never-smokers or adolescents with HIV have shown that both airway disease in general as well as small airway disease specifically were more frequently observed in HIV-positive participants compared with controls.[19,20,21] Importantly, these studies have been cross-sectional in design and reflect not only recent and ongoing HIV-related damage, but also historical tissue damage. This historical damage includes insults from previous HIV-associated pulmonary infections, lengthy exposure to HIV viraemia, and the cumulative exposures to inhaled toxic substances, such as those from smoking. Since spirometry can definitively distinguish pulmonary pathology only in the medium-sized and large airways, the exact aetiology of accelerated declines in both $FEV_1$ and FVC in our virally-suppressed

HIV-positive participants remains unclear. Differences between historical and ongoing damage could also explain the contrast between our current findings and findings from our baseline analysis (ie, lower FVC in our baseline analysis but similar $FEV_1$ in HIV-positive vs HIV-negative participants without heavy smoking experience). The characteristics of historical cumulative damage might be phenotypically different from current ongoing HIV-related pulmonary damage during cART. Given that neither lower nadir CD4 count nor previous Pneumocystis jirovecii pneumonia or pulmonary tuberculosis was associated with faster declines in $FEV_1$ or FVC during follow-up, our findings instead suggest that ongoing pulmonary damage underlies these declines.

We explored whether the faster $FEV_1$ and FVC declines in our participants were associated with higher concentrations of several inflammatory markers. Higher concentrations of high-sensitivity CRP were strongly associated with faster declines in both $FEV_1$ and FVC among HIV-positive participants, but there was no association among HIV-positive never-smokers. Smoking has been associated with increased CRP, which could partly have driven the association between higher CRP concentrations and faster $FEV_1$ and FVC declines in our participants. Moreover, people living with HIV remain more prone to bacterial pneumonias compared with the general population even when they are virally suppressed, especially among smokers.[22] Repeated insults from infections might result in both increased CRP and faster declines in spirometry indices. Although IL-6 and CRP are on similar inflammatory pathways, we found no association between IL-6 concentrations and declines in $FEV_1$ and FVC. IL-6 and CRP are more strongly correlated in cases of high levels of inflammation. Perhaps CRP, specifically during low-level inflammation, might be more linked to accelerated decline in lung function compared with IL-6. Higher concentrations of the monocyte activation marker soluble CD14, but not soluble CD163, were associated with a faster FVC decline among HIV-positive participants. Monocyte activation in people living with HIV is suggested to be chronically elevated via increased microbial translocation from the gut, leading to a state of chronic systemic inflammation and end-organ damage. As such, soluble CD14 has been associated with more frequent emphysema as detected by CT imaging, and soluble CD163 has been associated with a lower $FEV_1$/FVC ratio and lower diffusion capacity in people living with HIV.[23,24,25] In our study, I-FABP, a marker of intestinal permeability, was not associated with faster declines in pulmonary function among HIV-positive participants. This finding suggests that monocyte activation could be driven by other processes, including pulmonary tissue damage itself or exposure to infections. Importantly, these inflammatory markers were measured in plasma, which might poorly correlate with marker concentrations in the lung compartment.[26] Furthermore, indications for pathophysiological processes in the lungs of people with HIV on antiretroviral therapy other than chronic inflammation have recently been reported; Chelvanambi and colleagues[27] have shown the presence of

HIV-Nef protein in bronchoalveolar fluid in people living with HIV with suppressed viraemia and its link to surface expression of proapoptotic endothelial-monocyte-activating polypeptide II, which is implicated in the progression of pulmonary emphysema. This finding could indicate that local pulmonary tissue damage by HIV itself can still occur even when HIV is undetectable in plasma.

The extent to which HIV-positive participants in our study had functional impairments related to the measured accelerated decline in pulmonary function remains unclear, since pulmonary symptoms and related functional decline were not assessed. However, since the effect size of HIV status on the additional decline in $FEV_1$ was two-thirds of that of smoking, suppressed HIV infection might become associated with clinically relevant functional impairments, especially over the longer term. We believe our results are illustrative of the independent effects of HIV infection and as such are generalizable to all people living with HIV. However, pulmonary function might differ in populations of people living with HIV with different risk profiles for lung disease (eg, differing smoking rates or history of pulmonary co-infections like tuberculosis). Few women and people of non-white ethnicity were included in the $AGE_hIV$ cohort and in this analysis. Generalizability of our results could thus be poor among these specific groups of people living with HIV. HIV-positive participants were more frequently excluded from the analysis due to missing data and had less follow-up time compared with HIV-negative participants. Since HIV-positive participants are at increased risk of developing comorbidities,[28] a health-related differential rate of drop-out might have resulted in an underestimated effect of HIV infection on lung function. Further limitations of our analysis were the lack of a post-bronchodilator measurement, little data on marijuana or e-cigarette use, and potential self-reporting bias of risk behaviours.

In conclusion, despite adequate antiretroviral treatment, HIV infection is associated with a faster decline in pulmonary function as measured by spirometry, independent of tobacco smoking. Future studies are needed to evaluate the aetiology and clinical consequences of these changes. Since the $FEV_1$/FVC ratio appears mostly unaffected by HIV status and the underlying cause of these HIV-specific pulmonary changes cannot be identified by spirometry alone, clinicians should be aware of the limitations of using spirometry as a single diagnostic tool for pulmonary disease in people living with HIV on cART. Both diffusion capacity testing and CT imaging would prove particularly valuable in identifying interstitial pulmonary disease, aiding in distinguishing interstitial disease from the presence of small airway disease. In the absence of specific treatments for the long-term pulmonary sequelae of HIV infection, smoking cessation should remain the mainstay of improving pulmonary health in people living with HIV.

## AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualization and design of the study. SOV and EV performed data curation and contributed to data collection and coordination of the study. SOV and AB designed the methodology of the study and conducted the formal analysis. PR obtained funding and supervised the conduct of the study. SOV provided the original draft of the manuscript including figures. All authors contributed to reviewing and editing the manuscript to its final form. SOV, EV and AB had access to, and verified, the underlying data.

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# SUPPLEMENTARY MATERIAL

**SUPPLEMENTARY TEXT 3.1:** $AGE_hIV$ Cohort study design

From Schouten J, Wit FW, Stolte IG, et al. Cross-sectional Comparison of the Prevalence of Age-Associated Comorbidities and Their Risk Factors Between HIV-Infected and Uninfected Individuals: The AGEhIV Cohort Study. CID 2014:59 (15 Dec):

**Study Design and Data Collection**

HIV-1–infected participants were recruited from the HIV outpatient clinic of the Academic Medical Center in Amsterdam, and HIV-uninfected participants (controls) were recruited from the sexual health clinic of the Amsterdam Public Health Service or among uninfected participants in the existing Amsterdam Cohort Studies on HIV/AIDS [1]. To ensure comparability of the HIV-infected and HIV-uninfected study groups, we regularly monitored age, sex, and ethnicity in both study groups, and adjusted enrolment of underrepresented categories among HIV-uninfected participants accordingly. All participants were aged ≥45 years with laboratory confirmed presence or absence of HIV-1 infection. All subjects who provided written informed consent within the 2-year enrolment period were included. Of 1100 eligible patients from the HIV outpatient clinic, between 600 and 800 were expected to be enrolled, and we therefore aimed to include a similar number of uninfected controls. This sample size would provide sufficient statistical power to investigate associations between a broad range of AANCCs and potential risk factors. At baseline, 2 years later, and depending on sufficient resources every 2 years thereafter, participants undergo standardized screening for AANCCs and organ dysfunction. Participants are requested to complete a standardized questionnaire concerning demographics, (family) medical history, use of medications (both prescribed and over-the-counter), participation in population screening programs, substance use, quality of life, depression, sexual orientation/behaviour/dysfunction, cognitive complaints, calcium/vitamin D intake, physical exercise, social behaviour, and work participation/income. All participants undergo measurements of blood pressure, height, weight, and hip/waist circumference, as well as electrocardiography, measurement of vascular elasticity, spirometry, screening cognitive tests, frailty, bone densitometry, and quantification of advanced glycation end products in the skin. Blood and urine samples are obtained for extensive laboratory testing, and cryopreserved for future analyses. Detailed information concerning HIV and ART history is obtained from the Dutch HIV Monitoring Foundation, formally responsible for capturing detailed HIV/ART-related data from all individuals in care for HIV at an HIV treatment facility in the Netherlands [2]. The study protocol was approved by the local ethics review committee and registered at www.clinicaltrials.gov (identifier NCT01466582). All participants provided written informed consent.

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**SUPPLEMENTARY TABLE 3.1** | Baseline demographic, behavioural and clinical characteristics of excluded participants compared to included participants

| | Excluded participants (N=167) | Included participants (N=981) | P |
|---|---|---|---|
| HIV-positive | 99 (59%) | 449 (51%) | 0.044 |
| Male sex at birth | 131 (78%) | 858 (88%) | 0.0018 |
| Age in years (median, IQR) | 51 (48, 56) | 53 (48, 59) | 0.0070 [1] |
| Ethnic descent | | | <0.0001 |
| White | 131 (78%) | 889 (92%) | |
| Black | 33 (20%) | 64 (7%) | |
| Asian | 1 (1%) | 18 (2%) | |
| Unknown | 2 (1%) | 0 (0%) | |
| Smoke-status | | | 0.0061 |
| Never | 28 (17%) | 343 (35%) | |
| Former | 58 (35%) | 337 (34%) | |
| Current | 40 (24%) | 263 (27%) | |
| Unknown | 41 (25%) | 38 (4%) | |
| 1 successful spirometry measurement available for analysis | 141 (84%) | 981 (100%) | N/A |
| Baseline $FEV_1$ (mean % predicted [2], SD) | 90.6 (15.9) | 92.4 (15.1) | 0.19 [3] |
| Baseline FVC (mean % predicted [2], SD) | 95.0 (16.3) | 97.4 (14.3) | 0.073 [3] |
| Baseline $FEV_1$/FVC (mean %, SD) | 75.5 (9.2) | 74.4 (8.3) | 0.16 [3] |

*Numbers are N (%) and P-values based on Pearson $\chi$ tests unless stated otherwise. 1. Wilcoxon rank-sum test. 2. Based on Global Lung Initiative reference calculations (i.e. predicted based on age, sex, ethnicity and height) 3. Student's t-test. Abbreviations: $FEV_1$, Forced 1-second expiratory volume; FVC, Forced vital capacity.*

**SUPPLEMENTARY TABLE 3.2** | Adjusted mean yearly declines in $FEV_1$, FVC and $FEV_1$/FVC by HIV-status and smoking behaviour

| | | Non-smoking during follow-up | | Smoking during follow-up | | All participants | |
|---|---|---|---|---|---|---|---|
| | | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI)** | **P** |
| **$FEV_1$** | | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI)** | **P** |
| Fully adjusted analysis [1] | HIV +<br>HIV - | 38.3 (34.0, 42.6)<br>28.3 (24.4, 32.3) | 0.00066 | 54.7 (47.7, 61.7)<br>43.7 (36.0, 51.3) | 0.036 | 10.5 (4.7, 16.3) | 0.00040 |
| Sensitivity analysis 1; High quality measurements only [1,2] | HIV +<br>HIV - | 40.2 (35.3, 45.2)<br>27.0 (22.8, 31.3) | <0.0001 | 58.1 (50.4, 65.8)<br>42.9 (34.5, 51.2) | 0.0085 | 14.3 (7.8, 20.7) | <0.0001 |
| Sensitivity analysis 2; HIV-undetectable participants only [1,3] | HIV +<br>HIV - | 38.7 (34.3, 43.1)<br>28.5 (24.6, 32.4) | 0.00058 | 54.1 (47.0, 61.3)<br>43.8 (36.2, 51.4) | 0.051 | 10.3 (4.4, 16.1) | 0.00058 |
| Sensitivity analysis 3; limited to participants who had never smoked [4] | HIV +<br>HIV - | 35.8 (29.9, 41.7)<br>29.8 (24.6, 35.0) | 0.13 | N/A | N/A | 6.0 (-1.8, 13.7) | 0.13 |
| **FVC** | | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI)** | **P** |
| Fully adjusted analysis [1] | HIV +<br>HIV - | 24.9 (18.5, 31.3)<br>9.5 (3.6, 15.4) | 0.00046 | 17.7 (7.2, 28.2)<br>10.0 (1.4, 21.5) | 0.33 | 11.5 (2.8, 20.3) | 0.0096 |
| Sensitivity analysis 1; High quality measurements only [1,2] | HIV +<br>HIV - | 24.0 (16.6, 31.4)<br>8.5 (2.1, 14.9) | 0.0017 | 19.6 (8.0, 31.3)<br>11.7 (-0.93, 24.4) | 0.37 | 11.7 (2.0, 21.5) | 0.018 |
| Sensitivity analysis 2; HIV-undetectable participants [1,3] | HIV +<br>HIV - | 25.5 (18.8, 32.1)<br>9.8 (3.9, 15.7) | 0.00050 | 16.7 (5.8, 27.6)<br>9.8 (1.7, 21.3) | 0.40 | 11.3 (2.4, 20.2) | 0.013 |
| Sensitivity analysis 3; limited to participants who had never smoked [4] | HIV +<br>HIV - | 23.7 (14.5, 32.8)<br>14.6 (6.5, 22.7) | 0.14 | N/A | N/A | 9.1 (-3.0, 21.1) | 0.14 |

**SUPPLEMENTARY TABLE 3.2 *(continued)*** | Adjusted mean yearly declines in $FEV_1$, FVC and $FEV_1$/FVC by HIV-status and smoking behaviour

| | | Non-smoking during follow-up | | Smoking during follow-up | | All participants | |
|---|---|---|---|---|---|---|---|
| | | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI)** | **P** |
| **$FEV_1$/FVC** | | **Mean %/year decline (95% CI)** | **P HIV+ vs HIV-** | **Mean %/year decline (95% CI)** | **P HIV+ vs HIV-** | **Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI)** | **P** |
| Fully adjusted analysis [1] | HIV +<br>HIV - | 0.43 (0.34, 0.52)<br>0.42 (0.33, 0.50) | 0.81 | 0.94 (0.79, 1.09)<br>0.81 (0.65, 0.98) | 0.26 | 0.07 (-0.05, 0.19) | 0.26 |
| Sensitivity analysis 1; High quality measurements only [1,2] | HIV +<br>HIV - | 0.51 (0.41, 0.61)<br>0.41 (0.32, 0.49) | 0.12 | 0.97 (0.81, 1.13)<br>0.75 (0.57, 0.92) | 0.059 | 0.17 (0.03, 0.30) | 0.014 |
| Sensitivity analysis 2; HIV-undetectable participants [1,3] | HIV +<br>HIV - | 0.44 (0.34, 0.53)<br>0.41 (0.33, 0.50) | 0.72 | 0.96 (0.80, 1.11)<br>0.82 (0.66, 0.98) | 0.22 | 0.08 (-0.04, 0.20) | 0.21 |
| Sensitivity analysis 3; limited to participants who had never smoked [4] | HIV +<br>HIV - | 0.38 (0.24, 0.51)<br>0.37 (0.25, 0.50) | 0.97 | N/A | N/A | 0.00 (-0.18, 0.18) | 0.97 |

*1. Adjusted for: (time-updated) smoking behaviour 1 month prior to spirometry; age at baseline; sex at birth; ethnicity; (time-updated) weight; (time-updated) height; baseline number of pack-years smoking; baseline number of years since cessation of smoking; interaction pack-years with smoking-cessation years; daily use of marijuana; prior injecting drug use; (time-updated) bronchodilator use 24h prior to spirometry measurements. 2. Including only spirometry measurements with ≥3 ATS-qualifying efforts (includes 938 participants with 2707 measurements). 3. Including only spirometry measurements while participants were HIV-undetectable (<40 c/mL) at time of the spirometry measurement (includes 971 participants with 3336 measurements). 4. Only participants who never smoked (includes 322 participants with 1150 measurements), these models were adjusted for the same covariates as the main model except for covariates regarding smoking or marijuana use. Abbreviations: $FEV_1$, 1-second forced expiratory volume, FVC, forced vital capacity; N/A, not applicable.*

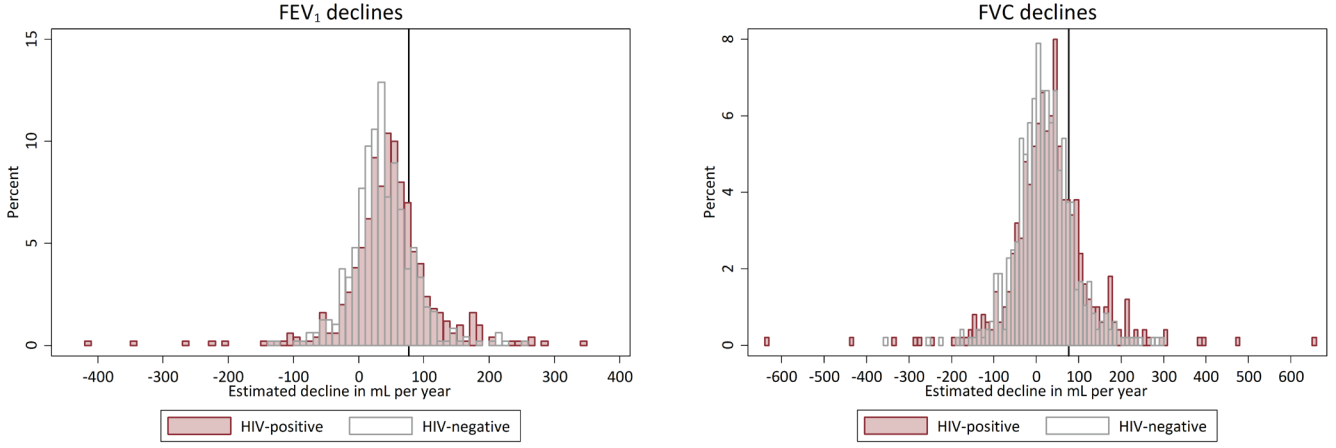
**SUPPLEMENTARY TABLE 3.3** | Sensitivity analysis using a weighted approach to account for covariate imbalances between HIV-positive individuals and their controls

| | | Non-smoking during follow-up | | Smoking during follow-up | | All participants | |
|---|---|---|---|---|---|---|---|
| | | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI)** | **P** |
| **$FEV_1$** | | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI)** | **P** |
| Weighted sensitivity analysis | HIV +<br>HIV - | 43.9 (38.6, 49.3)<br>31.7 (27.4, 36.0) | 0.00045 | 57.8 (48.4, 67.2)<br>46.1 (36.8, 55.3) | 0.080 | 11.9 (4.6, 19.3) | 0.0014 |
| Main primary analysis | HIV +<br>HIV - | 38.3 (34.0, 42.6)<br>28.3 (24.4, 32.3) | 0.00066 | 54.7 (47.7, 61.7)<br>43.7 (36.0, 51.3) | 0.036 | 10.5 (4.7, 16.3) | 0.00040 |
| **FVC** | | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Mean mL/year decline (95% CI)** | **P HIV+ vs HIV-** | **Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI)** | **P** |
| Weighted sensitivity analysis | HIV +<br>HIV - | 31.9 (24.0, 39.9)<br>16.2 (9.6, 22.8) | 0.0028 | 23.9 (10.8, 36.9)<br>14.2 (3.6, 24.8) | 0.26 | 12.7 (3.0, 22.4) | 0.010 |
| Main primary analysis | HIV +<br>HIV - | 24.9 (18.5, 31.3)<br>9.5 (3.6, 15.4) | 0.00046 | 17.7 (7.2, 28.2)<br>10.0 (1.4, 21.5) | 0.33 | 11.5 (2.8, 20.3) | 0.0096 |

**SUPPLEMENTARY TABLE 3.3 *(continued)*** | Sensitivity analysis using a weighted approach to account for covariate imbalances between HIV-positive individuals and their controls

| | | Non-smoking during follow-up | | Smoking during follow-up | | All participants | |
|---|---|---|---|---|---|---|---|
| | | Mean mL/year decline (95% CI) | P HIV+ vs HIV- | Mean mL/year decline (95% CI) | P HIV+ vs HIV- | Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI) | P |
| **$FEV_1$/FVC** | | **Mean %/year decline (95% CI)** | **P HIV+ vs HIV-** | **Mean %/year decline (95% CI)** | **P HIV+ vs HIV-** | **Overall mL/year difference in mean decline HIV+ vs HIV- (95% CI)** | **P** |
| Weighted sensitivity analysis | HIV +<br>HIV - | 0.44 (0.34, 0.54)<br>0.40 (0.31, 0.48) | 0.49 | 0.89 (0.72, 1.07)<br>0.80 (0.61, 0.99) | 0.48 | 0.07 (-0.07, 0.21) | 0.34 |
| Main primary analysis | HIV +<br>HIV - | 0.43 (0.34, 0.52)<br>0.42 (0.33, 0.50) | 0.81 | 0.94 (0.79, 1.09)<br>0.81 (0.65, 0.98) | 0.26 | 0.07 (-0.05, 0.19) | 0.26 |

*The table reports results from a sensitivity analysis using a weighted approach to account for covariate imbalances between HIV-positive individuals and their controls at baseline. Results of the main primary non-weighted adjusted analysis are included for comparison. In order to assign weights, we used a logistic regression model with HIV-status as the outcome and the following baseline variables as predictors: age, sex, ethnicity, weight, height, number of pack-years smoking, years since cessation of smoking, interaction between pack-years smoking and years since cessation of smoking and former injection drug use. Note that we did not include current smoking, daily marijuana use or bronchodilator use as predictors, as these are potential important confounders during follow-up and require time-updated adjustment, which are not ideal for this weighted approach (i.e. weights representing probability of inclusion). Instead of BMI, we included height and bodyweight in the model, as height is required to adjust for physiological differences in lung-volumes and BMI would be collinear with this variable. Weights were derived as the inverse of individual predicted probabilities from this model, which were then standardized to a mean of 1. We then ran linear mixed effects models with $FEV_1$, FVC and the $FEV_1$/FVC ratio as outcomes and HIV-status, time-updated smoking-status (yes or no), time (and interaction-terms between these variables), daily marijuana use, and bronchodilator use as predictor variables, while weighting the analysis using the calculated weights described above. From this model, we calculated expected declines within HIV- and smoking categories, similar to the main primary analysis of the paper.*




**SUPPLEMENTARY FIGURE 3.1**. | Distribution of individual $FEV_1$ and FVC declines comparing HIV-positive and HIV-negative participants

*Individual declines in spirometry indices were calculated as the linear slope from regressing the 2-4 longitudinal spirometry measurements on follow-up time for each individual. The frequency distribution of the individual linear slopes of all those with >1 spirometry measurements are plotted in the figures. The black vertical lines indicate the 80$^{th}$ percentile of decline rates ($FEV_1$: 77 mL/year, FVC: 76 mL/year)*

**SUPPLEMENTARY TABLE 3.4** | Differences in yearly $FEV_1$ and FVC decline with respect to covariables

| | $FEV_1$ (mL) | | FVC (mL) | |
|---|---|---|---|---|
| | ***mL/y additional decline* (95% CI)** | ***P*** | ***mL/y additional decline* (95% CI)** | ***P*** |
| Current smoking (yes vs. no, time-updated) | **16.9 (10.9, 22.9)** | **<0.0001** | -2.1 (-11.1, 6.9) | 0.65 |
| Baseline age (years) | | | | |
| 45-54 | *Ref* | *Ref* | *Ref* | *Ref* |
| 55-64 | 4.3 (-1.2, 9.8) | 0.12 | 6.5 (-1.7, 14.6) | 0.12 |
| 65-74 | 0.2 (-9.2, 9.7) | 0.96 | 7.7 (-6.4. 21.8) | 0.29 |
| >75 | -17.1 (-48.6, 14.5) | 0.29 | 6.7 (-40.2, 53.6) | 0.78 |
| Male vs. female sex | **13.4 (5.9, 21.0)** | **<0.00047** | 7.9 (-3.3, 19.2) | 0.17 |
| Ethnicity | | | | |
| White | *Ref* | *Ref* | *Ref* | *Ref* |
| Black | -6.2 (-17.2, 4.8) | 0.27 | 9.7 (-6.7, 26.1) | 0.25 |
| Asian | -6.8 (-24.5, 10.9) | 0.45 | -19.2 (-45.5, 7.1) | 0.15 |
| BMI ($kg/m^2$, time-updated) [1] | | | | |
| <18.5 | **28.4 (3.2, 53.5)** | **0.027** | 14.4 (-22.8, 51.6) | 0.45 |
| 18.5 - <25 | *Ref* | *Ref* | *Ref* | *Ref* |
| 25 - <30 | -2.2 (-7.9, 3.5) | 0.45 | 3.7 (-4.7, 12.1) | 0.39 |
| ≥30 | 1.6 (-7.5, 10.8) | 0.73 | **14.6 (1.0, 28.2)** | **0.035** |
| No. of baseline pack-years smoking[2] | | | | |
| *Among former smokers:* | | | | |
| >0 - <10 | *Ref* | *Ref* | *Ref* | *Ref* |
| 10 - <25 | 6.0 (-4.1, 16.1) | 0.24 | -15.2 (-30.6, 0.2) | 0.053 |
| 25 - <40 | -0.6 (-4.1, 16.1) | 0.92 | 5.8 (-14.2, 25.8) | 0.57 |
| ≥40 | **18.9 (5.1, 32.6)** | **0.0071** | 20.7 (-1.0, 42.5) | 0.062 |
| *Among current smokers:* | | | | |
| >0 - <10 | *Ref* | *Ref* | *Ref* | *Ref* |
| 10 - <25 | -3.2 (-18.5, 12.0) | 0.68 | -5.3 (26.9, 16.2) | 0.63 |
| 25 - <40 | 4.7 (-12.0, 21.4) | 0.58 | -2.5 (-26.1, 21.2) | 0.84 |
| ≥40 | **28.0 (12.2, 43.8)** | **0.0005** | 15.4 (-7.1, 37.8) | 0.18 |
| No. of years since cessation of smoking at baseline (*among former smokers only*) | | | | |
| >0 - <5 | *Ref* | *Ref* | *Ref* | *Ref* |
| 5 - <20 | 1.8 (-8.4, 12.0) | 0.73 | 3.0 (-12.5, 18.5) | 0.70 |
| ≥20 | -0.7 (-11.2, 9.8) | 0.90 | 2.0 (-14.0, 17.9) | 0.81 |
| Former injecting drug use (yes vs. no) | -7.1 (-23.0, 8.9) | 0.38 | 4.5 (-19.2, 28.2) | 0.71 |

**SUPPLEMENTARY TABLE 3.4 *(continued)*** | Differences in yearly $FEV_1$ and FVC decline with respect to covariables

| | $FEV_1$ (mL) | | FVC (mL) | |
|---|---|---|---|---|
| | ***mL/y additional decline* (95% CI)** | ***P*** | ***mL/y additional decline* (95% CI)** | ***P*** |
| Marijuana use [3] | | | | |
| No or less than weekly use | *Ref* | *Ref* | *Ref* | *Ref* |
| Weekly use | 6.2 (-5.5, 17.9) | 0.30 | -3.0 (-20.5, 14.5) | 0.74 |
| Daily use | **27.0 (13.2, 40.9)** | **<0.00013** | 19.3 (-1.4, 40.0) | 0.067 |
| Bronchodilator use (time-updated) | **-20.6 (-37.5, -3.7)** | **0.017** | -11.8 (-37.0, 13.3) | 0.36 |

*The additional change in $FEV_1$ or FCV at a given level of covariable (compared to a reference group) was calculated from the two-way interaction term of a mixed-effects model including time in years and the indicated variable as a covariable. Models were adjusted for all other variables in the table and HIV-status. The models only included one interaction term at a time, meaning that each parameter estimate was obtained from separately run models. 1. To be consistent with the model used in the primary analysis, we included height and weight as covariables for all models. However, these covariables were replaced with BMI in this particular model to study the effects of obesity. 2. We included baseline pack-years smoking, regardless of current smoking status, for all models. However, baseline pack-years smoking was evaluated in former and current smoker sub-groups separately in this model to evaluate differences in historical smoking exposure. 3. We included daily marijuana use in all models. However, we included weekly and daily use in this model to determine the effect of marijuana smoking frequency. Results are bold when P<0.05.*

**SUPPLEMENTARY TEXT 3.2** Evaluation of covariables driving accelerated $FEV_1$ or FVC decline

Supplementary table 3.4 shows that the rate of $FEV_1$ decline was estimated to be faster in participants who were male, had ≥40 pack-years smoking history, had a BMI <18 kg/$m^2$, used marijuana daily or did *not* use a bronchodilator prior to spirometry measurements. The rate of FVC decline was steeper in participants with a BMI ≥30 kg/$m^2$ and almost significantly steeper among participants with ≥40 pack-years smoking history.

We further explored the influence of these covariates by running sensitivity analyses of the main model after excluding participants from the above mentioned categories and determined whether HIV-status remained significantly associated with a faster decline in $FEV_1$ or FVC.

$FEV_1$ decline remained significantly faster among HIV-positive vs. HIV-negative participants after (1) excluding female participants (additional decline: 12.0 mL/y, 95%CI 5.6-18.4, P=0.00024), (2) excluding participants with ≥40 pack-years smoking history (additional decline: 11.8 mL/y, 95%CI 5.5-18.2, P=0.00025), (3) excluding participants with a BMI <18 kg/$m^2$ (additional decline: 10.6 mL/y, 95%CI 4.8-16.5, P=0.00036), (4) excluding participants who smoked marijuana daily (additional decline: 9.7 mL/y, 95%CI 3.6-15.8, P=0.0018), and (5) excluding participants who used a bronchodilator prior to spirometry measurements (additional decline: 10.0 mL/y, 95%CI 4.1-15.8, P=0.00086).

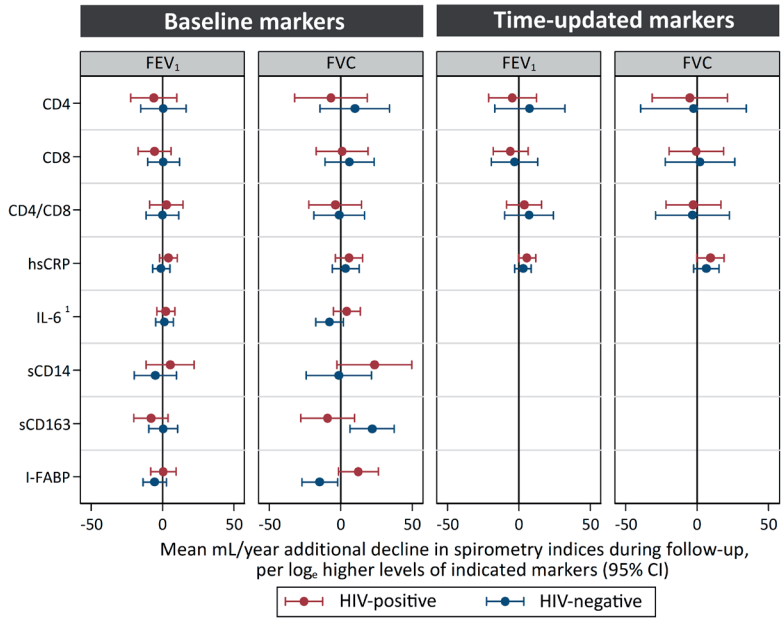
FVC decline remained significantly faster among HIV-positive vs. HIV-negative participants after (1) excluding participants with a BMI >30 kg/$m^2$ (additional decline: 12.5 mL/y, 95%CI 3.2-21.7, P=0.0081), and (2) when excluding participants with ≥40 pack-years smoking history (additional decline: 12.2 mL/y, 95%CI 2.7-21.7, P=0.011).

These results provide evidence that the covariables identified as potential drivers of accelerated declines in $FEV_1$ and FCV do not influence the association between HIV-status and lung function.

