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Low-level HIV viremia during antiretroviral therapy

OLOF ELVSTAM

DEPARTMENT OF TRANSLATIONAL MEDICINE | LUND UNIVERSITY





OLOF ELVSTAM graduated as a medical doctor from Lund University in 2017 and currently works at the Infectious Disease Clinic at Central Hospital Växjö, Sweden.

While undetectable plasma level of HIV ribonucleic acid is achieved in most recipients of antiretroviral therapy, up to 25% may have low-level viremia at some time point. This doctoral thesis explores clinical aspects of low-level viremia through three register-based epidemiological studies and two laboratory investigations.



Low-level HIV viremia during antiretroviral therapy

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Olof Elvstam



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DOCTORAL DISSERTATION

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Abstract <p>In most cases, antiretroviral therapy (ART) results in undetectable plasma HIV viral load (VL). Still, up to 25% of ART recipients may have detectable low-level viremia (LLV) of different amplitude and persistence. This thesis explores the impact of LLV during ART on virologic and clinical outcomes.</p> <p>Paper I–III are retrospective analyses based on InfCare HIV, a national quality register for people with HIV in Sweden. Time-updated viremia categories were used. Paper I included all participants in Malmö and Gothenburg between 1996 and 2016. Compared with viral suppression, individuals with LLV of 200–999 c/mL, but not 50–199 c/mL, had increased risk of future virologic failure (adjusted hazard ratio [aHR], 3.1; 95% confidence interval [CI], 1.4–7.0). LLV was associated with increased all-cause mortality, although this was not statistically significant in multivariable analysis.</p> <p>For paper II and III, we linked the nationwide InfCare HIV cohort (1996–2017) to national health registers. After 49 986 person-years of follow-up (median 5.7 years), 4177/6956 (60%) were classified as viral suppression, 339 (5%) as LLV of 50–199 c/mL, 258 (4%) as LLV of 200–999 c/mL, and 2182 (31%) as non-suppression. LLV of 50–999 c/mL was associated with increased all-cause mortality when compared with viral suppression (aHR, 2.2; 95% CI, 1.3–3.6). In subanalysis, LLV of 50–199 c/mL had an aHR of 2.2 (95% CI, 1.3–3.8) and LLV of 200–999 c/mL of 2.1 (95% CI, 0.96–4.7). LLV was not associated with AIDS, but individuals with LLV of 200–999 c/mL had increased risk of serious non-AIDS events (SNAE; cardiovascular disease being the most common diagnosis). Neither time-updated viremia category nor cumulative viremia during ART had statistically significant associations with cancer incidence. Higher pre-ART VL was associated with cancer (adjusted subhazard ratio, 1.4; 95% CI, 1.0–1.8). In subanalysis, the association between pre-ART VL and cancer was restricted to AIDS-defining malignancies and infection-related non-AIDS-defining cancer.</p> <p>In paper IV, we measured the levels of nine biomarkers in people with LLV (≥ 3 VLs in the range 50–999 c/mL) and matched controls with viral suppression. We found no difference in markers of inflammation and immune activation, but patients with LLV had higher levels of growth differentiation factor 15 (GDF-15) and D-dimer.</p> <p>Lastly, we analyzed 21 blood biomarkers and measures of cardiovascular function and structure in participants of a South African research cohort (paper V). We observed similar cardiovascular profiles among individuals with detectable viremia (50–999 c/mL in one measurement) and those with viral suppression (<50 c/mL).</p> <p>In conclusion, this thesis adds to mounting evidence that LLV is associated with inferior clinical outcomes in ART recipients. Specifically, we observed associations between LLV and virologic failure, all-cause mortality, and SNAE, respectively. Our findings suggest that this is likely not mediated through inflammation or immune activation, but elevated GDF-15 and D-dimer for people with LLV in repeated VL measurements could suggest higher cardiovascular risk. We found no evidence of increased risk of cancer or AIDS for people with LLV.</p>		
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Front cover photo by National Institute of Allergy and Infectious Diseases (NIAID) depicting budding of HIV from a human immune cell.

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- I. Elvstam O, Medstrand P, Yilmaz A, Isberg PE, Gisslén M, Björkman P. **Virological failure and all-cause mortality in HIV-positive adults with low-level viremia during antiretroviral treatment.** *PLOS One.* 2017; 12(7). doi: 10.1371/journal.pone.0180761.
- II. Elvstam O, Marrone G, Medstrand P, Treutiger CJ, Sönnnerborg A, Gisslén M, Björkman P. **All-Cause Mortality and Serious Non-AIDS Events in Adults with Low-level Human Immunodeficiency Virus Viremia During Combination Antiretroviral Therapy: Results From a Swedish Nationwide Observational Study.** *Clinical Infectious Diseases.* Published online April 9, 2020. doi: 10.1093/cid/ciaa413 [Epub ahead of print].
- III. Elvstam O, Marrone G, Medstrand P, Treutiger CJ, Svedhem V, Gisslén M, Björkman P. **Associations between Plasma HIV RNA Levels and Incidence of Invasive Cancer in People with HIV after Initiation of Combination Antiretroviral Therapy.** *Open Forum Infectious Diseases.* Published online March 17, 2021. doi: 10.1093/ofid/ofab131 [Accepted manuscript].
- IV. Elvstam O, Medstrand P, Jansson M, Isberg PE, Gisslén M, Björkman P. **Is low-level HIV-1 viraemia associated with elevated levels of markers of immune activation, coagulation and cardiovascular disease?** *HIV Medicine.* 2019; 20(9): 571-80. doi: 10.1111/hiv.12756.
- V. Botha-Le Roux S, Elvstam O, De Boever P, Goswami N, Magnusson M, Nilsson PM, Strijdom H, Björkman P, Fourie CMT. **Cardiovascular profile of South African adults with low-level HIV-1 viraemia during antiretroviral therapy.** Manuscript in preparation.

Populärvetenskaplig sammanfattning

Kombinationsbehandling mot hiv (humant immunbristvirus) är en av den moderna medicinens stora framgångar. Medan hiv kunde innebära en dödsdom fram till mitten av 1990-talet, kan personer med hiv och kombinationsbehandling idag leva länge utan att ha några symtom orsakade av viruset och är inte heller smittsamma. I genomsnitt har dock personer med hiv fortfarande ökad sjuklighet och dödlighet och det är ofullständigt känt vad detta beror på. Det kan delvis förklaras av att personer med hiv har ökad förekomst av traditionella riskfaktorer för sjukdom, till exempel rökning. Troligen finns även andra samband mellan hiv och sjukdomar som hjärt- och kärlsjukdom och cancer.

De flesta patienter som får kombinationsbehandling har omätbara nivåer av hiv-RNA (*ribonukleinsyra*, virusets arvs massa) i blodet, och detta mäts regelbundet för att utvärdera behandlingseffekten. I takt med att känsligare mätmetoder för hiv-RNA har utvecklats har det visat sig att upp till en fjärdedel av alla patienter någon gång har låga – men mätbara – nivåer av *viremi* (förekomst av virus i blod). Man vet att höga nivåer av hiv-RNA (behandlingssvikt) är förenat med ökad dödlighet och allvarlig sjukdom, men det är fortfarande osäkert om låggradig viremi innebär ökad risk. I denna doktorsavhandling har jag studerat långtidseffekter av låggradig viremi genom analys av den svenska hivkohorten. För att vidare undersöka vilka mekanismer som kan ligga bakom ökad sjuklighet hos personer med låggradig viremi har vi även genomfört två laboratoriestudier.

I delarbete I analyserade vi risken för behandlingssvikt (definierat som minst 1000 kopior hiv-RNA per ml i upprepade mätningar) hos personer med hiv från Malmö och Göteborg mellan åren 1996 och 2016. Vi fann ett samband mellan låggradig viremi mellan 200 och 999 kopior/ml och ökad risk för senare behandlingssvikt, jämfört med omätbart virus. Vi observerade även tecken till ökad dödlighet hos personer med låggradig viremi (både 50–199 och 200–999 kopior/ml) jämfört med omätbart virus, även om det inte kan uteslutas att skillnaden berodde på slumpen.

För att vidare kartlägga sambandet mellan låggradig viremi och dödlighet utvidgade vi analysen till hela den svenska hivkohorten mellan åren 1996 och 2017 baserat på InfCare HIV, ett nationellt kvalitetsregister för personer med hiv i Sverige. Information om dödsorsaker och sjukdomar inhämtades från Socialstyrelsens register. I delarbete II

fann vi ett samband mellan låggradig viremi (mellan 50 och 999 kopior/ml) och dödlighet, motsvarande att personer med låggradig viremi hade drygt dubbelt så hög risk för död vid varje given tidpunkt jämfört med personer med omätbart virus. Det är viktigt att betona att den ökade dödligheten alltså är i jämförelse med välbehandlade hivpatienter, som har jämförbar överlevnad med bakgrundsbefolkningen. Vi noterade även ett samband mellan låggradig viremi och risk för ett antal icke-smittsamma sjukdomar (vanligast hjärt- och kärlsjukdomar), men endast för personer med låggradig viremi mellan 200 och 999 kopior/ml.

I delarbete III undersökte vi hur risken för cancer påverkas av viremi före och efter start av kombinationsbehandling, med hjälp av data från Cancerregistret. Vi kunde inte påvisa något samband mellan viremi under pågående kombinationsbehandling och risk för cancer. Däremot hade personer med hög viremi innan start av behandling en ökad risk för cancerformer som orsakas av andra virus (de två vanligaste i vårt material var lymfom [lymfkörtelcancer] och livmoderhalscancer).

För att bättre förstå vad som kan orsaka sambandet mellan låggradig viremi och högre risk för sjukdom och död undersökte vi om personer med låggradig viremi har påverkade nivåer av ämnen kopplade till aktivering av immunförsvaret och risk för hjärt- och kärlsjukdom (delarbete IV). Vi analyserade sparade blodprover hos 34 personer med låggradig viremi respektive omätbart virus och fann högre nivåer av två ämnen som kallas GDF-15 och D-dimer hos personer med låggradig viremi, vilket kan tala för en ökad risk för hjärt- och kärlsjukdom. Däremot fann vi ingen skillnad avseende sju andra markörer som speglar inflammation, aktivering av immunförsvaret respektive risk för hjärt- och kärlsjukdom.

Slutligen fortsatte vi undersökningen av en möjlig koppling mellan låggradig viremi och hjärt- och kärlsjukdom med att jämföra 95 sydafrikanska personer med hiv-RNA mellan 50 och 999 kopior/ml med 113 personer med mindre än 50 kopior/ml (delarbete V). Vi mätte 21 olika ämnen i blod och undersökte även mått på åderförkalkning, blodtryck och grad av kärlstelhet. Vi fann liknande nivåer av alla ämnen och mått hos personer med låggradig viremi och omätbart virus. Skillnaden avseende GDF-15 från delarbete IV förelåg inte i detta material. Till skillnad från övriga delarbeten hade vi bara tillgång till ett måttillfälle i denna studie, och vi kan alltså inte avgöra om studiepersonerna hade tillfällig eller långvarig viremi.

Sammanfattningsvis ger denna avhandling stöd för att personer med låggradig viremi har ökad risk för behandlingssvikt, allvarlig sjukdom och död. Samtidigt såg vi inga tecken till att personer med låggradig viremi skulle ha ökad inflammation eller försämrad kärlfunktion. Det är viktigt att påpeka att även om sambanden mellan låggradig viremi och behandlingssvikt, sjukdom respektive död är intressanta, är det

inte säkert att de är orsakssamband. Våra resultat stöder att låggradig viremi är ett viktigt fenomen som dels bör beaktas vid klinisk handläggning av personer med hiv, dels är ett relevant ämne för vidare forskning. Framtida forskning kan inriktas på att 1) se om sambandet mellan låggradig viremi och sjukdom respektive död kan upprepas i andra material, 2) undersöka risken för behandlingssvikt vid låggradig viremi på en nivå lägre än 200 kopior/ml eller kortvariga episoder av låggradig viremi, 3) upprepa analysen av mått på hjärt- och kärlsjukdom med längre uppföljning eller fler virusmätningar, 4) kartlägga vilka faktorer som är kopplade till ökad risk hos personer med låggradig viremi samt 5) undersöka betydelsen av låggradig viremi hos personer som påbörjar kombinationsbehandling idag, med moderna läkemedel.

Abbreviations

3TC	lamivudine
ABC	abacavir
ADAMTS13	a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
aHR	adjusted hazard ratio
AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
ART-CC	Antiretroviral Therapy Cohort Collaboration
aSHR	adjusted subhazard ratio
AZT	zidovudine
CDC	Centers for Disease Control and Prevention
CI	confidence interval
c/mL	copies/mL
CMV	cytomegalovirus
CRP	C-reactive protein
CVD	cardiovascular disease
D:A:D	Data Collection on Adverse Events of Anti-HIV Drugs
DNA	deoxyribonucleic acid
DRV	darunavir
DTG	dolutegravir
EACS	European AIDS Clinical Society
EFV	efavirenz
FTC	emtricitabine
GDF-15	growth differentiation factor 15
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HPV	human papillomavirus

HR	hazard ratio
ICAM-1	intercellular adhesion molecule 1
ICD	International Classification of Diseases
INSTI	integrase strand transfer inhibitor
IL	interleukin
IP-10	interferon- γ -induced protein
IQR	interquartile range
LLV	low-level viremia
LPV	lopinavir
MSM	men who have sex with men
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside/nucleotide reverse transcriptase inhibitor
NT-proBNP	N-terminal pro B-type natriuretic peptide
PI	protease inhibitor
PYFU	person-years of follow-up
PWH	people with HIV
RAL	raltegravir
RNA	ribonucleic acid
SMART	Strategies for Management of Antiretroviral Therapy
SNAE	serious non-AIDS events
ST2	suppression of tumorigenicity 2
START	Strategic Timing of Antiretroviral Therapy
VL	viral load
TDF	tenofovir disoproxil fumarate
UNAIDS	Joint United Nations Programme on HIV/AIDS
VCAM-1	vascular cell adhesion molecule 1
WHO	World Health Organization

Introduction

Many diseases are as old as humanity itself, but human immunodeficiency virus (HIV) infection is, in comparison, a recent condition. First transferred to humans from African primates around one hundred years ago, it is only since the 1980s that the virus has been spreading more widely. Thus, the story of HIV is in many ways that of our modern society, including scientific innovation but also deep inequalities and societal barriers like discrimination and stigmatization. The last decades' advances in HIV treatment are a great accomplishment, but much work remains, especially on a global scale. The topic discussed in this thesis—low-level viremia (LLV)—is only relevant for people with access to antiretroviral therapy (ART) and the infrastructure around it. In December 2019, it was estimated that 33% of people with HIV (PWH) globally do not have that [1]. With 690 000 yearly deaths due to acquired immunodeficiency syndrome (AIDS) and, recently, decreases in global HIV funding, it is our collective task to improve this situation.

For people receiving ART, life expectancy is approaching that of HIV-negative individuals, but significant gaps remain, both in total life expectancy and—even more pronounced—when only counting comorbidity-free years [2]. Non-AIDS-defining cancers and cardiovascular disease (CVD) are of increasing importance for an aging population of PWH, and it has been estimated that PWH have an approximately twofold increase in the risk of CVD [3] and 69% increased cancer risk, with some cancer forms such as cervix cancer, anal cancer, and certain types of lymphoma being markedly more common [4]. PWH have increased prevalence of traditional risk factors, such as smoking, but this does not explain all of the increased risk of non-AIDS morbidity.

Having suppressed HIV ribonucleic acid (RNA) in plasma is a sign of well-functioning treatment and good prognosis. Still, following the development of more sensitive viral load (VL) assays, it has become apparent that as many as one in four ART recipients have low-level but detectable VL at some time point [5]. While it is undisputed that VLs in a higher range indicate treatment failure that could have serious clinical consequences, it remains largely unknown whether LLV is linked to adverse outcomes. In a way, the ability to detect and quantify LLV has developed faster than the knowledge of its clinical significance.