**SUPPLEMENTARY TABLE 3.5** | Baseline determinants of rapid $FEV_1$ and FVC decline

| | Highest quintile of FEV1 decline (>77 mL/year) | | | | Highest quintile of FVC decline (>76 mL/year) | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariable OR (95%CI) | P | Multivariable OR (95%CI) | P | Univariable OR (95%CI) | P | Multivariable OR (95%CI) | P |
| HIV-positive vs. HIV-negative | 1.7 (1.2, 2.3) | 0.0018 | 1.5 (1.1, 2.1) | 0.014 | 1.6 (1.2, 2.2) | 0.0025 | 1.6 (1.1, 2.2) | 0.0068 |
| Age (per 5 year) | 1.1 (1.0, 1.3) | 0.0098 | 1.2 (1.0, 1.3) | 0.010 | 1.2 (1.0, 1.3) | 0.0040 | 1.1 (1.0, 1.3) | 0.012 |
| Male vs. female sex | 2.5 (1.4, 4.7) | 0.0031 | 1.5 (0.7, 3.0) | 0.27 | 1.5 (0.9, 2.6) | 0.10 | - | - |
| Ethnicity | | | | | | | | |
| White | Ref | Ref | - | - | Ref | Ref | - | - |
| Black | 0.5 (0.3, 1.2) | 0.12 | - | - | 1.0 (0.5, 1.9) | 0.99 | - | - |
| Asian | 0.5 (0.1, 2.1) | 0.33 | - | - | 0.5 (0.1, 2.1) | 0.34 | - | - |
| Weight (per 10 kg) | 1.0 (0.9, 1.2) | 0.43 | - | - | 1.1 (1.0, 1.2) | 0.21 | - | - |
| Height (per 10 cm) | 1.3 (1.1, 1.6) | 0.0025 | 1.3 (1.1, 1.7) | 0.012 | 1.0 (0.9, 1.2) | 0.86 | - | - |
| Current smoking (yes vs. no) | 1.9 (1.3, 2.6) | 0.00018 | 1.7 (1.2, 2.5) | 0.0042 | 0.9 (0.6, 1.3) | 0.63 | - | - |
| Pack-years smoking (per additional 5 year) | 1.1 (1.0, 1.1) | <0.0001 | 1.0 (1.0, 1.1) | 0.038 | 1.0 (1.0, 1.1) | 0.012 | 1.0 (1.0, 1.1) | 0.098 |
| Former injecting drug use | 0.7 (0.2, 2.3) | 0.51 | - | - | 0.9 (0.3, 2.8) | 0.88 | - | - |
| Daily marijuana use | 2.1 (1.1, 4.0) | 0.034 | 1.5 (0.8, 3.1) | 0.24 | 1.8 (0.9, 3.6) | 0.084 | 1.7 (0.9, 3.4) | 0.13 |
| Bronchodilator use | 0.8 (0.2, 2.8) | 0.71 | - | - | 0.5 (0.1, 2.1) | 0.34 | - | - |

*Univariable and multivariable odds ratios (OR) and 95% confidence intervals (CI) of the associations between baseline covariables and rapid decline in $FEV_1$ (i.e. >77 ml/y $FEV_1$) and in FVC (76 ml/y FVC) during follow-up were calculated using logistic regression models. Individual declines in spirometry indices were calculated as the linear slope from regressing the 2-4 longitudinal spirometry measurements on follow-up time for each individual. Cut-offs for rapid decline were chosen based on the $80^{th}$ percentile. Variables were included in the multivariable model if their univariable association was at P<0.1. Results are bold when P<0.05. Abbreviations: OR, Odds Ratio; CI, Confidence Interval.*




**SUPPLEMENTARY FIGURE 3.2** | Associations between markers of chronic inflammation and rate of yearly decline in $FEV_1$ and FVC among participants who had never smoked.

*1. Log-log-transformation. Abbreviations: hsCRP, highly sensitive C-reactive protein; IL-6, Interleukin-6, sCD14, soluble CD14; sCD163, soluble CD163; I-FABP, Intestinal fatty acid binding protein.*

# 4

# Generally rare but occasionally severe weight gain after switching to an integrase inhibitor in virally suppressed AGE$_h$IV cohort participants

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## ABSTRACT

**Objectives:** Recent studies have reported disproportionate weight gain associated with integrase strand transfer inhibitor (INSTI) initiation in antiretroviral therapy(ART)-naive people with HIV (PLWH), particularly among black women. We investigated if HIV-positive AGE$_h$IV participants with suppressed viremia switching to INSTI-containing ART experienced more weight gain compared to HIV-positive virally-suppressed non-switching and HIV-negative controls.

**Methods:** In the AGE$_h$IV cohort, standardized weight measurements were performed biennially. Participants switching to INSTI-containing ART were 1:2:2 propensity score-matched with controls by age, gender, ethnicity and body mass index. Mean weight changes and proportions experiencing >5% or >10% weight gain were compared between study-groups using linear mixed-effects models and logistic regression, respectively.

**Results:** 121 INSTI-switching participants and 242 participants from each of the control groups were selected. Across groups, median age was 53-55 years, 83-91% were male and 88-93% white. Mean weight change after switch among INSTI-switching participants was +0.14 kg/year (95%CI -0.25, +0.54) and similar among HIV-positive [+0.13 kg/year (95%CI +0.07, +0.33; P=.9)] and HIV-negative [+0.18 kg/year (95%CI 0.00, +0.37; P=.9)] controls. Weight gain >5% occurred in 28 (23.1%) INSTI-switching, 38 HIV-positive (15.7%, P=.085) and 32 HIV-negative controls (13.2%, P=.018). Weight gain >10% was rare.

**Conclusions:** Switching to INSTI-containing ART in our cohort of predominantly white men on long-term ART was not associated with greater mean weight gain, but >5% weight gain was more common than in controls. These results suggest that not all, but only certain, PLWH may be particularly prone to gain a clinically significant amount of weight as a result of switching to INSTI.

## INTRODUCTION

International guidelines currently position integrase strand transfer inhibitors (INSTIs) as preferred agents for people with HIV (PLWH) initiating combined antiretroviral therapy (cART), and ART-experienced PLWH are frequently being switched to INSTIs from protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) based regimens.[1,2] Most INSTIs are considered to have favorable pharmacological and toxicity profiles. However, multiple post-marketing studies have reported greater-than-expected weight gain among both cART-naive-[3-10] and treatment-experienced[11-16] PLWH initiating an INSTI. Whether this will result in a similarly increased risk of obesity-related complications, like are found in the general population[17,18] remains currently unclear.

The most striking data thus far were reported by the ADVANCE study[8-10], a randomized-controlled trial comparing the use of three cART regimens in ART-naive black South-African PLWH: dolutegravir/tenofovir alafenamide(TAF)/emtricitabine, dolutegravir/tenofovir disoproxil/ emtricitabine, and efavirenz/tenofovir disoproxil/emtricitabine. Use of the INSTI dolutegravir was significantly associated with greater weight gain, the effect of which was strongest in women and more prominent with concomitant use of TAF. Other studies reporting significantly greater mean weight gain in PLWH initiating an INSTI generally describe more modest degrees of weight gain and INSTI-related weight gain to be particularly observed in specific sub-groups (i.e. women and people of black African descent).[3-7,11-16] A recent meta-analysis of randomized controlled trials conducted in ART-naive PLWH reported that within the class of INSTIs, there was greater weight gain with the newer generation INSTIs dolutegravir and bictegravir compared to two previously licensed agents of this drug class, raltegravir and elvitegravir.[6] Some other studies, however, did not observe any above-normal weight gain among PLWH initiating INSTIs.[19-22]

The majority of studies thus far have examined weight gain in ART-naive PLWH,[3-10,21] for whom any INSTI-specific effect on weight is difficult to disentangle from the weight gain coinciding with initial suppression of HIV, as part of a 'return-to-health' phenomenon. Fewer studies have been published assessing PLWH with suppressed viremia *switching* to an INSTI-containing regimen, in whom such a phenomenon is absent.[11,15,22] Only one of these studies included standardized weight measurements, with 62% and 25% of participants being overweight and obese prior to switch, respectively.[15] In order to expand our knowledge in persons switching to INSTI whilst already suppressed, we took advantage of data obtained in the AGE$_h$IV cohort study population, with 38% and 8%, respectively, of participants switching to INSTI being overweight and obese prior to switch. Furthermore, the AGE$_h$IV cohort only includes middle-aged and older PLWH,

in whom age-associated comorbidities were found to be more prevalent among PLWH compared to people without HIV of similar age.[23] INSTI-associated weight gain could therefore be particularly relevant for this older sub-population of PLWH, as it could further increase risks of developing comorbidities, such as cardiovascular diseases or malignancies. Our aim was to determine if virally-suppressed HIV-positive participants switching to INSTI-containing cART experienced more weight gain compared to two propensity score matched control groups: (1) PLWH with suppressed viremia who did not alter their cART regimen, and (2) HIV-negative participants.

## MATERIALS AND METHODS

### Study participants and data collection

The AGE$_h$IV Cohort Study is an ongoing prospective cohort study evaluating the occurrence of age-related comorbidities in 598 HIV-1-positive and 550 HIV-negative participants. HIV-positive participants were recruited from the HIV outpatient clinic of the Amsterdam University Medical Centers, location Academic Medical Center. HIV-negative participants were recruited from the sexual health clinic at the Amsterdam Public Health Service and from the Amsterdam Cohort Studies on HIV/AIDS.[24] Participants were enrolled in 2010-2012 and included if at least 45 years of age. At each biennial study visit participants receive a standardized screening for age-related comorbidities and HIV-negative participants a 4$^{th}$ generation HIV-antibody test. Details about the study protocol have previously been published.[23] Detailed information on recent and historical HIV characteristics prior to, and during, study follow-up were obtained from the Dutch HIV Monitoring Foundation registry. This includes prospectively collected detailed data on prior and current use of ART, as well as reasons for regimen alterations.[25] Written informed consent was obtained from all participants and the study was approved by the ethical review board of the Amsterdam UMC and registered at ClinicalTrials.gov (identifier NCT01466582).

At each study visit, body weight was measured using electronic scales (Seca® type 877, Seca, Germany), which were calibrated annually. Participants were explicitly instructed to undress to underwear and socks and remove any heavy jewelry.

### Study group selection through propensity score matching

We identified all participants who switched to an INSTI-containing regimen during follow-up and had never used an INSTI before. Participants were subsequently included in the index group if (1) they had ≥1 weight measurement prior to and ≥1 weight measurement after switch, and (2) had an undetectable (<40 copies/mL) HIV-1 viral load ≥1

year prior to switch (allowing for isolated 'blips' up to 200 copies/mL). For comparison, we selected two separate control groups. Each index participant was matched to two HIV-positive non-switching and two HIV-negative control participants. Eligible HIV-positive non-switching controls were those who continued a PI- and/or NNRTI-based ART regimen (permitting changes in antiretroviral agents within classes and NRTI backbone). Potential HIV-negative controls were participants who remained HIV-negative during study follow-up. Controls with ≤1 weight measurement were excluded. (see Supplementary Figure 4.1)

As participants switched to INSTI-based cART at various time-points during follow-up, the goal was to identify a time-point at which subjects from the control group most closely resembled the index participant at the moment they had switched to INSTI. To accomplish this, we used a time-dependent propensity score[26] derived from the time-fixed covariates "ethnicity" (based on region of origin), gender, and the time-varying covariates age and body mass index (BMI). (see Supplementary Figure 4.2) These variables were selected a priori based on their known association with weight or risk of weight gain. A risk set was constructed in which the hazards of switching to INSTI were modelled for all participants using a Cox proportional hazards model with the matching criteria as independent variables. Predicted hazards were estimated at the visit prior to switch for participants switching to INSTI and at each study visit during follow-up for control participants. Matched pairs were chosen by the smallest total distance in predicted hazards within matched sets. Controls were allowed to be matched with only one index-participant, and a match with an HIV-positive non-switching control was only allowed to occur if they had an HIV-RNA <40 copies/mL for >1 year while on cART.

Risk sets were constructed on information available at study visits. The actual date of switch occurred in-between study visits for the majority of participants in the group switching to INSTI. To select a date for matching controls, the number of days between date of study visit prior to switch and date of switch was first calculated in index participants. This offset was added to the date of the matched study visit in the control participants and was used as the hypothetical date of switch in these participants.

## Statistical analysis

Baseline was defined as the date of switch to INSTI-containing cART in the index participants and the date of hypothetical switch for control participants. We defined two follow-up periods: (1) *pre-baseline*, from the date of enrolment into the $AGE_hIV$ cohort to baseline; and (2) *post-baseline*, from baseline until the last available $AGE_hIV$ weight measurement, INSTI-discontinuation (for index participants), loss of HIV-RNA suppression (>40 copies/mL; excluding isolated blips <200 copies/mL), or death, whichever occurred

first. The pre-baseline follow-up of HIV-positive participants started at the visit where a weight measurement was done and the HIV-RNA was suppressed, and all later visits also had HIV-RNA <40 copies/mL (excluding isolated blips up to 200 copies/mL).

We first used absolute body weight as an outcome, modelling mean yearly changes during follow-up by mixed-effects linear regression, in which between-participant variability at baseline and over time was accounted for by including a random intercept and slope, respectively. Mean changes in weight (i.e. interaction with time) between study-groups and pre- and post-baseline were directly calculated via a three-way interaction term. The differences in weight change slopes between study groups were statistically tested with a joint test using the 'contrast' command in Stata.

Subsequently, we used more prominent and potentially clinically-relevant weight gains as outcomes. These were defined as >5% (thus including those with >10%) or only >10% weight gain at the first weight measurement after baseline, using the last weight measured prior to baseline as comparison. As weight was measured biennially in all participants, choosing these measurements ensured comparable time-intervals during which weight gains could have occurred between study groups. The probability of this outcome was modeled using logistic regression and differences between study groups were tested using a Wald $X^2$ test.

Finally, we compared demographic and ART regimen-specific characteristics between groups of participants switching to INSTI experiencing three discrete categories of weight gain; those with 5-10% or >10% weight gain were compared to those with ≤5% weight gain using Fisher's exact and Wilcoxon rank-sum tests as appropriate. All statistical analyses were performed using Stata software (v12.0, College Station, TX, USA).

## RESULTS

### Study group characteristics

From the 598 HIV-positive $AGE_hIV$ participants, 212 had ever used an INSTI-containing regimen before their last available $AGE_hIV$ cohort weight measurement. Of them, 121 fulfilled criteria of an index participant switching to INSTI. Of these 121 participants, 64 (53%) switched to dolutegravir, 41 (34%) to elvitegravir and 16 (13%) to raltegravir during study follow-up. At switch, 60 (50%) participants also changed the nucleos(t)ide reverse transcriptase inhibitor (NRTI) backbone of their regimen (see Supplementary Table 4.1). Of note, no participants in the $AGE_hIV$ cohort switched to a regimen including both dolutegravir and tenofovir alafenamide (TAF). The reasons for switching to INSTI

differed, with regimen simplification being the most common (n=41, 34%; see Supplementary Table 4.2).

From the eligible HIV-positive (N=271) and HIV-negative (N=488) controls, 242 HIV-positive non-switching participants and 242 HIV-negative participants were matched with index participants. Propensity score matching resulted in three comparable study groups with respect to age, body mass index (BMI), gender and ethnicity at the study visit prior to baseline (Table 4.1). The only significant difference was found in

**TABLE 4.1** | Characteristics of INSTI-switching, HIV-positive non-switching and HIV-negative selected study groups at baseline

| | INSTI-switching | HIV-positive non-switching | HIV-negative | *P* INSTI switching vs. HIV-positive non-switchers | *P* INSTI switching vs. HIV-negative |
|---|---|---|---|---|---|
| N | 121 | 242 | 242 | | |
| Time before baseline (yr) | 4.2 (3.6, 5.2) | 2.7 (1.2, 4.3) | 2.9 (1.5, 4.7) | <.001 | <.001 |
| Time after baseline (yr) | 1.9 (0.9, 2.8) | 2.2 (1.1, 4.0) | 3.0 (1.3, 4.6) | .007 | <.001 |
| Age (yr) | 55 (51, 61) | 54 (51, 61) | 53 (50, 59) | .7 | .02 |
| Male gender | 106 (88%) | 221 (91%) | 200 (83%) | .3 | .2 |
| MSM | 92 (79%) | 182 (79%) | 170 (71%) | .9 | .1 |
| Ethnic descent | | | | | |
| White[1] | 107 (88%) | 217 (90%) | 224 (93%) | .9 | .4 |
| African | 13 (11%) | 23 (10%) | 17 (7%) | | |
| Asian | 1 (1%) | 2 (1%) | 1 (0%) | | |
| BMI (kg/m$^2$) | 24.3 (22.4, 26.1) | 24.0 (22.2, 27.2) | 23.9 (22.5, 26.2) | .8 | .8 |
| BMI (kg/m$^2$) categories | | | | | |
| Underweight: <18.5 | 0 (0%) | 9 (4%) | 1 (0%) | .2 | .9 |
| Normal: 18.5–<25 | 75 (62%) | 137 (57%) | 152 (63%) | | |
| Overweight: 25-<30 | 36 (30%) | 74 (31%) | 70 (29%) | | |
| Obese: ≥30 | 10 (8%) | 22 (9%) | 19 (8%) | | |
| Smoking status | | | | | |
| Never | 38 (32%) | 64 (28%) | 95 (40%) | .07 | .4 |
| Former | 51 (43%) | 82 (36%) | 89 (37%) | | |
| Current | 29 (25% | 85 (37%) | 54 (23% | | |
| Latest CD4 count (cells/mm$^3$) | 640 (500, 790) | 630 (500, 840) | 870 (640, 1060) | .9 | <.001 |
| CD4 nadir (cells/mm$^3$) | 190 (75, 270) | 175 (90, 250) | … | .7 | … |
| Time since HIV diagnosis (yr) | 14 (8, 19) | 14 (10, 19) | … | .4 | … |
| Time since ART initiation (yr) | 12 (7, 17) | 13 (8, 16) | … | .9 | … |

*Values are median (interquartile range) or n (%). [1]Including Hispanic ethnicity. P values were calculated using the Wilcoxon rank-sum test for continuous variables and the $X^2$ test for categorical variables. Abbreviations: BMI, body mass index; MSM, men who have sex with men; ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor.*

median age of HIV-negative controls compared to index-group participants (53 vs. 55 years respectively, *P*=.02). HIV-specific characteristics, such as current and nadir CD4 count and time between diagnosis and ART initiation, were not significantly different between HIV-positive study groups. Median follow-up after baseline was 1.9 (IQR=0.9, 2.8), 2.2 (IQR=1.1, 4.0) and 3.0 (IQR=1.3, 4.6) years for HIV-positive index participants, HIV-positive controls and HIV-negative controls, respectively. Among the HIV-positive controls, 65 (27%) continued using a PI-, 168 (69%) an NNRTI-, and 9 (4%) a PI- plus NNRTI-containing regimen during follow-up.

## Mean weight changes before and after baseline

Figure 4.1 depicts both the body weight trajectories of each participant and the median body weight trajectories of the study groups, which were overall comparable. In the index group (Table 4.2), yearly changes in predicted mean weight were not significantly different from zero during both the pre- and post-baseline periods (0.15 kg/year, P=.2 and 0.14 kg/year, P=.5 respectively), nor statistically different between these follow-up periods (P=0.9). Similarly, no significant differences in yearly changes of predicted mean weight were found between pre- versus post-baseline periods in the HIV-positive non-switching controls (0.08 vs. 0.13 kg/year respectively, P=.7). In the HIV-negative controls, mean yearly increase in weight was borderline significantly lower before baseline compared to after baseline (-0.06 vs. 0.18 kg/year respectively, P=.05). During the post-baseline follow-up period, the yearly changes in predicted mean weight were not significantly different when comparing the index group to either of the control groups (HIV-positive non-switching controls, P =.9; and HIV-negative controls, P=.9).

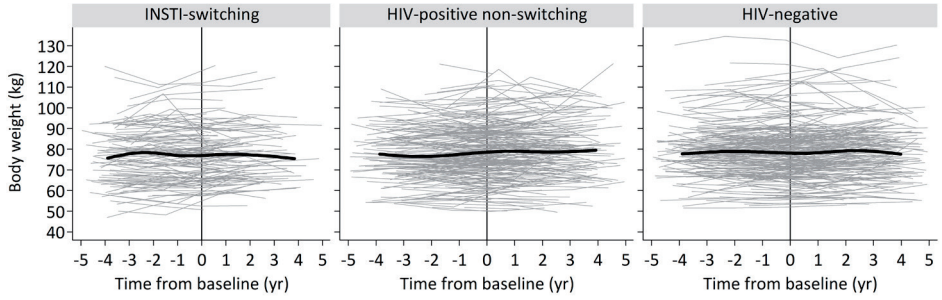


**FIGURE 4.1** | Median and per participant body weight trajectories of INSTI-switching, HIV-positive non-switching and HIV-negative selected participants

*Grey lines demonstrate individual body mass index trajectories before and after baseline (i.e. moment of switch in INSTI-switching participants or at assigned hypothetical moment of switch in controls). Black lines are median splines (6 knots each) within each study group.*

**TABLE 4.2** | Comparison of mean yearly changes in bodyweight by study group, before and after baseline

| | | Mean yearly change in body weight during follow-up periods | | | | Within group comparison, after vs. before baseline | |
|---|---|---|---|---|---|---|---|
| | | ***Before* baseline** | | ***After* baseline** | | | |
| | N | kg/year | 95% CI | kg/year | 95% CI | Δ kg/year | 95% CI |
| INSTI-switching | 121 | 0.15 | -0.08, 0.39 | 0.14 | -0.25, 0.54 | -0.01 | -0.45, 0.43 |
| HIV-positive non-switching | 242 | 0.08 | -0.13, 0.29 | 0.13 | 0.07, 0.33 | 0.05 | -0.22, 0.32 |
| HIV-negative | 242 | -0.06 | -0.26, 0.13 | 0.18 | 0.00, 0.37 | 0.25 | 0.00, 0.50 |
| *Between group comparison* | | *Before* baseline | | *After* baseline | | | |
| | | Δ kg/year | 95% CI | Δ kg/year | 95% CI | | |
| Switch to INSTI vs | 121 | Ref | | Ref | | | |
| HIV-positive non-switching | 242 | -0.07 | -0.39, 0.25 | -0.01 | -0.46, 0.43 | | |
| HIV-negative | 242 | -0.22 | -0.53, 0.09 | 0.04 | -0.40, 0.48 | | |

*Reported results were calculated from a linear mixed-effects model with bodyweight as the dependent variable and a three-way interaction of study group x time x follow-up period (along the with the separate individual variables and two-way interactions between these variables) as independent variables. Abbreviations: CI, Confidence Interval; Δ, difference*

## Probability of experiencing >5% or >10% weight gain

Figure 4.2 illustrates the distribution of proportional weight changes. In this analysis, median time between baseline and post-switch weight measurement was 0.9 (IQR 0.4-1.5) years in the index group, 0.9 (IQR 0.4-1.6) years for HIV-positive controls (P=.7 vs. the index group) and 0.8 (IQR 0.4-1.5) years for HIV-negative controls (P=0.2 vs. the index group). The probability of a >5% increase in weight after baseline was greater in participants switching to INSTI (*n*=28, 23.1%) than in both HIV-negative (*n*=32, 13.2%, P=.018) and in HIV-positive controls (*n*=38, 15.7%, P=.085), with the latter comparison not reaching statistical significance. The probability of a >10% increase in weight was 5.0% (*n*=6) for index participants, 3.7%, (*n*=9, P=.6) for HIV-positive controls, and 2.5% (*n*=5, P=.1) for HIV-negative controls.

## Participants with ≤5%, 5-10% and >10% weight gain after INSTI switch

Three (50%) of the 6 index participants experiencing a >10% weight gain after baseline were black women, while there were only 2 (2%) black women among the 91 index participants with ≤5% weight gain after switch (P=.005, Table 4.3). In comparison, black women were not significantly more likely to have >10% versus ≤5% weight gain, respectively, in any of the control groups: 2 (7%) vs. 4 (2%) in HIV-positive non-switching controls (P=.3); and 1 (4%) vs. 7 (3%) in HIV-negative controls (P=.4). No specific INSTIs, or NRTIs were switched to more often among participants with >10% or 5-10% weight

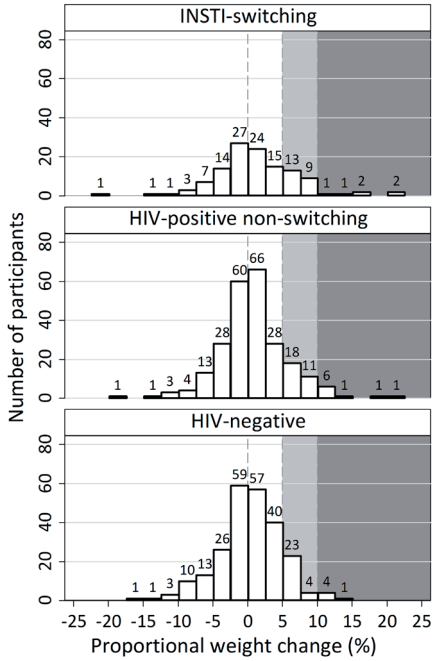


**FIGURE 4.2** | Proportional weight change at first visit after baseline compared to last visit before baseline**.**

*Change compares the first weight measurement after baseline with the last weight measured prior to baseline. Light and dark grey fields indicate participants with a proportional weight increase of >5% (light + dark grey) and >10% (dark grey). Numbers above bars indicate absolute number of participants per bin*

gain compared to those with ≤5% weight gain, nor were there any differences between having a PI- vs. NNRTI-based regimen prior to switch.

## DISCUSSION

In our longitudinal analysis with standardized weight measurements, when judged by mean weight changes participants switching to INSTI-containing cART did not experience significantly greater weight gain compared to HIV-positive individuals who continued their non-INSTI-containing cART or HIV-negative individuals. Whereas other studies have reported significantly greater weight gains 0.9 to 2.4 years after switching to INSTI-containing cART compared to controls, the additional mean weight gain in these studies was limited to 0.05-2.2 kg.[11,12,14-16,22]

Of note, these previous studies focused on relatively small mean differences, rather than on the possible occurrence of more pronounced weight gain in a subset of participants. In our study, a 5% or greater weight gain after a median of 1 year was more likely among those who switched to INSTI-containing cART.

**TABLE 4.3** | Characteristics of INSTI-switching participants with ≤5%, 5-10% and >10% weight gain after INSTI switch

| | ≤5% weight gain | 5-10% weight gain | >10% weight gain | *P* 5-10% vs. ≤5% weight gain | *P* >10% vs. ≤5% weight gain |
|---|---|---|---|---|---|
| N | 93 | 22 | 6 | | |
| Age at baseline (years) | 55 (50, 62) | 54 (52, 59) | 54 (53, 56) | .8 | 1.0 |
| Gender & Ethnicity | | | | | |
| Non-black male | 78 (85%) | 16 (73%) | 3 (50%) | .7 | .005 |
| Non-black female | 7 (8%) | 3 (14%) | 0 (0%) | | |
| Black male | 5 (5%) | 3 (14%) | 0 (0%) | | |
| Black female | 2 (2%) | 0 (0%) | 3 (50%) | | |
| BMI at baseline (kg/m$^2$) | 24.6 (22.8, 26.2) | 23.2 (21.1, 24.6) | 22.0 (19.8, 22.9) | .019 | .04 |
| INSTI initiated | | | | | |
| Dolutegravir | 46 (49%) | 14 (64%) | 4 (67%) | .5 | .9 |
| Elvitegravir | 34 (37%) | 5 (23%) | 2 (33%) | | |
| Raltegravir | 13 (14%) | 3 (14%) | 0 (0%) | | |
| Regimen prior to switch | | | | | |
| PI based | 39 (42%) | 13 (59%) | 3 (50%) | .5 | 1.0 |
| NNRTI based | 46 (49%) | 9 (41%) | 5 (50%) | | |
| Both NNRTI/PI | 5 (5%) | 0 (0%) | 0 (0%) | | |
| No NNRTI/PI | 3 (3%) | 0 (0%) | 0 (0%) | | |
| TDF after switch | 34 (37%) | 6 (27%) | 2 (33%) | .5 | 1.0 |
| TAF after switch | 19 (20%) | 4 (18%) | 2 (33%) | 1.0 | .6 |
| ABC after switch | 30 (32%) | 9 (41%) | 1 (17%) | .5 | .7 |

*Comparisons were made using Wilcoxon rank-sum and Fisher's exact tests. Abbreviations: BMI, body mass index; INSTI, integrase-strand transfer inhibitor; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; TDF; tenofovir disoproxil; TAF, tenofovir alafenamide; ABC, abacavir.*

Hill et al have proposed to consider >5% weight gain a clinically relevant threshold when reporting INSTI and more generally ART-associated increases in weight,[27] mirroring 'clinically relevant' definitions for weight loss interventions by the United States Food and Drug Administration.[28]

Our findings suggest INSTI-associated weight change to be heterogeneous, and provides support for the hypothesis that some individuals may be more prone to develop prominent INSTI-associated weight gain than others. The results of the ADVANCE trial and other studies which have shown that people of black African descent, particularly

women, and women in general were at increased risk of INSTI-related weight gain and treatment-emergent obesity.[8-10,13,15] Interestingly, although black African women were clearly underrepresented in our study, they were overrepresented in the subgroup with more than 10% weight gain after switching to INSTI.

A mechanism underlying a specific susceptibility to prominent INSTI-associated weight gain is currently unknown, but it seems reasonable to speculate that pharmacogenetics may play a role. As stated in the European Medicines Agency assessment report for dolutegravir, dolutegravir *in vitro* was shown to inhibit binding of radiolabeled α-melanocyte-stimulating hormone (MSH) to the human recombinant melanocortin 4 receptor (MC4R) by 64% at a concentration equal to the clinical Cmax.[29] Whereas a more recent report largely confirmed this observation and in fact demonstrated a class-wide ability of INSTIs to bind MC4R, functional antagonistic effects *in vitro* were only observed at concentrations substantially greater than the therapeutic plasma concentrations of each drug.[30]

The melanocortin system plays an important role in the regulation of food intake and body weight by the hypothalamus, and particular mutations and polymorphisms in the MC4R receptor gene have been demonstrated to increase the risk of obesity.[31,32] Furthermore, individuals with a recessive single nucleotide polymorphism rs489693 near the MC4R gene, as well as those with polymorphisms in different genes expressed in other areas of the brain have been shown to increase susceptibility to extreme weight gain associated with the use of anti-psychotics.[33,34] Whether particular variants of the gene encoding MC4R, or other genes involved in food intake and weight regulation, may be differentially present according to ethnicity and sex, and render individuals particularly susceptible to the potential effects of INSTI on proteins expressed by these genes at clinically relevant concentrations merits further investigation. A higher rate of genetic variation in the MC4R gene in people with African ancestry has previously been reported.[35]

Importantly, the impact of a more pronounced weight gain on an individual's health can be expected to be greater, also dependent on prior weight and preexisting conditions, such as obesity and diabetes. Furthermore, the impact may well be influenced by other than biological factors. For example, African women have been shown to perceive obesity as less of a health threat than African men, and being obese can have more severe psychosocial effects for some ethnic groups than others.[36,37]. Thus, the extent to which individuals would be inclined to implement behavioural countermeasures such as diet and exercise, can be expected to be associated with socio-cultural perceptions of body

weight. Further research is required to delineate the potential adverse cardiometabolic and psychological impact of INSTI-associated weight gain.

An important strength of this study was that body weight was measured in a standardized manner at pre-defined intervals both before and after switch, as opposed to studies which rely on weights captured using not regularly calibrated and varying scales, with inconsistent instructions to patients concerning disrobing. In addition, our study was not subject to bias which may occur when clinicians nowadays could be more inclined to measure weight (more frequently) in patients using INSTIs, given the increased interest in the subject of weight gain potentially associated with INSTIs. Finally, we were able to carefully match participants initiating INSTIs to both treated HIV-positive and HIV-negative control groups in a time-dependent fashion, further minimizing potential biases between comparison groups.

Our study nonetheless also has a number of limitations. First, the number of women, particularly black African women, and black African participants in general was limited, which precluded us from performing adequately powered analyses concerning the influence of gender and ethnic descent. Second, our sample size did not allow meaningful analyses into any differential effects of individual INSTIs including dolutegravir and bictegravir, or of the (concomitant) use of TAF as were observed in the ADVANCE trial and other studies.[9,10,38] Third, follow-up after switch to INSTI may have been insufficient for part of our study participants to allow INSTI-related weight gain to be observed. Finally, changes in weight can be caused by a host of other factors (e.g. smoking, smoking cessation, socio-economic status, mental health problems, etc.) and since many of these factors were either not collected or were fairly homogenous in our study population, they were not adjusted for in our analysis. Residual confounding from these factors could be present.

Generally speaking, these results are reassuring for the majority of PLWH who consider switching their regimen to include an INSTI, in a country like the Netherlands with a largely white male HIV epidemic. In such a population the likelihood for someone to experience a prominent gain in weight appears to be a relatively rare event. This finding is relevant for older PLWH, well-represented in our cohort, who are inclined to switch to an INSTI-containing regimen, for example to prevent potential drug-drug interactions with other co-medication taken for age-associated comorbidities. Nonetheless clinicians should remain vigilant in monitoring weight, particularly in women and black people. Assessing the mechanism by which some people are specifically prone to develop prominent weight gain on INSTI should be prioritized for further research.

## AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualization and design of the study. SOV and EV performed data curation and contributed to data collection and coordination of the study. SOV and AB designed the methodology of the study and SOV conducted the formal analysis. PR obtained funding and supervised the conduct of the study. SOV provided the original draft of the manuscript including figures and tables. All authors critically revised and approved the final version for publication.

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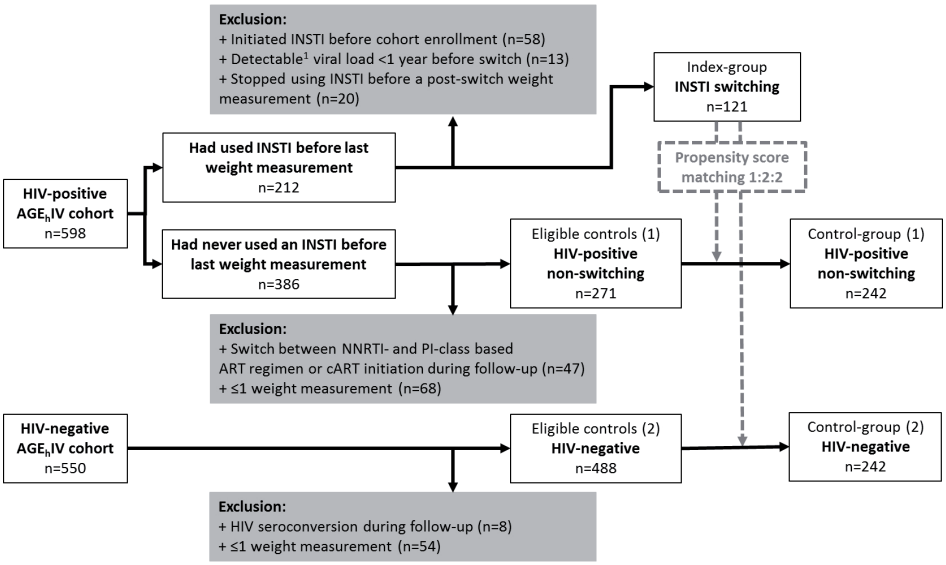
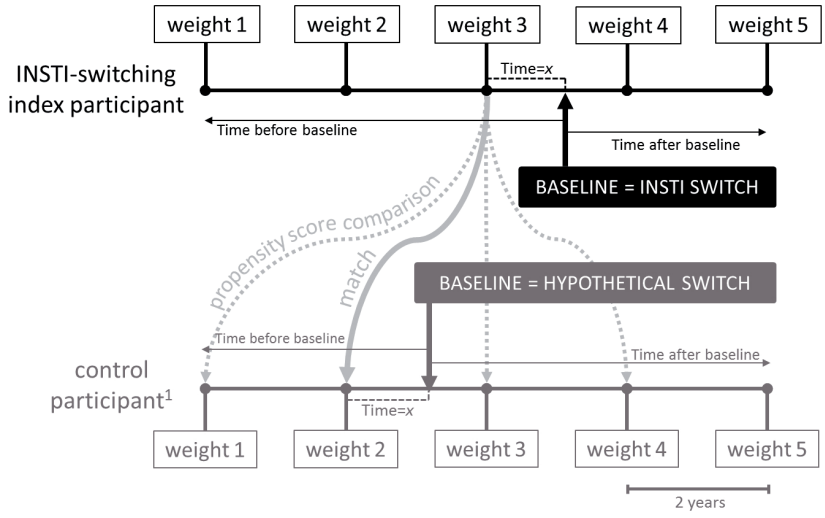
## SUPPLEMENTARY MATERIAL




**SUPPLEMENTARY FIGURE 4.1** | Flow-chart illustrating the selection of index and control participants from the AGE$_h$IV cohort

*[1]HIV viral load >40 copies/mL excluding 'blips' up to 200 copies/mL. Abbreviations: INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; cART, combination antiretroviral therapy; PI, protease inhibitor.*




**SUPPLEMENTARY FIGURE 4.2** | Time-dependent propensity score matching of control participants to index-participants and determining their hypothetical moment of switch

*[1]HIV-positive non-switching or HIV-negative control. Propensity scores were calculated using a Cox proportional hazard model, including time-updated age and body mass index, and time-fixed gender and ethnicity. Abbreviations: INSTI, integrase strand transfer inhibitor*

**SUPPLEMENTARY TABLE 4.1** | Nucleos(t)ide reverse transcriptase inhibitor use before and after switch to INSTI

| | | NRTI use *after switch* to INSTI | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **ABC/3TC** | **TDF** | **TDF/FTC** | **3TC** | **3TC/AZT** | **TAF/FTC** | **no NRTI** |
| **NRTI use *before switch* to INSTI** | ABC/3TC | 20 (17%) | … | …. | 1 (1%) | … | 3 (2%) | 1 (1%) |
| | ABC/3TC/AZT | 3 (2%) | … | 1 (1%) | … | … | … | 1 (1%) |
| | TDF | … | 1 (1%) | … | … | … | … | … |
| | TDF/ABC/3TC | … | 1 (1%) | … | … | … | … | … |
| | TDF/FTC | 14 (12%) | … | 38 (32%) | 3 (2%) | 1 (1%) | 21 (17%) | 3 (2%) |
| | TDF/3TC | 2 (2%) | … | 1 (1%) | … | … | … | … |
| | 3TC/AZT | 1 (1%) | … | … | 1 (1%) | 1 (1%) | 1 (1%) | 1 (1%) |
| | no NRTI | … | … | … | … | … | … | 1 (1%) |

*Numbers are n (%). Abbreviations: ABC, abacavir; 3TC, lamivudine; TDF, tenofovir disoproxil; FTC, emtricitabine; AZT, zidovudine; TAF, tenofovir alafenamide; NRTI, nucleoside reverse transcriptase inhibitor; INSTI, integrase strand transfer inhibitor.*

**SUPPLEMENTARY TABLE 4.2** | Primary reasons for switching to INSTI

| | **N (%)** |
|---|---|
| Simplification of regimen | 41 (34%) |
| Neuro-/psychological side-effects from previous regime | 26 (21%) |
| Somatic side-effects from previous regime | 31 (26%)[1] |
| Interaction of co-medication with previous regime | 9 (7%) |
| Unknown | 14 (12%) |

[1]*of which 6 (5% of total) were gastro-intestinal complaints*

5

# Patterns of Co-occurring Comorbidities in People Living With HIV

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## ABSTRACT

**Aims** To identify common patterns of comorbidities observed in people living with HIV (PLWH), using a data-driven approach, and evaluate associations between patterns identified.

**Methods** A wide range of comorbidities were assessed in PLWH participating in two independent cohorts (POPPY: UK/Ireland, $AGE_hIV$: Netherlands). Presence/absence of each comorbidity was determined using a mix of self-reported medical history, concomitant medications, healthcare resource use and laboratory parameters. Principal component analysis (PCA) based on Somers' D statistic was applied to identify patterns of comorbidities.

**Results** PCA identified six patterns among the 1073 POPPY PLWH [85.2% male, median (IQR) age 52 (47-59) years]: cardiovascular diseases (CVDs), sexually transmitted diseases (STDs), mental health problems, cancers, metabolic disorders, chest/other infections. The CVDs pattern was positively associated with the cancers (r=0.32), metabolic disorders (r=0.38), mental health (r=0.16) and chest/other infections (r=0.17) patterns (all p's<0.001). The mental health pattern was correlated with all the other patterns (in particular cancers: r=0.20 and chest/other infections: r=0.27, both p's<0.001). In the 598 $AGE_hIV$ PLWH [87.6% male, median (IQR) age 53 (48-59) years], six patterns were identified: CVDs, chest/liver, HIV/AIDS events, mental health/neurological problems, STDs, and general health. The general health pattern was correlated with all the other patterns (in particular CVDs: r=0.14; chest/liver: r=0.15; HIV/AIDS events: r=0.31, all p's<0.001), except STDs (r=-0.02, p=0.64).

**Conclusion** Comorbidities in PLWH tend to occur in non-random patterns reflecting known pathological mechanisms and shared risk factors, but also suggesting potential previously-unknown mechanisms. Their identification may assist in adequately addressing the pathophysiology of increasingly prevalent multimorbidity in PLWH.

## INTRODUCTION

The widespread access to combination antiretroviral therapy (cART) has led to a marked improvement in survival and life expectancy of people living with HIV (PLWH)[1,2] and to an increase of proportions of PLWH over the age of 50 years in most settings.[3] As a result of this demographic shift, a high prevalence of multimorbidity, defined as the occurrence of multiple comorbidities within the same individual, has been reported among PLWH with implications for health outcomes and functional status.[4-6] However, knowledge about how individual comorbidities distribute or co-occur in the same individual among PLWH remains limited.

Managing individuals with multiple acute or chronic comorbidities is typically more challenging than managing individuals with a single condition.[7,8] Current HIV guidelines,[9,10] recommend close monitoring of cardiovascular, metabolic, liver, kidney, and bone health and regular assessment of drug-drug interactions. Investigating common patterns, associations, interactions and possible synergies between comorbidities, could support further development of targeted interventions and guidelines for prevention and management of PLWH experiencing multiple comorbidities.

Assessment of patterns of co-morbidities is complicated by 'coincidental comorbidity' – the co-occurrence of two or more comorbidities by chance.[11] The identification of comorbidities that are more likely to occur together than would be expected by chance can reveal disease-disease interactions, or indicate shared etiologies between comorbidities. It is therefore important to separate coincident (random) comorbidity from non-random comorbidity. Modern statistical methods allow the exploration of the underlying structure in the distribution of comorbidities giving an overall picture of the broad pattern of how comorbidities cluster in a particular population.

The recent report from the HIV and Aging Working Group called for the collection of better observational data to identify common clusters (patterns) of comorbidities among PLWH and their impact on treatment and disease outcomes.[12] Whilst some studies have attempted to do so in the general population using statistical approaches without any *a priori* hypothesis,[13] to the best of our knowledge, only two studies have focused on PLWH.[14,15] These studies considered up to 15 comorbidities, but were unable to include the broad spectrum of comorbidities and medical conditions that are commonly reported by PLWH and, therefore, the patterns among a more comprehensive set of comorbidities observed in PLWH remains unclear.

The aims of this study were (i) to explore (non-coincidental) associations between co-morbidities in two independent cohorts of PLWH, (ii) to investigate common patterns of comorbidities using a data-driven statistical approach and (iii) to evaluate associations between the patterns identified.

## METHODS

This study is based on data from two European cohorts of PLWH: the POPPY study in the UK/Ireland and the AGE$_h$IV study in the Netherlands. Both studies aim to investigate the effects of ageing and comorbidities in PLWH and assessed a wide range of comorbidities as detailed below.

### The POPPY study

#### *Study participants*

The POPPY study recruited two cohorts of PLWH: an 'older' group of PLWH aged ≥50 years and a younger group of PLWH aged between 18 and 50 years, as described previously.[16] Inclusion criteria were: documented presence of HIV infection, white or black-African ethnicity, likely route of HIV acquisition via sexual exposure and ability to comprehend the study information leaflet. The younger group of PLWH was frequency matched on gender, ethnicity, sexual orientation and location (in or out of London) to the older PLWH. In addition, the study recruited a group of HIV-negative individuals aged ≥50 years which was not included in the present analysis. Participants were recruited from HIV outpatient clinics between April 2013 and January 2016. The study was approved by the UK National Research Ethics Service (NRES; Fulham London, UK number 12/LO/1409). All participants provided written informed consent.

#### *Data collection*

At study entry, a full clinical history of participants was captured detailing comorbidities and clinical conditions but also medications and healthcare resources used over the previous year. This information was self-reported by the study participants through a structured interview with trained staff who, where possible, also reviewed hospital notes to validate the presence of comorbidities. Participants were asked whether they ever experienced any of the comorbidities or medical conditions from a detailed list (see Supplementary Table 5.1). For each organ system/pathophysiological group, participants were also asked to report a history of any other relevant comorbidity not included in the study protocol. Answers to these free-text questions were examined to update the existing list of comorbidities or to include additional ones. Reasons for any healthcare

utilization over the previous year (including general practitioner visit, hospital visit, use of ambulance or hospital transport, psychiatrist/psychologist visit, nurse visit, specialist visit, hospital procedure and healthcare provider) and information regarding any other (non-antiretroviral) medication received in the previous year were also scrutinised and the presence of each comorbidity was then updated accordingly. Congenital diseases and conditions with a prevalence <1.5% in the study population were subsequently excluded, yielding a total of 65 individual comorbidities from 19 organ system/pathogenic groups (Table 5.1).

## The $AGE_hIV$ study

### *Study participants*

The AGEhIV study recruited PLWH aged ≥45 years from the HIV outpatient clinic of the Academic Medical Centre in Amsterdam, the Netherlands,[17] between October 2010 and September 2012. Inclusion criteria were: age ≥45 years and laboratory-confirmed presence of HIV infection. Whilst a control group of HIV-negative individuals was also enrolled into the study, this was not considered in the present analysis. The study protocol was approved by the local ethics review committee (ClinicalTrial.gov identifier NCT01466582). All participants provided written informed consent.

### *Data collection*

Participants underwent standardized screening for several comorbidities, including a questionnaire concerning personal medical history, use of medications (both prescribed and over-the-counter), and participation in population screening programs. Information concerning blood pressure, anthropometrics, vascular elasticity, respiratory function (via spirometry test), cognitive function, frailty, bone densitometry (using DXA scan) were also obtained, as well as blood and urine samples for extensive laboratory testing and historical HIV characteristics (from the Dutch HIV Monitoring Foundation registry).

Information collected were used to derive the presence/absence of 42 of the 65 comorbidities in Table 5.1, as well as a further 4 (oesophageal candidiasis, hyperparathyroidism, liver problems and thrombocytopenia). Whenever possible, patient-reported comorbidities were validated using hospital records. The list of comorbidities obtained and the source of information for each comorbidity are reported in Supplementary Table 5.2.

## Statistical analysis

Comparison of participants' characteristics between the older POPPY PLWH and $AGE_hIV$ PLWH were performed using Chi-square or Wilcoxon rank-sum test, as appropriate. Pair-

**TABLE 5.1** | List of comorbidities considered by organ system/photogenic group in the POPPY study with prevalence in the all POPPY PLWH sample (n=1073)

| Organ system/ pathogenic group | Comorbidities | Prevalence n (%) | Collected in $AGE_hIV$ |
|---|---|---|---|
| AIDS events | Tuberculosis (TB) | 83 (7.7%) | ✓ |
| | Cytomegalovirus (CMV) | 28 (2.6%) | |
| | Pneumocystis pneumonia | 94 (8.8%) | ✓ |
| | Kaposi's sarcoma | 70 (6.5%) | ✓ |
| | Other AIDS events | 124 (11.6%) | ✓ |
| Infections | Varicella zoster virus (VZV) | 155 (14.4%) | ✓ |
| | Fungal infection | 54 (5.0%) | |
| Endocrine diseases | Type 2 diabetes | 62 (5.8%) | ✓ |
| | Lipodystrophy/Lipoatrophy | 24 (2.2%) | ✓ |
| | Dyslipidaemia | 293 (27.3%) | ✓ |
| | Hypothyroidism | 22 (2.1%) | |
| Mental health problems | Depression | 367 (34.2%) | ✓ |
| | Anxiety | 67 (6.2%) | |
| | Panic attacks | 20 (1.9%) | |
| | Sleeping problems | 71 (6.6%) | ✓ |
| Nervous system problems | Dizziness/Vertigo | 116 (10.8%) | ✓ |
| Cardiovascular diseases (CVDs) | Myocardial infarction (MI) | 41 (3.8%) | ✓ |
| | Angina pectoris | 34 (3.2%) | ✓ |
| | Peripheral vascular disease (PVD) | 19 (1.7%) | ✓ |
| | Hypertension | 229 (21.3%) | ✓ |
| | Transient ischemic attack (TIA) | 31 (2.9%) | ✓ |
| | Coronary artery bypass grafting (CABG) | 24 (2.2%) | |
| | Heart failure | 25 (2.3%) | ✓ |
| Bones and joint disorders | Joint inflammation/Arthritis | 224 (20.9%) | ✓ |
| | Joint replacement | 24 (2.2%) | |
| | Osteopenia/Osteoporosis | 69 (6.4%) | ✓ |
| | Joint/Back pain | 118 (11.0%) | |
| | Osteoporotic fracture | 136 (12.7%) | ✓ |
| Skin disorders | Eczema/Dermatitis | 109 (10.2%) | |
| | Psoriasis | 47 (4.4%) | |
| Sexually transmitted diseases (STDs) | Chlamydia | 245 (22.8%) | ✓ |
| | Gonorrhoea | 457 (42.6%) | ✓ |

**TABLE 5.1** ***(continued)*** | List of comorbidities considered by organ system/photogenic group in the POPPY study with prevalence in the all POPPY PLWH sample (n=1073)

| Organ system/ pathogenic group | Comorbidities | Prevalence n (%) | Collected in $AGE_hIV$ |
|---|---|---|---|
| | Loss of consciousness | 31 (2.9%) | ✓ |
| | Epilepsy | 45 (4.2%) | ✓ |
| | Encephalitis | 16 (1.5%) | ✓ |
| | Peripheral neuropathy | 43 (4.0%) | ✓ |
| | Migraine/Headache | 33 (3.1%) | |
| Respiratory diseases | Asthma/Bronchitis/Chronic obstructive pulmonary disease (COPD) | 264 (24.6%) | ✓ |
| | Pneumonia | 44 (4.1%) | ✓ |
| | Chest infection | 114 (10.6%) | |
| | Hay fever/Allergy | 77 (7.2%) | ✓ |
| Hepatitis | Hepatitis A | 44 (4.1%) | |
| | Hepatitis B | 147 (13.7%) | ✓ |
| | Hepatitis C | 75 (7.0%) | ✓ |
| Renal problem | Renal problem | 28 (2.6%) | ✓ |
| Cancer | Skin cancer | 67 (6.2%) | ✓ |
| | Haematological cancer | 22 (2.1%) | ✓ |
| | Solid organs cancer | 75 (7.0%) | ✓ |
| Anaemia | Anaemia | 28 (2.6%) | ✓ |
| | Human papilloma virus (HPV) | 99 (9.2%) | |
| | Herpes simplex virus (HSV) | 122 (11.4%) | |
| | Lymphogranuloma venereum (LGV) | 46 (4.3%) | |
| | Syphilis | 326 (30.4%) | ✓ |
| Gastro-intestinal disorders | Hernia | 27 (2.5%) | |
| | Gastro-oesophageal reflux disease (GORD) | 70 (6.5%) | |
| | Irritable bowel syndrome (IBS) | 30 (2.8%) | |
| Genitourinary disorders | Urinary incontinence | 62 (5.8%) | ✓ |
| | Urinary tract infections (UTI) | 31 (2.9%) | |
| | Erectile dysfunction | 75 (7.0%) | ✓ |
| | Non-specific Urethritis | 58 (5.4%) | |
| | Prostate dysfunction | 31 (2.9%) | |
| | Kidney stones | 28 (2.6%) | |
| Ear dysfunction | Ear dysfunction | 40 (3.7%) | |
| Eye problem | Eye problem | 81 (7.5%) | ✓ |
| Vitamin D deficiency | Vitamin D deficiency | 19 (1.8%) | ✓ |

wise associations between comorbidities were assessed using the Somers' *D* statistic for binary variables as proposed by Ng et al.[18] and shown to perform better than other measures of agreement in detecting non-random comorbidity.[19] Briefly, the Somers' *D* measures the degree of association between two comorbidities other than that given by chance alone (the product of the prevalence of the individual comorbidities). Somers' *D* ranges from -1 when there is perfect disagreement between the two comorbidities (i.e. all individuals have either one or the other comorbidity) to 1 when there is perfect agreement (i.e. all individuals either have both or don't have either comorbidities), with 0 indicating agreement equals to that given by chance alone. Significance of the Somers' *D* statistics were evaluated using permutation tests with $p<0.001$ indicative of significant non-random association (reflecting the high number of pairwise associations tested, i.e. 2080).

Principal component analysis (PCA) was applied to the matrix containing the pairwise associations (as measured by the Somers' *D*) between the comorbidities. PCA is a data-reduction method which transforms the original set of variables into a smaller set of principal components (PCs), which are linear combinations of the original variables. These PCs are determined so that they retain as much of the variability in the dataset as possible, with the first component retaining the most of the variation present and the other components progressively retaining a decreasing amount of variation.[20] PCs can be interpreted as patterns of comorbidities, i.e. groups of comorbidities frequently associated with each other. A comorbidity was regarded to be associated with a pattern if its correlation with the pattern was >0.40. We adopted an *oblimin* rotation to allow PCs (patterns) to be associated within each other, therefore allowing the possibility of multiple patterns being present in the same individual. The number of PCs to be extracted was determined using the scree plot and the very simple structure criterion.[21]

For each participant and each pattern, a severity score for that pattern was obtained using data on the presence/absence of comorbidities and coefficients returned by the PCA. Correlations between patterns' severity scores were evaluated using the Spearman's rank correlation coefficient (*r*). All the analyses were performed separately in all POPPY PLWH, in older POPPY PLWH only and in $AGE_hIV$ PLWH using the statistical software R v3.3.3.

# RESULTS

## Characteristics of the POPPY and AGE$_h$IV study participants

The POPPY study recruited 699 older and 374 younger PLWH; 598 PLWH were recruited into the AGE$_h$IV cohort study. Socio-demographic and HIV-related characteristics are summarised in Table 5.2. POPPY participants were predominantly male (85.2%), of white ethnicity (84.1%) and men who have sex with men (MSM: 76.0%). The median (interquartile range: IQR) CD4$^+$ T-cell count was 624 (475, 811) cells/L and 89.9% had a suppressed viral load (<50 copies/mL).

Similarly, the majority of PLWH recruited in the AGE$_h$IV study were male (87.6%), of Dutch origin (85.8%) and MSM (70.4%). When compared to the older POPPY PLWH, they were younger (p<0.001) and had a lower body mass index (BMI, p<0.001), however there

**TABLE 5.2** | Characteristics of POPPY and AGE$_h$IV PLWH

| n (%) or median (IQR) | POPPY | | | AGE$_h$IV |
|---|---|---|---|---|
| | *All PLWH (n=1073)* | *PLWH ≥50 (n=699)* | *PLWH <50 (n=374)* | *PLWH ≥45 (n= 598)* |
| Gender | | | | |
| Male | 914 (85.2%) | 612 (87.5%) | 302 (80.8%) | 524 (87.6%) |
| Female | 159 (14.8%) | 87 (12.5%) | 72 (19.2%) | 74 (12.4%) |
| Age [years] | 52 (47, 59) | 57 (53, 62) | 43 (37, 47) | 53 (48, 59) |
| Ethnicity[a] | | | | |
| Black-African | 171 (15.9%) | 96 (13.7%) | 75 (19.8%) | 74 (12.4%) |
| White | 902 (84.1%) | 603 (86.3%) | 299 (80.2%) | 513 (85.8%) |
| Other/Unknown | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 11 (1.8%) |
| Sexual orientation | | | | |
| MSM/homosexual | 816 (76.0%) | 548 (78.4%) | 268 (71.7%) | 369 (70.4%) |
| Heterosexual | 257 (24.0%) | 151 (21.6%) | 106 (28.3%) | 177 (29.6%) |
| BMI [kg/m$^2$] | 25.5 (23.2, 28.2) | 25.7 (23.4, 28.5) | 25.2 (23.0, 27.8) | 24.3 (22.4, 26.7) |
| Duration of HIV [years] | 13.2 (7.8, 20.5) | 15.8 (9.8, 22.4) | 9.7 (5.5, 15.2) | 12.0 (6.6, 17.0) |
| CD4$^+$ T cell count [cells/mm$^3$] | 624 (476, 811) | 610 (468, 792) | 661 (500, 847) | 565 (433, 740) |
| Nadir CD4$^+$ count [cells/mm$^3$] | 202 (101, 304) | 180 (85, 273) | 253 (152, 376) | 170 (70, 260) |
| On ART | 1046 (97.5%) | 690 (98.7%) | 356 (95.2%) | 573 (95.8%) |
| HIV RNA <50 copies/ml | 965 (89.9%) | 644 (91.8%) | 323 (86.4%) | 545 (91.6%) |

*[a]For the AGEhIV participants white refers to Dutch origin, black-African to African origin. IQR: interquartile range; MSM: men having sex with men; BMI: body-mass index; ART: antiretroviral therapy*

were no significant differences in terms of gender (p=0.91), ethnicity (p=0.61) or sexual orientation (p=0.09).

## Individual comorbidities

In POPPY PLWH, prevalence of comorbidities ranged from 1.6% (encephalitis) to 42.6% (gonorrhoea, Table 5.1) with also depression (34.2%), syphilis (30.4%) and dyslipidaemia (27.3%) among the most prevalent comorbidities. In total, 97.2% of all POPPY PLWH and 98.6% of older POPPY PLWH reported ≥1 comorbidity with a median (IQR) of 5 (3, 7) and 6 (3, 8) comorbidities per individual, respectively. Of the comorbidities considered in $AGE_hIV$ PLWH, hypertension (43.1%), osteopenia/osteoporosis (42.6%), lipodystrophy/lipoatrophy (32.1%) and candidiasis (31.9%) were the most common (Supplementary Table 5.2). Overall, 98.7% had ≥1 comorbidity with a median (IQR) of 5 (3, 7) comorbidities per individual.

## Non-random associations between comorbidities

Significant non-random associations, based on the Somers' D statistic, among POPPY PLWH are depicted in Figure 5.1. Non-random associations within several cardiovascular diseases (CVDs) were significant (top-right part of Figure 5.1); these included hypertension, angina, heart failure, type 2 diabetes, lipodystrophy/lipoatrophy, dyslipidaemia, transient ischemic attack (TIA), coronary artery bypass graft (CABG), myocardial infarction (MI) and renal problems. Other strong non-random associations were identified within mental health problems (depression, anxiety and panic attacks) with depression also associated with sleeping problems and irritable bowel syndrome (IBS), cancers (haematological cancer with both solid organ and skin cancer) and sexually transmitted diseases (STDs, in particular gonorrhoea, chlamydia, lymphogranuloma venereum (LGV), syphilis and hepatitis C virus).

Most of these non-random associations were maintained when the analysis was restricted to older POPPY PLWH (Figure 5.2A). In addition, a strong non-random positive association was found between panic attacks and asthma/bronchitis/chronic obstructive pulmonary disease (COPD) and a significantly lower than expected co-occurrence of hypertension and LGV. A few of these significant non-random associations were also found in the $AGE_hIV$ PLWH (Figure 5.2B); namely the associations between MI, angina and hypertension, those of dyslipidaemia with TIA, MI and type 2 diabetes, and those between gonorrhoea and chlamydia and between angina and heart failure.

## Patterns of comorbidities

The PCA in POPPY PLWH yielded six components explaining 24.4% of the total variation in the original 65 comorbidities. Correlations between comorbidities and each pattern

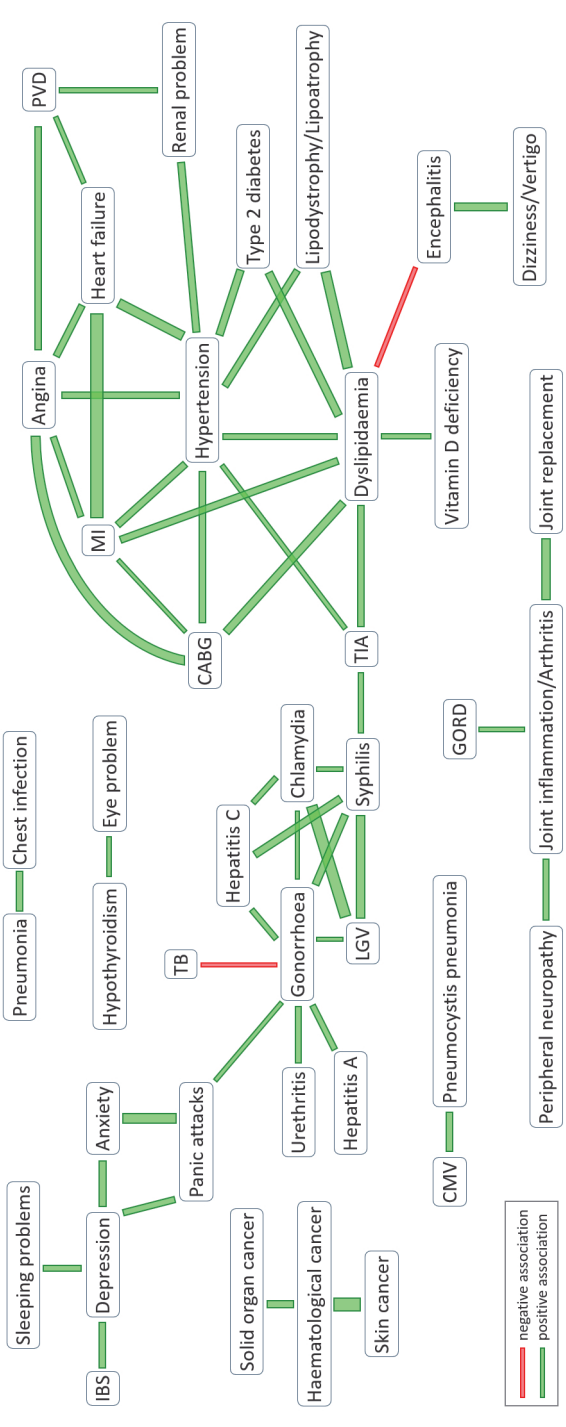


**FIGURE 5.1** | Significant non-random associations between comorbidities (as indicated by a significant Somers' D at the 0.1% significance level) in all POPPY PLWH (n=1073). The thickness of the line is directly proportional to the absolute value of the Somers' D.

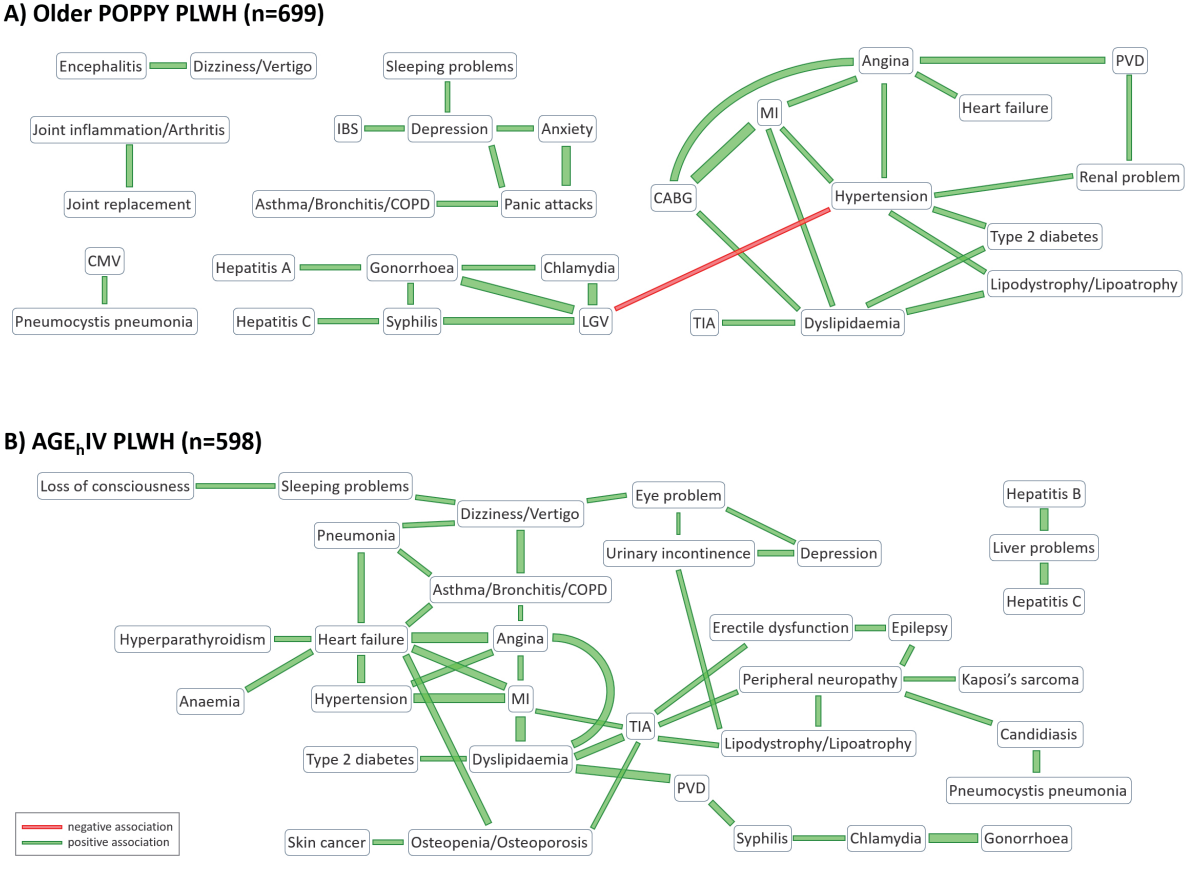


**FIGURE 5.2** | Significant non-random associations between comorbidities (significant Somers' D at the 0.1% significance level) in all older POPPY PLWH (A) and AGEhIV PLWH (B). The thickness of the line is directly proportional to the absolute value of the Somers' D.

are reported in Supplementary Table 5.3 with Table 5.3 reporting only comorbidities with a correlation >0.4 with each pattern. The first PC accounted for 6.2% of the variance and encompassed CVDs such as angina, CABG, MI, heart failure, hypertension, peripheral vascular disease (PVD) and renal problems. The second PC, with strong correlations with STDs (gonorrhoea, chlamydia, LGV, syphilis and hepatitis C), accounted for 5.0% of the variability. The third PC strongly correlated with mental health problems (depression, anxiety and panic attacks). The fourth and fifth PCs included cancers (haematological, skin and solid organ cancer) and metabolic disorders (dyslipidaemia, lipodystrophy/lipoatrophy and hypertension), respectively. Finally, the sixth PC had the strongest

**TABLE 5.3** | PC extracted by the PCA in all the POPPY PLWH (A, n=1073), older POPPY PLWH only (B, n=699) and AGE$_h$IV PLWH (C, n=598). For each PC, comorbidities with a high correlation (>0.4) are reported.