Many important questions remain open: Is the presence of LLV predictive of future loss of control of viral replication and virologic failure? Is LLV associated with increased risk of clinical events such as death, AIDS, and serious non-AIDS events (SNAE)? What could be the mechanism behind these possible associations? Do people with LLV have a different profile of plasma biomarkers reflecting immune activation and cardiovascular risk? Is there a difference between people with LLV and viral suppression when comparing measures of subclinical CVD and atherosclerosis? In this doctoral thesis, these questions are explored by analysis of the Swedish HIV cohort, InfCare HIV, as well as a research cohort based in the North West province of South Africa.

Background

In 1981, the U.S. Centers for Disease Control and Prevention (CDC) reported five unusual cases of the opportunistic infection *Pneumocystis pneumonia* in Los Angeles, California. All patients were previously healthy men who have sex with men (MSM) and all had candida and cytomegalovirus (CMV) infections [6]. This was the first description of what would subsequently be termed AIDS, a new and devastating disease that was transmitted through sexual contact, in blood products, by contaminated injection equipment, and from pregnant women to their children.

Two years later, French researchers reported isolation of a novel retrovirus from the lymph node of an AIDS patient [7]. This virus was confirmed to be the causative agent of AIDS. After several names had been used for closely related isolates—lymphadenopathy-associated virus, human T-lymphotropic lymphotropic virus type III, immunodeficiency-associated virus, and AIDS-associated retrovirus—the International Committee on the Taxonomy of Viruses proposed the name HIV in 1986 [8].

Human immunodeficiency virus (HIV)

HIV belongs to the genus *Lentivirus* which is part of the family *Retroviridae*. As other retroviruses, it has an RNA genome and the characteristic ability to integrate its viral genome into the host cell. The prefix “retro” refers to the action of the viral enzyme reverse transcriptase, which produces deoxyribonucleic acid (DNA) from RNA templates.

Two types of HIV are recognized, HIV-1 and HIV-2. HIV-2 is mainly spread in West Africa and is characterized by a slower progression to AIDS [9]. HIV-1 is spread throughout the world and will be the focus of the rest of this thesis. Different zoonotic transmissions have resulted in four separate groups of HIV-1 (hereafter referred to as HIV): M (“major”), N (“non-M, non-O”), O (“outlier”), and the most recently described P group [10]. The N, O, and P groups have caused a small number of infections in Central Africa, but the M group is globally widespread and can be further subdivided into subtypes A–L as well as circulating recombinant forms [11]. In Sweden, as in the rest of Europe and North America, subtype B is hitherto the most prevalent

form. Subtype A is the dominant subtype in parts of East Africa and Russia, and subtype C in Southern Africa and parts of Asia [12].

The HIV genome consists of nine genes that encode fifteen proteins (Figure 1). *Gag* codes for the matrix and capsid proteins, *pol* encodes the viral enzymes reverse transcriptase, integrase, and protease, and *env* codes for the surface glycoproteins gp120 and gp41. *Vif*, *vpr*, *vpu*, *tat*, *rev*, and *nef* all encode regulatory and accessory proteins [13].

The main components of the HIV virion are the envelope (composed of a bilipid layer derived from the host cell and the *env* glycoproteins) and a cone-shaped capsid core which contains the RNA genome, the *pol* enzymes, as well as regulatory viral proteins [13].

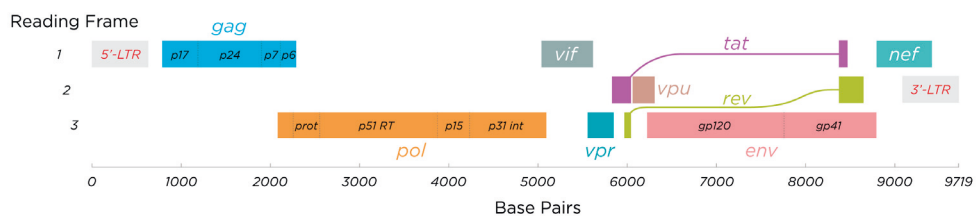


Figure 1. The structure of the HIV genome. Credit: Thomas Splettstoesser (www.scistyle.com), CC BY-SA 3.0.

The replication cycle

Infection of a new cell begins with binding of gp120 to the cellular receptor CD4 and coreceptors (Figure 2). The most important coreceptors are the chemokine receptors CCR5 and CXCR4. After fusion between the viral particle and the host cell, the components of the virus enter the cytoplasm. The viral reverse transcriptase retrotranscribes the viral genome into a double-stranded DNA which is transported into the nucleus and ultimately integrated into the host DNA [13]. The error-prone transcription of the reverse transcriptase is the main reason behind the rapid mutation rate of HIV that helps the virus evade immune control and antiviral drugs [14]. After the viral integrase integrates proviral DNA into the host chromosome, cellular factors start producing new viral RNA and viral proteins. Immature virions are formed after budding, and the viral protease cleaves polyproteins to create a mature and infectious virion [13].

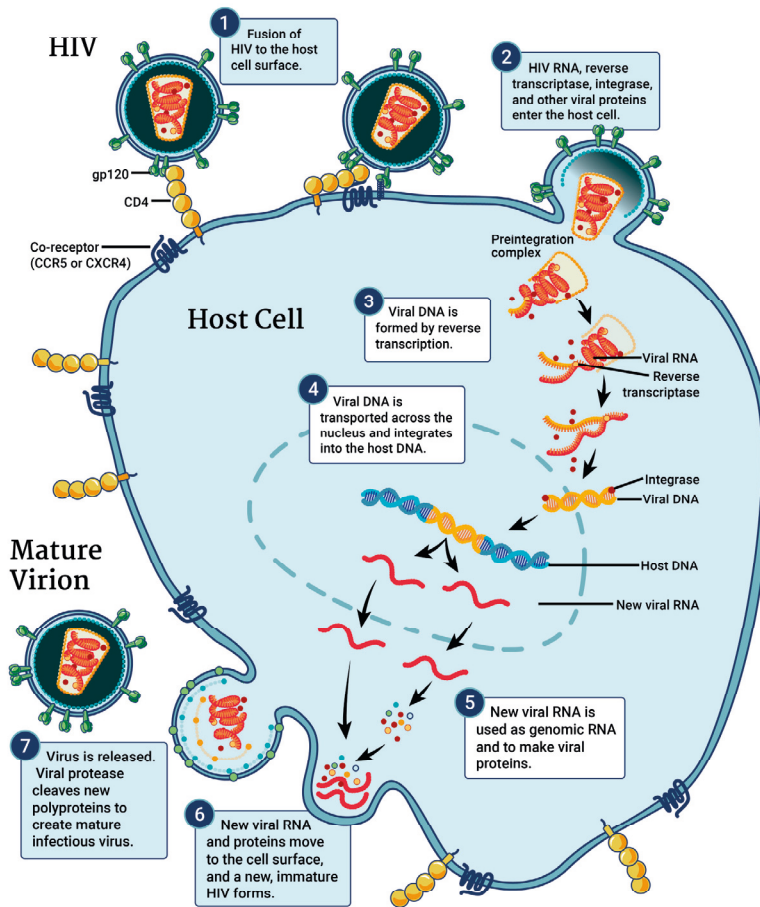


Figure 2. HIV replication cycle. Credit: National Institute of Allergy and Infectious Diseases (NIAID), CC BY 2.0.

Pathogenesis

HIV is transmitted by sexual contact, from mother to infant during pregnancy, delivery, and breastfeeding, or by bloodborne routes. Importantly, large trials have demonstrated that the transmission risk through condomless sex is effectively zero from a person with suppressed VL during ART [15, 16].

After exposure, the virus infects cells expressing the CD4 receptor, including CD4 T lymphocytes, resting CD4 T cells, monocytes, macrophages, and dendritic cells. The founder virus (typically using CCR5 as coreceptor) establishes the infection, after which rapid viral replication occurs [17]. It has been estimated that 40–90% of people with

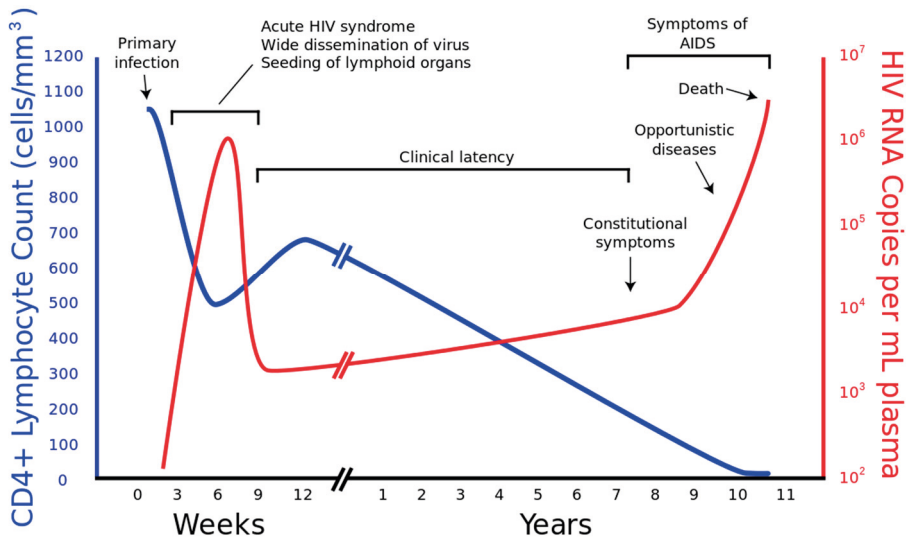


Figure 3. Viral loads and CD4 cell counts in untreated HIV infection. Credit: Jurema Oliveira, CC BY-SA 3.0, <https://commons.wikimedia.org/wiki/File:Hiv-timecourse.png>.

primary HIV infection experience transient symptoms of acute retroviral syndrome, commonly with flu-like illness and lymphadenopathy. VLs are very high at this stage, often $>1\,000\,000$ copies/mL (c/mL) [18]. After this initial peak, the VL decreases as a result of the host immune response. The level of this steady-state viremia—called the set point—is highly variable between patients and predictive of progression to AIDS [19]. Rare patients ($<1\%$) maintain undetectable VL without treatment; these are referred to as “HIV controllers” [20]. During the natural course of infection, CD4 T cell counts progressively diminish, resulting in increasing susceptibility to opportunistic infections (Figure 3). Most CD4 T cells are not lost due to direct HIV infection, but the main mechanism behind CD4 T cell death is caspase-1–mediated pyroptosis (a highly inflammatory type of programmed cell death) [21]. Although it normally takes several years for systemic CD4 T cell counts to drop to levels conferring high risk of development of AIDS, a massive reduction of CD4 T cells occurs in the gastrointestinal tract early in the course of infection, with limited recovery after start of treatment [17]. Latency of the HIV infection is established by survival of a stable reservoir of HIV-infected resting CD4 memory T cells. These are transcriptionally silent (thus evading antivirals and immune control), but capable of producing virus when the host cell is activated [22].

Apart from progressive immune suppression by reduction of CD4 cells, HIV infection is also characterized by increased immune activation. Several mechanisms behind the HIV-related chronic immune activation have been proposed (reviewed in [23]), including

recognition of HIV antigen by B and T lymphocytes as well as Toll-like receptors of the innate immune system; immune stimulation by viral proteins, such as Nef; chronic CMV infection or CMV reactivation, as well as other chronic viral infections; microbial translocation in the intestinal tract enabled by the local depletion of CD4 cells, resulting in stimulation of pro-inflammatory cytokines such as tumor necrosis factor alpha and interleukin (IL)-6 by fungal and bacterial products; altered homeostasis of different CD4 cell subsets, e.g. preferential loss of Th17 cells; T cell proliferation in response to loss of central memory CD4 T cells; and increased production of pro-inflammatory molecules, such as type I interferon (Figure 4). Two ways of assessing immune activation in PWH are by measuring activation of T cells and other immune cells or by plasma biomarkers. After start of ART, immune activation as measured by T cell activation declines, although generally not reaching the level of HIV-negative individuals [24]. Markers of residual inflammation (such as C-reactive protein [CRP] and IL-6) often remain elevated even in patients with effective ART and CD4 recovery and have been linked to increased mortality, CVD, and cancer [17]. As discussed below, markers of monocyte activation (such as soluble CD14 and CD163) and the coagulation cascade (such as D-dimer) are also predictive of clinical outcomes in PWH.

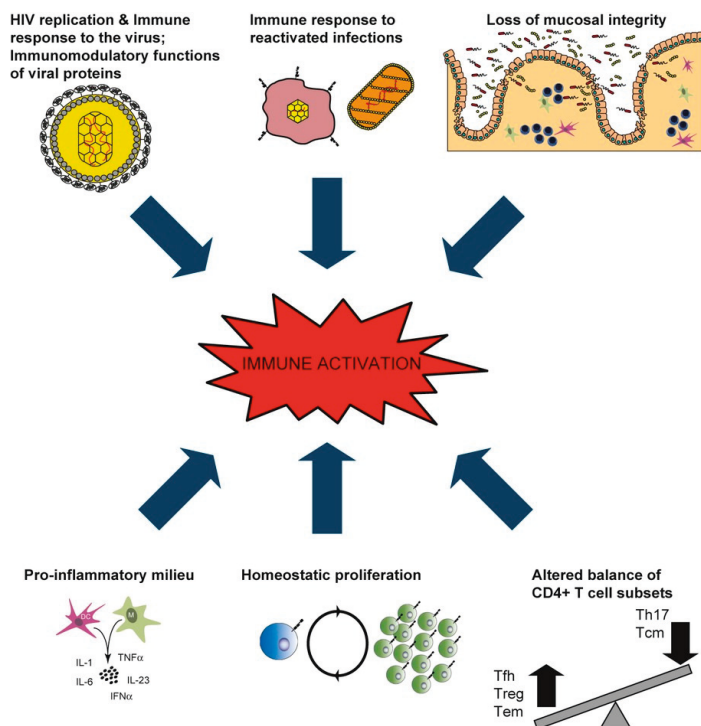


Figure 4. Factors that contribute to HIV-associated chronic immune activation. From Paiardini, *Immunol Rev.* 2013 [23]. Reprinted with permission from John Wiley and Sons.

The HIV epidemic

When the virus was first isolated in 1983, around 170 000 people had been infected [25]. In the latest Joint United Nations Programme on HIV/AIDS (UNAIDS) global report, it was estimated that 38 million people (plausibility bounds, 32–45 million) are currently living with HIV, and each year there are 1.7 million new infections and 690 000 AIDS-related deaths. The improved survival of PWH has contributed to a growing total number of cases, and more people are living with HIV today than ever before. While a majority of global cases are found in Eastern and Southern Africa (21 million), this region has seen a decrease in the number of new infections by 38% since 2010. Decreasing incidence has also been observed in other parts of the world. In contrast, however, there are regions where the number of new cases has increased since 2010: by 72% in Eastern Europe and Central Asia, by 22% in the Middle East and North Africa, and by 21% in Latin America [26]. South Africa remains the country with the highest case count (7.8 million people in 2020) [27]. The specific risk for different subpopulations varies by region. In Western and Central Europe and North America, MSM accounted for 64% of new infections in 2019, while young women are at highest risk in Eastern and Southern Africa. Globally, 10% of new cases are in people who inject drugs, but this subpopulation account for almost half of the new cases in Eastern Europe and Central Asia [26].

Despite decreased mortality and morbidity due to improved access to ART, HIV remains an important contributor to burden of disease. In the Global Burden of Disease Study 2019, HIV was the second cause of disability-adjusted life-years (after road traffic injuries) in adults aged 25–49 years, and number nine in people aged 10–24 years [28].

In 2014, United Nations articulated the 90–90–90 targets as a step towards ending the HIV pandemic by 2030. The aim was that by the end of 2020, at least 90% of all PWH should know their HIV status, at least 90% of known cases should receive ART, and at least 90% of treated individuals should be virally suppressed. While this has been achieved in some countries (Australia, Botswana, Cambodia, Denmark, Eswatini, France, Germany, Iceland, Ireland, Namibia, the Netherlands, Rwanda, Spain, Sweden, Switzerland, Thailand, Zambia, Zimbabwe, and the United Kingdom [1, 29, 30]), UNAIDS estimated in September 2020 that the global targets were unlikely to be met [31].

HIV in Sweden

The prevalence of HIV in Sweden is low; 8020 (0.08% of the entire population) were diagnosed with HIV as of 2019 [32]. The number of new cases per year has been relatively constant over the recent 10 years, with 449 new cases being reported in 2019. Among these, 17% (n = 78) were infected in Sweden, with 53% (41/78) having acquired HIV through male-to-male sex, 35% (27/78) through heterosexual contact, and 10% (8/78) through injecting drug use. Among cases infected outside Sweden, heterosexual contact was the main route of transmission (49%, 173/352) [33]. In 2016, Sweden was claimed to be the first country to achieve the 90–90–90 targets, with a total of 78% of the estimated population of PWH being virally suppressed [30].

HIV care is provided at infectious disease clinics at 29 sites throughout Sweden. By the Communicable Diseases Act, all new cases are reported to the Public Health Agency both by the clinical laboratory and the treating physician. PWH are required to keep appointments, and ART is provided free of charge [34].

Clinical management of HIV infection

Staging of HIV infection

Without ART, the progressive loss of CD4 cells leads to impaired cellular immunity, susceptibility to opportunistic infections and certain malignancies, and development of AIDS. The median duration from HIV acquisition to onset of AIDS is 10 years, with large inter-individual variations [35]. According to the CDC staging system, an adult person meets the case definition of AIDS (stage 3) if CD4 cell counts are below 200 cells/ μ L or the patient has at least one of 23 defined clinical conditions [36]. The other staging system, by the World Health Organization (WHO), does not include a CD4-based criterion, and is more suited for low- and middle-income settings, where access to laboratory monitoring is generally limited [37].

Diagnostic testing

In a study from 2015, 58% of new HIV cases in Sweden were late presenters, meaning that CD4 cell counts were <350 cells/ μ L or that AIDS-defining conditions were present at the time of HIV diagnosis [38]. Globally, it is estimated that 81% of PWH know their HIV status [31]. Given the benefit of early diagnosis and treatment, increased HIV testing could thus have a positive impact on individual and public health.

HIV tests are either antibody tests or combined antigen–antibody tests that test for HIV antibodies as well as the *gag* protein p24 (which increases sensitivity during acute infection). Antigen–antibody tests are used for screening at all Swedish laboratories, and reactive tests are verified by confirmatory testing [39]. A window period of 6 weeks after exposure is recommended to rule out infection, although approximately half test positive after 2 weeks. In some situations, rapid tests for HIV antibodies are used; these tests have a window period of 8 weeks [39]. Opt-out testing is implemented for certain populations with increased HIV prevalence (such as immigrants from high-endemic countries) and in populations where an HIV diagnosis would have significant adverse implications (such as pregnant women). Furthermore, testing based on indicator conditions is recommended in Europe [40]. These are conditions with an estimated HIV prevalence of >0.1%, and some examples (from a Swedish perspective) are sexually transmitted disease of any type, tuberculosis, all types of lymphoma, cervix and anal cancer, herpes zoster in people <65 years old, and hepatitis B and C virus (HBV, HCV) infections [41].

Globally, unequal or limited access to HIV testing remains a barrier to control of the pandemic. A study from 2020 showed that while uptake of HIV testing has increased in sub-Saharan Africa between 2008 and 2016, large absolute and relative inequalities remain, with lower access for people living in poverty and for those with low education [42].

Antiretroviral therapy (ART)

The development and successive improvement of ART has transformed HIV infection from a deadly disease to a chronic manageable condition. In 1987, zidovudine (AZT), a nucleoside reverse transcriptase inhibitor (NRTI), became the first approved treatment for HIV-infection, followed by other NRTIs [43]. Treatment with AZT improved short-term survival and decreased the risk of opportunistic infections in people with symptomatic HIV infection [44]. However, these early NRTIs had considerable toxicity, and it soon became clear that both monotherapy and early types of combination regimens (such as double NRTI) led to rapid development of drug resistance [45]. An important milestone in the history of ART was the Vancouver AIDS conference in 1996, where the International AIDS Society–USA first recommended triple therapy, combining two NRTIs with one protease inhibitor (PI) or one non-nucleoside reverse transcriptase inhibitor (NNRTI) [43].

Since the introduction of the first antiretrovirals, several new agents have been developed and approved, all targeting different steps in the replication cycle of HIV. NRTIs and NNRTIs both target the viral reverse transcriptase, PIs inhibit the viral

Table 1. List of antiretroviral agents available in Sweden in January 2019 [41]. Reproduced with permission.

Generic name	Abbreviation
Nucleoside/nucleotide reverse transcriptase inhibitors (NRTI)	
abacavir	ABC
emtricitabine	FTC
lamivudine	3TC
tenofovir disoproxil fumarate	TDF
tenofovir alafenamide	TAF
zidovudine	AZT
Non-nucleoside reverse transcriptase inhibitors (NNRTI)	
efavirenz	EFV
nevirapine	NVP
etravirine	ETR
rilpivirine	RPV
doravirine	DOR
Protease inhibitors (PI)	
atazanavir	ATV
darunavir	DRV
fosamprenavir	fAPV
indinavir	IDV
lopinavir/ritonavir	LPV/r
ritonavir	RTV
tipranavir	TPV
Integrase strand transfer inhibitors (INSTI)	
raltegravir	RAL
dolutegravir	DTG
elvitegravir	EVG
bictegravir	BIC
Fusion inhibitors (FI)	
enfuvirtide	T-20
CCR5 inhibitors	
maraviroc	MVC

protease, and integrase strand transfer inhibitors (INSTI) block the action of the viral integrase. Drugs with other mechanisms of action are more rarely used (enfuvirtide, which binds to the *env* glycoprotein gp41 that is required for infection of new cells, and maraviroc, which binds to the coreceptor CCR5). A list of antiretroviral agents available in Sweden in January 2019 is presented in Table 1. Since then, fostemsavir (which blocks gp120) has also been approved.

Despite achievement of viral suppression during effective ART, latent HIV that persists in resting CD4 cells constitutes a pool for reactivation following treatment interruptions [46, 47]. Lifelong treatment is thus needed.

Typically, a backbone of two NRTIs is combined with one anchoring agent from another class. The recommended first-line regimens for treatment-naïve individuals in Sweden are currently emtricitabine (FTC) + tenofovir disoproxil fumarate (TDF) + dolutegravir (DTG) or abacavir (ABC) + lamivudine (3TC) + ritonavir-boosted darunavir (DRV/r) [41]. Nevertheless, many other regimens are possible and could be preferred in certain situations; aspects to consider in the choice of antiretroviral regimen include comorbidities and opportunistic infections, other medications (with possible drug–drug interactions), potential to become pregnant, HBV coinfection, VL and CD4 cell counts, presence of genotypic resistance, previous ART experience, anticipated adherence, and cost-effectiveness. In 2018, WHO global guidelines were updated to recommend a DTG-based regimen as preferred first-line regimen, instead of an efavirenz (EFV)-based that was previously recommended [48].

Growing evidence suggests that certain two-drug regimens are effective in maintaining viral suppression in patients who have suppression on their current regimen. This has been shown in large trials for DTG + rilpivirine [49], DTG + 3TC [50], ritonavir-boosted PI + 3TC [51, 52], and in a smaller trial for DRV/r + DTG [53]. Moreover, DTG + 3TC was non-inferior to DTG + FTC + TDF as initial treatment in the GEMINI-1 and GEMINI-2 trials [54]. Following these results, two-drug regimens are now recommended in select cases [41, 55].

The benefit of starting ART early, as opposed to deferring treatment until CD4 cell counts decrease below 500 cells/ μ L, has been shown in two large randomized controlled trials: Strategic Timing of Antiretroviral Therapy (START) [56] and TEMPRANO [57]. In START, patients starting ART with CD4 cell counts of >500 cells/ μ L had a hazard ratio (HR) of 0.43 (95% confidence interval [CI], 0.30–0.62) for the primary composite endpoint (death, AIDS, or SNAE). For the individual components of the composite endpoint, the immediate-initiation group had significantly lower risks of serious AIDS-events, SNAE, tuberculosis, and Kaposi sarcoma [56]. Moreover, early start of ART could be associated with lower chronic immune activation and smaller reservoir size [58].

ART is also used for prevention of HIV in different contexts. As virally suppressed individuals cannot transmit HIV to others, high treatment coverage in a population will reduce the incidence of HIV infection—a concept referred to as “treatment as prevention” [26]. Treatment of women during pregnancy and delivery reduces mother-to-child transmission [59]. Post-exposure prophylaxis with e.g. FTC + TDF + raltegravir (RAL) is recommended in some situations to decrease the risk of acquiring HIV after an exposure [41]. Furthermore, pre-exposure prophylaxis with FTC + TDF has been demonstrated effective in people with high risk of HIV acquisition (such as certain MSM or transgender individuals) [60, 61], and this strategy is currently recommended in Swedish, European, American, and WHO guidelines [41, 55, 62, 63].

Laboratory monitoring during treatment

Two biomarkers are central in the monitoring of PWH: plasma VL and CD4 cell counts. CD4 cell counts reflect the degree of immunosuppression and guide the need of prophylaxis to prevent opportunistic infections. In a European cohort of patients with viral suppression during ART, the mean increase in CD4 counts was 100 cells/ μ L during the first year after ART initiation. Thereafter, lower but still significant yearly increases were observed [64].

VL has been shown to be a better predictor of progression to AIDS and death than CD4 cell counts in the natural course of HIV infection [19]. In patients initiating ART, plasma VL is the most important biomarker of treatment success; the proportion of time a patient has undetectable VL during 6–18 months after ART initiation is predictive of future virologic outcome, CD4 cell counts, and all-cause mortality [65]. Normally, ART initiation is followed by undetectable plasma VL and recovery of CD4 cell counts, but other trajectories are possible. So called “discordant immune response”, with unsatisfactory CD4 reconstitution despite viral suppression is linked to increased mortality [66]. As discussed below, different patterns of detectable viremia during ART can be a sign of treatment failure and predictive of worse outcomes.

Viral load assays

The development of new assays for VL monitoring has lowered the limit of detectable viremia. The Roche Molecular Systems (Basel, Switzerland) Amplicor HIV-1 Monitor Test was the first widely available assay (approved by the U.S. Food and Drug Administration in 1996). This test was the gold standard for viral suppression in many clinical trials, and the lower limit of detection was first 400 c/mL and, in a later update, 50 c/mL. Since then, several more sensitive assays have been developed, such as the Roche Cobas TaqMan version 2.0, with a detection limit of 20 c/mL and Abbot (North Chicago, IL) RealTime RT-PCR, with a detection limit of 40 c/mL [67].

For the topic of this thesis, the comparability between assays in the low detectable range is highly relevant. This was explored in a large international collaboration, which found lower inter-assay correlation for samples with VL <1000 c/mL compared with the full range. Concordance between assays was greater when using <200 c/mL as a threshold, compared with <50 c/mL. Furthermore, this study showed that VLs of 125 and 100 c/mL with the newer assays TaqMan version 1.0 and 2.0, respectively, correspond to <50 c/mL with the earlier widely used Amplicor test [68]. In another study, 3.4% of samples with <50 c/mL with the Amplicor test had a VL \geq 50 c/mL with TaqMan version 2.0 [69]. The frequency and relevance of detectable VL <200 c/mL could thus depend on the assay.

Drug resistance

As HIV replicates, mutations arise randomly. An infected individual thus harbors a large number of genetically different variants or quasispecies [14]. Some of these mutations could alter the virus' susceptibility to antiretroviral agents. The approximate number of mutations needed to confer resistance differ between different drugs, often called the genetic barrier to resistance. First-generation NNRTIs such as EFV and nevirapine (NVP) and the INSTIs RAL and elvitegravir all have a low barrier, with only one mutation required for high-grade resistance. Ritonavir-boosted lopinavir (LPV/r) and DRV/r, on the other hand, generally require 3–4 mutations, and the second-generation INSTI DTG also has a high barrier to resistance [70].

Drug resistant viruses can be transmitted (transmitted resistance is one form of pre-treatment drug resistance, which also includes resistance acquired during previous exposure to antiretroviral drugs, such as prevention of mother-to-child transmission), but resistance can also emerge in an infected individual (acquired drug resistance). In Sweden, the prevalence of transmitted drug resistance increased from 5.6% in 2003–2010 to 7.1% in 2010–2016, mainly driven by an increase in NNRTI-resistance in migrants from sub-Saharan Africa [71, 72]. In many low- and middle-income countries, the pre-treatment drug resistance currently exceeds 10% [73].

Resistance testing can be performed by genotypic or phenotypic methods; genotypic testing is used in clinical routine. The most common type of resistance testing is population sequencing (Sanger) that detects known resistance mutations if present in >20% of the viral population. More sensitive methods are needed to find less frequent minority variants [70]. In high-income settings, resistance testing is recommended before treatment initiation and in case of virologic failure [41, 55, 63]. A reference database, such as the Stanford HIV Drug Resistance Database, can be used to interpret the results from resistance testing [74]. The success rate of resistance testing is greatest in samples with higher VL, but genotypic resistance testing can often also be done at VL <1000 c/mL [75–77].

Mortality and morbidity in the ART era

Mortality and causes of death

Following the introduction of combination ART, HIV-related mortality declined sharply in high-income settings (Figure 5). In a U.S. study, the death rate decreased from 29.4 to 8.8 per 100 person-years between 1995 and 1997; during the same

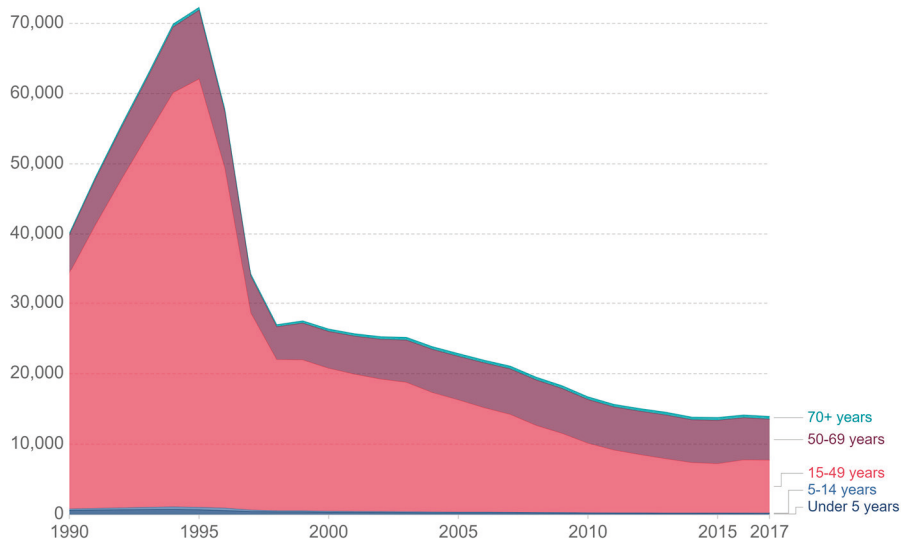


Figure 5. Number of yearly deaths from HIV/AIDS in high-income countries 1990–2017. Source: Institute for Health Metrics and Evaluation, Global Burden of Disease. From: OurWorldInData.org/hiv-aids, CC-BY [78].

period, the incidence of AIDS declined from 50 to 13.3 per 100 person-years [79]. Still, PWH have increased mortality compared with the general population. A recent large cohort study from the United States found a 9.1-year difference in overall life expectancy between PWH and matched uninfected controls (6.8 years for PWH initiating ART with CD4 cell counts of >500 cells/ μ L); matching was based on age, sex, race/ethnicity, medical center, and calendar year. When comparing comorbidity-free years, the difference was 16.3 years [2]. Increased mortality for PWH despite effective ART has also been observed in other cohorts [80–83], although some reports suggest that patients who achieve immune recovery and viral suppression after treatment initiation have normal or near-normal life expectancy [82, 84].