**A) All POPPY PLWH (n=1073)**

| PC (% of variance explained) | Meaning | Comorbidities with correlation >0.4 (correlation with the PC) |
|---|---|---|
| 1 (6.2%) | CVDs | Angina (0.66), CABG (0.66), MI (0.64), Heart failure (0.59), Hypertension (0.54), PVD (0.53), Renal problem (0.42) |
| 2 (4.9%) | STDs | Gonorrhoea (0.77), Syphilis (0.67), LGV (0.66), Chlamydia (0.64), Hepatitis C (0.40) |
| 3 (3.9%) | Mental health | Depression (0.79), Anxiety (0.58), Panic attacks (0.50) |
| 4 (3.5%) | Cancers | Haematological cancer (0.75), Skin cancer (0.64), Solid organ cancer (0.49) |
| 5 (3.1%) | Metabolic | Dyslipidaemia (0.71), Lipodystrophy/Lipoatrophy (0.57), Hypertension (0.44) |
| 6 (2.8%) | Chest and other infections | CMV (0.49), Pneumonia (0.49), Dizziness/Vertigo (0.44), Asthma/Bronchitis/COPD (0.42), Chest infection (0.41) |

**B) Older POPPY PLWH (n=699)**

| PC (% of variance explained) | Meaning | Comorbidities with correlation >0.4 (correlation with the PC) |
|---|---|---|
| 1 (5.7%) | CVDs | Angina (0.67), Heart failure (0.65), PVD (0.62), CABG (0.58), MI (0.57), Hypertension (0.54), Renal problems (0.44) |
| 2 (5.0%) | STDs | Gonorrhoea (0.74), LGV (0.72), Syphilis (0.69), Chlamydia (0.63), Hepatitis C (0.46) |
| 3 (4.1%) | Mental health and asthma | Depression (0.79), Anxiety (0.58), Panic attacks (0.50), Asthma/Bronchitis/COPD (0.45), Dizziness/Vertigo (0.40) |
| 4 (3.7%) | Cancers | Haematological cancer (0.78), Skin cancer (0.64), Solid organ cancer (0.54) |
| 5 (3.3%) | Metabolic | Dyslipidaemia (0.71), Lipodystrophy/Lipoatrophy (0.43) |
| 6 (3.0%) | Other | Hypothyroidism (0.46), Other AIDS events (0.46) |

**TABLE 5.3** ***(continued)*** | PC extracted by the PCA in all the POPPY PLWH (A, n=1073), older POPPY PLWH only (B, n=699) and AGE$_h$IV PLWH (C, n=598). For each PC, comorbidities with a high correlation (>0.4) are reported.

**C) AGE$_h$IV PLWH (n=598)**

| PC (% of variance explained) | Meaning | Comorbidities with correlation >0.4 (correlation with the PC) |
|---|---|---|
| 1 (9.7%) | CVDs | Dyslipidaemia (0.73), Hypertension (0.64), PVD (0.60), Angina pectoris (0.54), MI (0.51) |
| 2 (6.2%) | Chest/liver | Asthma/Bronchitis/COPD (0.59), Liver problems (0.55), Hepatitis B (0.47), Dizziness/Vertigo (0.43) |
| 3 (4.9%) | HIV/AIDS events | Peripheral neuropathy (0.63), Candidiasis (0.62) , Lipodystrophy (0.53), Other AIDS event (0.48) |
| 4 (4.9%) | Mental health & neurological problems | Eye problem (0.64), Depression (0.63), Urinary incontinence (0.50), Loss of consciousness (0.48), Sleeping problems (0.42), Dizziness/Vertigo (0.40) |
| 5 (4.4%) | STDs | Chlamydia (0.70), Gonorrhoea (0.67), Syphilis (0.62) |
| 6 (4.3%) | General health | Heart failure (0.88), Hyperparathyroidism (0.54), Vitamin D deficiency (0.48), Anaemia (0.45) |

correlation with chest problems (pneumonia, asthma/bronchitis/COPD and chest infection), CMV and dizziness/vertigo.

In the older POPPY PLWH the PCA returned 6 components explaining 24.8% of the total variability (Supplementary Table 5.4 and Table 5.3). Several components were similar to those found among all POPPY PLWH: CVDs (first PC), STDs (second PC), cancers (fourth PC) and metabolic disorders (fifth PC). Similar to the 'mental health' pattern in all POPPY PLWH, the third PC included mental health problems but with the addition of asthma/bronchitis/COPD and dizziness/vertigo. Finally, the sixth PC correlated with hypothyroidism (0.46) and other AIDS events.

Some of the six patterns returned by the PCA in the AGE$_h$IV PLWH, explaining 34.2% of the total variance, resembled those identified in the older POPPY PLWH. The first PC correlated with CVDs (dyslipidaemia, hypertension, PVD, angina pectoris and MI). The second PC included chest (asthma/bronchitis/COPD) and liver problems (with also hepatitis B). The third and fourth PCs were related to HIV/AIDS events and mental health/neurological problems, respectively. The fifth included STDs (gonorrhoea, chlamydia and syphilis) and the sixth encompassed disorders associated with general health such as anaemia, vitamin D deficiency, hyperparathyroidism as well as heart failure.

## Correlations between patterns

Correlations between patterns' severity scores are reported in Table 5.4. In all POPPY PLWH, positive correlations of severity scores in the CVDs pattern were strongest with scores in the cancers ($r$=0.32) and metabolic patterns $r$=0.38) and moderate with those in the mental health ($r$=0.16) and chest and other infections ($r$=0.17) patterns (all p's<0.001). A significant negative correlation was also found between the CVDs and the STDs patterns ($r$=-0.10, p=0.001), suggesting that PLWH with a higher burden of CVDs tend to

**TABLE 5.4** | Correlation between patterns' severity scores in all the POPPY PLWH (A, n=1073), older POPPY PLWH only (B, n=699) and AGE$_h$IV PLWH (C, n=598)

**A) All POPPY PLWH (n=1073)**

| | STDs | Mental health | Cancers | Metabolic | Chest & other infections |
|---|---|---|---|---|---|
| CVDs | -0.10 (p=0.001) | 0.16 (p<0.001) | 0.32 (p<0.001) | 0.38 (p<0.001) | 0.17 (p<0.001) |
| STDs | | 0.11 (p<0.001) | 0.07 (p=0.01) | 0.05 (p=0.13) | 0.03 (p=0.27) |
| Mental health | | | 0.20 (p<0.001) | 0.16 (p<0.001) | 0.27 (p<0.001) |
| Cancers | | | | 0.21 (p<0.001) | 0.27 (p<0.001) |
| Metabolic | | | | | 0.25 (p<0.001) |

**B) Older POPPY PLWH (n=699)**

| | STDs | Mental health & asthma | Cancers | Metabolic | Other |
|---|---|---|---|---|---|
| CVDs | 0.05 (p=0.18) | 0.22 (p<0.001) | 0.26 (p<0.001) | 0.34 (p<0.001) | -0.04 (p=0.24) |
| STDs | | 0.11 (p=0.004) | 0.07 (p=0.06) | 0.09 (p=0.02) | 0.08 (p=0.03) |
| Mental health and asthma | | | 0.18 (p<0.001) | 0.12 (p=0.002) | 0.25 (p<0.001) |
| Cancers | | | | 0.11 (p=0.005) | 0.14 (p<0.001) |
| Metabolic | | | | | -0.10 (p=0.008) |

**C) AGEhIV PLWH (n=598)**

| | Chest/Liver | HIV/AIDS events | Mental health/ neurological problems | STDs | General health |
|---|---|---|---|---|---|
| CVDs | 0.02 (p=0.70) | 0.18 (p<0.001) | 0.05 (p=0.21) | -0.07 (p=0.11) | 0.14 (p<0.001) |
| Chest/Liver | | 0.21 (p<0.001) | 0.05 (p=0.23) | -0.06 (p=0.14) | 0.15 (p<0.001) |
| HIV/AIDS events | | | 0.10 (p=0.02) | 0.04 (p=0.30) | 0.31 (p<0.001) |
| Mental health/ neurological problems | | | | -0.01 (p=0.90) | 0.08 (p=0.05) |
| STDs | | | | | -0.02 (p=0.64) |

have a lower number of STDs and vice versa. Generally, the severity of the mental health pattern was positively correlated with the severity of all other patterns, with strongest evidence for cancers ($r$=0.20, p<0.001) and chest and other infections ($r$=0.27, p<0.001). Chest/other infections was also positively associated with cancers and metabolic patterns ($r$=0.27 and $r$=0.25, respectively, both p's<0.001).

In the older POPPY PLWH, higher CVDs severity scores were correlated with higher mental health/asthma ($r$=0.22), cancers ($r$=0.26) and metabolic ($r$=0.34) scores (all p's<0.001). Mental health /asthma scores also correlated with all the remaining patterns with correlations ranging between 0.11 (with STDs) and 0.25 (with other). Among $AGE_hIV$ PLWH association of CVDs was strongest with HIV/AIDS events ($r$=0.18, p<0.001) and general health problems ($r$=0.14, p<0.001) but weak with other patterns. A higher severity of the general health pattern was correlated with higher severity in all the other patterns (CVDs; chest/liver: r=0.15, p<0.001; HIV/AIDS events: r=0.31, p<0.001; mental health/neurological problems: $r$=0.08, p=0.05), but not with STDs ($r$=-0.02, p=0.64).

## DISCUSSION

This study explored associations between a wide range of comorbidities and identified common patterns occurring in two independent cohorts of PLWH. Our findings suggest that, in PLWH, comorbidities do not co-occur at random and, in general, are likely to cluster in specific patterns, some of which are consistent across different cohorts. In particular, we found that patterns of CVDs, metabolic disorders, STDs and mental health problems are present in treated PLWH from both the UK/Ireland and the Netherlands. Our study adds to the two previous studies reporting patterns of comorbidities identified through purely statistical approaches[14,15] by considering a wider range of comorbidities and validating the results in two independent cohorts.

Non-random associations between CVDs such as angina, hypertension, MI, CABG and heart failure (which formed one of the patterns identified) reflect previously known pathological mechanisms and were also previously reported in HIV-positive veterans[14] and in the general population.[22-24] Similarly, patterns of metabolic disorders are often reported in conjunction with CVDs in both PLWH[15] and the general population[22,23]. On the other hand, contrary to other studies that used a similar data-driven approach in PLWH[14,15] and in the general population,[22-24] we found frequent co-occurrence of STDs, likely due to exposure to some shared risk factors (i.e. risk-taking sexual behaviours) that are highly prevalent among populations of PLWH.[25] Moreover, in one of the two cohorts analysed, we found links between several opportunistic infections (i.e. candi-

diasis, pneumocystis pneumonia and other AIDS-defining events). These links likely reflect past immunosuppression and were mainly present in long-term survivors as also indicated by the association of the pattern's severity score with age and time since HIV diagnosis (data not shown).

Whilst associations between mental health problems like depression, anxiety and panic attacks can reflect true underlying psychological distress and co-occurrence patterns have been reported in other studies,[23,24] it may also highlight a monitoring bias. Individuals reporting one of the problems tend to be more likely to also report the others. Moreover, in our study, mental health disorders were associated not only with each other, but also with neurological problems, especially in older PLWH. These results were also reported by Kirchberger et al[26] and are consistent with the growing evidence about the bidirectional link between mental health and neurological disorders.[27]

Interestingly, there was some overlap between patterns as suggested by significant correlations between patterns' severity scores. In particular mental health problems appeared to be associated with almost all other patterns, including those of CVDs, STDs and metabolic disorders. Whilst these findings are in line with reports of the strong link between physical and mental health,[28,29] the nature of these associations is likely to be bi-directional. Poor physical health can lead to an increased risk of developing mental health problems, but, at the same time, individuals with several mental health disorders are often more likely to experience physical health conditions.[30]

There are some limitations to our study that need to be considered. First, not all the comorbidities considered required a medical diagnosis and some consisted more of symptoms or treatments rather than actual diseases. Whilst no uniform list comorbidities and medical conditions exists to define multimorbidity, the list of comorbidities considered here aimed to capture the broad spectrum of conditions affecting PLWH. Nevertheless the use of a more standardised list of conditions and criteria for the ascertainment of the presence of conditions (for example ICD codes) could have provided a more uniform set of conditions and more replicable results. Second, the self-reported nature of data collection may have led to under or over reporting of some comorbidities. Whilst the two cohort studies (POPPY and $AGE_hIV$) were conducted following similar protocols, not all comorbidities were assessed by both studies and differences across studies exist in how the presence/absence of some comorbidities was defined, which may have resulted in the differences in the patterns identified. Moreover, both the POPPY and the $AGE_hIV$ cohorts were designed to be representative of the population of PLWH seen in care in the respective countries, where the majority of PLWH are white MSM; therefore, results

could be less generalizable to cohorts of PLWH that include larger proportions of women, people of black-African ethnicity or to cohorts in different HIV epidemic settings.

Our findings could be useful for both research and clinical purposes. With an increasingly aging population of PLWH[3] and the consequent increase in the prevalence of multimorbidity and its associated health care costs,[31] a better understanding of how comorbidities cluster together would enable to develop targeted interventions and appropriate guidelines addressing the needs of PLWH with multiple comorbidities. Further studies may help to elucidate the possible pathophysiological pathways linking conditions with demonstrated co-occurrence prevalences higher than those expected by chance alone, and their impact on health and treatment outcomes.

## AUTHOR CONTRIBUTIONS

DDF, SOV, JU, PR and CAS contributed to the conceptualization and design of the study. DDF and SOV performed data curation. DDF and CAS designed the methodology of the study and DDF conducted the formal analysis. CAS and PR obtained funding and supervised the conduct of the study. DDF provided the original draft of the manuscript including figures and tables. All authors critically revised and approved the final version for publication.

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## SUPPLEMENTARY MATERIAL

**SUPPLEMENTARY TABLE 5.1** | list of comorbidities and medical conditions assessed via structured questionnaire in the POPPY study

| Organ system/pathogenic group | Comorbidity |
|---|---|
| AIDS defining events | Tuberculosis (TB) |
| | Other AIDS events |
| Infections and paracytic | Hepatitis B |
| | Hepatitis C |
| | Other Infections |
| Sexually transmitted diseases (STDs) | Syphilis |
| | Gonorrhoea |
| | Chlamydia |
| | Lymphogranuloma venereum (LGV) |
| | Other STD |
| Endocrine disease | Type 1 Diabetes |
| | Type 2 Diabetes |
| | Thyroid disease |
| | Other endocrine diseases |
| Blood diseases | Blood and blood forming organ events |
| Mental health | Depression |
| | Diagnosed depression (treated by a doctor) |
| | Other mental disorders |
| Nervous system | Parkinson's disease or any other movement disorder |
| | Dizziness or vertigo |
| | Loss of consciousness for 30 minutes or more |
| | Brain surgery |
| | Encephalitis |
| | Epilepsy |
| | Peripheral neuropathy |
| | Other nervous system disorders |
| Cardiovascular disease | Myocardial infarction (MI) |
| | Coronary artery bypass surgery or percutaneous transluminal coronary angioplasty (CABG/PCTA) |
| | Cerebrovascular accident or transient ischemic attack (CVA/TIA) |
| | Other cardiovascular disease |

**SUPPLEMENTARY TABLE 5.1 *(continued)*** | list of comorbidities and medical conditions assessed via structured questionnaire in the POPPY study

| Organ system/pathogenic group | Comorbidity |
|---|---|
| | Heart Failure |
| | Angina Pectoris |
| | Narrowed blood vessels in legs or abdomen |
| Chest disease | Asthma, bronchitis, pulmonary emphysema or chronic obstructive pulmonary disease (COPD) |
| | Other chest disease |
| Gastro-intestinal | Persistent bowel disorder over last 3 months |
| | Other liver disease |
| | Other gastro-intestinal disorders |
| Renal/Urinary/Reproductive | End-stage renal disease (receipt of dialysis or renal transplant) |
| | Urinary incontinence requiring treatment |
| | Other genitourinary disorders |
| Skin | Any skin disorders |
| Joint, bone and connective tissue | Joint inflammation or rheumatoid or osteoarthritis |
| | Arthritis of knee or hip |
| | Joint replacement |
| | Fractures |
| | Congenital bone disease (osteogenesis imperfecta) |
| | Other joint, bone or connective tissue disorders |
| Congenital | Congenital disorders |
| Injury and poisoning | Injury or poisoning |
| Cancer | Any form of cancer (if not covered elsewhere) |
| Chronic disease | Any chronic diseases not recorded elsewhere |

**SUPPLEMENTARY TABLE 5.2** | List of comorbidities with source of information and prevalence in the $AGE_hIV$ study (n=598)

| Organ system/ pathogenic group | Comorbidities | Source of information | Prevalence n (%) |
|---|---|---|---|
| AIDS events | Tuberculosis (TB) | Hospital records | 17 (2.8%) |
| | Pneumocystis pneumonia | Hospital records | 53 (8.9%) |
| | Kaposi's sarcoma | Hospital records | 47 (7.8%) |
| | Candidiasis | Hospital records | 191 (31.9%) |
| | Other AIDS events | Hospital records | 159 (26.6%) |
| Infections | Varicella zoster virus (VZV) | Hospital/GP records | 122 (20.4%) |
| Endocrine diseases | Type 2 diabetes | Medical history \| $HbA_{1c}$ ≥ 48 mmol/l \| blood glucose (≥11.1 non-fasting, ≥7 fasting) \| Medication | 37 (6.2%) |
| | Lipodystrophy/Lipoatrophy | Hospital records | 192 (32.1%) |
| | Dyslipidaemia | Total cholesterol/HDL >7.0 \| Medication | 100 (16.7%) |
| | Hypothyroidism | Thyroid-stimulating hormone >4 \| Medication | 20 (3.3%) |
| | Hyperparathyroidism | Parathyroid hormone >6.7 | 85 (14.2%) |
| Mental health problems | Depression | Medical history | 172 (28.8%) |
| | Sleeping problems | Medical history \| Medication | 98 (16.4%) |
| Nervous system problems | Dizziness/Vertigo | Medical history | 13 (2.2%) |
| | Loss of consciousness | Medical history | 27 (4.5%) |
| | Epilepsy | Medical history \| Medication | 16 (2.7%) |
| | Encephalitis | Medical history | 24 (4.0%) |
| | Peripheral neuropathy | Hospital records | 98 (16.4%) |
| Respiratory diseases | Asthma/Bronchitis/Chronic obstructive pulmonary disease (COPD) | Medical history | 186 (31.1%) |
| | Pneumonia | Hospital records | 56 (9.4%) |
| | Hay fever/Allergy | Medication | 21 (3.5%) |
| Hepatitis | Hepatitis B | HBV Surface Antigen >0 | 39 (6.5%) |
| | Hepatitis C | HCV RNA >0 | 22 (3.7%) |
| Renal problem | Renal problem | eGFR <60 ml/min (MDRD estimation) | 28 (4.7%) |
| Cancer | Skin cancer | Medical history (validated in hospital/GP records) | 21 (3.5%) |
| | Haematological cancer | Medical history (validated in hospital/GP records) | 7 (1.2%) |

**SUPPLEMENTARY TABLE 5.2 *(continued)*** | List of comorbidities with source of information and prevalence in the $AGE_hIV$ study (n=598)

| Organ system/ pathogenic group | Comorbidities | Source of information | Prevalence n (%) |
|---|---|---|---|
| | Solid organs cancer | Medical history (validated in hospital/GP records) | 12 (2.0%) |
| Blood conditions | Anaemia | Haemoglobin <8.5 (men) <7.5 (women) \| medication | 71 (11.9%) |
| | Thrombocytopenia | Platelets <150 x $10^{-9}$ | 44 (7.4%) |
| Cardiovascular | Myocardial infarction (MI) | Medical history (validated in hospital/GP records) | 21 (3.5%) |
| Diseases (CVDs) | Angina pectoris | Medical history (validated in hospital/GP records) | 21 (3.5%) |
| | Peripheral vascular disease (PVD) | Medical history (validated in hospital/GP records) | 13 (2.2%) |
| | Hypertension | Systolic BP ≥140 \| Diastolic BP ≥ 90 \| Medication | 258 (43.1%) |
| | Transient ischemic attack (TIA) | Medical history (validated in hospital/GP records) | 13 (2.2%) |
| | Heart failure | Medical history (validated in hospital/GP records) | 6 (1.0%) |
| Bones and joint disorders | Joint inflammation/Arthritis | Medical history | 50 (8.4%) |
| | Osteopenia/Osteoporosis | DXA T-score <-1 \| Medication | 255 (42.6%) |
| | Osteoporotic fracture | Medical history (validated in hospital/GP records) | 91 (15.2%) |
| Sexually transmitted diseases (STDs) | Chlamydia | Hospital records | 54 (9.0%) |
| | Gonorrhoea | Hospital records | 25 (4.2%) |
| | Syphilis | Positive syphilis serology | 184 (30.8%) |
| Genitourinary disorders | Urinary incontinence | Medical history | 30 (5.0%) |
| | Erectile dysfunction | Medical history \| Medication | 87 (14.5%) |
| | Liver problems | Medical history | 42 (7.0%) |
| Eye problem | Eye problem | Medical history | 108 (18.1%) |
| Vitamin D deficiency | Vitamin D deficiency | 25-hydroxy vitamin D <25 nmol/l \| Medication | 108 (18.1%) |

**SUPPLEMENTARY TABLE 5.3 A** | Correlation between the six PCs extracted by the PCA in all POPPY PLWH (n=1073) and individual comorbidities

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| **TB** | 0.12 | -0.23 | -0.10 | -0.01 | -0.11 | 0.27 |
| **CMV** | -0.08 | -0.19 | -0.17 | 0.16 | -0.03 | **0.49** |
| **Pneumocystis pneumonia** | 0.00 | -0.11 | 0.06 | 0.33 | -0.10 | 0.25 |
| **Kaposi's sarcoma** | 0.00 | 0.16 | -0.14 | 0.22 | 0.08 | 0.19 |
| **Other AIDS events** | 0.24 | 0.04 | 0.25 | 0.32 | -0.24 | 0.21 |
| **Fungal infection** | 0.04 | 0.14 | 0.01 | -0.01 | 0.10 | 0.14 |
| **VZV** | 0.16 | 0.28 | 0.08 | 0.32 | 0.02 | -0.04 |
| **Type 2 diabetes** | 0.12 | -0.12 | 0.08 | 0.01 | 0.28 | 0.18 |
| **Hypothyroidism** | -0.02 | -0.10 | 0.16 | 0.10 | -0.10 | 0.27 |
| **Dyslipidaemia** | 0.25 | 0.00 | 0.08 | 0.11 | **0.71** | 0.00 |
| **Lipodystrophy/Lipoatrophy** | -0.10 | -0.05 | 0.19 | -0.04 | **0.57** | 0.12 |
| **Anaemia** | 0.12 | 0.00 | 0.24 | -0.10 | -0.10 | -0.02 |
| **Depression** | -0.03 | -0.01 | **0.79** | -0.02 | 0.00 | -0.03 |
| **Panic attacks** | -0.06 | 0.24 | **0.50** | -0.11 | 0.03 | 0.03 |
| **Anxiety** | -0.06 | 0.04 | **0.58** | -0.12 | 0.18 | -0.02 |
| **Sleeping problems** | 0.00 | 0.02 | 0.28 | 0.03 | 0.16 | -0.06 |
| **Dizziness/Vertigo** | 0.08 | 0.04 | 0.27 | -0.03 | -0.28 | **0.44** |
| **Loss of consciousness** | 0.20 | -0.10 | 0.23 | 0.05 | -0.23 | 0.16 |
| **Encephalitis** | 0.06 | -0.11 | 0.21 | -0.10 | **-0.41** | 0.29 |
| **Epilepsy** | 0.23 | -0.06 | -0.04 | -0.07 | -0.11 | 0.06 |
| **Peripheral neuropathy** | 0.19 | -0.09 | 0.16 | 0.06 | 0.04 | 0.29 |
| **Migraine/Headaches** | 0.00 | 0.08 | 0.11 | 0.10 | -0.13 | 0.06 |
| **MI** | **0.64** | 0.03 | 0.09 | 0.00 | 0.10 | -0.17 |
| **Angina pectoris** | **0.66** | 0.06 | -0.11 | 0.05 | -0.04 | 0.10 |
| **PVD** | **0.53** | 0.04 | 0.02 | -0.14 | -0.12 | 0.05 |
| **Heart failure** | **0.59** | 0.01 | -0.12 | -0.20 | -0.01 | 0.21 |
| **Hypertension** | **0.54** | -0.15 | 0.02 | -0.08 | **0.44** | 0.11 |
| **TIA** | 0.28 | 0.03 | 0.04 | 0.14 | 0.10 | -0.01 |
| **CABG** | **0.66** | 0.04 | 0.00 | 0.12 | 0.09 | -0.21 |
| **Asthma/Bronchitis/COPD** | -0.15 | 0.22 | 0.20 | 0.02 | 0.18 | **0.42** |
| **Pneumonia** | 0.01 | 0.11 | -0.18 | -0.08 | 0.23 | **0.49** |
| **Chest infection** | 0.00 | 0.01 | 0.05 | -0.20 | 0.11 | **0.41** |
| **Hay fever/Allergy** | 0.01 | -0.05 | 0.12 | 0.01 | 0.04 | -0.08 |
| **IBS** | 0.00 | 0.17 | 0.36 | 0.03 | -0.02 | 0.01 |

**SUPPLEMENTARY TABLE 5.3 A *(continued)*** | Correlation between the six PCs extracted by the PCA in all POPPY PLWH (n=1073) and individual comorbidities

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| **Hernia** | 0.21 | 0.00 | 0.06 | 0.28 | -0.20 | -0.04 |
| **GORD** | -0.01 | -0.08 | 0.26 | 0.02 | 0.27 | -0.03 |
| **HBV** | 0.12 | -0.02 | 0.22 | 0.04 | -0.17 | -0.15 |
| **HCV** | 0.01 | **0.40** | 0.13 | 0.01 | -0.17 | -0.04 |
| **HAV** | 0.03 | 0.33 | -0.12 | -0.06 | 0.10 | 0.13 |
| **Urinary Incontinence** | 0.05 | 0.09 | -0.04 | -0.08 | 0.10 | 0.28 |
| **Erectile dysfunction** | 0.00 | -0.04 | 0.15 | 0.00 | 0.24 | 0.01 |
| **Urethritis** | -0.01 | 0.38 | -0.12 | -0.04 | 0.10 | 0.04 |
| **Renal problem** | **0.42** | -0.07 | -0.01 | 0.14 | 0.00 | 0.02 |
| **Prostate dysfunction** | -0.01 | 0.18 | -0.02 | 0.14 | 0.13 | 0.17 |
| **Kidney stones** | -0.04 | 0.04 | 0.03 | 0.11 | 0.15 | 0.14 |
| **UTI** | 0.01 | 0.01 | -0.12 | 0.05 | 0.15 | 0.26 |
| **Joint inflammation/Arthritis** | 0.08 | -0.09 | 0.40 | 0.36 | 0.08 | 0.11 |
| **Joint replacement** | -0.08 | -0.19 | 0.20 | 0.27 | 0.14 | -0.02 |
| **Osteopenia/Osteoporosis** | 0.09 | -0.11 | 0.19 | 0.08 | 0.03 | 0.13 |
| **Joint/Back pain** | -0.03 | -0.08 | 0.24 | -0.07 | 0.11 | 0.01 |
| **Osteoporotic fracture** | 0.06 | -0.04 | 0.09 | 0.00 | -0.06 | -0.01 |
| **Syphilis** | 0.12 | **0.67** | -0.08 | 0.11 | 0.01 | -0.04 |
| **Gonorrhoea** | -0.01 | **0.77** | 0.05 | 0.01 | 0.14 | 0.02 |
| **Chlamydia** | -0.06 | **0.64** | 0.08 | -0.11 | -0.17 | -0.05 |
| **LGV** | 0.01 | **0.66** | -0.01 | -0.01 | -0.16 | 0.06 |
| **HPV** | -0.24 | 0.17 | 0.01 | 0.06 | 0.08 | 0.20 |
| **HSV** | -0.10 | 0.24 | -0.04 | 0.20 | 0.19 | 0.12 |
| **Psoriasis** | -0.01 | 0.15 | 0.03 | 0.01 | 0.13 | 0.07 |
| **Eczema/Dermatitis** | -0.04 | 0.10 | 0.18 | -0.02 | 0.15 | 0.03 |
| **Eye problem** | 0.03 | 0.02 | 0.03 | 0.27 | -0.03 | 0.32 |
| **Ear dysfunction** | -0.08 | 0.02 | 0.05 | -0.04 | 0.03 | 0.26 |
| **Vitamin D deficiency** | -0.02 | -0.07 | -0.07 | -0.14 | 0.30 | 0.00 |
| **Skin cancer** | -0.01 | 0.01 | -0.02 | **0.64** | 0.09 | -0.02 |
| **Haematological cancer** | -0.05 | 0.00 | -0.08 | **0.75** | -0.02 | -0.03 |
| **Solid organ cancer** | -0.02 | 0.01 | -0.08 | **0.49** | 0.06 | 0.04 |

*Bold signifies correlation ≥0.40*

**SUPPLEMENTARY TABLE 5.3 B** | Correlation between the six PCs extracted by the PCA in older POPPY PLWH (n=699) and individual comorbidities

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| **TB** | 0.19 | -0.15 | 0.00 | 0.00 | -0.17 | 0.13 |
| **CMV** | 0.04 | -0.22 | -0.01 | 0.19 | -0.17 | 0.19 |
| **Pneumocystis pneumonia** | -0.01 | -0.14 | 0.04 | 0.23 | -0.10 | 0.37 |
| **Kaposi's sarcoma** | 0.03 | 0.13 | -0.12 | 0.10 | 0.10 | 0.34 |
| **Other AIDS events** | 0.21 | 0.06 | 0.12 | 0.25 | -0.23 | **0.46** |
| **Fungal infection** | 0.11 | 0.17 | 0.02 | -0.07 | 0.07 | 0.15 |
| **VZV** | 0.08 | 0.23 | 0.04 | 0.26 | 0.18 | 0.21 |
| **Type 2 diabetes** | 0.16 | -0.13 | 0.13 | 0.00 | 0.23 | 0.04 |
| **Hypothyroidism** | -0.01 | -0.11 | 0.10 | -0.03 | 0.02 | **0.46** |
| **Dyslipidaemia** | 0.24 | -0.04 | 0.11 | 0.06 | **0.71** | -0.08 |
| **Lipodystrophy/Lipoatrophy** | 0.00 | -0.08 | 0.24 | -0.09 | **0.43** | 0.16 |
| **Anaemia** | 0.16 | 0.08 | 0.21 | 0.00 | -0.28 | -0.19 |
| **Depression** | -0.05 | 0.03 | **0.72** | -0.04 | -0.04 | 0.03 |
| **Panic attacks** | -0.10 | 0.17 | **0.61** | -0.01 | 0.17 | -0.14 |
| **Anxiety** | -0.08 | -0.05 | **0.53** | -0.07 | 0.30 | 0.06 |
| **Sleeping problems** | -0.07 | 0.05 | 0.26 | 0.09 | 0.20 | 0.08 |
| **Dizziness/Vertigo** | 0.08 | -0.01 | **0.40** | -0.04 | -0.28 | 0.23 |
| **Loss of consciousness** | 0.16 | -0.10 | 0.20 | 0.12 | -0.17 | -0.02 |
| **Encephalitis** | 0.07 | -0.03 | 0.29 | -0.13 | **-0.47** | 0.06 |
| **Epilepsy** | 0.28 | 0.04 | -0.06 | 0.05 | -0.15 | -0.21 |
| **Peripheral neuropathy** | 0.32 | -0.12 | 0.24 | -0.03 | -0.17 | 0.24 |
| **Migraine/Headaches** | -0.06 | 0.16 | -0.02 | 0.11 | 0.00 | 0.40 |
| **MI** | **0.57** | 0.07 | -0.04 | -0.09 | 0.24 | 0.06 |
| **Angina pectoris** | **0.67** | 0.08 | -0.08 | 0.08 | -0.03 | 0.09 |
| **PVD** | **0.62** | 0.10 | -0.03 | -0.08 | -0.15 | -0.03 |
| **Heart failure** | **0.65** | -0.03 | 0.04 | -0.10 | -0.03 | -0.03 |
| **Hypertension** | **0.54** | -0.21 | 0.17 | 0.00 | 0.33 | -0.18 |
| **TIA** | 0.20 | 0.04 | 0.03 | 0.17 | 0.17 | -0.01 |
| **CABG** | **0.58** | 0.08 | -0.17 | 0.06 | 0.19 | 0.02 |
| **Asthma/Bronchitis/COPD** | 0.01 | 0.20 | **0.45** | -0.02 | 0.05 | 0.23 |
| **Pneumonia** | 0.20 | 0.05 | 0.20 | 0.04 | -0.05 | -0.12 |
| **Chest infection** | 0.16 | -0.05 | 0.39 | -0.12 | -0.16 | -0.11 |
| **Hay fever/Allergy** | 0.03 | -0.06 | 0.20 | 0.07 | 0.00 | -0.11 |
| **IBS** | -0.07 | 0.16 | 0.33 | 0.02 | 0.01 | 0.00 |

**SUPPLEMENTARY TABLE 5.3 B *(continued)*** | Correlation between the six PCs extracted by the PCA in older POPPY PLWH (n=699) and individual comorbidities