With declining incidence of AIDS and an aging population of PWH, the panorama of causes of death has changed. Important causes of death among PWH now include non-AIDS-defining cancer, CVD, and liver disease [80, 85–87]. Still, AIDS remained the most common cause of death among PWH in a recent study from the United Kingdom; of people who died from AIDS in this material, 87% were late presenters and 29% were never linked to care [80]. The relative contribution of AIDS to overall mortality among PWH decreases substantially during the first year after ART initiation; correspondingly, the importance of non-AIDS morbidity increases in long-term ART recipients [88].

Cardiovascular disease among people with HIV

CVDs, and most importantly ischemic heart disease and stroke, are major contributors to the global disease burden [28], and it is estimated that PWH have a more than twofold increase in the risk of CVD compared with uninfected people [3]. Following this observation, it has been proposed that HIV should be regarded as a major cardiovascular risk factor, along with smoking, hypertension, diabetes mellitus, and dyslipidemia [89].

Specifically, PWH have increased risk of acute myocardial infarction [90], sudden cardiac death [91], congestive heart failure [92], peripheral artery disease [93], stroke [94], and venous thromboembolic diseases [95]. The proposed mechanisms behind the increased risk of coronary heart disease for PWH are discussed in a recent review [96] and can include higher prevalence of traditional risk factors, cellular immunodeficiency, immune activation and chronic inflammation, as well as cumulative exposure to ART. A study from the Danish HIV cohort suggested that the population-attributable factor (a measure of the reduction that would occur if a risk factor were modified to its ideal status, e.g. no smoking) of smoking for myocardial infarction among PWH is 72%, underscoring the importance of reducing tobacco smoking in HIV-positive populations [97]. The relationship between antiretroviral drugs and cardiovascular events has been extensively studied. In a systematic review, an increased CVD risk was observed for PI-including regimens and recent exposure to ABC [98]. For the more recent PIs, increased incidence of CVD has been associated with cumulative use of DRV/r, but not with ritonavir-boosted atazanavir [99]. Concerns about ART toxicity were addressed in a large randomized trial, in which patients with stable ART were randomized to continued treatment or structured treatment interruptions, where ART was restarted when CD4 cell counts decreased below 250 cells/ μ L—the Strategies for Management of Antiretroviral Therapy (SMART) trial. Patients with treatment interruptions had increased mortality and 70% increased hazards of major cardiovascular, renal, and hepatic disease, rejecting the hypothesis that treatment interruptions could lower CVD risk [100]. Instead, the results from the SMART trial stress the importance of continuous suppression of viral replication for optimizing treatment outcomes in ART recipients.

Inflammation—a general term for multiple biological processes—plays a crucial role in the development and progression of atherosclerosis and CVD, both in PWH and the general population. It is important to note that systemic levels of inflammation markers may not always give relevant insight to local inflammatory processes in endothelial cells or the myocardium. Nevertheless, the plasma biomarkers IL-6, CRP, and D-dimer predict CVD among PWH receiving ART (as in the general population) [101–104]. Other markers of immune activation that have been studied among PWH are the monocyte markers soluble CD14 and CD163 [105]. Since these previous biomarkers

are mainly secreted outside the cardiovascular system, there has also been an interest in more specific biomarkers expressed by cardiovascular tissue. Suppression of tumorigenicity 2 (ST2), growth differentiation factor 15 (GDF-15), N-terminal pro B-type natriuretic peptide (NT-proBNP), and high-sensitivity troponin have all been shown to predict CVD risk in the general population [106]. Still, few studies have hitherto evaluated these more cardiovascular-specific markers in PWH [107–109].

Cancer risk among people with HIV

PWH have increased cancer risk compared with HIV-negative individuals, and a list of cancer forms with increased prevalence in PWH is presented in Table 2. Following the introduction of ART, the risks of several types of virus-related cancer as well as lung cancer have decreased but remain elevated compared with uninfected controls [4].

Table 2. List of malignancies with increased prevalence among people with HIV. Based on [4, 110].

Cancer form	Related microorganism	Note
AIDS-defining cancer		
Kaposi sarcoma	HHV-8	
Non-Hodgkin lymphoma	EBV (not all types)	
Cervix cancer	HPV	
Infection-related non-AIDS-defining cancer		
Hodgkin lymphoma	EBV	
Hepatocellular carcinoma	HBV, HCV	
Stomach cancer	<i>Helicobacter pylori</i>	Increased risk in [110] but not in [4]
Vulva and vagina cancer	HPV	
Penis cancer	HPV	
Anal cancer	HPV	
Oral cavity and pharynx squamous cell carcinoma	HPV	
Non-melanoma skin cancer	HPV (possibly)	
Lip cancer	HPV (possibly)	
Esophagus cancer	HPV (possibly)	
Larynx cancer	HPV (possibly)	
Eye cancer	HPV (possibly)	
Non-AIDS-defining cancer with no known association with infectious agents		
Trachea, bronchus, and lung cancer		
Kidney cancer		Increased risk in [110] but not in [4]
Multiple myeloma		
Leukemia		
Malignant melanoma		
Brain cancer		Increased risk in [110] but not in [4]
Testis cancer		

Abbreviations: HHV-8, human herpesvirus 8; EBV, Epstein-Barr virus.

Some of the increased cancer risk can be due to increased prevalence of recognized risk factors for different types of cancer among PWH; a meta-analysis of studies on adults in high-income settings observed increased smoking and increased prevalence of high-risk human papillomavirus (HPV), HBV, and HCV infections in PWH compared with uninfected controls [111]. Nevertheless, directly HIV-related effects likely contribute: immunodeficiency, reflected in lower CD4 cell counts, as well as immune activation and inflammation are possible mechanisms in which HIV infection may increase cancer risk [112]. Interestingly, the risk of lung cancer among PWH was found to be increased even after adjustment for smoking in a large U.S. study [113]. Higher levels of certain blood biomarkers (IL-6, CRP, and D-dimer) are all associated with higher risk of developing cancer in ART recipients [114].

The SMART and START trials both shed light on the pathogenesis of cancer among PWH. Patients with structured treatment interruptions in the SMART trial had a significantly higher risk of AIDS-defining cancer but not of non-AIDS-defining cancer [115]. In the START trial, reduced risks of infection-related cancer (including Kaposi sarcoma, non-Hodgkin lymphoma, Hodgkin lymphoma, anal cancer, and cervical cancer) were reported for individuals randomized to immediate ART. There was also a reduction in infection-unrelated cancer, albeit not statistically significant [116].

The increased risk of cervical cancer among women with HIV highlights the importance of screening, which should be performed in the same age groups as for the general population [41]. Long-term follow-up of Danish women with HIV suggested that women with normal cytology at baseline who attended regular screening did not have increased risk compared with HIV-negative controls [117].

A global perspective

Globally, severely limited access to ART resulted in a continued increase in HIV-related deaths until 2006, after which it has declined in many world regions, especially sub-Saharan Africa [78]. Tuberculosis remains the most important cause of death among PWH, but with increased rollout of ART and an aging population, non-communicable diseases is already a major threat to the health of PWH, and this problem is likely to grow [26]. Furthermore, HIV can impact the epidemiology of non-communicable diseases. In some countries with high HIV prevalence, HIV is a major contributor to atherosclerotic CVD; the population-attributable factor is estimated to be >15% in Swaziland, Botswana, Lesotho, and South Africa [3].

Low-level viremia (LLV)

Most ART recipients achieve persistent viral suppression with undetectable VL in plasma, but some individuals have low levels of detectable HIV RNA, commonly referred to as LLV (Figure 6). There is no standard definition of LLV, but the term is usually reserved for detectable VL in repeated measurements (as opposed to transient episodes of viremia, “blips”) below the threshold for virologic failure [118, 119].

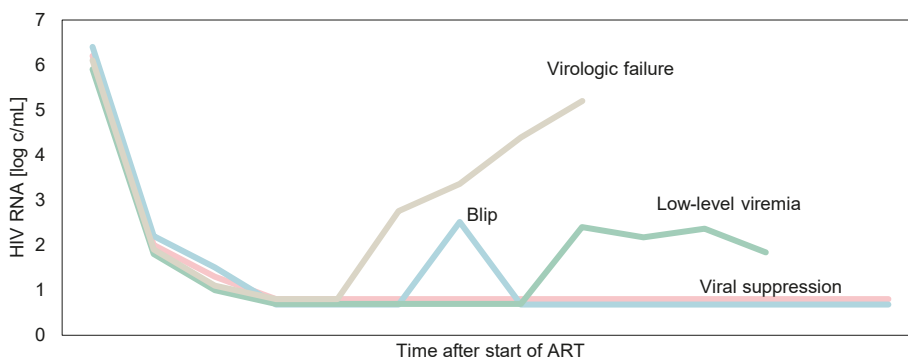


Figure 6. Different viremia profiles during antiretroviral therapy.

Swedish, British, and U.S. guidelines all currently define virologic failure as repeated VL >200 c/mL (Table 3); the definition of LLV is thus persistently detectable viremia below this threshold [41, 63, 120]. European AIDS Clinical Society (EACS) has a stricter definition of failure: confirmed VL >50 c/mL [55]. WHO consolidated guidelines, which are widely used in low- and middle-income countries, define failure as repeated VL >1000 c/mL and LLV as 50–1000 c/mL [121]. Since the material analyzed in this thesis dates back to 1996, historic versions of these guidelines are also relevant. Until 2007, Swedish guidelines recommended considering 50 c/mL as the threshold for virologic failure and did not include any specific recommendations for LLV [122]. In the guidelines from the U.S. Department of Health and Human Services, >50 c/mL was the definition of failure until 2009, although the authors stated that the best management for LLV of 50–1000 c/mL is not clear and that many experts would continue current therapy with close monitoring [123]. EACS has defined failure as >50 c/mL back to 2003, but the threshold at which immediate regimen switch is recommended has been revised from confirmed VL of 500 or 1000 c/mL in 2003 to 200 c/mL in the latest update [55, 124].

Table 3. Summary of guideline recommendations regarding LLV.

	Definition of viral suppression	Definition of virologic failure	Definition of LLV	Management of LLV
RAV, 2019 [41]	Below the limit of detection with commercial assay.	Repeated VL >200 c/mL.	20–200 c/mL, if adherence and treatment are considered adequate.	Not specified. If VL >200 c/mL: consider adherence, interactions, TDM, resistance testing, consulting a specialist, and change treatment if needed.
EACS 10.1, 2020 [55]	VL <50 c/mL for at least 6 months.	Incomplete suppression: VL >200 c/mL at 6 months after starting ART. Rebound: confirmed VL >50 c/mL if previously undetectable.	Not defined.	If VL 50–200 c/mL: check adherence, recheck VL in 1–2 months, consider regimen switch. If VL confirmed >200 c/mL: change regimen as soon as possible, resistance testing, consider TDM.
DHSS, 2019 [63]	VL <20–75 c/mL, depending on the assay.	Confirmed VL >200 c/mL.	Confirmed detectable VL <200 c/mL.	If VL <200 c/mL: continue current regimen and monitor VL every 3 months. If VL 200–1000 c/mL: resistance testing (especially >500 c/mL), consider adherence, interactions, TDM, change treatment if needed.
BHIVA, 2016 [120]	VL <50 c/mL.	Incomplete response: repeated VL >200 c/mL after 24 weeks without any VL <50 c/mL. Rebound: >50 c/mL in two consecutive measurements.	Persistent VL 50–200 c/mL	If VL 50–200 c/mL: resistance testing if feasible. If LLV on a regimen with low genetic barrier, consider regimen change. Consider adherence, tolerability, interactions, mental health/drug dependency.
WHO, 2016 [121]	Below the limit of detection with commercial assay.	Persistently detectable VL >1000 c/mL (for ≥3 months) ≥6 months after starting ART.	50–1000 c/mL.	No specific management unless VL >1000 c/mL.

Abbreviations: RAV, The Swedish Reference Group for Antiviral Therapy; TDM, therapeutic drug monitoring; DHSS, Department of Health and Human Services; BHIVA, British HIV Association.

Virologic blips

An isolated detectable VL, preceded and followed by undetectable values, is called a virologic blip. Blips could represent random statistical and biological variation, and several studies have not observed any association between blips and adverse outcomes [125–128]. Yet, increased risk of virologic failure has been observed in other studies [129–132]. Furthermore, blips are associated with the size of the latent HIV reservoir, as measured by HIV DNA in peripheral blood mononuclear cells [133]. Among Swedish adults starting ART 2007–2013, 10% experienced at least one blip during a median follow-up of 170 weeks [131].

To determine the longevity of virologic blips, frequent VL sampling is needed. In a study from 2001, ten patients underwent intensive sampling (one sample every 2–3 days during 3–4 months). Nine patients had at least one blip, and the median duration of blips was 2.5 days (range, 2–11.5) [125]. With sampling frequency around once every 3–6 months in clinical practice, it is thus not possible to distinguish between short-lived blips and more prolonged episodes of viremia in studies based on VL samples obtained in clinical routine.

Mechanisms of LLV

At least two separate mechanisms contribute to the occurrence of LLV. Phylogenetic analysis of samples from eleven children with LLV showed six specimens with multiple identical sequences (suggesting clonal outgrowth from latently infected cells, Figure 7) and three specimens with signs of viral evolution (suggesting ongoing replication) [134]. In another study, the *env* sequences were found to be diverse in 11 of 28 (39%) adults with LLV and monotypic in 17 (61%). Interestingly, blood samples from people with monotypic versus diverse LLV showed distinct cytokine profiles (higher CRP and soluble CD163 in monotypic LLV; higher soluble CD14 in diverse) and monotypic LLV was also linked to a higher frequency of CXCR4-using variants, which could be related to starting ART at an advanced disease stage [135]. Ongoing replication during ART (as in diverse LLV) could be caused by inadequate antiretroviral drug concentrations in plasma (e.g. caused by drug–drug interactions or insufficient adherence), lower local drug exposure in anatomical sanctuary sites (such as the central nervous system), or the inability of ART to prevent cell-to-cell spread of HIV [136]. The respective importance of monotypic versus diverse LLV in larger populations remains unknown, although smaller studies suggest that monotypic LLV is the dominant type [125, 134, 135, 137–139].

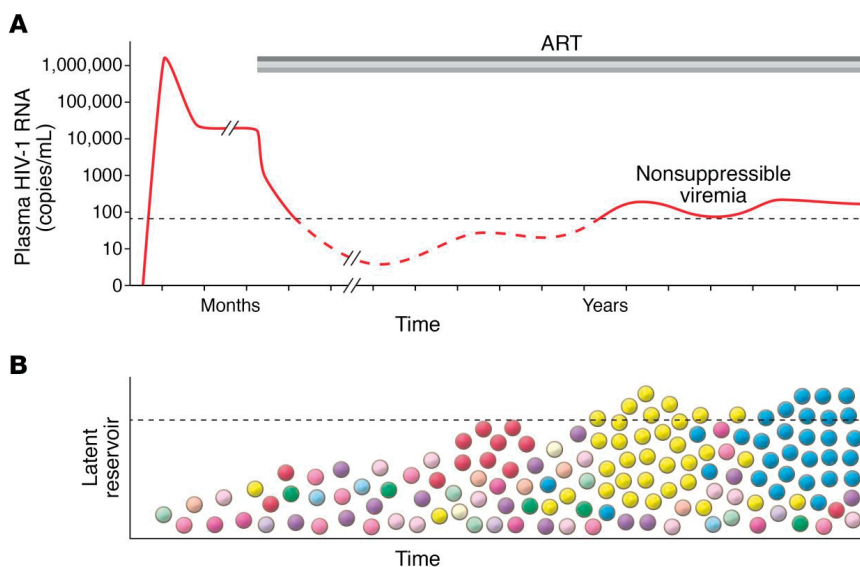


Figure 7. A schematic example of how clonal outgrowth of latently infected CD4 T cells can contribute to LLV during ART without ongoing viral replication. (A) Initiation of ART results in suppression of plasma VL below the limit of detection. Later, however, the example patient develops LLV as a result of virus release from the reservoir. **(B)** Each color represents a clonal lineage of latently infected CD4 T cells. In this individual, expansion of the yellow and blue clones is sufficient to produce detectable viremia. From Siliciano, J Clin Invest. 2020 [140]. Reprinted with permission from American Society for Clinical Investigation.