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| **Hernia** | 0.15 | 0.03 | -0.07 | 0.31 | -0.11 | 0.03 |
| **GORD** | 0.01 | -0.08 | 0.22 | 0.08 | 0.20 | -0.08 |
| **HBV** | 0.01 | -0.02 | 0.01 | -0.02 | -0.06 | 0.14 |
| **HCV** | -0.01 | **0.46** | 0.22 | 0.00 | -0.26 | -0.16 |
| **HAV** | 0.11 | 0.32 | -0.09 | -0.13 | 0.10 | 0.06 |
| **Urinary Incontinence** | 0.16 | 0.09 | 0.17 | 0.00 | -0.17 | -0.13 |
| **Erectile dysfunction** | 0.05 | 0.04 | 0.12 | -0.03 | 0.09 | -0.02 |
| **Urethritis** | -0.03 | 0.34 | -0.16 | -0.15 | 0.17 | 0.08 |
| **Renal problem** | **0.44** | -0.07 | -0.08 | 0.13 | 0.00 | 0.15 |
| **Prostate dysfunction** | 0.00 | 0.14 | 0.14 | 0.17 | 0.02 | -0.06 |
| **Kidney stones** | -0.01 | -0.04 | -0.03 | 0.01 | 0.19 | 0.36 |
| **UTI** | 0.10 | -0.01 | -0.01 | 0.00 | 0.00 | 0.14 |
| **Joint inflammation/Arthritis** | 0.07 | -0.14 | 0.35 | 0.33 | -0.05 | 0.11 |
| **Joint replacement** | -0.04 | -0.20 | 0.21 | 0.29 | -0.11 | -0.14 |
| **Osteopenia/Osteoporosis** | 0.10 | -0.17 | 0.23 | -0.02 | -0.04 | 0.17 |
| **Joint/Back pain** | -0.05 | -0.08 | 0.17 | -0.13 | 0.21 | 0.09 |
| **Osteoporotic fracture** | -0.03 | -0.02 | 0.03 | -0.14 | -0.05 | 0.17 |
| **Syphilis** | 0.03 | **0.69** | 0.05 | 0.25 | -0.02 | -0.29 |
| **Gonorrhoea** | 0.08 | **0.74** | 0.03 | 0.00 | 0.13 | 0.07 |
| **Chlamydia** | -0.05 | **0.63** | -0.08 | -0.21 | 0.00 | 0.22 |
| **LGV** | -0.01 | **0.72** | 0.07 | -0.02 | -0.16 | 0.02 |
| **HPV** | -0.22 | 0.06 | 0.10 | 0.02 | 0.20 | 0.15 |
| **HSV** | -0.15 | 0.20 | 0.11 | 0.18 | 0.27 | 0.15 |
| **Psoriasis** | 0.00 | 0.11 | 0.00 | -0.07 | 0.22 | 0.15 |
| **Eczema/Dermatitis** | -0.04 | 0.11 | 0.19 | -0.12 | 0.19 | 0.23 |
| **Eye problem** | 0.06 | 0.02 | 0.10 | 0.25 | -0.05 | 0.28 |
| **Ear dysfunction** | -0.06 | 0.03 | 0.12 | -0.03 | 0.06 | 0.02 |
| **Vitamin D deficiency** | 0.02 | 0.01 | 0.08 | 0.06 | 0.13 | -0.37 |
| **Skin cancer** | 0.00 | 0.02 | 0.01 | **0.64** | 0.11 | 0.03 |
| **Haematological cancer** | -0.07 | 0.01 | -0.09 | **0.78** | 0.01 | 0.04 |
| **Solid organ cancer** | 0.02 | -0.03 | 0.00 | **0.54** | 0.00 | -0.04 |

*Bold signifies correlation ≥0.40*

**SUPPLEMENTARY TABLE 5.3 C** | Correlation between the six PCs extracted by the PCA in $AGE_hIV$ PLWH (n=598) and individual comorbidities

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| **TB** | -0.25 | -0.33 | 0.22 | 0.02 | -0.01 | 0.30 |
| **Pneumocystis pneumonia** | 0.08 | 0.00 | 0.37 | -0.02 | -0.25 | 0.01 |
| **Kaposi's sarcoma** | 0.10 | 0.16 | 0.35 | -0.01 | 0.07 | -0.04 |
| **Candidiasis** | -0.14 | 0.01 | **0.63** | 0.08 | -0.06 | 0.16 |
| **Other AIDS events** | -0.20 | -0.12 | **0.48** | -0.04 | 0.32 | -0.04 |
| **Lipodystrophy** | 0.34 | -0.01 | **0.53** | 0.07 | -0.08 | -0.08 |
| **VZV** | -0.08 | 0.12 | 0.33 | -0.15 | 0.22 | 0.23 |
| **Type 2 diabetes** | 0.28 | -0.13 | 0.21 | 0.08 | 0.06 | -0.06 |
| **Erectile dysfunction** | 0.36 | 0.01 | 0.09 | 0.26 | 0.17 | -0.06 |
| **Dyslipidaemia** | **0.73** | -0.14 | 0.19 | -0.02 | 0.00 | 0.05 |
| **Hypothyroidism** | -0.09 | 0.15 | 0.04 | -0.13 | -0.20 | 0.01 |
| **Hyperparathyroidism** | -0.13 | -0.11 | 0.07 | -0.04 | -0.23 | **0.54** |
| **Anaemia** | -0.04 | -0.19 | 0.08 | -0.06 | 0.05 | **0.45** |
| **Thrombocytopenia** | 0.01 | 0.19 | 0.01 | -0.26 | 0.03 | -0.09 |
| **Depression** | 0.03 | 0.11 | -0.13 | **0.63** | 0.01 | 0.01 |
| **Sleeping problems** | 0.22 | 0.06 | 0.02 | **0.42** | 0.06 | -0.18 |
| **Dizziness/Vertigo** | -0.05 | **0.43** | 0.13 | **0.40** | 0.11 | -0.08 |
| **Loss of consciousness** | -0.06 | -0.01 | 0.10 | **0.48** | 0.10 | -0.09 |
| **Epilepsy** | 0.18 | 0.06 | 0.27 | 0.33 | -0.15 | -0.25 |
| **Encephalitis** | -0.16 | -0.22 | 0.22 | 0.22 | 0.23 | 0.26 |
| **Peripheral neuropathy** | 0.13 | 0.13 | **0.63** | 0.09 | -0.06 | -0.13 |
| **Asthma/Bronchitis/COPD** | 0.10 | **0.59** | 0.07 | 0.10 | 0.08 | 0.25 |
| **Pneumonia** | -0.05 | 0.37 | -0.03 | 0.11 | 0.15 | 0.39 |
| **Hay fever/Allergy** | -0.18 | 0.33 | 0.30 | 0.06 | -0.14 | -0.02 |
| **HBV** | -0.12 | **0.47** | -0.02 | -0.08 | -0.12 | -0.05 |
| **HCV** | -0.30 | 0.40 | 0.01 | 0.04 | 0.03 | 0.06 |
| **MI** | **0.51** | -0.07 | -0.12 | 0.08 | -0.10 | 0.35 |
| **Angina pectoris** | **0.54** | 0.15 | -0.16 | 0.15 | 0.14 | 0.34 |
| **PVD** | **0.60** | 0.08 | 0.13 | -0.08 | 0.17 | -0.19 |
| **Hypertension** | **0.64** | -0.01 | -0.11 | -0.06 | -0.17 | 0.15 |
| **TIA** | 0.33 | -0.16 | 0.40 | -0.06 | -0.05 | 0.08 |
| **Heart failure** | 0.13 | 0.15 | -0.01 | 0.04 | 0.02 | **0.88** |

**SUPPLEMENTARY TABLE 5.3 C *(continued)*** | Correlation between the six PCs extracted by the PCA in AGE-$_{h}$IV PLWH (n=598) and individual comorbidities

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| **Liver problems** | -0.28 | **0.55** | 0.02 | 0.05 | -0.06 | 0.05 |
| **Joint inflammation/Arthritis** | 0.02 | 0.16 | 0.05 | 0.33 | -0.25 | -0.04 |
| **Osteopenia/Osteoporosis** | 0.10 | 0.37 | 0.31 | -0.18 | -0.07 | 0.30 |
| **Osteoporotic fracture** | 0.06 | -0.08 | -0.05 | 0.17 | -0.06 | 0.05 |
| **Chlamydia** | -0.03 | 0.00 | -0.12 | 0.00 | **0.70** | -0.08 |
| **Gonorrhoea** | -0.06 | 0.05 | -0.04 | -0.01 | **0.67** | 0.02 |
| **Syphilis** | 0.17 | -0.08 | 0.08 | 0.00 | **0.62** | 0.08 |
| **Urinary Incontinence** | 0.10 | 0.09 | 0.11 | **0.50** | 0.08 | 0.07 |
| **Renal problem** | 0.30 | 0.07 | 0.11 | -0.05 | -0.01 | 0.18 |
| **Eye problem** | -0.14 | -0.11 | 0.09 | **0.64** | -0.09 | 0.19 |
| **Vitamin D deficiency** | -0.02 | -0.38 | 0.05 | 0.05 | 0.00 | **0.48** |
| **Skin cancer** | 0.15 | 0.18 | 0.30 | -0.38 | 0.04 | 0.07 |
| **Solid organ cancer** | 0.34 | 0.25 | -0.15 | -0.10 | -0.15 | 0.13 |
| **Haematological cancer** | -0.02 | 0.14 | 0.14 | -0.31 | 0.27 | 0.07 |

*Bold signifies correlation ≥0.40*

# 6

# HIV-negative Men Who Have Sex with Men have higher $CD8^+$ T-cell Counts and Lower $CD4^+/CD8^+$ T-cell Ratios compared to HIV-negative Heterosexual Men

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## ABSTRACT

**Background**. We reported T-cell senescence to be similar in people living with HIV (PLWH) with suppressed viremia (predominantly men who have sex with men (MSM)) and HIV-negative otherwise comparable controls, but greater than in healthy blood donors. This lead us to compare $CD4^+$ and $CD8^+$ T-cell counts and $CD4^+/CD8^+$ ratios between HIV-negative MSM and men who only have sex with women (MSW), and relate observed differences to behavioural factors and infectious exposures, including cytomegalovirus (CMV) infection.

**Methods**. In 368 HIV-negative MSM and 72 HIV-negative MSW T-lymphocyte phenotyping was performed 3 times biennially. Baseline CMV serology, and STI-incidence/-seroprevalence, sexual and substance-use behaviour data were collected during study visits.

**Results**. MSM, compared to MSW, had higher $CD8^+$ counts (551 vs. 437 cells/$mm^3$, P<.001), similar $CD4^+$ counts (864 vs. 880 cells/$mm^3$, P=.5) and lower $CD4^+/CD8^+$ ratios (1.84 vs. 2.47, P<.001). Differences were most pronounced for MSM with >10 recent sex partners, and partly explained by higher CMV seroprevalence in MSM.

**Discussion**. These findings suggest that factors other than HIV may, both in PLWH and certain HIV-negative MSM, contribute to a low $CD4^+/CD8^+$ ratio. Whether this, like in PLWH, contributes to comorbidity risk in HIV-negative MSM requires further study.

## BACKGROUND

Since the beginning of the HIV epidemic, $CD4^+$ (CD4) and $CD8^+$ T-lymphocyte (CD8) counts, and the CD4/CD8 ratio, have been studied as potential predictive markers of disease progression in people with HIV (PLWH). We now know that CD4 count restoration following combination antiretroviral therapy (cART) initiation is strongly correlated not only with a lower likelihood of developing AIDS, but also non-AIDS-associated comorbidities.[1,2] Furthermore, although evidence remains inconclusive,[3] studies have suggested a low CD4/CD8 ratio during long-term cART to be predictive for the development of non-AIDS-associated comorbidities.[4-9] PLWH remain at increased risk of such comorbidities despite sustained viral suppression on cART.[10,11] In HIV-negative populations a low CD4/CD8 ratio with an expansion of $CD8^+$ T cells that express CD57 or lack expression of CD28, has been described as reflecting part of an "immune risk phenotype".[12,13] Thus, low CD4/CD8 ratios, both in HIV-positive and -negative populations, have been associated with morbidity [14] and mortality.[15-17] An in-depth analysis of T-cell senescence and activation was performed on a limited sample of $AGE_h$IV participants,[18] and in those enrolled in the COBRA (COmorBidity in Relation to AIDS) study linked to it.[19] Both cohorts include an HIV-negative control group, with a majority of MSM, to ensure good comparability with the HIV-positive index group. Only within the COBRA cohort a second control group of blood bank donors was included, at low risk of blood-born infectious diseases. $CD4^+$ and $CD8^+$ T-cell senescence as measured by the expression of CD57 or loss of CD27 and CD28 expression was comparable between HIV-positive and HIV-negative participants, but for both higher compared to blood bank donors. This suggests increased T-cell senescence also in HIV-negative COBRA/$AGE_h$IV participants. Cytomegalovirus (CMV)-seropositivity was an important driver of this increased T-cell senescence in both HIV-positive and HIV-negative COBRA participants. Similarly, CMV-seropositivity has also been associated with high CD8 counts and low CD4/CD8 ratios in PLWH.[20]

In many countries, including the Netherlands, the majority of PLWH are MSM.[21] MSM are not only at increased risk of acquiring HIV, but also of acquiring a wide range of other sexually transmitted infections (STIs). These include not only treatable bacterial STIs such as syphilis, gonorrhea, and chlamydia, but also chronic viral infections like viral hepatitis and herpesvirus infections, including CMV.[22] CMV-seropositivity in particular[23], but also transient bacterial STIs,[24,25] have each been associated with a decreased CD4/CD8 ratio and an increased CD8 count.

In view of our earlier observation, we evaluated CD4 and CD8 counts, and CD4/CD8 ratios, in a larger group of HIV-negative individuals, comparing MSM and men who only

have sex with women (MSW). We evaluated to what extent any observed differences were related to sexual and behavioural factors, exposure to STIs and CMV infection.

## METHODS

### Study population

The prospective $AGE_hIV$ Cohort Study investigates age-related comorbidities and their risk factors in HIV-positive and HIV-negative individuals, aged ≥45 years.[10] HIV-positive participants were recruited from the Academic Medical Center's ambulatory HIV-clinic and HIV-negative participants from the sexual health clinic and the Amsterdam Cohort Studies on HIV/AIDS at the Public Health Service of Amsterdam.[26] Biennially, participants undergo standardized screening to evaluate the presence of (risk factors for) age-associated comorbidities. Most participants in the HIV-negative cohort are MSM, with a limited number of MSW and women. For the current analysis, only HIV-negative men with data available on sexual behaviour were included (Figure 6.1). HIV-positive participants and women were not included to allow evaluating the effects of being an MSM independent from the effects of gender and HIV. Participants who HIV-seroconverted during follow-up were excluded from the time of seroconversion onward. All participants provided written informed consent; the study protocol was approved by the local ethics review committee and is registered at www.clinicaltrials.gov (identifier NCT01466582).

### Measurements

Data from baseline and 2 follow-up study visits were available for analysis, comprising 4 years of follow-up. Each study visit, information about sexual behaviour, smoking, alcohol use and recreational drug use was obtained through standardized questionnaires inquiring about lifetime exposure and exposure during the 6 months prior to the study visits. Each study visit, percentages and absolute counts of T, B, and natural killer (NK) cells, and CD4 and CD8 subpopulations of T cells in peripheral blood were determined using BD Multitest™ 6-color TBNK reagent with BD Trucount™ tubes (BD Biosciences) by flow cytometry (BD FACSCanto™ II, BD Biosciences). Serology for HIV (4$^{th}$ generation antibody/antigen test) and syphilis (Treponema pallidum particle agglutination assay and rapid plasma reagin) were also performed at each visit. Chronic hepatitis B (HBsAg positive) and C (HCV RNA positive) status were determined only at baseline. In stored baseline plasma samples CMV-IgG titer (ELISA-VIDITEST; VIDIA, Praha, Czech republic) and levels of Intestinal Fatty Acid Binding Protein (I-FABP; a biomarker for intestinal integrity), soluble CD14, soluble CD163 (markers of monocyte activation) and interleukin-6 (marker of inflammation) were determined using enzyme-linked immunosorbent assays (DuoSet ELISA; R&D systems). Data on syphilis, chlamydia and gonorrhea

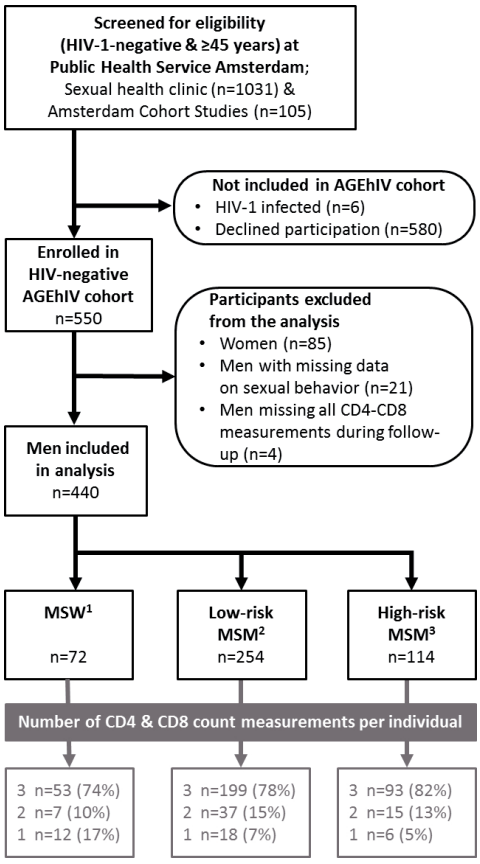


**FIGURE 6.1** | Flow diagram of included participants and measurements

*[1]MSW = Men who have sex with women and reported <2 male sex partner in their lifetime [2]low-risk MSM = Men with ≥2 male sex partner in their lifetime and on average ≤10 sex partners during 6 months prior to study visits; includes one male-to-female transgender individual. [3] high-risk MSM = Men with ≥2 male sex partner in their lifetime and on average >10 sex partners during 6 months prior to study visits.*

diagnosed at the municipal sexual health clinic in between study visits were extracted from clinical records. For a subset of participants (n=92, Supplementary Table 6.1) data regarding percentages of activated ($CD38^+/HLA\text{-}DR^+$) and senescent ($CD27^-/CD28^-$) T-cells within total CD4 and CD8 T-cell populations were available from analyses we previously published.[18,19]

## Definitions

MSM were defined as men who reported having had ≥2 male lifetime sex partners, and MSW as men with <2 lifetime male sex partner, given that repeated exposures were the primary focus of interest. After finding large differences in CD4/CD8 ratios between MSW and MSM, but also between MSM with high versus low numbers of recent sex partners in a preliminary analysis, we decided for this analysis to further stratify MSM into high and low risk categories for the exploration of potential factors driving differences between study groups. High- as opposed to low-risk MSM were defined as MSM who reported a

mean of >10 vs. ≤10 sex partners in the 6 months prior to each of the three study visits, respectively, using the number of sex partners as proxy for the risk of both STIs and HIV.

### Statistical analysis

Characteristics of MSW, low-risk MSM and high-risk MSM were compared using Wilcoxon rank sum and chi-squared tests as appropriate. Differences in $\log_e$-transformed CD4/CD8 ratios and $\log_e$-transformed CD4 and CD8 counts between study groups were determined using linear mixed-effects models to account for repeated measurements. Models included a random intercept and random slope for time since baseline for each participant. Subsequently, we included potential confounding and/or mediating factors in the models following a step-wise forward variable selection process to evaluate which factors were driving any observed differences in lymphocyte subsets between subgroups. The variables Body Mass Index (BMI), recent gonorrhea diagnosis, recent chlamydia diagnosis, and those concerning sexual and substance use behaviours were time-updated. To allow differentiating between effects caused by different behavioural patterns between participants (between-participant variation) versus changes in an individual's behaviour over time (within-participant variation) we used a technique called 'within subject centering'.[27] This means continuous predictor variables were split into two. One dummy variable was fixed in time and consisted of the participant's calculated mean of that variable over the three study visits, for the analysis of between-participant variation. A second dummy variable consisted of the deviation from the individual's mean at a particular study visit, for the analysis of within-participant variation. Since the covariates CMV-seropositivity, hepatitis C RNA status and I-FABP levels were only measured at the baseline visit, we also performed sensitivity analyses using baseline-data only. Additional mixed effects models were built to evaluate associations of activation and senescence markers with absolute T-cell counts and the mean differences of these markers between MSW, low-risk and high-risk MSM, for the subgroup of participants in whom these markers were available.[18,19]

## RESULTS

### Differences in participant characteristics

72 MSW, 254 low-risk MSM and 114 high-risk MSM were included in this analysis, of whom 74%, 78% and 82%, respectively, completed 4 years of follow-up (Figure 6.1). High-risk MSM were slightly younger than low-risk MSM (Table 6.1). MSM generally were more highly educated, used recreational drugs more often and smoked less frequently compared to MSW. Participants' estimated lifetime number of sex partners differed substantially: MSW had a median of 25 (IQR 15-50), compared to 200 (IQR 50-700) and

**TABLE 6.1** | Baseline characteristics of MSW, low- and high-risk MSM

| | MSW | Low-risk MSM (≤10 partners) | High-risk MSM (>10 partners) | P MSW vs. low-risk MSM[a] | P low-risk MSM vs. high-risk MSM[a] |
|---|---|---|---|---|---|
| N | 72 | 254 | 114 | | |
| Age in years | 52 (48-60) | 53 (48-59) | 51 (47, 56) | 1 | .02 |
| BMI (kg/m$^2$) | 25 (24-29) | 25 (23-27) | 24 (23, 26) | .009 | .06 |
| Born in the Netherlands | 55 (76.4%) | 218 (86.5%) | 93 (82.3%) | .04 | .3 |
| High educational attainment[b] | 32 (45.1%) | 157 (62.5%) | 70 (64.2%) | .008 | .8 |
| Estimated[c] number of lifetime male sex partners | 0 (0-0) | 200 (50-700) | 1000 (500-2000) | <.001 | <.001 |
| Estimated[c] number of lifetime female sex partners | 25 (15-50) | 2 (0-5) | 2 (0-5) | <.001 | .7 |
| Number of male sex partners prior 6 months | 0 (0-0) | 2 (1-5) | 20 (10-30) | <.001 | <.001 |
| Number of recent female sex partners prior 6 months | 2 (1-3) | 0 (0-0) | 0 (0-0) | <.001 | .5 |
| Tobacco smoking during last month | 25 (34.7%) | 56 (22.2%) | 22 (19.5%) | .03 | .6 |
| Heavy daily drinking [d,e] | 7 (9.7%) | 12 (4.8%) | 6 (5.4%) | .1 | .8 |
| Recreational drug use [e] | 11 (15.5%) | 68 (27.1%) | 47 (41.6%) | .05 | .006 |
| Daily marijuana use [e] | 4 (5.6%) | 11 (4.4%) | 2 (1.8%) | .7 | .2 |
| Use of MDMA, monthly or more frequent [e] | 3 (4.2%) | 18 (7.2%) | 14 (12.4%) | .4 | .1 |
| *N. gonorrhoeae* diagnosis at recruitment visit | 1 (1.4%) | 9 (3.6%) | 11 (9.6%) | .4 | .02 |
| *C. trachomatis* diagnosis at recruitment visit | 2 (2.8%) | 17 (6.7%) | 10 (8.8%) | .2 | .5 |
| Ever contracted syphilis [f] | 1 (1.4%) | 53 (20.9%) | 32 (28.1%) | <.001 | .1 |
| Syphilis diagnosis at recruitment visit [g] | 1 (1.4%) | 2 (0.8%) | 5 (4.4%) | .6 | .02 |
| HCV RNA positive | 0 (0.0%) | 2 (0.8%) | 2 (1.8%) | .5 | .4 |
| HBsAg positive | 0 (0.0%) | 1 (0.4%) | 2 (1.8%) | .6 | .2 |
| CMV IgG-seropositive | 39 (54.2%) | 193 (76.3%) | 102 (89.5%) | <.001 | .003 |

*Given values are median (IQR) or n (%). a. $\chi^2$ tests were used for comparison, except for age, BMI and numbers of sex partners where Wilcoxon rank-sum tests were used. b. finished vocational level education or higher c. estimated by participant himself. d. drinking >4 International Units of alcohol daily or almost daily. e. during prior 6 months. f. Treponema pallidum particle agglutination assay positive. g. rapid plasma reagin >1:4. Abbreviations: BMI, Body Mass Index; MSW, Men who only have sex with women; MSM, Men who have sex with men; P, p-value; MDMA, 3,4-Methylenedioxymethamphetamine; HCV, Hepatitis C Virus; RNA, Ribonucleic acid; HbsAg, Hepatitis B surface antigen; CMV, Cytomegalovirus.*

1000 (IQR 500-2000) estimated lifetime sex partners in low-risk MSM and high-risk MSM, respectively. All STI rates were higher in MSM, especially in high-risk MSM, however chronic hepatitis B and C were rare (prevalence <2% for both in all groups). Baseline CMV seroprevalence was higher in low-risk MSM compared to MSW (76% vs. 54%, P<.001), and highest in high-risk MSM (90%, $P_{\text{high-risk MSM vs low-risk MSM}}$ =.003). During follow-up, none of the MSW, but two low-risk MSM and four high-risk MSM seroconverted for HIV (incidence rate 0, 2.1, and 9.3 per 1000 person years of follow-up, respectively).

## Higher CD8 counts and lower CD4/CD8 ratios in MSM, especially in high-risk MSM

Including all measurements from all three study visits, MSM, compared to MSW, had higher mean CD8 counts (551 vs. 437 cells/mm$^3$, P<.001), similar mean CD4 counts (864 vs. 880 cells/mm$^3$, P=.5) and lower mean CD4/CD8 ratios (1.84 vs. 2.47, P<.001), respectively. Furthermore, high- compared to low-risk MSM, had higher mean CD8 counts (599 vs. 529 cells/mm$^3$, P=0.013), similar mean CD4 counts (824 vs. 881, P=.097) and mean lower CD4/CD8 ratios (1.59 vs. 1.96, P<.001), respectively. We calculated for each participant separately the within-subject mean CD4 count, mean CD8 count, and mean CD4/CD8 ratio, based on all his available measurements (from at most 3 study visits). Figure 6.2 shows the distribution of these mean CD4/CD8 ratios and mean CD4 and CD8 counts, stratified by MSW, low-risk MSM and high-risk MSM. Mean CD8 counts were higher and mean CD4/CD8 ratios lower with higher numbers of recent sex partners, with 5.6% of MSW having a mean CD4/CD8 ratio below 1, as opposed to 9.1% of low-risk MSM and 21.9% of high-risk MSM. A CD4/CD8 ratio <0.5, observed at just a single study

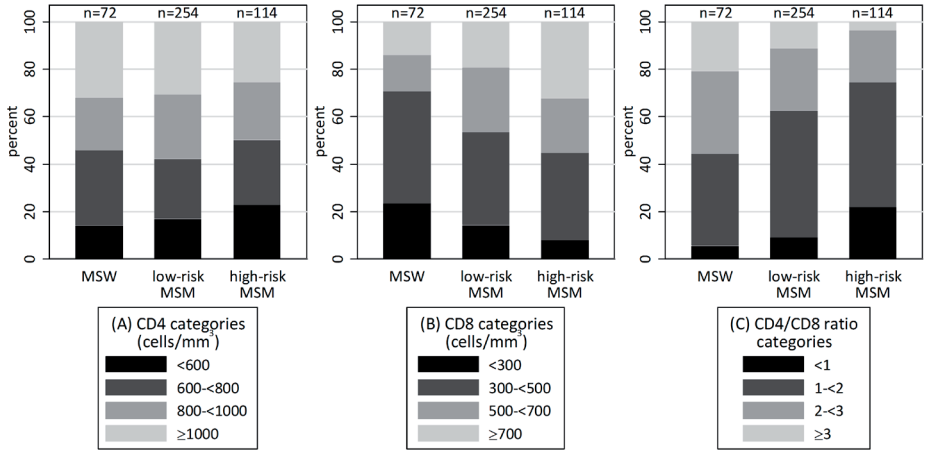


**FIGURE 6.2** | Distribution of per participant mean absolute CD4 and CD8 counts and mean CD4/CD8 ratios stratified by risk group

*Abbreviations: MSW, men who only have sex with women; MSM, men who have sex with men;*

visit, was found in four (2%) low-risk MSM, one (1%) high-risk MSM and no (0%) MSW. The cut-off for having *on average* >10 recent sex partners (i.e. 'high-risk') was derived from the analysis shown in Table 6.2. Three separate multivariable linear mixed-effects models were constructed to analyze predictors of CD4 count, CD8 count, and CD4/CD8 ratio. The models include, in addition to two potential confounding variables (time since baseline and time in the day of blood draw), the following three predictor variables of interest: (1) MSW vs. MSM; (2) mean number of recent sex partners over all study visits of the participant; and (3) variation in number of recent sex partners between the mean and the actual visit. Being MSM and having either >10 or >30 recent sex partners *on average during study follow-up*, each were independently associated with higher CD8 counts ($P_{\text{MSM vs. MSW}}$ 0.003, $P_{>10 \text{ vs } \leq 2 \text{ partners}}$ 0.028, $P_{>30 \text{ vs } \leq 2 \text{ partners}}$ 0.14) and lower CD4/CD8 ratios ($P_{\text{MSM vs. MSW}}$ 0.001, $P_{>10 \text{ vs } \leq 2 \text{ partners}}$ 0.003, $P_{>30 \text{ vs } \leq 2 \text{ partners}}$ 0.017). Changes in an individual's number of

**TABLE 6.2** | Associations of being MSM and the reported number of sex partners in the six months prior to study visits with the absolute CD4 and CD8 counts and CD4/CD8 ratio. Results of multivariable linear mixed-effects models.

| | N[a] | $\text{Log}_e$(CD4) | | $\text{Log}_e$(CD8) | | $\text{Log}_e$(CD4/CD8) | |
|---|---|---|---|---|---|---|---|
| | | Coeff | 95% CI | Coeff | 95% CI | Coeff | 95% CI |
| <2 lifetime male-male sexual contact (MSW) | 181 | *ref* | *ref* | *ref* | *ref* | *ref* | *ref* |
| ≥2 lifetime male-male sexual contact (MSM) | 968 | -0.01 | -0.10, 0.07 | 0.19** | 0.06, 0.31 | -0.20** | -0.32, -0.08 |
| Mean number of sex partners during 6 months prior to study visits, per participant *(between participant variation)* | | | | | | | |
| ≤2 sex partners | 410 | *ref* | *ref* | *ref* | *ref* | *ref* | *ref* |
| >2-≤10 sex partners | 432 | -0.01 | -0.09, 0.06 | 0.02 | -0.08, 0.12 | -0.03 | -0.13, 0.07 |
| >10-≤30 sex partners | 225 | -0.05 | -0.14, 0.04 | 0.14* | 0.02, 0.27 | -0.19** | -0.31, -0.07 |
| >30 sex partners | 82 | -0.08 | -0.21, 0.05 | 0.14 | -0.05, 0.32 | -0.21* | -0.39, -0.04 |
| Deviation from individual's mean number of sex partners reported at current visit *(within participant variation)* | | | | | | | |
| ≤5 sex partners higher or lower | 931 | *ref* | *ref* | *ref* | *ref* | *ref* | *ref* |
| >5 sex partners higher | 105 | -0.02 | -0.07, 0.03 | -0.01 | -0.08, 0.05 | -0.00 | -0.05, 0.04 |
| >5 sex partners lower | 113 | -0.02 | -0.07, 0.03 | -0.02 | -0.09, 0.05 | 0.00 | -0.04, 0.05 |

*** P < .01 * P < .05 a. Number of measurements per variable category. Estimates are derived from three multivariable linear mixed-effects models with the $\log_e$ transformed absolute CD4 and CD8 counts and $\log_e$ transformed CD4/CD8 ratio as outcome variables. The models include a per participant random intercept and random slope for time since baseline, using an unstructured covariance matrix. Models also included adjustment for time since baseline and time in the day of blood draw. Abbreviations: Coeff, coefficient; CI, Confidence interval; Ref, reference category.*

recent sex partners during study follow-up were not associated with differences in CD4 or CD8 counts.

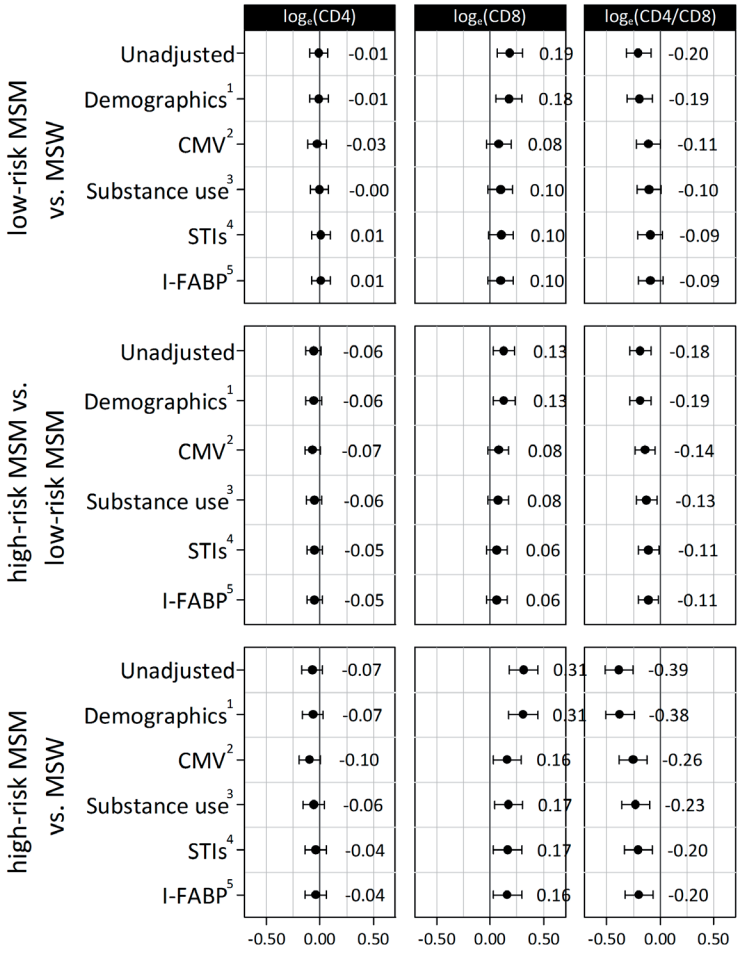


**FIGURE 6.3** | Differences in CD4- and CD8-counts and CD4/CD8 ratios between MSW, low-risk MSM and high-risk MSM, before and after stepwise forward adjustment for additional potential mediating factors.

*Values are the coefficients from differences between risk groups with 95% Confidence Intervals extracted from linear mixed-effects models. Adjustment was performed for: [1]educational level, time of blood draw, time since baseline [2]demographics plus CMV-serostatus at baseline [3]demographics, CMV, plus pack-years smoking, recent (<6 months) MDMA-use [4]demographics, CMV, substance use, plus recent (<1-2yr) syphilis or gonorrhea infections, hepatitis C RNA positivity at baseline [5]demographics, CMV, substance use, STIs, plus Intestinal fatty acid-binding protein level (i.e. marker of microbial translocation). Not independently associated with the CD4/CD8 ratio and therefore not included in the models: age, BMI, country of origin, current smoking status, recent (<6 months), GHB-, popper-, cocaine-use, daily marijuana use, Hepatitis B surface antigen positivity at baseline, recent (<1-2yr) chlamydia infection. Abbreviations: MSW, men who only have sex with women; MSM, men who have sex with men.*

## Lower CD4/CD8 ratio for a large part explained by higher CMV-seroprevalence in MSM

Figure 6.3 shows results of multivariable models adjusted both for potential confounders and mediators of the differences between MSW, low-risk MSM and high-risk MSM. The differences in CD4/CD8 ratios and absolute CD8 counts between groups were reduced after addition of baseline CMV-serostatus to the models, with the difference in CD4/CD8 ratio between MSW and low-risk MSM remaining borderline statistically significant (P=0.051). The differences in CD4/CD8 ratios between high-risk MSM and both low-risk MSM and MSW were also attenuated by 26% and 32% respectively, but remained statistically significant (P=.003 and P<.001 respectively). Supplementary Figure 6.1 illustrates differences in CD4/CD8 ratios in both CMV-positive and –negative subgroups. CMV IgG titers were not associated with a lower CD4/CD8 ratio, independently of CMV-serostatus, and therefore not included in the models. While other factors were independently associated with the CD4/CD8 ratio, they had little to no effect on the low-risk MSM and high-risk MSM coefficients. Table 6.3 shows the associations of the included covariates

**TABLE 6.3** | Associations of factors potentially driving differences in CD4/CD8 ratios, CD4 and CD8 counts, between high- and low-risk MSM and MSW. Results of multivariable linear mixed-effects models

| | FX/ TU[a] | $Log_e$ (CD4) *All measurements* | | $Log_e$ (CD8) *All measurements* | | $Log_e$ (CD4/CD8) *All measurements* | |
|---|---|---|---|---|---|---|---|
| | | *coeff* | *P Value* | *coeff* | *P Value* | *coeff* | *P Value* |
| **Including all follow-up measurements** | | | | | | | |
| Risk category | | | | | | | |
| MSW | FX | *ref* | *ref* | *ref* | *ref* | *ref* | *ref* |
| Low-risk MSM | FX | 0.01 | .8 | 0.10 | .10 | -0.09 | .12 |
| High-risk MSM | FX | -0.04 | .4 | **0.16** | **.018** | **-0.20** | **.003** |
| High educational attainment[b] | FX | -0.01 | .8 | 0.07 | .1 | -0.08 | .05 |
| Time of blood draw (per h later in the day) | TU | **0.02** | **<.001** | 0.01 | .1 | **0.01** | **.009** |
| CMV-seropositive at baseline | FX | **0.09** | **.02** | **0.41** | **<.001** | **-0.32** | **<.001** |
| Pack-years smoking per 5 pack-years [c] | TU | **0.03** | **<.001** | **0.01** | **.02** | **0.01** | **.01** |
| At least monthly use of MDMA during last 6 months | TU | -0.01 | .7 | 0.06 | .2 | **-0.09** | **.02** |
| Recent syphilis diagnosis [d] | TU | -0.06 | .2 | 0.05 | .4 | **-0.12** | **.01** |
| Recent gonorrhea diagnosis [e] | TU | **-0.07** | **.01** | 0.01 | .8 | **-0.08** | **.005** |
| Hepatitis C RNA positive at baseline | FX | 0.16 | .3 | **0.68** | **.001** | **-0.52** | **.01** |
| I-FABP (ng/mL, per $log_e$ increase) | FX | -0.02 | .5 | 0.04 | .2 | -0.05 | .09 |
| Time since baseline in years | TU | -0.00 | .6 | **-0.02** | **<.001** | **0.02** | **<.001** |

**TABLE 6.3 *(continued)*** | Associations of factors potentially driving differences in CD4/CD8 ratios, CD4 and CD8 counts, between high- and low-risk MSM and MSW. Results of multivariable linear mixed-effects models

| | FX/ TU[a] | $Log_e$ (CD4) *All measurements* | | $Log_e$ (CD8) *All measurements* | | $Log_e$ (CD4/CD8) *All measurements* | |
|---|---|---|---|---|---|---|---|
| | | *coeff* | *P Value* | *coeff* | *P Value* | *coeff* | *P Value* |
| **Sensitivity analyses including baseline measurements only** | | | | | | | |
| Risk category | | | | | | | |
| MSW | FX | *ref* | *ref* | *ref* | *ref* | *ref* | *ref* |
| Low-risk MSM | FX | 0.00 | .9 | 0.10 | .1 | -0.10 | .1 |
| High-risk MSM | FX | -0.06 | .3 | 0.13 | .06 | **-0.19** | **.006** |
| High educational attainment[b] | FX | 0.01 | .9 | **0.09** | **.04** | **-0.08** | **.05** |
| Time of blood draw (per h later in the day) | FX | **0.02** | **.05** | 0.00 | 1.0 | 0.02 | .2 |
| CMV-seropositive at baseline | FX | **0.08** | **.04** | **0.43** | **<.001** | **-0.35** | **<.001** |
| Pack-years smoking per 5 pack-years [c] | FX | **0.03** | **<.001** | 0.01 | 0.05 | **0.01** | **.02** |
| At least monthly use of MDMA during last 6 months | FX | 0.02 | .8 | 0.05 | .5 | -0.03 | .7 |
| Recent syphilis diagnosis [d] | FX | -0.06 | .6 | 0.13 | .4 | -0.19 | .2 |
| Recent gonorrhea diagnosis [e] | FX | -0.4 | .6 | -0.02 | .8 | -0.02 | .9 |
| Hepatitis C RNA positive at baseline | FX | 0.23 | .2 | **0.74** | **.001** | **-0.52** | **.014** |
| I-FABP (ng/mL, per $log_e$ increase) | FX | -0.02 | .4 | 0.03 | .4 | -0.05 | .1 |

*a. Variables are fixed over time (FX) or time-updated (TU). b. higher vocational education or university degree. c. 1 pack-year = 20 cigarettes/day during one year. d. rapid plasma reagin > 1:4, syphilis diagnosis at STI clinic in between visits or <1yr before baseline. e. diagnosis at recruitment visit or in between follow-up visits at the STI clinic. Abbreviations: coeff, coefficient; MSW, Men who only have sex with women; MSM, Men who have sex with men; CMV, cytomegalovirus; MDMA, 3,4-Methylenedioxy methamphetamine; RNA, Ribonucleic acid; I-FABP, Intestinal-Fatty Acid Binding Protein; ref, reference category.*

on the lymphocyte counts in the final multivariable models from Figure 6.3. Other than baseline CMV-serostatus, recent 3,4-methyleendioxymethamfetamine (MDMA; P=.02) use, a recent diagnosis of syphilis (P=.01) or gonorrhea (P=.005) and having chronic hepatitis C at baseline (P=.01) were also significantly associated with lower CD4/CD8 ratios. A history of tobacco smoking (expressed as pack-years smoking, P=.01), a blood draw later in the day (i.e. diurnal effect, P=.009) and time since baseline (P<.001) were associated with a higher CD4/CD8 ratio. Higher I-FABP levels were univariably associated with a lower CD4/CD8 ratio (P=.009), but no longer in the multivariable model (P=.09). sCD14, sCD163, IL-6, hs-CRP and D-dimer were not associated with the CD4/CD8 ratio (Supplementary Table 6.4). Sensitivity analyses limited to cross-sectional data from just the baseline visit showed similar results: after adjustment for CMV-serostatus and other covariates high-risk MSM still had a significantly lower CD4/CD8 ratio (P=.006), and higher CD8 count (P=.06) compared to MSW. Effect sizes were comparable to those found

in the full models [$\log_e$(CD4/CD8)high-risk MSM vs MSW -0.19 (baseline only) and -0.20 (full model); $\log_e$(CD8) high-risk MSM vs MSW 0.13 (baseline only) and 0.16 (full model)].

### High CD8 count and low CD4/CD8 ratio associated with T-cell activation and senescence

In the sub-group of participants with available data, both high CD8 counts and low CD4/CD8 ratios were strongly associated with higher percentages of senescent and activated T-cells, in both the CD4 and CD8 compartments (Supplementary Table 6.2). Moreover, the percentages of activated CD4 and CD8 T-cells were significantly higher among high-risk MSM compared to MSW and low-risk MSM (Supplementary Table 6.3). Albeit proportions of senescent CD4 and CD8 T-cells were also numerically higher in high-risk MSM compared to MSW and low-risk MSM, these differences were not statistically significant.

## DISCUSSION

In our analysis middle-aged HIV-negative MSM, especially MSM with a higher number of sex partners, had lower CD4/CD8 ratios, mostly driven by higher CD8 counts, compared to HIV-negative MSW. These differences for a large part were explained by a higher CMV-seroprevalence among MSM and high-risk MSM. The remainder of the differences in relative CD4 and CD8 counts could not fully be explained by other factors measured in our study. Several behavioural characteristics and recent STIs were more prevalent among MSM and also independently associated with a lower CD4/CD8 ratio, but did not influence the low-risk MSM and high-risk MSM coefficients. The independence of these factors (e.g. recent syphilis, gonorrhea or MDMA use) should be interpreted with caution. These factors are also strongly correlated with, and therefore a proxy of, high risk sexual behaviour which might not be fully adjusted for through the other variables in the models. Vieira et al also reported lower CD4 and higher CD8 counts in pre-exposure prophylaxis-using MSM diagnosed with asymptomatic chlamydia and/or gonorrhea, which was related to increased CD8 T-cell activation, similar to our findings. [25] Furthermore, repeated infection with different CMV strains could be responsible for increased CD8 counts in MSM. CMV-superinfection with a different strain has been demonstrated to occur in humans, most definitively in the context of women already known to be CMV-seropositive before becoming pregnant, delivering a child with symptomatic congenital CMV disease [28,29]. Similarly, in animal models CMV superinfection was demonstrated and was related to CD8 T-cell expansion.[30,31]. Other viruses, like herpes simplex virus 1/2, Epstein-Barr virus and human herpesvirus 8, are also more prevalent among MSM and could contribute to differences in immunological phenotypes.[22,32,33] Finally, Noguera-Julian et al showed MSM to have a different gut microbiome compared to MSW irrespective

of their HIV-status.[34] We found a trend of higher I-FABP (i.e. intestinal integrity marker) in those with lower CD4/CD8 ratios. Differences in the composition of the gut microbiome, for example caused by specific sexual practices or frequent antibiotic use, could hypothetically contribute to the lower CD4/CD8 ratios we observed in MSM. Contrary to our findings, previous studies, but conducted only in HIV-positive persons, have reported higher sCD14, sCD163, hs-CRP and IL-6 levels associated with a lower CD4/CD8 ratio.[4,35] Specifically, Serrano-Villar et al,[4] reported such associations related only to lower CD4 counts but not higher CD8 counts, and to be absent in HIV-positive participants with >500 CD4 cells/mm$^3$. In PLWH the association between lower CD4/CD8 ratios and increased innate immune activation markers therefore seems predominantly related to HIV-specific factors, in conjunction with poor CD4 count recovery after cART initiation. The association between a lower CD4/CD8 ratio and higher CD8 T-cell activation in the subgroup of our HIV-negative participants, with higher degrees of T-cell activation in high-risk MSM, resembles findings concerning T-cell activation in HIV-positive individuals.[4] Our subgroup however is insufficiently large and lacks the required follow-up time to demonstrate whether these changes in HIV-negative MSM are associated with increased risk of non-AIDS comorbidity and mortality as was shown in PLWH.[4]

## Strengths and limitations

The strengths of this study were the relatively large sample of MSM with a sexual risk behaviour profile comparable to that of many HIV-positive MSM, and the measurement of CD4 and CD8 counts at multiple time points, allowing us to account for the high variability of these indices over time. The extensive data collection including STI-positivity, immune activation markers, and data on sexual behaviour and recreational drug use allowed us to explore a multitude of potential factors driving the observed differences in T-lymphocyte counts. Important limitations include that data on chlamydia and gonorrhea infections were not systematically collected, implying that reported diagnosed STIs had been treated at the time of lymphocyte phenotyping, and those asymptomatically present during study visits may have remained undiagnosed. Furthermore, CMV and hepatitis B and C status were only determined at baseline. Thus incident super- or (re)infections with these viruses during follow-up could not be accounted for. Any such undiagnosed infections could have influenced our results, potentially explaining the remainder of the observed effect of being MSM. The cross-sectional baseline-only sensitivity analysis however suggested other causative mechanisms, as STI data at baseline were complete. Furthermore, the use of self-reported sexual behaviour data may potentially under- or overestimate true exposures, as participants may be inclined to provide socially acceptable answers and be subject to recall bias. The strong correlation of self-reported data with biomarkers of sexual risk such as STI incidence suggests that such bias may be limited in our study. Generalizability of these findings to the general

population of MSW and MSM is limited given recruitment of participants at a sexual health clinic, who are more likely to have exhibited more sexual risk behaviour. Finally, the classification of low- and high-risk behaviour based on ≤/> 10 sex partners was data-driven and might therefore not be reproducible in other cohorts.

### Conclusion and implications for future research

We observed lower CD4/CD8 ratios, mainly resulting from higher CD8 counts, in HIV-negative MSM compared to MSW. We show these differences could at least partially be explained by higher CMV-seroprevalence in MSM. MSM have high rates of STIs, but also psycho-social stress due to a social minority position,[36] often in conjunction with higher rates of recreational drug use. Future studies should attempt to evaluate the possible influence of such factors on immune system changes. In turn, these changes may be related to increased cardiovascular disease risk observed in both HIV-positive and –negative MSM.[37,38] For example, CMV-seroprevalence was much higher amongst MSM in our study and has been associated with cardiovascular disease development, frailty and mortality.[39-46] Our findings contribute to the understanding of why in many HIV-positive MSM who are treated with cART and have suppressed viremia the CD4/CD8 ratio and CD8 counts do not return to general population-based normal values. Many PLWH differ in their lifestyle compared to people from the general population. We show that such factors or exposures can impact their immune system. These immunological differences should thus not mistakenly be attributed only to HIV infection or its treatment in studies using general population control subjects. Our results also warrant further investigation into the effects of (CMV-induced) immunological differences on the occurrence of age-related comorbidities not only in HIV-positive but also HIV-negative MSM.

## AUTHOR CONTRIBUTIONS

SOV, FWW, EV, RvZ, NAK, MvdV, MFSvdL and PR contributed to the conceptualization and design of the study. SOV and EV performed data curation and contributed to data collection and coordination of the study. SOV and FWW designed the methodology of the study and conducted the formal analysis. PR obtained funding and supervised the conduct of the study. SOV provided the original draft of the manuscript including figures and tables. All authors critically revised and approved the final version for publication.

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# SUPPLEMENTARY MATERIAL

**SUPPLEMENTARY TABLE 6.1** | Overview of the number of available in-depth T-cell phenotyping in the HIV-negative participants included in the main analysis.

| | MSW | Low-risk MSM | High-risk MSM | Total |
|---|---|---|---|---|
| No. of participants<br>(% of total in main analysis) | 9<br>(12.5%) | 54<br>(21.2%) | 29<br>(25.2%) | 92<br>(20.9%) |
| No. of measurements | 18 | 132 | 76 | 226 |
| No. of measurements per participant | | | | |
| 0 | 64 (89%) | 200 (79%) | 85 (75%) | 349 (79%) |
| 1 | 2 (3%) | 6 (2%) | 3 (3%) | 11 (3%) |
| 2 | 2 (3%) | 18 (7%) | 5 (4%) | 25 (6%) |
| 3 | 4 (6%) | 30 (12%) | 21 (18%) | 55 (13%) |

**SUPPLEMENTARY TABLE 6.2** | Sub-group analysis (n=92) evaluating the associations of percentages of T-cell activation and senescence within the CD4 and CD8 T-cell compartments with the absolute CD4 and CD8 count and the CD4/CD8 ratio.

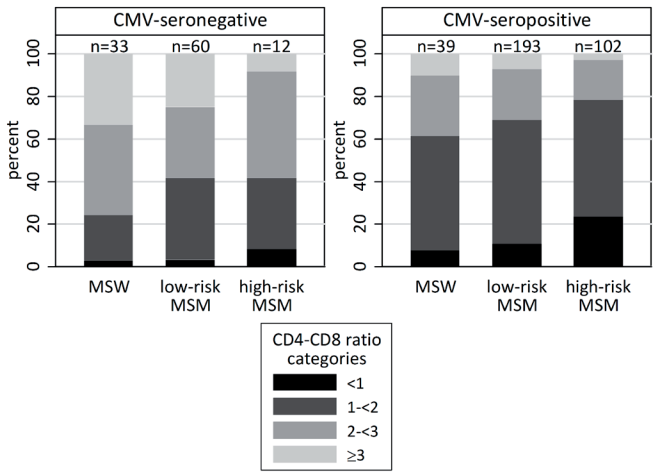
| | $Log_e$(CD4) | | $Log_e$(CD8) | | $Log_e$(CD4/CD8) | |
|---|---|---|---|---|---|---|
| | **Coeff[1] ($x10^3$)** | ***P*** | **Coeff[1] ($x10^3$)** | ***P*** | **Coeff[1] ($x10^3$)** | ***P*** |
| % Activated[2] CD4 cells | 7.19 | .6 | **62.32** | **.001** | **-41.73** | **.004** |
| % Senescence[3] in CD4 cells | **7.95** | **.001** | **21.51** | **<.001** | **-13.15** | **<.001** |
| % Activated[2] CD8 cells | 6.79 | .06 | **14.65** | **.001** | **-7.03** | **.04** |
| % Senescence[3] in CD8 cells | 2.51 | .05 | **10.63** | **<.001** | **-7.76** | **<.001** |

*1. Coefficient from univariable linear mixed effects models including a random intercept for each participant to adjust for repeated measures; 2. Percentage of CD38/HLA-DR double positive cells within the total CD4 or CD8 T cell population. 3. Percentage of CD27/CD28 double negative cells within the total CD4 or CD8 T cell populations (i.e. terminal differentiation of effector memory cells); Bold signifies P<.05.*

**SUPPLEMENTARY TABLE 6.3** | Sub-group analysis (n=92) evaluating differences in percentages activated and senescent CD4 and CD8 T-cells between MSW, and low- and high-risk MSM.

| | Low-risk MSM vs. MSW | | High-risk MSM vs. low-risk MSM | | High-risk MSM vs. MSW | |
|---|---|---|---|---|---|---|
| | % difference[1] | *P* | % difference[1] | *P* | % difference[1] | *P* |
| % Activated[2] CD4 cells | 0.4 | .2 | **0.6** | **.001** | **0.9** | **.004** |
| % Senescence[3] in CD4 cells | 2.4 | .5 | 1.1 | .6 | 3.4 | .3 |
| % Activated[2] CD8 cells | 0.8 | .5 | **2.5** | **<.001** | **3.3** | **.007** |
| % Senescence[3] in CD8 cells | 1.1 | .9 | 2.6 | .5 | 3.7 | .6 |

*1. Mean difference in percentage points between groups derived from univariable linear mixed effects models including a random intercept for each participant to adjust for repeated measures; 2. Percentage of CD38/HLA-DR double positive cells within the total CD4 or CD8 T cell population. 3. Percentage of CD27/CD28 double negative cells within the total CD4 or CD8 T cell population (i.e. terminal differentiation of effector memory cells); Bold signifies P<.05*




**SUPPLEMENTARY FIGURE 6.1** | Distribution of per participant mean CD4/CD8 ratio during follow-up stratified by baseline CMV-serostatus and risk group.

*CMV serostatus was missing for 1 low-risk MSM. Abbreviations: MSW, men who only have sex with women; MSM, men who have sex with men.*

**SUPPLEMENTARY TABLE 6.4** | Associations of factors potentially driving differences in CD4/CD8 ratios between MSW, low- and high-risk MSM. Results of univariable linear mixed-effects models.

| | | Coeff | 95% CI | *P* |
|---|---|---|---|---|
| Demographics | Baseline age per y | 0.08 | -0.00 , 0.05 | **.07** |
| | BMI in (per kg/m$^2$ increase) | 0.05 | -0.00 , 0.02 | .2 |
| | Ethnicity | | | |
| | Caucasian | *ref* | | *ref* |
| | African | 0.07 | -0.04 , 0.60 | **.09** |
| | Asian | 0.02 | -0.24 , 0.41 | .6 |
| | High educational attainment[a] | -0.11 | -0.20 , -0.02 | **.02** |
| Substance use | Daily alcohol use | 0.02 | -0.02 , 0.06 | .4 |
| | Heavy daily alcohol use[b] | 0.03 | -0.00 , 0.13 | **.08** |
| | Smoke status | | | |
| | Never smoker | *ref* | -0.05 , 0.12 | *ref* |
| | Former | 0.04 | -0.01 , 0.15 | .4 |
| | Current | 0.06 | 0.00 , 0.02 | .1 |
| | Pack-years[c] per 5 y | 0.10 | -0.07 , 0.01 | **.02** |
| | Recreational drug use (any drug) | -0.03 | -0.05 , 0.12 | .2 |
| | ≥monthly use of: | | | |
| | Marijuana | 0.00 | -0.05 , 0.06 | .9 |
| | MDMA | -0.06 | -0.17 , -0.04 | **.002** |
| | GHB | -0.02 | -0.14 , 0.04 | .3 |
| | Cocaine | -0.01 | -0.14 , 0.08 | .6 |
| | Poppers | 0.01 | -0.05 , 0.07 | .7 |
| | Daily marijuana use | 0.01 | -0.06 , 0.13 | .5 |
| | Former intravenous drug use | -0.06 | -0.73 , 0.17 | .2 |

**SUPPLEMENTARY TABLE 6.4 *(continued)*** | Associations of factors potentially driving differences in CD4/CD8 ratios between MSW, low- and high-risk MSM. Results of univariable linear mixed-effects models.

| | | Coeff | 95% CI | *P* |
|---|---|---|---|---|
| Coinfections | CMV-seropositive | -0.33 | -0.47 , -0.28 | **<.001** |
| | CMV-IgG titer (CMV-positive only) per $log_e$ increase | -0.03 | -0.09, 0.02 | .2 |
| | (History of) syphilis | | | |
| | Seronegative | *ref* | -0.12 , 0.04 | *ref* |
| | TPHA positive | -0.04 | -0.25 , -0.04 | .3 |
| | RPR >1:4 or recent diagnosis[d] | -0.05 | -0.14 , -0.02 | **.008** |
| | Gonorrhoea diagnosis[d] | -0.05 | -0.09 , 0.02 | **.006** |
| | Chlamydia diagnosis[d] | -0.02 | -0.12 , 0.04 | .2 |
| | Hepatitis B serostatus | | | |
| | No antibodies | *ref* | | *ref* |
| | Vaccinated | -0.03 | -0.13 , 0.07 | .6 |
| | Past infection | -0.14 | -0.26 , -0.04 | **.008** |
| | Chronic infection | -0.04 | -0.76 , 0.28 | .4 |
| | HCV RNA positive at baseline | -0.11 | -0.96 , -0.06 | **.03** |
| Bio-markers associated with inflammation | IFABP per $log_e$ increase | -0.12 | -0.16 , -0.02 | **.009** |
| | sCD14 per $log_e$ increase | -0.06 | -0.20 , 0.035 | .2 |
| | sCD163 per $log_e$ increase | 0.01 | -0.08 , 0.11 | .8 |
| | IL-6 (pg/mL) | | | |
| | ≤1 | *ref* | | *ref* |
| | 1-≤2 | 0.01 | -0.12 , 0.13 | .9 |
| | 2-≤10 | 0.02 | -0.11 , 0.14 | .8 |
| | >10 | -0.02 | -0.21 , 0.13 | .7 |
| | High sensitivity CRP (per quartile increase) | 0.02 | -0.02 , 0.06 | .4 |
| | D-dimer (per quartile increase) | 0.02 | -0.01 , 0.06 | .2 |

*Coefficient are derived from univariable linear mixed-effects models. a. higher vocational education or university degree b. for men > 4/day and woman >2/day International Units alcohol daily or almost daily c. 1 pack-year = 20 cigarettes/day during one year. d. diagnosis at recruitment visit or in between follow-up visits at the STI clinic. The models include a per participant random intercept and random slope for time since baseline, using an unstructured covariance matrix. Abbreviations: Coeff, coefficient; MDMA, 3,4-Methylenedioxymethamphetamine; GHB, gamma-Hydroxybutyric acid; CMV, cytomegalovirus; TPHA, Treponema pallidum particle agglutination assay; HCV, Hepatitis C Virus; RNA, Ribonucleic acid; IFABP, Intestinal fatty acid binding protein; IL-6, Interleukine-6; CRP, C-reactive protein.*

# 7

# General Discussion

## INTRODUCTION

With the use of highly effective combination antiretroviral therapy (cART), a growing number of people living with HIV (PLWH) is now able to reach advanced age. As part of the ageing process, PLWH who are living longer are also becoming at risk of developing ageing-related comorbidities (AACs). However, AACs have been shown to occur more frequently and possibly at younger ages in PLWH compared to the general population, even when HIV is adequately suppressed by treatment.[1,2] Aside from increasing age, developing AACs can be influenced by a multitude of potential risk factors, which can *only* (e.g. related to the presence of HIV infection or the use of antiretroviral drugs) or *more frequently* (e.g. substance use or having coinfections) be present among PLWH compared to the general population, as was already described in the introduction of this thesis (**Chapter 1**).

In this thesis, data are presented in which the *pulmonary function* of people ageing with HIV is evaluated. Furthermore, mechanisms that may contribute to the overall development of AACs among ageing PLWH are studied, specifically on *weight gain related to the use of integrase strand transfer inhibitors (INSTIs)*, the *co-occurrence of comorbidities* in PLWH, and *immunological characteristics of HIV-negative men who have sex with men (MSM)*. The latter is also relevant for PLWH since the majority of PLWH in the Netherlands are MSM.

The next paragraphs will first focus on these four themes separately, followed by a more general evaluation of what, in my view, the data from this thesis signify when considered together, and how they could guide future research in the field of ageing with HIV.

## HIV AND LONG-TERM PULMONARY HEALTH

### Pulmonary function of HIV-positive AGE$_h$IV participants

In chapters two and three, spirometry data collected as part of the AGE$_h$IV cohort study are used to evaluate the pulmonary function of PLWH aged 45 years and older, while predominantly being virally suppressed on cART. Several studies have reported the prevalence of *chronic obstructive pulmonary disease* (COPD) to be higher among PLWH compared to people without HIV, independent of current and prior smoking behaviour.[3] On the basis of these reports, we evaluated the prevalence of airway obstruction in HIV-positive vs. HIV-negative participants at study entry defined as [forced 1-second expiratory volume ($FEV_1$) / forced vital capacity (FVC) <70%], following the Global Initiative for Chronic Obstructive Lung Disease classification (**Chapter 2**).[4] Unexpectedly, the

prevalence of airway obstruction was similar in both study groups. In fact, when taking current and prior smoking behaviour into account, among those with a limited history of smoking (<25 pack-years) the prevalence of airway obstruction was *lower* in HIV-positive than HIV-negative participants, as indicated by a higher $FEV_1$/FVC ratio. Upon further analysis of the data, the difference in $FEV_1$/FVC appeared to be driven by a *lower FVC* among HIV-positive participants, with the $FEV_1$ being similar to that of HIV-negative participants. These results suggest that the impact of HIV per se on the lung may be more restrictive (i.e. interstitial) rather than obstructive in nature.[5]

We subsequently evaluated differences in spirometry indices between HIV-positive and HIV-negative study participants over time (**Chapter 3**). We found a faster decline in both $FEV_1$ and FVC over a period of about six years among HIV-positive than HIV-negative AGE$_h$IV participants, independent of smoking behaviour, with the $FEV_1$/FVC ratio declining at similar rates in the two study groups. This finding was in sharp contrast with the effect of smoking, which was associated with a steep decline in the $FEV_1$ and $FEV_1$/FVC ratio, but without affecting the rate of FVC decline. Once again, these arguably HIV-specific findings were not typical for the development of obstructive lung disease, but rather more indicative of either restrictive lung pathology or small-airway disease.[6]

## Pulmonary function of HIV-negative AGE$_h$IV controls

Similar to HIV-positive participants, almost one in four of the HIV-negative AGE$_h$IV 'lifestyle-comparable' control participants had airway obstruction at study entry. In a study similarly including Dutch participants of 45 years of age or older from the general population, the prevalence of airway obstruction was estimated at 4.7%.[7] Hence, the observed differences in lung function declines described above could have been strongly influenced by the control group. Had we compared our HIV-positive AGE$_h$IV participants with people from the general Dutch population, in whom airway obstruction is much less common, we would have likely found even larger differences between people with and without HIV.

COPD is characterized by an abnormal inflammatory response in pulmonary tissue as a result of inhaled particles, with exposure to tobacco smoke being by far the most important risk factor for COPD development in high income countries.[4] The resulting damage leads to both the *narrowing* of airways due to chronic airway inflammation as well as to the *dynamic collapse* of airways due to the loss of pulmonary elastic recoil as a result of parenchymal tissue damage (i.e. emphysema).[4] Since airway obstruction was also highly prevalent among never-smoking HIV-negative controls, lifetime exposures to inhaled particles other than first-hand cigarette smoke likely contributed to decreased pulmonary function in HIV-negative AGE$_h$IV participants. This may include, but not be

limited to, exposure to second-hand cigarette smoke. Since $FEV_1$ was similar in both study groups and both groups consisted of mostly MSM, these results could implicate that MSM in general may be at increased risk of developing obstructive lung disease, irrespective of their HIV-status. A similar prevalence of obstructive pulmonary disease between HIV-positive and HIV-negative MSM (9.7% and 8.7% respectively) from the US was also reported by the Multicenter AIDS Cohort Study (MACS) . Albeit lower than in $AGE_hIV$ participants, this prevalence was nevertheless remarkably high.[8] These findings merit further research into the pulmonary function of these MSM populations.

## HIV-associated chronic pulmonary disease

By using our 'lifestyle-comparable' control group, we were able to uncover characteristic differences attributable to HIV specifically. Singhvi et al have suggested that HIV is causative of a specific subtype of COPD, most distinctly characterized by low diffusion capacity.[9] It is recognized that COPD has multiple phenotypes and in some instances also involving interstitial and small-airway pathology.[10,11] However, since HIV-specific pulmonary changes have a different aetiology in comparison to 'classical' COPD (i.e. HIV infection vs. inhaled particles) and are not typically characterized by airway *obstruction*, as measured by spirometry[8,12], it can be questioned whether these HIV-specific pathophysiological changes should be called "Chronic *Obstructive* Pulmonary Disease". Assigning a different name to this potential, long-term, HIV-related comorbidity would aid in honing its characterization in future research (e.g. by differentiating it from smoking-related COPD), and in turn may also stimulate investigation of specific treatment and prevention strategies. Thus far, the term *HIV-associated chronic lung disease* has been used in several publications, primarily in studies evaluating chronic pulmonary changes in children with HIV, where the term 'COPD' would be out of place.[13,14] However, its definition currently lacks consensus and it is unclear whether the HIV-associated pulmonary changes seen in children with HIV are comparable to those seen in adult PLWH.