Risk factors for LLV are incompletely characterized. Several studies (but not all [141]) have reported higher incidence of LLV in people with high pre-ART VL [142–144]. As high pre-ART VL is related to large HIV reservoir size, this is consistent with the hypothesis of a large reservoir as the source of LLV [133]. PI-based regimens have been associated with higher risks of LLV compared with NNRTI-based ART [132, 142–144]; however, this could be confounded by indication, as providers may be more prone to prescribe PI-based regimens to patients with anticipated lower adherence and/or high baseline VL. Still, this relationship between PIs and LLV has also been observed in retrospective analysis of two randomized trials, with lower risk of indication bias [145]. Limited data indicate that INSTI-based regimens are associated with lower risk of LLV [146]. The association between LLV and adherence is not clear, partly because adherence is difficult to measure accurately. Increased risk of LLV has been reported in patients with lower adherence to ART, as measured by unannounced pill counts and prescription refill rates [147, 148], but not in a study using a combination of self-reported adherence and plasma drug concentrations [135]. Several socio-demographic risk factors for LLV have been described, such as male sex, injecting drug use, and social deprivation [142, 143, 146, 148]. Furthermore, shorter ART duration, non-B subtype, HIV RNA quantification by Roche TaqMan (compared with Abbot RealTime), low CD4 nadir, type 2 diabetes, and subtherapeutic plasma drug concentrations have all been associated with LLV in different studies [142–144, 146, 149–152].

A related phenomenon—although not the focus of this thesis—is the presence of residual viremia below the level of detection with routine assays. In fact, highly sensitive single-copy assays have revealed that >80% of ART recipients with undetectable VL with routine assays have residual viremia of ≥ 1 c/mL [153]. The mechanisms behind this residual viremia are reviewed in [154], and while clonal expansion from latently infected cells likely is the dominant mechanism, some degree of persistent replication cannot be ruled out. Residual viremia could be predictive of future higher viremia; in one study, the risk of a subsequent VL >400 c/mL was 13% for individuals with a VL of 40–49 c/mL, 3.8% for detectable VL <40 c/mL, and 1.2% for undetectable VL [155].

Virologic consequences of LLV

Future virologic failure

A large body of evidence from observational studies in high-income settings supports an association between LLV and subsequent virologic failure, although the exact amplitude at which LLV becomes significant is controversial. This could also depend on the definition of virologic failure. An analysis of 17 902 PWH from the Antiretroviral Therapy Cohort Collaboration (ART-CC; from the United States,

Canada, and several European countries) showed a strong association (adjusted hazard ratio [aHR], 3.97; 95% CI, 3.05–5.17) between LLV in the range 200–499 c/mL and virologic failure (defined as at least two consecutive VLs ≥ 500 c/mL), and a non-significantly increased risk for LLV of 50–199 c/mL (aHR, 1.38; 95% CI, 0.96–2.00) [156]. Several other studies have reported increased risk of virologic failure (defined as consecutive VLs ≥ 400 , ≥ 500 , or ≥ 1000 c/mL, respectively) for LLV in the ranges 50–1000, 200–500, 200–400, 50–500, and 50–400 c/mL [129, 141, 146, 157–160].

For LLV of < 200 c/mL, the association with future virologic failure is less clear. An increased risk has been observed in some [5, 160–163] but not all [146, 156] studies focusing on this group. In two of these studies, LLV of 50–200 c/mL was only linked to risk of failure in treatment-experienced individuals [162, 163].

In sub-Saharan Africa, recent evidence from two large observational cohorts [164, 165] indicates that LLV of < 1000 c/mL is linked to increased risk of virologic failure also in low- and middle-income settings. Worse virologic outcome was observed also for LLV of < 200 c/mL in one of these studies [164]. These results imply that a lower threshold than 1000 c/mL for defining virologic failure in WHO guidelines should be considered.

Antiretroviral drug resistance

Although many available genotypic resistance testing assays require at least 1000 c/mL, it has been shown that resistance testing can be successfully performed in LLV samples with lower HIV RNA levels, and that the results are predictive of virologic outcome [77]. Resistance has been reported in association with LLV (in the ranges 20–500, 40–500, and 50–1000 c/mL) in several studies, both using RNA and proviral DNA, and also in patients without drug resistance in pre-ART samples [166–168]. For LLV at lower levels, the association with resistance is largely unknown. One study of 18 patients with LLV between 20 and 250 c/mL found no drug resistance after a median follow-up of 4.8 years [138]. Another study did not find any signs of resistance (i.e. genotypic susceptibility score of 3) in 46/57 (81%) of patients with LLV in the range 21–200 c/mL [169].

Immunologic consequences of LLV

LLV does not seem to be associated with progressive loss of CD4 T cells [145, 169], although higher HIV RNA during follow-up was associated with lower increase in CD4 cell counts in one Danish study (all 101 participants had a VL of < 200 c/mL at inclusion, the median increase at times with virologic rebound was 81 c/mL, and four participants had episodes with confirmed VL $> 10\,000$ c/mL) [170]. It has been suggested that LLV might stimulate immune activation, which could be a mechanism

contributing to non-AIDS morbidity [118, 171], but hitherto, this hypothesis has not been confirmed. Some studies have shown that individuals with LLV have higher levels of CD8 T cell activation than those with permanent suppression [170, 172]. Investigations of plasma markers of immune activation have yielded inconsistent results. One study with 1116 participants showed no increase in IL-6, CRP, or fibrinogen for any viremia category <10 000 c/mL [173]. In contrast, two other studies observed an association between LLV and IL-6 [174, 175]. LLV (37–200 c/mL) has also been linked to higher levels of soluble CD14 compared with suppressed viremia [176], contradicting two previous studies [174, 175]. Lastly, one study observed no significant differences in levels of CRP, IL-6, D-dimer, soluble CD14, β -2-microglobulin, or cystatin C in well-treated patients with VL >50 and <50 c/mL, respectively [177].

Clinical consequences of LLV

AIDS and mortality

Apart from increased risk of virologic failure, it has also been hypothesized that LLV may be associated with adverse clinical outcomes. Data on this issue are hitherto few and conflicting. Viral suppression (<400 c/mL) has been shown to have a positive impact on the lifespan of PWH [65, 84], but the specific importance of LLV is less explored. Nevertheless, some evidence indicates that also viremia in the LLV range could have a negative impact on long-term survival; Lee et al. found a continuous relationship between a single VL at six months after starting ART and all-cause 10-years mortality, which was statistically significant at a level of 130 c/mL [178].

An increased risk of AIDS in relation to LLV has been reported in a conference abstract from an Italian cohort [179], but in other studies, LLV does not predict AIDS [145, 156].

No association between LLV and AIDS/death (also analyzed separately) was observed in the large ART-CC study [156]. Similarly, no increased mortality for people with LLV compared with viral suppression has been found in several other cohorts [173, 180–182]. Zhang et al. explored the association between LLV (defined as an episode with a median VL in the range 50–400 c/mL) in the ATHENA cohort (the Netherlands); there was no association with mortality in the main results, but in a sensitivity analysis expanding the definition of LLV up to 1000 c/mL, LLV had a risk ratio for death of 3.3 (95% CI, 1.26–9.87) [182]. Time-updated VL in the range 201–999 c/mL was also significantly associated with all-cause mortality in a U.S. study [183]. In 2018, increased risk of AIDS/death was observed for LLV of 200–499 c/mL in a Spanish study with similar methodology as the ART-CC study [146]. A common

weakness of many of these studies is limited time of follow-up, which could decrease the chance of detecting a potential difference in mortality (Table 4).

In 2009, Cole et al. developed a measure of cumulative exposure to viremia: viremia-copy-years (VCY) [184]. This is mathematically defined as number of HIV-RNA *c*/mL integrated over the number of years from start of follow-up, and higher VCY has been linked to increased mortality in several studies [185–189]. Still, only one study has specifically analyzed VCY in the LLV range; in this report, only individuals with VCY >3 log₁₀ copies × years/mL (which corresponds to 200 *c*/mL for a mean follow-up of 4.95 years) had increased mortality. The authors concluded that the risk of death was not increased in PWH with low-level VCY [186].

Table 4. Studies on LLV or incomplete viral suppression and mortality.

Reference	Years studied	Setting	Sample size	Follow-up	Measure of viremia	Endpoint	Increased mortality for LLV?	Details (ranges in parentheses are 95% CI)
Low-level viremia								
Elvstam, 2020 [190] (Paper II)	1996–2017	Sweden	6956	49 986 PYFU, median 5.7 years	2 consecutive VLs ≥ 1 month apart, grouped into 50–199 and 200–999 c/mL	All-cause mortality	Yes	aHR 2.2 (1.3–3.6) for LLV 50–999 c/mL
Bernal, 2018 [146]	2004–2015	Spain	5986	19 117 PYFU, median follow-up 3.5 years for suppressed and 4.9 and 6.3 years, respectively, for LLV 50–199 and 200–499 c/mL	2 consecutive VLs ≥ 1 month apart, grouped into 50–199 and 200–499 c/mL	AIDS/all-cause mortality	Yes	aHR 1.44 (0.69–3.03) for LLV 50–200; aHR 2.89 (1.41–5.92) for LLV 200–499 c/mL
Elvstam, 2017 [191] (Paper I)	1996–2016	Sweden	1015	7812 PYFU, median 6.5 years	2 consecutive VLs ≥ 1 month apart, grouped into 50–199 and 200–999 c/mL	All-cause mortality	No (not significant)	aHR 2.19 (0.90–5.37) for LLV 50–199; aHR 2.29 (0.98–5.32) for LLV 200–999 c/mL; subset of the cohort in paper II
Salinas, 2016 [183]	1996–2012	U.S.	8168	53 861 PYFU	Three separate viremia metrics: baseline, time-updated (≤ 200 , 201–999, 1000–9999, >1000 c/mL), and cumulative	All-cause mortality (myocardial infarction also studied separately)	Yes	Time-updated VL 201–999 had an aHR of 1.40 (1.15–1.72)
ART-CC, 2015 [156]	1996–2012	Europe + North America	17 902	68 230 PYFU, median 3.1 years	2 consecutive VLs ≥ 1 month apart, grouped into 50–199 and 200–499 c/mL	AIDS/all-cause death, also analyzed separately	No	aHR 1.19 (0.78–1.82) for LLV 50–200, aHR 1.11 (0.72–1.71) for LLV 200–499 c/mL, also duration of LLV was not associated with AIDS/death
Costarelli, 2015 [180]	Not reported	Italy	4393	Not reported	50–400 c/mL in 2 consecutive VLs	1. CVD, AIDS, death 2. CVD, death (ignoring AIDS)	No	aHR 0.55 (0.25–1.2) for LLV regarding endpoint 2
Zhang, 2010 [182]	Not reported	The Netherlands	3321	11 165 PYFU, median 3.0 years	An episode of viremia started when VL >50 (24 weeks after start of ART) and ended when VL <50 , if the median VL was 50–400 c/mL the episode was regarded LLV	All-cause mortality	No	Risk ratio (RR) 1.63 (0.66–4.01) for LLV regarding mortality; in a sensitivity analysis, LLV up to 1000 c/mL had a RR for death of 3.53 (1.26–9.87)

Viremia-copy-years

Wang, 2018 [185]	1995–2015	U.S.	841	6739 PYFU, median 5 years	VCY counted from start of ART (VCY was then compared for different durations and temporalities)	All-cause mortality	Not studied	VCY based on the 3 recent years was most predictive of mortality, LLV not specifically studied (400 c/mL was lower limit of detection)
Quiros-Roldan, 2016 [186]	1998–2012	Italy	3217	16 197 PYFU, median 4.1 years	VCY counted from start of ART	All-cause mortality (not including the first 8 months after starting ART)	No	VCY was associated with death, but only for individuals with >3 log ₁₀ copies × years/mL (corresponding to 200 c/mL for a mean follow-up of 4.95 years)
Sempa, 2016 [187]	2004–2013	Uganda	489	Median 8.3 years	VCY counted from start of ART (linear and logarithmic scale, respectively)	Mortality (excluding accidents/violent death)	Not studied	VCY on a logarithmic scale was associated with death, but not on a linear scale, LLV not specifically studied (400 c/mL was lower limit of detection)
Chirouze, 2015 [188]	1997–2010	France	979	8112 PYFU, median 10 years	VCY counted from 8 months after start of ART	All-cause mortality	Not studied	VCY was associated with death, but LLV was not specifically studied
Mugavero, 2011 [189]	2000–2009	U.S.	2027	6579 PYFU, median 2.7 years	VCY counted from 24 weeks after ART initiation	All-cause mortality	Not studied	VCY was associated increased mortality, but LLV was not specifically studied

Reference	Years studied	Setting	Sample size	Follow-up	Measure of viremia	Endpoint	Increased mortality for LLV?	Details (ranges in parentheses are 95% CI)
Other measures of viremia (with relevance for LLV)								
Lee, 2017 [178]	1998–2014	U.S.	7944	49 118 PYFU	1 single VL 6 months after starting ART	All-cause year 10-year mortality	Yes	No clear threshold where LLV increased mortality, instead a continuous increase that became significant at 130 c/mL
Chao, 2012 [192]	2000–not reported	U.S.	4847	12 months (counting from 6 months after ART initiation)	1 single VL 6 months after ART initiation (grouped as <75, 75–5000, >5000 c/mL)	All-cause mortality	Yes (although defined as <5000 c/mL)	aHR 2.36 (1.11–5.02) for LLV of 75–5000 c/mL
Eastburn, 2011 [173]	2000–2007	U.S.	1116	5 years	Based on 1 VL sample (grouped as 0, 1–19, 20–399, 400–10 000, >10 000 c/mL)	All-cause mortality	No	VL was predictive of mortality, though attenuated by CD4, mainly VL >10 000 c/mL had this effect; this study also included inflammatory biomarkers
Zaccarelli, 2009 [193]	1999–2006	Italy	1389	Median 28 months	<50 c/mL (yes/no) as a time-varying covariate	All-cause mortality	Not studied	<50 c/mL had an aHR of 0.46 (0.26–0.76) for mortality; this study focused on patients failing on cART undergoing resistance testing
Murri, 2006 [181]	1997–2005	Italy	3023	11,447 PYFU, median 3.8 years	Cumulative time spent in each category (grouped as <500, 501–10 000, 10,001–100,000, >100,000 c/mL)	AIDS/all-cause mortality	No	501–10 000 had a non-significantly increased mortality, RR 1.77 (0.90–3.51) compared with <500 c/mL
Lohse, 2006 [65]	1995–2005	Denmark	2046	8898 PYFU	Percentage of time with undetectable (<400 c/mL) during 6–18 months after start of ART (grouped as 0%, 1–25%, 26–50%, 51–75%, 76–99%, 100%)	All-cause mortality (CD4 cell counts was also studied)	Not studied	1–25% had an adjusted mortality rate ratio of 2.63 (1.86–3.72) compared with 0% detectable

Serious non-AIDS events

Few studies have explored SNAE in relation to LLV, and the interpretation is hampered by discrepancies in the definitions of LLV and SNAE, as well as limited follow-up. Bernal et al. defined SNAE as non-AIDS-defining cancer, CVD, renal, or liver disease, and observed no significant associations with LLV of 50–199 or 200–499 c/mL [146]. Zhang et al. considered major CVD, liver fibrosis/cirrhosis, or chronic renal failure, and reported no increased risk in relation to LLV between 50 and 400 c/mL [194]. Furthermore, no increased risk of SNAE (major CVD, renal, or hepatic disease) or CVD alone, respectively, was reported for people with LLV in two conference abstracts from Italian cohorts [179, 180]. On the contrary, VL in the range 200–999 c/mL was predictive of acute myocardial infarction in a long-term follow-up of U.S. veterans; however, this study showed higher predictive value of baseline VL and cumulative viremia [183]. In the African Cohort Study (AFRICOS), with participants from Uganda, Kenya, Tanzania, and Nigeria, LLV of <1000 c/mL was significantly associated with non-communicable diseases (elevated blood pressure, hypercholesterolemia, hyperglycemia, and renal insufficiency) [195].