Future research will have to determine whether the HIV-associated accelerated declines in pulmonary function, which apparently can continue despite adequate viral suppression, will translate into distinct clinical signs and symptoms with time. It is imaginable that such HIV-associated pulmonary damage would only result in overt illness after prolonged exposure/infection, similar to how typically smoking-related COPD becomes clinically apparent only after decades of smoking. Studies using different diagnostic modalities (e.g. CT-imaging, diffusion capacity measurements and more experimental techniques to identify small-airway disease) should be used to better classify HIV-associated chronic lung abnormalities. In the meantime, clinicians should be made aware of the specific pulmonary changes that appear associated with HIV in order to

encourage careful diagnostic evaluations of their HIV-positive patients presenting with respiratory symptoms.

Our data show obstructive lung disease was highly prevalent among both HIV-positive and HIV-negative $AGE_hIV$ participants, which predominantly consisted of MSM. Future studies should evaluate whether women and heterosexual men with HIV are at similar risk for impairments in pulmonary function. Since smoking cessation remains the single most effective method of preventing COPD, relentless efforts should be made to encourage this intervention, as well as to provide smoke-free occupational and recreational environments to prevent damage from second-hand smoke.

## NOVEL ANTIRETROVIRALS AND BODYWEIGHT

Although antiretroviral drugs have greatly improved the life expectancy of PLWH, their long-term use may have adverse effects and thereby contribute to the development of AACs. We evaluated whether switch to an INSTI-containing cART regimen in virally-suppressed HIV-positive $AGE_hIV$ participants led to a greater increase in bodyweight compared to HIV-positive participants not switching to an INSTI-containing cART regimen, and also when compared to bodyweight changes in HIV-negative study participants (**Chapter 4**). Switch to INSTI was not associated with an increase in mean bodyweight, but prominent weight gain (>5%) did occur statistically more frequently among INSTI-switching participants compared to HIV-positive participants not switching to an INSTI.

### Inconclusive results

In recent years a substantial number of studies have evaluated the effects of the latest generations of antiretroviral drugs on bodyweight.[15,16] INSTIs as well as the most novel nucleotide reverse transcriptase inhibitor *tenofovir alafenamide* (TAF) have received the most attention.[15] Despite the large amount of data presented thus far, the exact extent and characteristics of this purported adverse effect - and which drugs are responsible for this effect - remain incompletely understood. The most reliable studies involve those in which PLWH already virally suppressed who *switch* their cART regimen are evaluated, thereby removing the impact of the well-known, return-to-health effect occurring in PLWH after initiating cART for the first time. Such switch studies have nonetheless led to conflicting results. When evaluating INSTIs, some studies have found associations with an increase in mean bodyweight (across the INSTI class, or only with specific drugs),[17,18] while others have been unable to replicate these findings[19,20]. Our own findings (**Chapter 4**) reported in this thesis are an example of the latter. It appears that INSTIs contribute to weight gain *only* or *predominantly* in specific people and under specific circumstances;

such as women, black people, when combined with TAF, or related to the type (or absence) of antiretrovirals used prior to switch.[15,16,18] Moreover, the *severity* of the associated weight gain has been inconsistent, with many of the studies finding statistically significant gains in mean weight, e.g in the order of approximately 2 kilogram; which are likely to be of little clinical relevance in individuals whose pre-INSTI body weight was within the normal range.[17]

In the ADVANCE trial (for a detailed description of this trial see chapter 4), however, cART-associated weight gains *were* of clinical relevance. In ART-naive Black female participants, treatment-emergent obesity at week 96 occurred in 28% of participants in the dolutegravir(DGT)/TAF/emtricitabine(FTC) study arm, compared to 18% in the DGT/tenofovir disoproxil(TDF)/FTC and 12% in the efavirenz/TDF/FTC study arms.[21] A subsequent sub-group analysis of participants from the efavirenz/TDF/FTC comparator-arm showed participants with slow metabolism of efavirenz due to a loss-of-function CYP2B6 polymorphism to have less weight gain compared to those with extensive metabolism of the drug.[22] Extensively metabolizing participants randomized to the efavirenz-containing regimen showed similarly large weight gains as participants in the dolutegravir/tenofovir disoproxil/emtricitabine arm. This would at least partly explain the ADVANCE trial results, since efavirenz may have been more likely to have *prevented* weight gain in some participants, for instance, by suppressing their appetite or by causing other gastro-intestinal symptoms, rather than, or in addition to, dolutegravir having *caused* it. The CYP2B6 slow metabolizer polymorphism is more frequent among people of African descent[23] and given that the ADVANCE trial was conducted in South Africa, this could also explain why the reported group differences were especially large in this particular setting.

CYP2B6 polymorphisms do not explain the effect of TAF in the ADVANCE trial. TAF has also been associated with greater-than-expected weight gain in other studies after switching from TDF to TAF in PLWH who are virally suppressed.[24,25] However, similar to efavirenz, the comparator TDF has also been suggested to have a weight suppressive effect. In randomized clinical studies of Pre-Exposure Prophylaxis (PrEP) in HIV-negative individuals, participants using TDF/FTC showed less age-associated weight gain when compared to placebo,[26,27] when compared to cabotegravir,[28] and when compared to TAF/FTC.[29]

A valuable next step would be to determine whether the *frequency of clinically relevant weight gain* (e.g. >10% of bodyweight) is increased with the use of TAF, and which PLWH would be at greatest risk. The approach taken in **Chapter 4** of this thesis, evaluating >5% and >10% weight gain as an outcome, would be suitable in studying TAF as well.

Relatively large study populations would be required to investigate this presumably rare outcome. As the ADVANCE trial results have illustrated, these study populations will also need to include adequate numbers of individuals with different ethnicity and gender, as effects may differ by these demographic characteristics.

### Weight gain and cART in clinical practice

The results regarding the effects of various antiretrovirals on bodyweight are still inconclusive and represent a considerable challenge for clinicians. Bodyweight fluctuates considerably (see Figure 4.2) and these fluctuations can be influenced by a variety of factors, many of which are directly (e.g. diet or exercise) or indirectly (e.g. psychological stress, cessation of smoking, etc.) related to behaviour that may be difficult to alter.[30] Attributing a patient's gain in bodyweight to the use of certain antiretrovirals and subsequently altering their cART regimen may, in such situations, be tempting for both the physician and patient. However, (1) reversibility of the purported antiretroviral-associated weight gain has not been demonstrated; (2) if the weight gain is caused by other factors, switching cART will not result in the desired weight loss; and (3) it will expose the patient to other antiretroviral drugs that may be less favourable in other ways (e.g. based on their toxicity profile). Nonetheless, both INSTIs and TAF have shown consistent associations with higher weight gain in the international literature. Therefore, in case of clinically significant weight gain following the initiation of an INSTI or TAF, and with other explanations for the weight gain excluded or unlikely, switching away from TAF or INSTIs could be considered, in addition to other measures such as providing dietary recommendations.

## COMORBIDITY CLUSTERING IN PLWH

### Comorbidity clustering

Ageing PLWH on long-term suppressive cART are observed to be at increased risk for many comorbidities, as discussed throughout this thesis. Understanding why or in whom certain comorbidities co-occur can potentially be used to identify common risk factors. Such common risk factors have been well-established in prior research as underlying reasons for the clusters reported, particularly regarding the co-occurrence of cardiovascular diseases (e.g. smoking, overweight, dyslipidemia, genetic predisposition, etc.), STIs (e.g. sexual risk behaviour, sexual networks, etc.) and AIDS-defining conditions (i.e. impaired immunity) (**Chapter 5**). Finding common risk factors can be of great value, since an intervention directed at *one* of these risk factors (e.g. to stop smoking) may decrease the risk for *multiple* comorbidities.

In that context, an intriguing finding was that, among POPPY participants, *mental health disorders* did not only cluster together, but the cluster of *mental health disorders* was also associated with every other cluster of comorbidities identified. It can be debated whether mental illness is a cause or consequence of these other comorbidities, or whether there only exists a correlation but no causal relationship between these comorbidities. However, these data suggest interventions improving mental health could potentially be beneficial for more than just mental health itself, but also for a host of other comorbidities. The correlation between mental health and comorbidity development is discussed in more detail in the later paragraphs.

## CHRONIC INFLAMMATION AND THE DEVELOPMENT OF AACS IN PLWH

### Low CD4/CD8 ratios among HIV-negative MSM

Increased levels of numerous markers of inflammation and immune activation have been associated with the development of AACs in both HIV-positive and -negative populations.[31,32] One marker of interest has been the CD4/CD8 T-cell ratio, which frequently remains low in PLWH despite viral suppression on cART.[33] A low CD4/CD8 ratio has been associated with the development of AACs in both people with and without HIV.[34,35] Similar to other markers, the CD4/CD8 ratio may be influenced by multiple factors, and especially by HIV and cytomegalovirus (CMV) infections.[36] Since MSM are at increased risk of both of these and other sexually transmitted infections, we studied the CD4/CD8 ratio and other T-cell markers among MSM (**Chapter 6**). To remove the well-known effects of HIV infection on both the CD4 and CD8 counts, we only compared HIV-negative MSM with HIV-negative heterosexual men included in the $AGE_hIV$ cohort. Our results show the CD4/CD8 ratio to be lower in HIV-negative MSM compared to HIV-negative heterosexual men, which was primarily driven by a higher absolute CD8 T-cell count in MSM. These differences remained statistically significant, even after adjustment for the CMV-serostatus.

### Immunological profile of HIV-positive and HIV-negative MSM

These results show that persisting low CD4/CD8 T-cell ratios in HIV-positive MSM, in spite of antiretroviral treatment, may in part be attributable to being MSM and belonging to the 'MSM community' rather than their HIV-status. Use of 'lifestyle-comparable' (i.e. predominantly MSM) HIV-negative controls in both the $AGE_hIV$ and COBRA (including $AGE_hIV$ and POPPY cohort study participants, see thesis introduction for cohort details) cohorts has resulted in several similar findings, where presumed HIV-related immunological and

other characteristics were also seen in the HIV-negative 'lifestyle-comparable' controls, or specifically among MSM and not among heterosexual participants:

In the COBRA cohort study (1) terminally differentiated ($CD57^+$ and $CD27^-CD28^-$) $CD4^+$ and $CD8^+$ T cells of HIV-positive participants were increased compared to HIV-negative blood donors, but similar to HIV-negative 'lifestyle-comparable' controls [37]; (2) a characteristic high monocyte activation marker profile was identified using a panel of cellular monocyte activation markers, which only (and equally often) occurred in HIV-positive and -negative COBRA participants, but not in HIV-negative blood donors[38]; and (3) biological age advancement (using a panel of markers previously associated with ageing (MARK-AGE panel) and which includes epigenetic, endocrine and other markers) of HIV-negative COBRA participants was estimated as lower compared to HIV-positive COBRA participants, but higher compared to blood donors.[39] Furthermore, in a subsample of the $AGE_hIV$ cohort, the gut microbiome of $AGE_hIV$ participants showed enrichment of *Prevotella* and increased *alpha diversity* both among HIV-positive and -negative MSM compared to both heterosexual HIV-positive and HIV-negative $AGE_hIV$ participants.[40] Microbial dysbiosis can contribute to increased levels of chronic inflammation.[41]

## Being MSM as a confounding or causal factor in the aetiology of AACs among PLWH

These results provide evidence for the concept that PLWH may display certain immunological characteristics by virtue of them frequently being MSM or belonging to the MSM community, rather than by virtue of having HIV. In conventional pathophysiological models, HIV is believed to cause AACs through chronic inflammation (Figure 7.1a). However, if being MSM is also - independent from HIV - associated with markers in these mechanistic pathways, two potential additional causal pathways for AAC development among PLWH (and MSM) should be considered. Importantly, since PLWH who are not MSM are also at increased risk of developing AACs, these additional pathways will not explain the entirety of HIV-associated comorbidity risk. These *additional* pathways therefore do not represent an alternative to the conventional hypothesized pathway, but should be viewed *in addition* to the conventional pathway.

*Additional hypothesized causal pathway 1: Chronic Inflammation as effect of being MSM and not an intermediate cause for AAC development (*Figure 7.1b*):* In this scenario, increased levels of a certain inflammatory marker are associated with both HIV and AACs, while the causal pathway between HIV infection and AACs is not mediated by elevated levels of the marker. In fact, the association between HIV-status and the inflammatory marker is confounded by the higher prevalence of PLWH being MSM in PLWH, where MSM are more likely to have increased levels of the inflammatory marker. The associa-

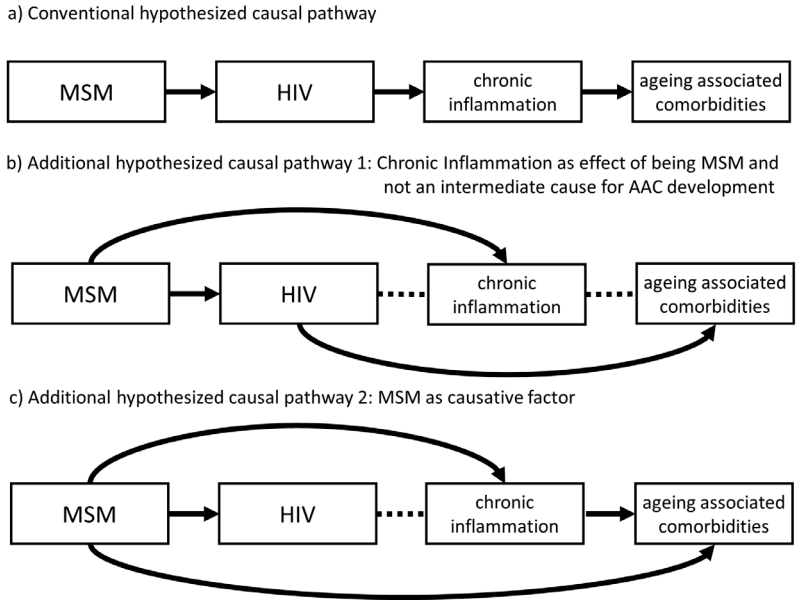



**FIGURE 7.1** | Additional causal pathways in the development of ageing associated comorbidities among PLWH in relation to chronic inflammation and men who have sex with men

tion between the inflammatory marker and AACs can be confounded by the fact that PLWH develop AACs more frequently through mechanisms other than inflammation, but are also more often MSM who have higher levels of the studied inflammatory marker. Studies comparing HIV-positive participants that include a majority of MSM with non-MSM HIV-negative controls are unable to distinguish between the conventional pathway (Figure 7.1a) and this potential additional confounding pathway (Figure 7.1b). This creates a risk for studies evaluating the effects of experimental interventions that target the identified inflammatory markers in aiming to reduce AAC risk in PLWH. If such a specific inflammatory marker is not on the causal pathway to AACs, these interventions are likely to be ineffective.

*Additional hypothesized causal pathway 2: MSM as causative factor (*Figure 7.1c*):* In this scenario, MSM, regardless of HIV-status, are at increased risk of AACs. This would mean that even if an HIV infection were to be cured, MSM formerly living with HIV would remain at increased risk of AACs. Moreover, it means that not only HIV-positive, but also HIV-negative MSM may be at increased risk of AACs, which requires further study and potentially tailored interventions in HIV-positive and -negative MSM. An example of this pathway is the increased prevalence of chronic hepatitis B infections among MSM, likely driving an increased risk of hepatocellular carcinoma among MSM.

In general, which combination of the hypothesized causal pathways in Figure 7.1 applies will most likely vary depending on the specific inflammatory marker/pathway, AAC and population studied. However, the concept could also be extended to other key populations at risk for HIV infection, such as transgender people, people who inject drugs, sex workers or migrants. Especially in high income countries, people at risk for HIV typically belong to these key populations with characteristics that differ from the general population (e.g. related to social or economic status or behavioural risk factors, such as substance use and sexual behaviour), which can influence inflammatory markers or contribute to AAC development. Not taking these factors into account introduces a risk of confounding (Figure 7.1b) and thereby misinterpretation of study results and likely increased error of not recognizing specific (HIV-negative) populations at risk for AACs (Figure 7.1c).

## THE NEED FOR A BIOPSYCHOSOCIAL PERSPECTIVE IN HIV & AGEING RESEARCH

### HIV and biological risk factors for AAC development

The findings from the research presented in this thesis suggest that treated HIV infection may be related to a specific phenotype of pulmonary function decline, and that the use of INSTIs in a small minority of PLWH may be related to a marked and clinically relevant increase in bodyweight. We were able to identify these specific HIV- and cART-related biological health effects through the use of a '*lifestyle-comparable*' control group without HIV. This allowed us to account for confounding risk factors biologically-unrelated to HIV infection and/or the use of cART. However, some of these biologically HIV and/or cART-unrelated risk factors *were* shown to play a role in the occurrence of AACs among both PLWH and those at risk of HIV, such as airway obstruction being highly frequent among both HIV-positive and -negative $AGE_hIV$ participants. Moreover, immunological characteristics related to the development of AACs in PLWH were *also* seen in the 'lifestyle-comparable' HIV-negative $AGE_hIV$ controls (e.g. low CD4/CD8 ratios).

### Lifestyle

*Lifestyle* is widely recognized as an important factor in determining the risk of developing AACs, and also associated with differences in immunological markers.[32,42] In this context, the term *lifestyle* typically refers to specific behavioural risk factors, namely diet, physical exercise and the use of substances, such as tobacco and alcohol. The results from chapter 6 suggest high numbers of sexual partners may have to be added to this list, especially in the context of ageing with - or whilst at risk of acquiring - HIV.

However, in studies investigating the occurrence of AAC among PLWH, separately adjusting for these single 'traditional' risk factors may not fully account for the entire spectrum of differences between the general population and people from key populations at risk for HIV, such as people from communities of MSM, sex workers or injection drug users. As discussed in the introduction of this thesis, people from these communities have often faced social adversities (e.g. stigma and discrimination) or other communal exposures (e.g. STI epidemics, trends in substance use) during their lifetime, which are likely to have contributed to an *exposome* (a broader term than lifestyle, characterizing the entirety of internal and external exposures accumulated during a lifetime) to be collectively different compared to people from the general population. These lifestyle-related differences - not captured by traditional risk factors - can lead to confounding in studies not using 'lifestyle-comparable' controls (i.e. people from similar communities). Both the high rate of airway obstruction measured in never-smoking HIV-negative participants and the lower CD4/CD8 ratios in HIV-negative MSM also after adjustment for CMV seropositivity are examples of lifestyle-related influences that would not have been captured by adjustment for known risk factors (i.e. smoking and CMV-seropositivity, respectively) related to the study outcomes.

For researchers in the field of HIV and ageing, lifestyle-related risk factors are often considered *confounders* in the effects that they are aiming to study.[43] Typically, attempts are made to distil the precise *biological* effects of HIV and then to *biologically* influence those effects through the use of *biomedical* interventions, e.g. developing an anti-inflammatory drug. In this process, the confounding described above can lead to erroneous conclusions and ineffective experimental interventions. Moreover, as results from this thesis show, even if all biological consequences of HIV itself were to be obviated, some PLWH may remain at increased risk of AACs compared to the general population. Thus, these biologically HIV-unrelated 'lifestyle' factors require attention from HIV researchers not only to reduce confounding in biomedical studies, but also to be included in their studies as potential targets if the aim is to provide full equality in health for PLWH compared to people from the general population.

When aiming to determine 'lifestyle differences' between PLWH and the general population and the consequences thereof, the term 'lifestyle' should be used with care. Often, the term implies a *free and conscious behavioural choice*. However, many of the circumstances giving rise to the lifestyle differences of PLWH and those at risk of acquiring HIV were not *chosen* by themselves, but rather *imposed,* either by nature (e.g. sexual orientation, the HIV epidemic) or society (e.g. social exclusion or stigma). To designate behaviour resulting from these circumstances 'poor lifestyle choices' would be - at least partly - inaccurate, since the reasons for the behaviour are likely to originate from factors

outside the individual's realm of influence. The term *health behaviours* more neutrally reflects that behaviours influencing health can be driven both by personal choices as well as the societal and natural circumstances of the individual.

## A biopsychosocial perspective

In 1980 the internist and psychiatrist G.L. Engel first introduced the *biopsychosocial model* to describe the multi-level impact somatic illnesses can have on a human being and their surroundings (i.e. from the level of molecules, proteins and cells to that of the mind/nervous system, interpersonal relationships, communities and societies) and how these are all interconnected.[44] With the introduction of this model, he challenged the traditional biomedical approach by showing how illnesses can develop and be prevented by factors in a context larger than simply from a biomedical perspective. Originally, Dr. Engel described and depicted the biopsychosocial model as a hierarchy of separate interconnected systems (Figure 7.2a), where each system exits within a larger system (Figure 7.2b). The model can be used to understand the full impact of a somatic illness. For example, pneumonia causes disturbances on the *tissue level* of the lungs, resulting in the lower *cell system* to initiate an inflammatory response with the production of cytokines (*molecular level*), while at the higher level systems of the *organ*, functional impairment occurs; of the *individual* (i.e. nervous system), subjective illness is experienced; and of the *interpersonal relationships*, care from others may be required. More recently, the biopsychosocial model has also been used to identify causal pathways of health disturbances, whereby the model is described and depicted as a trinity in which the biological, psychological and social domains can influence each other independently (Figure 7.2c).[45]

In my view, which of these models best represents reality is less important compared to their ability to successfully challenge the thinking of biomedical researchers and clinicians. In my own personal attempt to do so with regards to the development of AACs among PLWH, Figure 7.2d represents the biopsychosocial model as a multi-layered (semi)sphere, with at its core the development of AACs. Separations between systems are non-descriptive and blurred, illustrating the diffuse and intertwined relationships that all systems have with one another, while still maintaining the hierarchical order of each system functioning within the context of a higher ordered one. Behaviour is added as a separate system, while it can be recognized as a separate potential target to mitigate the occurrence of AACs and be influenced by – but not identical to – psychological risk factors. Psychological risk factors influence behaviour, but may also have direct consequences for biological health. For example, chronic psychological stress may - through activation of the immune system - independently affect AAC risk, which will be discussed in the next paragraph.

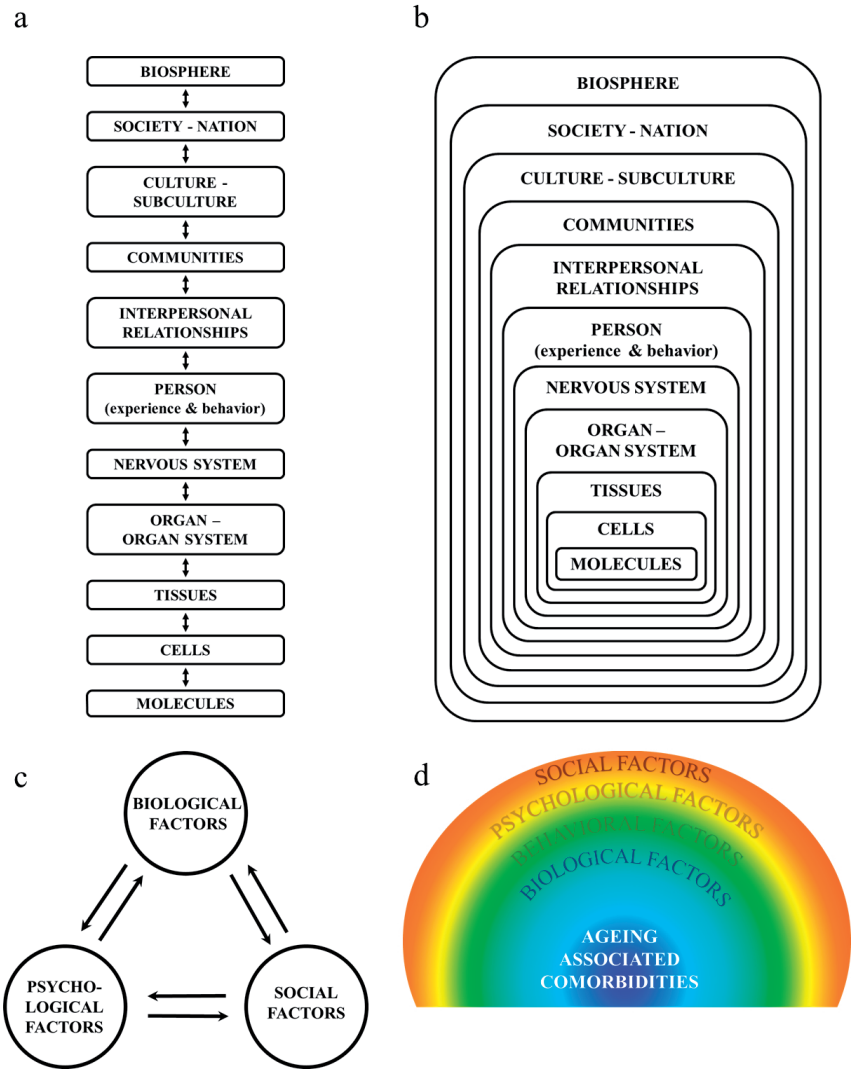



**FIGURE 7.2** | Various graphical representations of the biopsychosocial model.

*a. Simplified representation of the hierarchy of natural systems as described by G.L. Engel.[44] b. Simplified representation of the continuum of natural systems as described by G.L. Engel.[44] c. Contemporary representation of the biopsychosocial model.[45] d. Suggested conceptual illustration of the aetiology of ageing associated comorbidities (among PLWH and those at risk for HIV) from a biopsychosocial perspective (personal perspective).*

Using a biopsychosocial approach that recognizes the occurrence of illness as it originates from more than only biological factors may make the origin of illness seem too complex to study. Psychological and social risk factors are difficult to simulate in laboratory environments and in real-world data true causes are almost impossible to distinguish from confounding factors. Nevertheless, *disregarding* the biopsychosocial

perspective may lead to erroneous (i.e. confounded) conclusions in biomedical research, as has been illustrated earlier. For example, it can, in the case of AAC development in PLWH, lead to inflation of the number of comorbidities being associated with HIV (e.g. COPD), while these comorbidities may in fact be *biologically* unrelated. Additionally, it may result in spending a considerable amount of time and resources on relative trivialities (e.g. minor potential side-effects from antiretroviral drugs), while 'the elephant in the room' (e.g. modification of traditional risk factors) is being ignored.[46] *Adopting* the biopsychosocial model can inspire to create new research questions and approaches that may otherwise be overlooked and consequently given insufficient priority. Several of these questions are suggested in the following paragraphs.

## FUTURE OPPORTUNITIES

### Inflammation, chronic stress, HIV and sexual orientation

Systemic chronic inflammation (SCI) has been recognized as a contributing factor in the development of many AACs and can be caused by a variety of factors, including environmental and behavioural 'lifestyle' factors.[32] One additional factor thought to contribute to higher levels of SCI is that of *chronic stress*.[47] Stress is the body's physiological response to a threat, most distinctly characterized by activation of the hypothalamic-pituitary-adrenal (HPA)-axis and the autonomic nervous system.[48] Although initially described as the physiological response to *acute* threats (e.g. eliciting a fight or flight response), also chronic stressors, both physical and psychological, are able to induce chronic physiological stress responses.

Recently, a novel pathway from stress to inflammation and subsequent cardiovascular disease was visualized in humans with the use of PET-CT imaging.[49] Activity in the amygdala (i.e. area of the brain involved in the stress response) and high perceived stress levels were associated with increased metabolic activity in the bone marrow and the arterial wall (suggestive of vascular wall inflammation), as well as with the occurrence of cardiovascular events. Experimental murine models have shown stress to activate hematopoietic stem cells in the bone marrow via the $ß_3$ adrenergic receptor, resulting in increased production of inflammatory leukocytes.[50]

There are two reasons why these findings may be specifically relevant in the context of AAC development in people with and at risk of acquiring HIV:

1. Many PLWH are members of sexual or gender minorities who may be subjected to chronic *minority stress*.[51] Minority stress is believed to occur in response to adverse social conditions for people belonging to minority populations. This form of chronic

stress is related to the increased occurrence of mental health and substance use disorders.[51,52] However, the aforementioned findings[49] give rise to the question in what way chronic minority stress may also contribute to the occurrence of AACs, such as cardiovascular diseases, through the pathway of increased bone marrow activity and chronic inflammation.

2. In 2008, Savic et al studied the hemispheric asymmetry and functional connectivity using PET/MRI brain-imaging in homosexual and heterosexual men and women.[53] Cerebral hemisphere volumes were symmetrical among homosexual men and heterosexual women, while heterosexual men and homosexual women showed rightward cerebral asymmetry. Moreover, functional connectivity of the amygdala in homosexual men also resembled that of heterosexual women more than that of heterosexual men and homosexual women (i.e. connections were more widespread from the left vs. the right amygdala, and the amygdala was more often connected with the contralateral amygdala, the anterior cingulate, the subcallosum and the hypothalamus vs. the putamen and the orbitofrontal and prefrontal cortex in homosexual vs. heterosexual men, respectively). These findings give rise to the question whether stress (involving the amygdala) may be characterized or perceived differently in homosexual compared to heterosexual men.

*Note: Studies investigating the biological cause and consequences of homosexuality have been scarce due to their controversial nature, as they could undesirably pave the way to a potential 'cure' for homosexuality.[54] However, these data provide reason to think that there may also be biological, in addition to sociological, etiological risk factors for health problems among sexual minority populations. Studies further investigating this research question should - with care and in close collaboration with people from these minority populations - be considered in my view, as they could lead to tailored prevention and treatment strategies and thereby improvement of the quality of life for people from these minority populations.*

Together, these findings suggest that stress could, in a unique way, be a contributing risk factor for AAC development among both PLWH and MSM, mediated by social, psychological and biological factors. This potential pathway merits further investigation, especially since both psychological interventions (e.g. cognitive behavioural therapy) and several psychotropic drugs have been reported to have immunomodulatory effects, which could be beneficial in reducing AAC risk.[55,56]

## Queer health

Only since the 1960s have diversity in sexual orientation and gender identity become more openly accepted by the general Dutch population, just before - and also in tandem with - the beginning of the HIV epidemic (see Introduction). Now is not only the time that the first PLWH are reaching advanced age, but similarly, for the first people who

have lived lives in which they have freely and openly expressed their gender and sexual identities. Openly expressing one's sexual and gender identities for many signifies living a life different from the norm, i.e. having a *queer* 'lifestyle'. The term queer (meaning strange, odd or peculiar, and formerly used as a derogatory word for gay people) has been linguistically reappropriated to describe people accepting certain aspects of their own sexual and gender identities to be different from the norm.[57]

Being queer can have biological (e.g. STIs associated with having multiple sexual partners or biologically associated with homosexuality as discussed earlier), psychological (e.g. minority stress, alternative 'queer' families) and social (e.g. belonging to a community with characteristic traditions) consequences that can affect a person's health, both positively and negatively. This thesis has presented data that MSM may indeed be at increased risk of developing AACs, which merits further study.

One way to do so would be by including questions regarding sexual orientation, sexual behaviour and gender identity in large, health studies in the general population, similar to inquiring about age or income. It would allow researchers to identify which characteristics of queer people may have long-term positive or negative health consequences when compared to other groups in the general population, and thereby enable the development of tailored health interventions. This would also enrich research into the consequences of health risks related to PLWH, as these may overlap. Moreover, specifically in the context of HIV research, dual control groups could be considered. If both people from key populations at risk of HIV as well as people from the general population are included as controls, health effects associated with being part of the key population may be more easily disentangled from those biologically associated with HIV, and also interaction effects between these factors can be studied. Accordingly, both confounding effects can be adjusted for and potential health risks for those at increased risk for HIV (e.g. queer people) can be identified.

## CONCLUSIONS

In this thesis, I have presented data that identified two health concerns specifically, and most likely biologically, related to ageing with HIV, i.e. (1) the occurrence of HIV-associated decreased pulmonary function despite antiretroviral therapy, phenotypically suggestive of interstitial or small-airway pathology, and (2) the rare occurrence of severe weight gain related to the use of INSTIs. Moreover, I have presented data showing that people who are *at increased risk of acquiring,* but without, HIV may also be at increased risk of acquiring AACs through similar causal pathways potentially driving the increased

risk of AAC in PLWH, or that the characteristics of people from these communities may confound findings in biological studies. This means that researchers in the field of HIV and ageing may need to widen their scope and look at the characteristics and circumstances of those *at risk of acquiring* HIV to identify potential additional drivers of AAC risk among PLWH. In order to do so, a biopsychosocial, instead of a solely biomedical perspective, could provide guidance, as it is only with such a perspective that the health discrepancies between people ageing with and without HIV can be determined as a whole.

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# 8

# Summary & Nederlandse Samenvatting

## SUMMARY

**Title:** **Disentangling Health Risks for People Ageing with - or whilst at Risk of Acquiring - HIV in the Netherlands**

**Subtitle:** **Living with a Legacy**

Living with HIV fundamentally changed in 1996 with the introduction of Highly Active Antiretroviral Therapy (HAART), a treatment that transformed the infection from a fatal illness to a chronic condition. This scientific development has over the last decades enabled, and continues to enable, people living with HIV (PLWH) to age. Naturally, this puts an increasing number of PLWH at risk of developing ageing associated comorbidities (AACs), such as cardiovascular diseases and cancers. PLWH are reported to present more frequently, and possibly at younger ages, with such AACs. This thesis discusses in detail the pulmonary function of people ageing with HIV as well as several potential causes of the difference in observed AAC prevalence between people ageing with and without HIV.