Observational studies have reported an association between viral suppression and lower incidence of AIDS-defining cancer [196–202] and virus-related non-AIDS-defining cancer [198, 201, 203–205], but the specific impact of LLV has only been analyzed in one study. This study was restricted to non-Hodgkin lymphoma and observed a non-significantly increased incidence for people with LLV in the range 51–500 c/mL [202].

Management of LLV

The recommended clinical management of LLV is outlined in Table 3 (p. 36). In summary, providers are recommended to consider drug–drug and drug–food interactions, offer adherence counselling, and perform genotypic resistance testing. Swedish, U.S., and British guidelines all currently recommend considering treatment switch if VL >200 c/mL is confirmed, whereas EACS recommend considering switch also for people with LLV in the range 50–200 c/mL [41, 55, 63, 120].

It remains unclear whether ART modification improves the outcome for patients with LLV. For residual viremia of <50 c/mL as detected by single-copy assays, treatment intensification with RAL does not result in lower VLs [206, 207].

Several retrospective analyses have evaluated treatment modification in the context of LLV. A study from the United States found no statistically significant differences after 26/149 (17%) patients with LLV (50–1000 c/mL) switched treatment [208]. In contrast, a Spanish study reported 90% viral suppression one year after genotype-

guided treatment modification of 41 individuals with LLV of 20–1000 c/mL, compared with 26% suppression among the 51 participants who did not meet criteria for treatment optimization ($P < .001$; Fisher's exact test) [209]. In a study from Canada, 576/1702 (34%) patients with LLV (50–1000 c/mL) switched treatment. Those for whom the change resulted in a higher genotypic susceptibility score had lower risk of future virologic failure compared with those with a lower score for the new regimen [210]. A study performed in Taiwan reported higher rates of viral suppression after 48 weeks in 46/165 (28%) individuals with LLV (20–1000 c/mL) who were switched from an NNRTI-based or unboosted PI-based to regimens based on other anchor drugs [211]. Higher rates of suppression for people with LLV (21–400 c/mL) with treatment modification have also been observed in a Swiss cohort [158]. Lastly, an Italian study classified 21 patients with LLV (50–500 c/mL) into three groups after genotypic resistance testing: 1) those with inadequate ART regimen based on resistance testing, 2) those with inadequate drug exposure due to low adherence or interactions, and 3) LLV not explained by these factors. Treatment modification led to high rates of subsequent viral suppression for the first two categories. Of five patients in category 3, four intensified treatment, resulting in viral suppression for three cases and persistent LLV for one [212].

One randomized controlled trial has studied treatment switch for patients with LLV. It was based in Lesotho, and 80 individuals with LLV of 100–999 c/mL were randomized to continued first-line ART (EFV- or NVP-based) or switch to second-line regimen (LPV/r-based). 22/40 (55%) in the switch group had viral suppression at 36 weeks, compared with 10/40 (25%) in the control group (odds ratio, 3.55; 95% CI, 1.37–9.24) [213]. In this study, 31/37 (84%) of those with available sequence data (46% of the study population) had high-level resistance to at least two antiretrovirals used at enrollment, and none of the five participants with ongoing viremia after regimen switch and available sequence data had PI-resistance. Interestingly, 7/9 participants with VL between 50–199 c/mL at baseline had mutations associated with NRTI- or NNRTI-resistance, suggesting that LLV also in this low range may indicate antiretroviral resistance [214].

Collectively, these data suggest that switching to second-line ART may result in better virologic outcomes for patients with LLV of <1000 c/mL while on an NNRTI-based first-line regimen. For patients in high-income settings, some observational data indicate that regimen change guided by resistance testing could be beneficial, but these studies have included individuals with VL up to at least 400 c/mL. To the best of my knowledge, there is no data on whether any intervention for patients with LLV impacts the risk of clinical endpoints such as death, AIDS, or SNAE.

Aims of the present investigation

The overall aim of the studies presented in this thesis was to assess the associations between LLV during ART and virologic and clinical outcomes after long-term follow-up and to investigate the potential mechanisms involved. The Swedish nationwide cohort of PWH receiving ART was linked to national health registers, and plasma biomarkers were analyzed in a subset of participants. Furthermore, we studied cardiovascular measures and biomarkers in a South African research cohort.

Specific aims

- Investigate the association between different amplitudes of LLV during ART and subsequent virologic failure in a Swedish setting (paper I).
- Determine the relationship between LLV in Swedish ART recipients and all-cause mortality and explore the panorama of causes of death (paper I and II).
- Examine the risk of AIDS and SNAE in Swedish ART recipients with suppressed viremia, LLV, or non-suppressed viremia (paper II).
- Explore the associations between different viremia patterns and invasive cancer in Swedish PWH receiving ART (paper III).
- Compare the levels of plasma markers of immune activation and cardiovascular risk between people with LLV and viral suppression (paper IV and V).
- Compare the cardiovascular function and degree of subclinical atherosclerosis between people with LLV and viral suppression (paper V).

Materials and methods

The InfCare HIV cohort

InfCare HIV is a national quality assurance register for PWH in Sweden that also functions as an electronic clinical decision support system and a research database. It was established at Karolinska University Hospital, Stockholm and Sahlgrenska University Hospital, Gothenburg in 2003 and was fully implemented at all HIV clinics in Sweden in 2008 [215]. Data from the earlier years of the pandemic have since been added retrospectively, and at least the four largest centers (Karolinska, South Hospital/Venhälsan, Sahlgrenska, and Malmö) are considered to have complete data also before 2008 [216]. The current estimated coverage is 99.9% [32]. InfCare HIV contains demographic data (sex, year of birth, country of birth, estimated country of transmission, suspected route of transmission, and date of death), biologic data (date of first positive HIV serology, VL measurements, viral sequences, CD4 and CD8 cell counts, and HBV/HCV serostatus), and data on ART. Data from clinical laboratories are entered automatically, whereas ART data are manually registered by the provider [217]. Since 2011, a questionnaire based on self-reported data has been implemented that assesses patient-reported outcomes such as physical, psychological, and sexual health, adherence to treatment, side effects, feeling of involvement in care, and satisfaction with the provider [215, 217]. Data quality is controlled every year and the register holds the highest level of data quality (certification 1) by the Swedish Association of Local Authorities and Regions [218].

The version of the InfCare HIV database that was used for paper I was last updated in October 2016, whereas the version used for paper II and III was last updated in June 2017. Importantly, paper II and III were based on a linkage between InfCare HIV and national health registers, so only InfCare HIV participants with a Swedish personal identity number could be included. Of 11 030 InfCare HIV participants submitted for linkage, 10 855 (98%) could be successfully linked.

Swedish national registers

Sweden has a long history of population-based registers, and the use of personal identity numbers that are common across different sectors of the society enables linkage between registers. This constitutes a useful resource for research, especially for uncommon exposures or outcomes that require large materials. Paper II and III of this thesis use data from the following registers managed by the National Board of Health and Welfare:

National Inpatient Register. This subsection of the National Patient Register contains diagnoses from all hospital discharges in Sweden. It was established in 1964 and has complete national coverage since 1987. It includes primary and additional diagnoses classified according to a Swedish adaption of the International Classification of Disease (ICD) system, as well as procedures classified by the Swedish Classification of Surgical and Medical Procedures (Swedish: *klassifikation av vårdåtgärder, KVÅ*). ICD-10 was introduced in 1997. The accuracy of register data has been validated by several researchers and by the National Board of Health and Welfare. A review of the evidence suggests that the overall positive predictive value for diagnoses in the register is between 85 and 95% [219].

Cause of Death Register. The first Swedish nationwide register on causes of death was established in 1749, and in 1951, the classification system was adapted to an international standard. ICD-10 was implemented in 1997. The register includes all deceased registered Swedish citizens irrespective of where they died, but people who have migrated from Sweden are not included. Since 2012, all people who died in Sweden (irrespective of nationality) are included, but our studies only include people with a Swedish personal identity number. Deceased citizens without a cause of death certificate are also included since 1991. In 2008, 0.8% of all cases had a missing cause of death certificate. The data comprise date and cause of death (both underlying and multiple causes of death). The data quality is highly dependent on the quality of the cause of death certificate, which is issued by the responsible physician. In the latest survey from 1995, the underlying cause of death was correct in 77% of cases, but for some diagnoses (such as malignant tumors and ischemic heart disease) the accuracy was considerably higher [220].

Underlying cause of death is defined by WHO standards, and since 1987, a program developed by the U.S. National Center for Health Statistics (Automated Classification of Medical Entities) is used for classification in the Cause of Death Register [220]. The WHO system is known to overreport deaths as HIV-related in PWH, however, and another method has been developed specifically for HIV-infected, the Coding Causes

of Death in HIV (CoDe) project [221]. This classification is not implemented in the Swedish Cause of Death Register or InfCare HIV.

Swedish Cancer Register. The Cancer Register was founded in 1958 and includes patient data, data on the cancer diagnosis (date of diagnosis, anatomical site, histological type, and basis of diagnosis), and follow-up data. 99% of all cases are verified morphologically. The latest quality control found that 3.7% of Swedish cancer cases in 1998 were not reported to the register, and the authors concluded that the register has high completeness and that the underreporting is unlikely to have a major impact on most research or surveillance uses [222].

Lastly, the merged dataset was linked to the Population Register, which is managed by Statistics Sweden. In this way, we could check for reused or changed personal identity numbers.

EndoAfrica-NWU study

While paper I–IV is based on the InfCare HIV cohort, paper V is based on a South African material: the EndoAfrica-NWU (North-West University) study. In 2020, it was estimated that the overall HIV prevalence in South Africa was 13%; in adults aged 15–49 years it was 19%, and in women of the same age it was 23% [27]. South Africa has the largest national ART program in the world, with 5.2 million ART recipients (70% of all national HIV cases) [1]. In the 2020 update of the guidelines published by the Southern African HIV Clinical Society, the threshold for virologic failure was lowered from the WHO definition of 1000 c/mL to ≥ 50 c/mL in repeated samples at least 2–3 months apart [223].

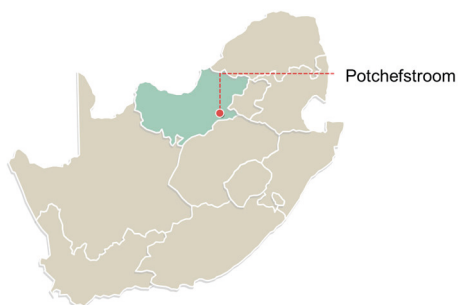


Figure 8. Potchefstroom is located in the North West province of South Africa. Credit: yourfreetemplates.com.

The EndoAfrica-NWU study was formed as an expansion of the parent EndoAfrica study which focuses on CVD in people with and without HIV in sub-Saharan Africa [224]. Participants were recruited in the North West province of South Africa, in and around Potchefstroom (Figure 8), a part of South Africa where 77% identify themselves as Black Africans. The study is described in detail in [225].

Study design

Paper I–III are observational cohort studies. The respective study designs are outlined in Table 5.

Table 5. Study design of paper I–III.

	Cohort	Exposure	Outcome	Data source
Paper I	PWH in Malmö and Gothenburg 1996–2016	Time-updated viremia category	<ul style="list-style-type: none"> • Virologic failure • All-cause mortality 	InfCare HIV
Paper II	PWH in Sweden 1996–2017	Time-updated viremia category	<ul style="list-style-type: none"> • All-cause mortality • AIDS • SNAE 	<ul style="list-style-type: none"> • InfCare HIV • National Inpatient Register • Cause of Death Register
Paper III	PWH in Sweden 1996–2017	<ul style="list-style-type: none"> • Pre-ART VL • Viremia during cART <ul style="list-style-type: none"> ◦ Time-updated viremia category ◦ VCY 	Invasive cancer	<ul style="list-style-type: none"> • InfCare HIV • Swedish Cancer Register • Cause of Death Register

Paper IV is a nested case-control study where cases with LLV from paper I were matched 1:1 to controls with viral suppression. Controls were matched for HCV serostatus, suspected route of transmission, sex, age, and sampling date. Biomarker levels were compared at three time points (Figure 9). Paper V has a cross-sectional design, where people with VL in the range 50–999 c/mL are compared with people with viral suppression.

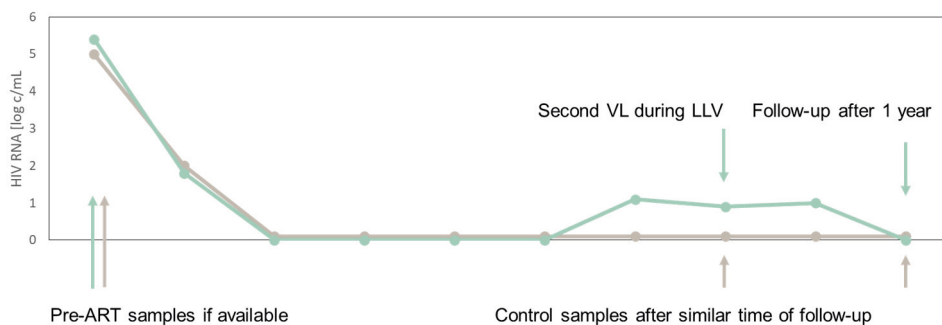


Figure 9. Timing of samples in paper IV.

Participants

For paper I, InfCare HIV participants registered in Gothenburg or Malmö (the second and third largest cities in Sweden) at any time between 1996 and 2016 were included if they met the following criteria: ≥ 15 years old, documented triple ART for ≥ 12 months, and a minimum of two VL measurements ≥ 12 months after treatment initiation. Follow-up started at the date of the first VL measurement ≥ 12 months after start of ART.

There are some differences in the inclusion criteria for paper II and III compared with paper I. Firstly, the cohort was expanded to include all sites in Sweden up until 2017. Secondly, the requirement of triple ART was changed to ART meeting certain criteria. The reason for this was that not all triple ART regimens are effective (e.g. triple NRTI) and some approved regimens only have two drugs. A complete list of regimens can be found in the respective papers. Furthermore, follow-up started at the first VL after six instead of twelve months of follow-up. Most previous studies on LLV have included participants 3–9 months after start of ART [146, 156, 182], and while INSTI-based regimens typically display the fastest decline in VL, the absolute majority of patients achieve undetectable VL after six months, even with non-INSTI regimens [63].

Participants of paper IV were selected from the cohort in paper I. All cases that had available plasma samples were included. Paper V included all participants of the EndoAfrica-NWU study who received ART and had available VL data. Non-pregnant individuals of African descent who were 18–60 years old were recruited by leaflets and by word-to-mouth. All received first-line ART with FTC + TDF + EFV [225].

Definitions

Measures of viremia

For paper I–III, participants were categorized by time-updated viremia category. Reclassification was only possible to a higher category.