**Chapter 1** of this thesis describes the historical background of the HIV epidemic, the evolving treatments for the infection as well as the background of key populations affected by HIV in the Netherlands. This is relevant as prior exposure to untreated HIV, AIDS-related illness, as well as to often toxic evolving therapeutic strategies for PLWH may have inflicted organ damage, which later on continues to influence the probability of AAC development. Moreover, the introduction of HIV in specific communities, such as those of men who have sex with men (MSM), did not occur in a historical vacuum but in tandem with other major societal developments. Civil rights and the societal position of sexual minorities have rapidly changed over the last decades. Social acceptance of sexual minorities as well as sexual behaviour have thereby changed over the years, also having had potential effects on the health of people with, and at risk for, HIV, currently ageing in the Netherlands.

In this thesis data are used from the longitudinal AGE$_h$IV and POPPY cohort studies. Both studies did not only include PLWH but also similarly-aged HIV-negative people belonging to communities at risk for HIV infection; in both cohorts most HIV-negative control participants are MSM. The inclusion of these control groups allows to disentangle health disparities directly related to HIV and its treatment from those associated with belonging to key populations at risk for HIV.

In **Chapter 2** spirometry data from the AGE$_h$IV cohort enrolment visit are used to cross-sectionally compare the pulmonary function of HIV-positive and HIV-negative participants. Prior research had suggested chronic obstructive pulmonary disease (COPD) to

be more prevalent among PLWH compared to people from the general population. However, we found a similarly high prevalence (23%) of obstructive lung disease (OLD, i.e. 1-second forced expiratory volume / forced vital capacity [$FEV_1$/FVC] <70%) among both study groups. In fact, when taking into account smoking history, among participants with limited smoking history (i.e. <25 pack-years) HIV-positive participants less frequently had obstructive lung disease compared to HIV-negative participants. However, this difference was found to be driven by a lower FVC among HIV-positive than HIV-negative participants, suggesting pulmonary function of HIV-positive participants to be impaired by restrictive/interstitial changes rather being better than that of HIV-negative participants. Moreover, higher plasma levels of IL-6 were associated with a lower FVC only among HIV-positive participants suggesting a potential role of HIV-associated chronic inflammation in the development of such restrictive pulmonary function impairment.

In **Chapter 3** we expand the prior analysis by evaluating the pulmonary function of AGE$_h$IV participants over approximately 6 years of follow-up, including up to 4 spirometry measurements obtained at 2-year intervals. After adjustment for smoking during follow-up, $FEV_1$ and FVC both declined significantly faster among HIV-positive compared to HIV-negative participants, while the $FEV_1$/FVC ratio declined at similar rates. In contrast, smoking during follow-up was associated with a faster decline in both $FEV_1$ and the $FEV_1$/FVC ratio, but not the FVC. Only among HIV-positive participants faster declines in both $FEV_1$ and FVC were associated with higher plasma levels of C-reactive protein. Again, these findings suggest HIV to be associated with the development of restrictive rather than obstructive pulmonary pathology, potentially driven by HIV-associated chronic inflammation. Spirometry cannot definitively determine the presence of interstitial lung disease or differentiate it from small-airway disease. Future studies should therefore use different diagnostic modalities (e.g. CT-imaging) to determine the exact characteristics and consequences of these potentially HIV-specific pulmonary changes.

Aside from its lifesaving properties, combination antiretroviral treatment (cART), also in the current treatment era, may continue to have adverse effects. New antiretroviral drugs are continuously being developed, aiming to increase efficacy while reducing toxicity. However, with the continuous introduction of new antiretrovirals comes the continuous risk for new side-effects, including those that have not yet emerged during phase III licensing clinical trials. One such unexpected side-effect emerging from post-marketing studies has been weight gain related to the use of integrase strand transfer inhibitors (INSTIs). This may be especially relevant in the context of ageing with HIV, since overweight may contribute to the development of AACs. In **Chapter 4** we therefore took advantage of weight data collected in a standardized manner in the AGE$_h$IV study and evaluated whether participants switching to an INSTI, while be-

ing virally suppressed, showed more weight gain during study follow-up compared to either HIV-positive participants not switching to an INSTI, or compared to HIV-negative participants. We did not observe differences in mean weight changes between these three selected study populations. More than 5% weight gain was however more frequent among INSTI -switching compared to non-switching HIV-positive participants. Together, this suggests INSTI-associated weight gain may occur in some PLWH. Other studies have similarly showed that predominantly people from specific populations (notably women and black people, both women and men) may be particularly at risk for this purported side-effect. The $AGE_hIV$ cohort includes only a limited number of women and black people. Moreover, INSTI-associated weight gain has been reported to be more pronounced with concomitant use of tenofovir alafenamide, which, during our period of observation, was rarely used in the selected $AGE_hIV$ study population. The relative underrepresentation of individuals with such characteristics may explain why in the $AGE_hIV$ cohort, no major differences in mean weight gain were found. Future studies will have to further evaluate the exact extent and causes of INSTI-associated weight gain in populations with more diversity with regards to gender and ethnic background.

In **Chapter 5** we studied the co-occurrence of comorbidities in PLWH, using cross-sectional data from both the $AGE_hIV$ and POPPY cohorts. Co-occurrence of comorbidities may imply the existence of common risk factors. By using a data-driven approach we evaluated which comorbidities were more likely to co-occur among *HIV-positive* participants from both cohorts. Several comorbidity patterns were identified in both cohorts and the patterns of cardiovascular diseases and sexually transmitted diseases occurred in both cohorts. These patterns reflect the existence of known common risk factors such as smoking, advanced age or sexual risk behaviour. Within the POPPY cohort the mental health comorbidity pattern was associated with all other comorbidity patterns identified, which may even suggest a potential causal relationship between mental and physical health. These findings can be used in future studies to further understand the underlying pathophysiology of comorbidities.

Chronic inflammation is currently believed to be one of the main drivers of AAC risk among both HIV-positive and –negative individuals. In the Netherlands, similar to other countries, MSM are at substantially increased risk of acquiring HIV as well as other sexually transmitted infections (STIs). Both HIV, also when suppressed by cART, and other STIs are able to cause changes in an individual's inflammatory profile. For example, cytomegalovirus (CMV) is known to cause chronic T-cell activation resulting in increased levels of $CD8^+$ T-cells and low $CD4^+/CD8^+$ T-cell ratios. In **Chapter 6** we therefore determined whether *HIV-negative* MSM showed differences in T-cell characteristics compared to *HIV-negative* heterosexual men. This is relevant since low $CD4^+/CD8^+$ ratios have been

associated with more frequent occurrence of AACs among people both with and without HIV. We found higher $CD8^+$ T-cell counts and lower $CD4^+/CD8^+$ T-cell ratios among MSM compared to heterosexual men, with largest differences found between heterosexual men and MSM with a history of >10 sex partners in the six months prior to study visits. These differences were attenuated but remained statistically significant after adjustment for CMV-serostatus, suggesting that other causes, in addition to CMV, led to the observed differences. These findings also contribute to further the understanding of persisting high $CD8^+$ T-cell counts and low $CD4^+/CD8^+$ T-cell ratios among *HIV-positive* MSM, as they suggest factors other than HIV to also be playing a role in those men. Importantly, our findings suggest that MSM may be at increased risk for AACs, regardless of HIV-status.

Throughout this thesis evidence is presented that HIV and its treatment can have specific consequences for the health of people ageing with HIV, i.e. HIV-specific pulmonary changes and INSTI-related weight gain. However, this thesis has also presented evidence that due to the fact that PLWH are from specific populations (in the Netherlands predominantly MSM), they may also be at increased risk of AACs through HIV-unrelated factors (e.g. high prevalence of obstructive lung disease and MSM-specific immunological characteristics). In **Chapter 8,** the general discussion, I therefore make a plea for a biopsychosocial approach to future research evaluating AAC development among PLWH, which as a result should also include those at risk for HIV. A biopsychosocial perspective could allow for new insights in the prevention and treatment of AACs, such as for example a closer evaluation of the connection between mind and body. Moreover, it can help to prevent confounding (e.g. wrongfully attributing inflammation to HIV instead of to being MSM) and thereby prevent the development of potentially ineffective interventions.

## NEDERLANDSE SAMENVATTING

**Titel:** **Ontrafelen van gezondheidsrisico's voor mensen die ouder worden met – of risico lopende op – hiv in Nederland**

**Subtitel:** **Leven met een nalatenschap**

Leven met hiv veranderde fundamenteel in 1996 door de introductie van "Highly Active Antiretroviral Therapy" (HAART), een behandeling die de infectie veranderde van een dodelijke ziekte in een chronische aandoening. Deze wetenschappelijke ontwikkeling heeft gedurende de afgelopen decennia ervoor gezorgd, en blijft er voor zorgen, dat mensen die leven met hiv (MLMH) oud kunnen worden. Vanzelfsprekend zorgt dit ervoor dat een toenemend aantal MLMH risico gaan lopen op het ontwikkelen van ouderdom-gerelateerde aandoeningen (OGA's), zoals hart- en vaatziekten en kanker. Er wordt echter beschreven dat deze OGA's zich vaker, en mogelijk op jongere leeftijd, voordoen bij MLMH. Dit proefschrift beschrijft in detail de longfunctie van mensen die ouder worden met hiv, als ook verscheidene potentiële oorzaken van het verschil in voorkomen van OGA's bij mensen die ouder worden met hiv in vergelijking met mensen zonder hiv.

**Hoofdstuk 1** van dit proefschrift beschrijft de historische achtergrond van de hiv epidemie, de ontwikkelingen in de behandeling voor de infectie alsook de achtergrond van de specifieke gemeenschappen getroffen door hiv in Nederland. Dit is relevant omdat voorafgaande blootstelling aan onbehandelde hiv infectie, aids-gerelateerde aandoeningen en aan vroegere vaak toxische behandelingen voor MLMH mogelijk hebben geresulteerd in orgaanschade die ook nu nog het risico op OGA's beïnvloedt. Tevens gebeurde de introductie van hiv in specifieke gemeenschappen zoals die van mannen-die-seks-hebben-met-mannen (MSM) niet in een historisch vacuüm, maar gelijktijdig met andere belangrijke maatschappelijke ontwikkelingen. Burgerrechten en de sociale positie van seksuele minderheden zijn snel veranderd gedurende de afgelopen decennia. Sociale acceptatie van seksuele minderheden en seksueel gedrag zijn hierdoor veranderd over de tijd, wat ook mogelijke gevolgen heeft gehad voor de gezondheid van mensen met hiv, of zij die daar risico op liepen, welke nu oud worden in Nederland.

In dit proefschrift zijn data van de longitudinale $AGE_{h}IV$ en POPPY cohort studies gebruikt. Beide studies hebben niet alleen MLMH geïncludeerd, maar ook hiv-negatieve controle-deelnemers uit gemeenschappen waarin hiv vaak voorkomt; in beide cohorten zijn de meeste hiv-negatieve controle-deelnemers MSM. De selectie van deze controlegroepen maakt het mogelijk om direct aan hiv gerelateerde gezondheidsrisico's te onderscheiden van gezondheidsrisico's geassocieerd met het behoren tot een gemeenschap waarin hiv vaak voorkomt.

In **Hoofdstuk 2** worden spirometrie data van het $AGE_hIV$ cohort, verkregen ten tijde van inclusie, gebruikt om de longfunctie van hiv-positieve en hiv-negatieve deelnemers cross-sectioneel te vergelijken. Voorgaand onderzoek heeft aangetoond dat "chronic obstructive lung disease" (COPD) onder MLMH vaker voorkomt vergeleken met de algemene bevolking. Wij vonden echter een gelijkwaardig maar hoog-frequent voorkomen (23%) van obstructieve longziekte (OLZ, 1-second forced expiratory volume / forced vital capacity [$FEV_1$/FVC] <70%) in beide studiegroepen. Onder de deelnemers met een beperkte rookgeschiedenis (<25 pak-jaren) hadden mensen met hiv zelfs minder vaak OLZ dan mensen zonder hiv. Dit verschil werd echter veroorzaakt door een lagere FVC bij hiv-positieve dan hiv-negatieve deelnemers, wat suggereert dat longfunctie verminderd is door restrictieve/interstitiële veranderingen bij hiv-positieve deelnemers en niet dat deze beter is dan die van hiv-negatieve deelnemers. Voorts waren alleen bij hiv-positieve deelnemers hogere plasmaspiegels van interleukine-6 geassocieerd met een lagere FVC, wat een potentiële rol van hiv-geassocieerde chronische ontsteking suggereert bij de ontwikkeling van wat oogt als een meer restrictieve longfunctiestoornis.

In **Hoofdstuk 3** zetten wij de voorgaande analyse voort en evalueerden de longfunctie van $AGE_hIV$ deelnemers over een periode van 6 jaar, bestaande uit maximaal 4 spirometrie metingen, verkregen in intervallen van twee jaar. Na statistisch rekening te hebben gehouden met rookgedrag gedurende follow-up, namen zowel $FEV_1$ als FVC significant sneller af bij hiv-positieve dan hiv-negatieve deelnemers, terwijl de $FEV_1$/FVC ratio even snel afnam. Roken gedurende follow-up daartegenover was geassocieerd met snellere afname van zowel de $FEV_1$ als de $FEV_1$/FVC ratio, maar niet van de FVC. Alleen bij hiv-positieve deelnemers waren snellere afname in $FEV_1$ en FVC geassocieerd met hogere plasmaspiegels van het "C-reactive protein". Ook deze bevindingen suggereren dat hiv geassocieerd is met de ontwikkeling van restrictieve i.p.v. obstructieve longpathologie, mogelijk gedreven door hiv-geassocieerde chronische ontsteking. Spirometrie kan niet definitief de aanwezigheid van interstitiële longziekte aantonen, noch dit differentiëren van ziekte van de kleine luchtwegen. Toekomstige studies zullen daarom met andere diagnostische onderzoeken (bijv. CT-scans) de exacte karakteristieken en consequenties van deze mogelijk hiv-specifieke veranderingen in de long moeten aantonen.

Naast de levensreddende eigenschappen van "combination antiretroviral treatment" (cART), kan deze behandeling, ook in het huidige behandeltijdperk, bijwerkingen hebben. Nieuwe antiretrovirale middelen worden nog steeds ontwikkeld, met als doel de effectiviteit te verhogen en de toxiciteit te verlagen. Met de gestage introductie van nieuwe middelen komt echter ook het risico op mogelijk nieuwe bijwerkingen, waaronder bijwerkingen die niet al naar voren zijn komen tijdens fase III registratietrials. Één van deze onverwachte bijwerkingen die naar voren is gekomen tijdens post-marketing

studies is gewichtstoename gerelateerd aan het gebruik van "integrase strand transfer inhibitors" (INSTIs). Dit is extra relevant in de context van ouder worden met hiv, aangezien overgewicht kan bijdragen aan de ontwikkeling van OGA's. Daarom hebben we in **Hoofdstuk 4** gebruik gemaakt van data omtrent gewicht zoals gestandaardiseerd verzameld in de AGE$_h$IV studie en geëvalueerd of hiv-positieve deelnemers bij wie de therapie gewijzigd was naar een INSTI, terwijl hiv bij hen al onderdrukt was, meer toenamen in gewicht vergeleken met hiv-positieve deelnemers die niet switchten naar een INSTI en in vergelijking met hiv-negatieve deelnemers. We vonden geen verschillen in gemiddelde veranderingen in gewicht tussen deze drie geselecteerde studie-populaties. Meer dan 5% gewichtstoename kwam echter wel vaker voor bij mensen die swichten naar een INSTI in vergelijking met hiv-positieve deelnemers die dit niet deden. Tezamen suggereert dit dat INSTI-geassocieerde gewichtstoename zich voor kan doen bij sommige MLMH. Andere studies hebben ook laten zien dat met name mensen uit specifieke populaties (bijv. vrouwen, zwarte mensen (zowel vrouwen als mannen)) risico lopen op deze verondersteld bijwerking. Het AGE$_h$IV cohort heeft maar een klein aantal vrouwen en zwarte mensen geïncludeerd. Daarbij is aangetoond dat INSTI-geassocieerde gewichtstoename meer uitgesproken is bij het gelijktijdig gebruik van tenofovir alafenamide, een middel dat in de geselecteerde AGE$_h$IV studie-populatie heel weinig werd gebruikt. Ondervertegenwoordiging van individuen met de genoemde karakteristieken in ons cohort kunnen mogelijk verklaren waarom in het AGE$_h$IV cohort geen verschillen in gemiddelde gewichtstoename werden gezien. Toekomstige studies zullen verder moeten evalueren wat de exacte omvang en oorzaak van INSTI-geassocieerde gewichtstoename is in populaties met meer diversiteit wat betreft gender en etnische achtergrond.

In **Hoofdstuk 5** hebben wij het gelijktijdig voorkomen van verschillende comorbiditeiten bij MLMH bestudeerd, gebruik makend van cross-sectionele data van zowel de AGE$_h$IV als POPPY cohorten. Gelijktijdig voorkomen van comorbiditeiten kan het bestaan van gemeenschappelijke risicofactoren impliceren. Door gebruik te maken van een "data-driven" benadering hebben wij onderzocht welke comorbiditeiten vaker gelijktijdig voorkwamen in beide cohorten. Verschillende comorbiditeit-patronen konden worden geïdentificeerd in beide cohorten, zoals de patronen van hart- en vaatziekten en seksueel overdraagbare aandoeningen. Deze patronen reflecteren het bestaan van bekende gemeenschappelijke risicofactoren, zoals roken, de toenemende leeftijd en seksueel risicogedrag. In het POPPY cohort was het mentale gezondheidsproblemen-patroon geassocieerd met alle andere comorbiditeit-patronen die in het POPPY cohort werden gezien, hetgeen een, mogelijk zelfs causaal, verband zou kunnen suggereren tussen de lichamelijke en geestelijke gezondheid. Deze bevindingen kunnen worden gebruikt in toekomstige studies om de onderliggende pathofysiologie van comorbiditeiten beter te begrijpen.

Chronische ontsteking wordt momenteel beschouwd als één van de voornaamste onderliggende oorzaken voor het ontwikkelen van OGA's bij zowel hiv-positieve als hiv-negatieve mensen. In Nederland, overeenkomend met andere landen, hebben MSM zowel een verhoogd risico op het krijgen van hiv als ook andere seksueel overdraagbare aandoeningen (SOA's). Zowel hiv, ook als dit onderdrukt is door cART, als andere SOA's zijn in staat om veranderingen in iemands ontstekingsprofiel te veroorzaken. Cytomegalovirus (CMV) kan bijv. chronische T-cel activatie veroorzaken, resulterende in verhoogde aantallen $CD8^+$ T-cellen en lage $CD4^+/CD8^+$ T-cel ratios. In **Hoofdstuk 6** hebben wij daarom onderzocht of *hiv-negatieve* MSM andere T-cel karakteristieken hadden dan *hiv-negatieve* heteroseksuele mannen. Dit is relevant aangezien lage $CD4^+/CD8^+$ ratio's geassocieerd zijn met OGA's bij zowel mensen met als zonder hiv. We zagen hogere $CD8^+$ T-cel aantallen en lagere $CD4^+/CD8^+$ T-cel ratio's bij MSM vergeleken met heteroseksuele mannen, met de grootste verschillen tussen heteroseksuele mannen en MSM met >10 sekspartners in de 6 maanden voor de studiebezoeken. Deze verschillen werden minder, maar bleven significant, als statistisch rekening werd gehouden met CMV-serostatus, wat suggereert dat (naast CMV) andere factoren ten grondslag liggen aan deze verschillen. Deze bevindingen dragen ook bij aan het verder begrijpen van persisterend hoge $CD8^+$ T-cel aantallen bij MSM met hiv, aangezien zij erop wijzen dat factoren anders dan hiv ook bij die mannen een rol kunnen spelen. Onze resultaten zouden er bovendien op kunnen wijzen dat MSM een verhoogd risico hebben op OGA's, onafhankelijk van hun hiv-status.

In dit proefschrift wordt bewijs geleverd voor het bestaan van specifieke consequenties van (de behandeling van) hiv voor de gezondheid van mensen die ouder worden met hiv, bijv. hiv-specifieke veranderingen in de long en INSTI-geassocieerde gewichtstoename. In dit proefschrift wordt echter ook bewijs geleverd voor het feit dat doordat MLMH van specifieke gemeenschappen komen (in Nederland voor het merendeel MSM) ze ook een verhoogd risico kunnen hebben door hiv-ongerelateerde factoren (bijv. op een hoge prevalentie van obstructieve longziekte en MSM-specifieke immunologische karakteristieken). In **Hoofdstuk 8**, de algemene beschouwing, pleit ik daarom voor een biopsychosociale benadering in toekomstig onderzoek dat de ontwikkeling van OGA's bestudeert. Een biopsychosociaal perspectief zou nieuwe inzichten kunnen bieden bij het voorkómen en behandelen van OGA's, zoals bijv. een grondiger evaluatie van de samenhang tussen de lichamelijke en mentale gezondheid. Ook kan het helpen om *confounding* (bijv. het onterecht geheel toewijzen van inflammatie aan hiv i.p.v. dit toewijzen aan het zijn van MSM) en daardoor de ontwikkeling van mogelijk ineffectieve interventies te voorkómen.

# Addendum

## CONTRIBUTING AUTHORS AND AFFILIATIONS


| | |
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M. Prins (co-principal investigator), M.F. Schim van der Loeff, L. del Grande, V. Olthof, I. Agard (Public Health Service of Amsterdam, Department of Infectious Diseases).

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## PHD PORTFOLIO

Name PhD student: Sebastiaan Verboeket

PhD period: October 2016 – June 2021

Name PhD supervisor: Prof. Peter Reiss

| 1. PhD training | Year | Workload (Hours/ ECTS) |
|---|---|---|
| **General courses** | | |
| Amsterdam UMC, University of Amsterdam, Graduate School for Medical Sciences, Amsterdam, the Netherlands | | |
| - Basic Course on Regulations and Organisation for Clinical Investigators (BROK®) | 2017 | 1.50 |
| - Practical Biostatistics | 2017 | 1.40 |
| - Oral Presentation in English | 2017 | 0.80 |
| - Endnote | 2017 | 0.80 |
| - Infectious Diseases | 2017 | 1.30 |
| - Observational Clinical Epidemiology Effects & Effectiveness | 2018 | 0.60 |
| **Specific courses** | | |
| Erasmus Summer Program 2017, Netherlands Institute for Health Sciences, Erasmus MC, Rotterdam, The Netherlands | | |
| - Linear Regression | 2017 | 1.40 |
| - Logistic Regression | 2017 | 1.40 |
| EpidM, Department of Epidemiology & Biostatistics, VU University Medical Center, Amsterdam, The Netherlands | | |
| - Mixed Models (WR05 | 2018 | 0.90 |
| VUmc academie, VU Medical Center, Amsterdam, The Netherlands | | |
| - PhD course Advanced Immunology 2019 | 2019 | 2.90 |
| **Seminars, workshops and master classes** | | |
| - HIV Masterclass, Virology Education, Utrecht, The Netherlands | 2016 | 1.00 |
| - GGD Amsterdam: Epidemiology masterclass, Amsterdam, the Netherlands. | 2017 | 1.00 |
| - Gilead, SHINE Clinical Research forum, Amsterdam, The Netherlands | 2017 | 0.60 |
| - Infectieziekten symposium Academic Medical Center, Amsterdam, The Netherlands | 2017 | 0.25 |

| | | |
|---|---|---|
| - State of Mind – Implementing and Integrating Mental Health in HIV Care | 2018 | 0.25 |
| - Amsterdam Infection & Immunity Institute PhD retreat, Heemskerk, The Netherlands | 2018 | 0.50 |

**Presentations**

Poster presentations

| | | |
|---|---|---|
| - Pulmonary function in controlled HIV-infection: lower Forced Vital Capacity but similar 1-second Forced Expiratory Volume among those with limited smoking experience, IWHOD, Fuengirola, Spain | 2018 | 0.50 |
| - Pulmonary function in Controlled HIV-infection: Lower Forced Vital Capacity but Similar 1-second Forced Expiratory Volume among never-smokers and (ex-)smokers with few smoking pack-years, International AIDS conference, Amsterdam, the Netherlands | 2018 | 0.50 |
| - Faster decline in lung function in treated HIV positive vs. HIV negative AGEhIV cohort participants independent of smoking behaviour, 13th conference on HIV pathogenesis, epidemiology, prevention and treatment, Amsterdam, The Netherlands | 2020 | 0.50 |

Oral Presentations

| | | |
|---|---|---|
| - HIV-negative men who have sex with men have lower CD4/CD8 ratios driven by higher absolute CD8 T-lymphocyte counts compared to HIV-negative heterosexual men, 20th International Workshop on Co-morbidities and Adverse Drug Reactions in HIV, New York, USA | 2018 | 0.50 |
| - Switchen naar een antiretroviraal regime met een integrase-remmer in het AGEhIV Cohort is niet geassocieerd met een bovengemiddelde toename in lichaamsgewicht, NVHB midzomer vergadering, Utrecht, The Netherlands. | 2019 | 0.50 |
| - Switching to an integrase inhibitor containing antiretroviral regimen is not associated with above-average weight gain in middle-aged people living with HIV on long-term suppressive antiretroviral therapy, the AGEhIV cohort study, 21st International Workshop on Co-morbidities and Adverse Drug Reactions in HIV, New York, USA | 2019 | 0.50 |

| | | |
|---|---|---|
| - Switching to an integrase inhibitor containing antiretroviral regimen is not associated with above-average weight gain in middle-aged people living with HIV on long-term suppressive antiretroviral therapy, the AGEhIV cohort study, 17th European AIDS Conference, Basel, Switserland | 2019 | 0.50 |
| - Faster decline in lung function in treated HIV-positive vs. HIV-negative AGEhIV cohort participants independent of smoking behaviour, 22nd International Workshop on Co-morbidities and Adverse Drug Reactions in HIV, New York, USA | 2020 | 0.50 |
| **(Inter)national conferences** | | |
| - HIV Glasgow conference, Glasgow, United Kingdom | 2016 | 1.00 |
| - 10th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV), Amsterdam, the Netherlands | 2016 | 0.25 |
| - KNAW-symposium: Vermoeidheid bij chronische ziekten, Amsterdam, the Netherlands | 2016 | 0.25 |
| - 11th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV) , Amsterdam, the Netherlands | 2017 | 0.25 |
| - COBRA symposium (Comorbidity in relation to AIDS), Amsterdam, The Netherlands | 2017 | 0.25 |
| - Soa*Hiv*Seks*2018 conference, Amsterdam, the Netherlands | 2018 | 0.25 |
| - PrEP in Europe Summit, Amsterdam, the Netherlands | 2018 | 0.25 |
| - IWHOD (International workshop on HIV and Hepatitis Observational Databases), Fuengirola, Spain | 2018 | 0.75 |
| - International AIDS conference 2018, Amsterdam, The Netherlands | 2018 | 1.00 |
| - 20th International Workshop on Co-morbidities and Adverse Drug Reactions in HIV, New York, USA | 2018 | 0.75 |
| - 21st International Workshop on Co-morbidities and Adverse Drug Reactions in HIV, New York, USA | 2019 | 0.75 |
| - 17th European AIDS Conference, Basel, Switserland | 2019 | 0.50 |
| - 12$^{th}$ Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment (NCHIV) | 2019 | 0.25 |
| - Soa*Hiv*Seks*2019 conference | 2019 | 0.25 |
| - 13th conference on HIV pathogenesis, epidemiology, prevention and treatment, Online conference | 2020 | 0.25 |

| | | |
|---|---|---|
| - 22nd International Workshop on Co-morbidities and Adverse Drug Reactions in HIV, Online conference | 2020 | 0.75 |
| - Soa*Hiv*Seks*2020, Online conference | 2020 | 0.25 |

| **2. Teaching** | **Year** | **Workload (Hours/ ECTS)** |
|---|---|---|
| **Tutoring, Mentoring** | | |
| - Direct supervisor of Eline Lap for her bachelor thesis: "Polypharmacy and adverse health outcomes in the aging HIV-population" | 2018 | 1.00 |

| **3. Parameters of Esteem** | **Year** |
|---|---|
| **Awards and Prizes** | |
| - Bob Munk award for "HIV-negative men who have sex with men have lower CD4/CD8 ratios driven by higher absolute CD8 T-lymphocyte counts compared to HIV-negative heterosexual men" at the 20th International Workshop on Co-morbidities and Adverse Drug Reactions in HIV, New York, USA | 2018 |

## LIST OF PUBLICATIONS

**Verboeket SO**, Boyd A, Wit FW, Verheij E, Schim van der Loeff MF, Kootstra N, et al. Changes in lung function among treated HIV-positive and HIV-negative individuals: analysis of the prospective AGEhIV cohort study. The Lancet Healthy Longevity. 2021;2(4):e202-e11.

Verheij E, Wit FW, **Verboeket SO**, Schim van der Loeff MF, Nellen JF, Reiss P, et al. Frequency, Risk Factors, and Mediators of Frailty Transitions During Long-Term Follow-Up Among People With HIV and HIV-Negative AGEhIV Cohort Participants. Journal of acquired immune deficiency syndromes (1999). 2021;86(1):110-8.

**Verboeket SO**, Boyd A, Wit FW, Verheij E, Schim van der Loeff MF, Kootstra N, et al. Generally rare but occasionally severe weight gain after switching to an integrase inhibitor in virally suppressed AGEhIV cohort participants. PloS one. 2021;16(5):e0251205.

Verheij E, Kirk GD, Wit FW, van Zoest RA, **Verboeket SO**, Lemkes BA, et al. Frailty Is Associated With Mortality and Incident Comorbidity Among Middle-Aged Human Immunodeficiency Virus (HIV)-Positive and HIV-Negative Participants. The Journal of infectious diseases. 2020;222(6):919-28.

**Verboeket SO**, Wit FW, Verheij E, van Zoest RA, Kootstra NA, van der Valk M, et al. HIV-negative Men Who Have Sex with Men have higher CD8+ T-cell Counts and Lower CD4+/CD8+ T-cell Ratios compared to HIV-negative Heterosexual Men. The Journal of infectious diseases. 2020 Jan 31;jiaa048

**Verboeket SO**, Wit FW, Kirk GD, Drummond MB, van Steenwijk RP, van Zoest RA, et al. Reduced Forced Vital Capacity Among Human Immunodeficiency Virus-Infected Middle-Aged Individuals. The Journal of infectious diseases. 2019;219(8):1274-84.

De Francesco D, **Verboeket SO**, Underwood J, Bagkeris E, Wit FW, Mallon PWG, et al. Patterns of Co-occurring Comorbidities in People Living With HIV. Open forum infectious diseases. 2018;5(11):ofy272.

**Verboeket SO**, van den Berk GE, Arends JE, van Dam AP, Peringa J, Jansen RR. Hookworm with hypereosinophilia: atypical presentation of a typical disease. Journal of travel medicine. 2013;20(4):265-7.

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**AGE$_h$IV Collega's**

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**Co-authors**

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**Members of my PhD committee**

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**AIGHD colleagues**

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## ABOUT THE AUTHOR

Sebastiaan Olaf Verboeket was born on July 1, 1987 in Breda. He grew up in the village of Prinsenbeek in the Dutch province of Noord-Brabant where he attended the Waldorf High School. After graduation he moved to Amsterdam where he obtained his medical degree at the University of Amsterdam. During his studies he wrote his scientific thesis at the University of Pittsburgh (USA) about the acceptability of rectal microbicide gels to prevent HIV transmission (if they were to be developed) among men who have sex with men. At the end of his studies he also completed an Intensive Care rotation at Hospital Italiano in Buenos Aires, Argentina.

After obtaining his medical license in 2014, he spent almost two years working as an Intensive Care resident at the Red Cross Hospital in Beverwijk and at the VU Medical Center in Amsterdam. In 2016 he started his PhD, working as research-physician at the $AGE_hIV$ cohort study and supervised by Peter Reiss, Maarten Schim van der Loeff, Ferdinand Wit and Anders Boyd. His PhD focused on studying the prevalence and incidence of - and risk factors for - ageing-associated comorbidities in people living with HIV. His specific areas of interest are pulmonary function in the context of ageing with HIV as well as the general health of people from key populations with, but also *at risk*, for HIV; in particular gay, bisexual and other men who have sex with men. During his PhD studies, and in his spare time, he has chaired the activist group PrEPnu, fighting for the availability and uptake of Pre-Exposure Prophylaxis (PrEP) in the Netherlands. In the capacity of PrEPnu chairman, Sebastiaan has acted as spokesperson to advocate for PrEP in the national media, given lectures to educate health care professionals about PrEP, supported PrEPnu awareness campaigns and outreach projects, and has been part of the WeArePrEPared working group, in which he represents the interests of people using PrEP in the Netherlands.

After finishing his PhD Sebastiaan will again be working as a clinician, moving to the field of addiction medicine. This will enable him to focus on preventing many of the ageing-associated comorbidities studied in his PhD thesis, as well as provide care for those in vulnerable and minority positions.