- **Viral suppression.** 50 c/mL was chosen as the threshold for suppression to account for less sensitive assays that were used during the first years of the study. This group also included cases with one or several episodes of a single VL (50–999 c/mL for paper II and III; no specific range for paper I), if preceded and followed by <50 c/mL.
- **LLV.** At least two consecutive VLs in the range 50–999 c/mL, at least one month apart. In all studies, LLV was also subdivided into 50–199 c/mL (two VLs in this range) and 200–999 c/mL (two VLs in the range 50–999 c/mL, of which at least one in the range 200–999 c/mL), to reflect current guideline recommendations in Sweden, the United Kingdom, and the United States.
- **Non-suppressed viremia.** In paper I, non-suppression was defined as two or more consecutive VLs ≥ 50 c/mL, of which at least one was ≥ 1000 c/mL. All participants with a documented treatment interruption (≥ 1 month) were also classified as non-suppressed viremia. In paper II and III, the definition was one or more VL ≥ 1000 c/mL.

Paper II and III also included pre-ART viremia as a separate variable. This was defined as the last VL before receiving any antiretroviral agent (not necessarily the same date as start of ART). Cumulative viremia exposure was calculated as VCY, using the trapezoidal rule [184]. Follow-up started at the first VL at least six months after ART initiation, and the copy-years variable was \log_{10} -transformed.

Stricter definitions of LLV and viral suppression were used in paper IV. Cases of LLV had at least three VLs in the range 50–999 c/mL, of which at least two were consecutive. Controls only had suppressed viremia <50 c/mL during the follow-up.

In paper V, participants were grouped by a single VL measurement into LLV (50–999 c/mL) or viral suppression (<50 c/mL).

Outcomes

Virologic failure was defined as two consecutive VLs ≥ 1000 c/mL in paper I. Mortality was defined as all-cause mortality in paper I and II.

We included the following conditions in the definition of SNAE: CVD, venous thromboembolic disease, pulmonary arterial hypertension, chronic kidney disease, decompensated liver disease, and non-AIDS-defining cancer. A complete list of ICD codes used for searching in the National Patient Register and Cause of Death Register can be found in the supplements to paper II.

In paper III, the main outcome was a composite of invasive cancer of the types recognized to have higher incidence among PWH [4, 110] (Table 2, p. 33). Details of the classification can be found in the paper.

A list of biomarkers compared between people with LLV and viral suppression is presented in Table 6.

Table 6. Some of the biomarkers and cardiovascular measures in paper IV and V.

	Paper IV	Paper V	Comment
Biomarkers			
ADAMTS13			Metalloprotease that cleaves von Willebrand factor and thereby decreases its adhesive activity [226]. (A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13)
β-2-microglobulin			A component of the major histocompatibility complex class I molecule that has been used as a marker of immune activation among PWH [227].
CD14 (soluble)			Marker of monocyte activation that has been associated with all-cause mortality and subclinical atherosclerosis among PWH [105, 228].
CD163 (soluble)			Marker of monocyte activation that has been associated with subclinical atherosclerosis among PWH [105].
CRP			Acute-phase protein that has been associated with occurrence of CVD and all-cause mortality among PWH [101, 102, 229]. (C-reactive protein)
D-dimer			Fibrin degradation product that has been associated with CVD and all-cause mortality among PWH [101, 102, 229].
GDF-15			Transforming growth factor β-related cytokine that has been associated with pulmonary hypertension and all-cause mortality among PWH [108]. (Growth differentiation factor 15)
ICAM-1			Adhesion molecules indicating vascular endothelial injury and dysfunction; higher levels have been observed in PWH [230]. (Intercellular adhesion molecule 1)
IL-6			Pro-inflammatory cytokine that has been associated with occurrence of CVD and all-cause mortality among PWH [101, 102, 229]. (Interleukin 6)
IP-10			Cytokine secreted in response to interferon-γ (also called CXCL10). Elevated in PWH and linked to VL and disease progression [231]. (Interferon-γ-induced protein)
Myeloperoxidase			Leukocyte enzyme that has been used as a marker of CVD risk [232].
NT-proBNP			Hormone secreted by cardiac myocytes that has been associated with CVD among PWH [107]. (N-terminal pro B-type natriuretic peptide)
P-selectin			Glycoprotein expressed by activated platelets [233].
Reactive oxygen species			Highly reactive compounds that are generated during normal cellular processes; important in the development of atherosclerosis. PWH have higher oxidative stress [234].
Troponin T			Marker of myocardial injury that has been associated with presence of coronary plaque among PHW [235].
VCAM-1			Adhesion molecules indicating vascular endothelial injury and dysfunction; higher levels have been observed in PWH [230]. (Vascular cell adhesion molecule 1)
Cardiovascular measures			
Blood pressure			Paper V includes brachial systolic and diastolic blood pressure, mean arterial pressure, central systolic blood pressure, and central pulse pressure.
Carotid intima-media thickness			Measure of atherosclerosis that has been used as a surrogate marker of CVD; HIV-infection has been associated with higher risk of focal plaque formation [236].
Carotid diameter distensibility			Measure of arterial stiffness, calculated as the difference between the maximum and minimum diameter divided by the minimum diameter.
Pulse wave velocity			Noninvasive measure of arterial stiffness that predicts CVD and all-cause mortality in the general population [237].
Pulse pressure amplification			Inversely related to subclinical and clinical CVD in the general population [238].

Laboratory procedures

Viral load measurements

During the long follow-up of the present investigation, several assays for VL monitoring have been used at different sites in Sweden. InfCare HIV does not contain data on which assay was used for a specific VL measurement.

At Karolinska University Laboratory, the Roche Amplicor was introduced in 1997, and since then, different versions of Cobas Amplicor, Cobas TaqMan, and Cobas HIV-1 assay have been used [239]. At Skåne University Hospital, TaqMan was introduced in 2006, TaqMan version 2.0 in 2010, and Cobas HIV-1 assay in 2016 (in an email from Dr. Löfgren [bengt.lofgren@skane.se] in September 2016).

Some VL measurements from the early years of follow-up were performed with an assay with a lower limit of detection of 500 c/mL. For paper I, participants started follow-up at the date of the first measurement with a more sensitive method, but for paper II and III, these measurements are regarded as viral suppression if the VL was <500 c/mL. Since type of assay is not reported, these measurements can only be identified in suppressed samples (which are then reported as 499 c/mL), so the strategy used in paper I could result in selection bias. In paper II, 1094 of 143 347 (0.8%) VL measurements were reported as 499 c/mL, so this issue is unlikely to have a substantial impact on the results.

In paper V, Cobas TaqMan version 2.0 was used for all VL measurements.

Biomarkers and cardiovascular measures

For paper IV, plasma samples were obtained from biobanks at the respective sites. All samples were stored at -20°C or colder and thawed right before analysis. A magnetic bead Luminex kit (Bio-Techne Ltd, Abingdon, United Kingdom) was used on a Bio-Plex Suspension Array Reader (Bio-Rad Laboratories, Hercules, CA). All samples were analyzed in duplicates, and only duplicate pairs with a coefficient of variation of <20% were included.

The samples in paper V were stored at -80°C until biochemical analysis (details of laboratory methods in the paper). The variables pulse wave velocity and pulse pressure amplification were based on pulse wave analysis using a SphygmoCor XCEL device (AtCor Medical, Sydney, Australia). Carotid intima-media thickness and diameter distensibility were based on sonographic carotid images.

Statistical analyses

In paper I and II, baseline variables were compared across viremia categories using Kruskal–Wallis and Pearson’s χ^2 tests, where appropriate. The study size of paper I–III was determined by the number of participants meeting inclusion criteria. For paper IV and V, we performed power calculations (see the original papers) and decided to include all LLV cases with available samples. In all papers, missing data were handled using a complete-case approach.

We used an extended version of the Kaplan–Meier estimator to assess temporal distribution of virologic failure and all-cause mortality in paper I. This extension, described by Snapinn et al., updates the cohort at all event times, and thus allows participants to change between strata during the follow-up [240]. In paper I and II, we fitted Cox proportional hazard models to analyze the risk of virologic failure, all-cause mortality, and SNAE after adjustment for potential confounders. Variables included as confounders were selected based on background knowledge rather than some significance or information criteria [241]. We tested the proportional hazard assumption graphically and by Schoenfeld residuals. Since the assumption was violated for non-suppressed viremia in the analysis of all-cause mortality in paper II, we included an interaction term between time and viremia category. In both paper I and II, we performed a subanalysis restricted to individuals starting ART January 2005 or later. In paper II, we further performed the following sensitivity analyses: modelling age with restricted cubic splines; a subanalysis restricted to participants not changing treatment, adjusted for type of regimen; and a subanalysis differentiating between people with <25% versus $\geq 25\%$ of VL measurements higher than 50 c/mL.

In paper III, we wanted to analyze the association between viremia and cancer in the presence of the competing risk of non-cancer death. The rationale is that if there were an association between the exposure (viremia) and death that was not mediated through the outcome (cancer), a standard Cox regression could yield biased results (since deceased people cannot reach the outcome). The Fine and Gray model for cause-specific hazard is one way to handle this; the results could be interpreted as the rate of developing the outcome (cancer) in those without previous cancer who are not lost to follow-up and including those who have died [242]. For this analysis, we tested the proportional subhazard assumption by fitting a separate model including time interaction for all variables. We assessed the association between both pre-ART VL and viremia during ART and development of invasive cancer. Subsequently, we subdivided the composite endpoint into 1) AIDS-defining cancer, 2) infection-related non–AIDS-defining cancer, and 3) non–AIDS-defining cancer not related to infectious agents.

Age-standardized incidence rates were calculated to the Segi's world population standard [243].

In paper IV, biomarker levels between cases and controls were compared using independent sample *t*-test after logarithmic transformation. Five biomarkers (CRP, D-dimer, GDF-15, soluble CD163, and β -2-microglobulin) had values higher than the upper limit of quantification; we also performed log-rank tests for these markers, right-censoring these values. Analysis of covariance was used to adjust for potential confounders.

Levels of plasma biomarkers in paper V were compared between groups using Mann-Whitney *U* test. We used backward multiple regression to analyze the relationships between viremia category (LLV or suppression) and cardiovascular outcomes. As a sensitivity analysis, all measures and biomarkers were categorized in quartiles and binomial logistic regression analyses were used to analyze the relationship between viremia category and having a measurement in the highest quartile, with adjustment for potential confounders.

We assessed normality by visual inspection and Shapiro-Wilk tests in paper IV and V. We made no mathematical correction for multiple comparisons.

We defined statistical significance as two-sided $P < .05$. We used the following software for statistical calculation and data management: IBM SPSS Statistics software for Windows (IBM Corp., Armonk, NY) for comparisons of baseline characteristics in paper I and all analyses in paper V; R version 3.3.1 [244] with the survival [245], ggplot2 [246], and survminer packages [247] for the survival analysis in paper I; and Stata SE 15 (StataCorp, College Station, TX) for all analyses in paper II–IV.

Ethical considerations

Ethical approval for paper I and IV was given by the Regional Ethical Review Board in Gothenburg (532-11) and for paper II and III by the regional board in Lund (2017/1023). Personal identity numbers were used for requesting biobank samples in paper IV, but in all other studies, the data were pseudo-anonymized. The register linkage was performed by Statistics Sweden and National Board of Health and Welfare.

All InfCare HIV participants are informed at inclusion that their data can be used for research, but no specific informed consent was obtained for paper I–IV. The arguments for not requesting informed consent in register studies in the Nordic countries are summarized in [248] and include a dramatic reduction of participation rates which would reduce the statistical power, risk of selection bias since consent could be more

difficult to obtain in certain groups, that some study participants will be dead at the time the research is conducted, and the substantial cost of obtaining consent from a large number of participants. Specifically for the studies in this thesis, attempts to contact PWH could pose greater threats to the participants' anonymity and integrity than using their pseudo-anonymized data in research.

The EndoAfrica-NWU study was approved by the Health Research Ethics Committee of the North-West University, the North West Department of Health, and Potchefstroom Patient Group. Written informed consent was obtained from all participants.

Results

Paper I

Of 1015 participants, 716 (71%) were classified as viral suppression at the end of follow-up, 46 (5%) as LLV of 50–199 c/mL, 52 (5%) as LLV of 200–999 c/mL, and 201 (20%) as non-suppressed viremia. The proportion of participants who later progressed to a higher category was 23% for viral suppression, 34% for LLV of 50–199 c/mL, and 42% for LLV of 200–999 c/mL.

During 5717 person-years of follow-up (PYFU), 39 cases of virologic failure were observed. Based on the extended Kaplan–Meier estimator, the likelihood of not having virologic failure after 5 years was 0.97 (95% CI, 0.96–0.98) for viral suppression, 0.96 (0.88–1.00) for LLV of 50–199 c/mL, and 0.83 (0.72–0.95) for LLV of 200–999 c/mL ($P < .01$). After adjustment for sex, age at inclusion (15–39, 40–59, and ≥ 60 years), transmission group (injecting drug use versus other), time of inclusion (before or after January 1, 2005), and CD4 nadir (0–199, 200–349, and ≥ 350 cells/ μ L), LLV of 200–999 c/mL, but not of 50–199 c/mL, had a statistically significant association with virologic failure (aHR, 3.1; 95% CI, 1.4–7.0).

Both LLV of 50–199 and 200–999 c/mL had elevated all-cause mortality in unadjusted analysis, but after adjustment for potential confounders, these associations were not statistically significant (aHR, 2.2; 95% CI, 0.90–5.4 for LLV of 50–199 c/mL and aHR, 2.3; 95% CI, 0.98–5.3 for LLV of 200–999 c/mL). Being classified as non-suppressed viremia (≥ 1000 c/mL) was not linked to increased mortality in this study.

Paper II

Of 10 855 individuals included in the merge, 6956 (64%) met the inclusion criteria. A majority of participants were male and the median age at inclusion was 37 years (Table 7). Individuals who were classified as LLV at the end of follow-up were more likely to have high pre-ART VL ($P < .001$). At the end of follow-up, 60% were

Table 7. Characteristics of study cohort based on InfCare HIV 1996–2017.

Total n = 6956	
Male sex	4396 (63%)
Age at start of ART [years]	37 (31–45)
Born in Sweden	2643 (38%)
Injecting drug use	396 (6%)
VL before start of ART [c/mL]	73 000 (18 050–242 000)
CD4 cell counts before start of ART [cells/ μ L]	240 (140–360)
Treatment experienced at start of ART	1331 (19%)
Viremia classification at end of follow-up	
Viral suppression	4177 (60%)
LLV 50–199 c/mL	339 (5%)
LLV 200–999 c/mL	258 (4%)
Non-suppressed viremia	2182 (31%)

Results are n (%) or median (IQR).

classified as viral suppression, 5% as LLV of 50–199 c/mL, 4% as LLV of 200–999 c/mL, and 31% as non-suppression.

459 deaths were observed during 49 986 PYFU and the most common stated causes of death were HIV/AIDS (31%), CVD (18%), and non-AIDS cancer (18%). LLV of 50–999 c/mL was associated with all-cause mortality in unadjusted analysis including an interaction term between viremia and time, and after adjustment for potential confounders (aHR, 2.2; 95% CI, 1.3–3.6) (Figure 10). In subanalysis, LLV of 50–199 c/mL had a statistically significant association with all-cause mortality (aHR, 2.2; 95% CI, 1.3–3.8), but for LLV of 200–999 c/mL, the association was not significant with a 95% confidence level (aHR, 2.1; 95% CI, 0.96–4.7). Participants with LLV of 50–199 c/mL with $\geq 25\%$ of VL measurements higher than 50 c/mL had increased mortality, but not those with $< 25\%$ of VL measurements higher than 50 c/mL. Non-suppressed viremia was associated with a statistically significant increase in mortality.

Of 684 SNAE observed during follow-up, 357 (52%) were CVD and 197 (29%) were non-AIDS cancer. Non-suppressed viremia was linked to higher risk of SNAE, but not LLV of 50–999 c/mL (Figure 10). When analyzing the two LLV categories separately, however, LLV of 200–999 c/mL had a statistically significant association with development of SNAE (aHR, 2.0; 95% CI, 1.2–3.6). This conclusion remained in sensitivity analyses restricted to participants starting ART January 2005 or later and when accounting for ART regimen type.

While non-suppressed viremia was associated with increased risk of AIDS, there were few cases of AIDS among participants with LLV and no indication of an increased risk.

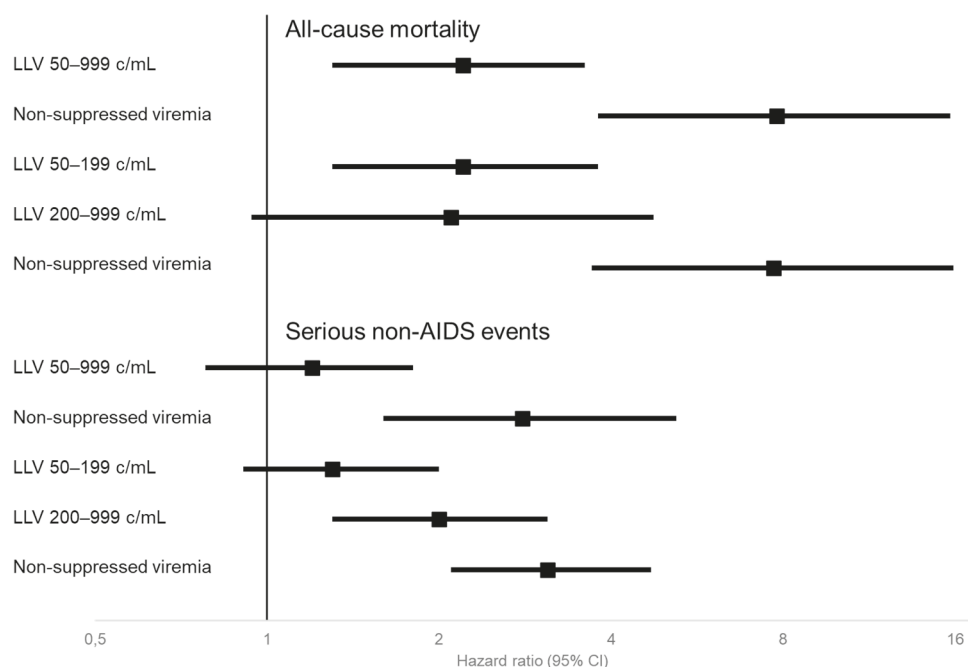


Figure 10. Cox regression models for all-cause mortality and SNAE by viremia category. The squares represent aHR (adjusted for age, sex, CD4 cell count and VL before start of ART, injecting drug use, born in Sweden, treatment experience, and treatment interruptions) and the whiskers represent 95% CI. Viral suppression (<50 c/mL) is the reference category.

Paper III

Since not having a pre-ART VL was an exclusion criterion for this study, the total number of participants was lower than in paper II, 4931 in the main analysis. Of the individuals excluded due to missing pre-ART VL measurement, 784 had otherwise complete data. These individuals had a median year of HIV diagnosis of 1990 (interquartile range [IQR], 1986–1993) and received their first antiretroviral treatment in 1994 (IQR, 1992–1996).

116 cases of invasive cancer (of the types recognized to have higher prevalence among PWH) were recorded during follow-up, and the most common diagnoses were non-Hodgkin lymphoma, cervix cancer, and lung cancer. The sex- and age-standardized incidence was higher compared with the general population for several diagnoses, especially non-Hodgkin lymphoma, Kaposi sarcoma, cervix cancer, Hodgkin lymphoma, and anal cancer.

Cumulative viremia, measured as VCY, or LLV during ART was not linked to increased cancer risk. Non-suppressed viremia during ART had no statistically significant association with the composite endpoint, but in subanalysis, there was an increased risk of AIDS-defining cancer, albeit not statistically significant (adjusted subhazard ratio [aSHR], 2.0; 95% CI, 0.92–4.2). Having a higher pre-ART VL was associated with increased cancer risk (aSHR for the composite outcome, 1.4; 95% CI 1.0–1.8). This association remained after inclusion of viremia during ART in the model (Table 8). In subanalysis, pre-ART VL was associated with increased risk of AIDS-defining cancer and infection-related non-AIDS-defining cancer, but not other cancer forms.

Table 8. Adjusted proportional subhazard model for the risk of invasive cancer accounting for the competing risk of non-cancer death (n = 4474).

	aSHR (95% CI)
Viremia during cART	
Viral suppression	1 (Ref)
LLV	0.67 (0.30–1.5)
Non-suppressed viremia	1.4 (0.89–2.4)
Pre-ART VL (per log ₁₀ c/mL)	1.4 (1.0–1.9)

Adjusted for sex, age, pre-ART CD4 cell count, and injecting drug use.

Paper IV

A majority of cases (n = 34) and controls (n = 34) were men, and the median age was 45 years (IQR, 38–50) for cases and 47 years (IQR, 43–53) for controls (Table 9). In cases and controls combined, 47% had a PI-based and 46% an NNRTI-based regimen. The median sampling date was October 26, 2008, for cases and October 25, 2011, for controls. There were no statistically significant differences between cases and controls regarding baseline variables, including matching variables (sex, age, HCV serostatus, transmission group, and sampling date), nadir CD4 cell count, pre-ART VL, and ART regimen type. Cases with LLV had a median VL of 107 c/mL (IQR, 69–229), and after one year of follow-up, 26/34 (76%) had suppressed viremia (<50 c/mL).

Table 9. Characteristics of study participants in paper IV and V.

	Paper IV (n = 68)	Paper V (n = 256)
Male sex	46 (68%)	70 (27%)
Age [years]	47 (40–52)	44 (36–50)
Type of ART		
NNRTI-based	31 (46%)	256 (100%)
PI-based	32 (47%)	0
Other	5 (7%)	0
VL at LLV sample* [c/mL]	107 (69–229)	60 (50–154)

Results are n (%) or median (IQR).

*For cases with LLV.

There were no differences between cases and controls regarding the values of CRP, vascular cell adhesion molecule 1 (VCAM-1), ST2, soluble CD14, soluble CD163, interferon- γ -induced protein (IP-10), or β -2-microglobulin. Cases with LLV had higher crude levels of both GDF-15 and D-dimer compared with controls with viral suppression (although not statistically significant for D-dimer in unadjusted analysis) (Table 10). After adjustment for age, type of ART, and nadir CD4, the association was statistically significant for both biomarkers. Eight GDF-15 values and one D-dimer value were higher than the quantification limit, and log-rank tests (right-censoring these values) resulted in $P = 0.050$ for GDF-15 and $P = 0.037$ for D-dimer.

After one year of follow-up, levels of GDF-15 were still slightly higher in cases with LLV compared with controls with viral suppression, but the difference was not statistically significant (geometric mean, 2536 pg/mL; 95% CI, 438–14 995 versus 1831 pg/mL; 95% CI, 467–7175; $P = 0.11$). There were no statistically significant differences between the other biomarkers in the follow-up sample.

Pre-ART samples were available in 16 cases (47%) and 26 controls (76%). Cases and controls had similar values of all nine biomarkers. When comparing biomarker levels between the LLV samples (which were obtained a median of 1621 days after the pre-ART sample for cases with LLV and 1510 days for controls), GDF-15 levels were higher ($P < .001$), and D-dimer, soluble CD163, VCAM-1, IP-10, and β -2-microglobulin levels were lower in samples obtained after ART initiation ($P < .05$).

Table 10. Levels of GDF-15 and D-dimer in 34 cases with LLV and matched controls with viral suppression.

	LLV (n = 34)	Viral suppression (n = 34)	Independent sample <i>t</i> -test, <i>P</i> value	ANCOVA, <i>P</i> value	Log-rank test, <i>P</i> value
GDF-15 [pg/mL]	3416 (804–14 516)	2002 (355–11 295)	0.0092	0.0026	0.050
D-dimer [ng/mL]	1114 (125–9917)	756 (157–3627)	0.11	0.036	0.037

Results are geometric mean (95% CI). *P* values are the result of 1) independent sample *t*-test after logarithmic transformation, 2) analysis of covariance (ANCOVA) adjusted for age, type of ART, and nadir CD4 and 3) log-rank test (right-censoring eight GDF-15 values and one D-dimer value).

Paper V

Of 256 participants, 113 (44%) had a VL of <50 c/mL, 95 (37%) of 50–999 c/mL, and 48 (19%) of ≥1000 c/mL. Participants had a median age of 44 years and 73% were women (Table 9). The median VL for individuals with LLV was 60 c/mL (IQR, 50–154). Of the total cohort, 58% were current or past smokers, and 65% reported alcohol consumption in the past 12 months.

The cardiovascular profile of people with a detectable VL of 50–999 c/mL was not different to those with viral suppression. The groups had similar values of both cardiovascular measures (blood pressure, pulse wave velocity, pulse pressure amplification, intima-media thickness, and carotid diameter distensibility) and blood biomarkers (troponin-T, NT-proBNP, CRP, IL-6, intracellular adhesion molecule 1 [ICAM-1], VCAM-1, P-selectin, myeloperoxidase, GDF-15, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 [ADAMTS13], and reactive oxygen species). Regression analyses did not result in any statistically significant associations between viremia category and cardiovascular measures or odds of having biomarker levels or cardiovascular measures in the highest quartile, respectively.

Discussion

This thesis explores clinical aspects of LLV in ART recipients. Specifically, the risks of future virologic failure, all-cause mortality, AIDS, and SNAE are studied in retrospective analysis of the Swedish nationwide HIV cohort. Furthermore, to investigate possible mechanisms behind the associations observed in epidemiological studies, we measured a range of blood biomarkers in one Swedish and one South African material of people with varying degree of detectable viremia, as well as cardiovascular measures in the South African cohort.

The main findings are that LLV of 200–999 c/mL (compared with viral suppression) is associated with increased risk of future virologic failure; there is a statistically significant association between LLV of 50–999 c/mL and all-cause mortality, and between LLV of 200–999 and SNAE; LLV is not associated with increased cancer incidence, but higher pre-ART VL is predictive of infection-related cancer; we observe no indication of increased inflammation, immune activation, or worse cardiovascular profile in people with LLV compared with those with viral suppression, but people in the Swedish cohort with persistent LLV have higher levels of GDF-15 and D-dimer.

The frequency of LLV in a population depends on the definitions, as well as time of follow-up. Furthermore, during a long life on ART, some patients will experience episodes of different levels of viremia, as well as prolonged periods of viral suppression. In paper II (the nationwide cohort), 14% of included participants had LLV of 50–999 c/mL during some point of the follow-up period, and 7.5% had LLV of 50–199 c/mL. Compared with previous studies, these proportions are relatively high. In the ART-CC study, only 3.5% experienced at least one episode of LLV of 50–199 c/mL [156], and Bernal et al. observed LLV of 50–199 c/mL in 4% of a Spanish cohort [146]. Two other studies reported LLV of 51–500 c/mL in 7.5% and LLV of 51–999 c/mL in 5.6% of participants, respectively [129, 145]. Importantly, all these studies have had relatively short follow-up (from a median of 18 months in [129] to a median of 3.5–6.3 years, depending on subgroup, in [146]). One study with a longer follow-up of eight years observed VLs in the range 50–500 c/mL in 28.6% of ART recipients; 20% of these (excluding those who switched treatment) had LLV in the subsequent sample, meaning that around 5% of the study population had LLV by our definition [128]. For

comparison, some people in our material were followed for over 20 years (median, 5.7). It is unlikely that Swedish PWH would have disproportionately high risk of detectable viremia during ART. Moreover, the requirement of two VLs in the LLV range (and a median time between VL samples of 120 days) likely results in an underestimated number of LLV cases, since more short-lived episodes will only be detectable in one sample and not captured by our sampling timepoints. In conclusion, we believe that the frequency of LLV in our nationwide material, although higher than in previous reports, likely is a conservative estimate of the probability of experiencing LLV during life-long ART, at least for patients starting ART during the period under study (1996–2017). This is also supported by a U.S. cohort (study period, 1996–2017; median follow-up, 7.8 years), where 7.5% had persistent LLV of 50–199 c/mL and 18.6% intermittent LLV in the same range (of whom 37% had more than one VL >50 c/mL) [5]. Whether the incidence of LLV is lower for PWH starting ART today, will have to be determined in future studies. In conclusion, LLV is relatively common, and knowledge of its possible implications for prognosis is much needed.

Associations between LLV and patient outcomes

Virologic failure

The rationale for the current definition of virologic failure in Swedish, British, and U.S. guidelines is an increased risk of loss of virologic control for people with repeated VL ≥ 200 c/mL [41, 63, 120]. The observed association between LLV over this level and virologic failure in paper I thus corroborates previous research and provides additional support for the current threshold. Like the large ART-CC study, we did not observe an increased risk of virologic failure for people with LLV of 50–199 c/mL [156]. Importantly, both in the ART-CC study and in paper I, participants with LLV of 50–199 c/mL were reclassified to LLV of 200–999 c/mL from the time when VLs in this higher range were recorded. To contribute to increased risk for the lower group, a participant therefore needed to develop virologic failure without any detected transitional LLV of 200–999 c/mL. Consequently, the risk of virologic failure for LLV of 50–199 c/mL might be underestimated in both these studies. In fact, our data suggest that the risk of progression to a higher viremia stratum could be higher for people with LLV of 50–199 c/mL than for people with viral suppression.

While not confirmed in our material, several reports have indicated an increased risk of virologic failure also for people with LLV of <200 c/mL, at least for individuals who are treatment-experienced when starting ART [5, 161, 162]. This implies that LLV in

this low range is not merely the result of assay variation or preparation tube handling, as has been suggested [249–251], but could represent a relevant entity with possible clinical consequences. Type of ART regimen at the time of LLV could also be relevant, since a small retrospective study from Taiwan did not observe increased risk of future virologic failure for people with LLV of 50–200 c/mL while receiving DTG-based or PI-based ART [252].

Participants with single detectable VLs (if preceded and followed by undetectable VL) were classified as having viral suppression in this thesis. It was recently suggested, however, that this group should be considered separately in studies on virologic failure [5]. Joya et al., surprisingly, found a lower risk of virologic failure for people with intermittent LLV of 50–199 c/mL (defined as <25% of measurements in this range) compared with viral suppression (aHR, 0.33; 95% CI, 0.21–0.52) [5]. The authors speculate that these patients could have higher retention in care or that intermittent viremia could have positive immunological effects. Still, other studies have reported increased risks of future virologic failure associated with intermittent viremia and/or blips [129, 131, 253], although these studies have included VLs >200 c/mL. Leierer et al. reported an increased risk of failure for patients with a single VL in the range 50–199 c/mL in an Austrian cohort [132]. The association between intermittent viremia and virologic failure thus remains unclear. Should the analysis from paper I be expanded to the nationwide cohort, intermittent viremia could be analyzed as a separate group to further explore these relationships.

Clinical endpoints

A main strength of this thesis is the analysis of clinical endpoints in long-term follow-up of people with LLV, which has been an area with limited data. Furthermore, we analyzed a nationwide cohort linked with national registers in a setting with relatively equal access to care [254]. In paper II, we found an association between LLV and increased mortality, which is in agreement with two previous studies [146, 183], although most previous reports have not found such an association [156, 173, 182, 186]. Our cohort had longer follow-up than these previous studies, which increases the chance of detecting a difference in mortality (Table 4, pp. 42–44). Interestingly, the association with mortality for LLV of 50–199 c/mL was restricted to individuals with ≥25% of VL measurements of ≥50 c/mL, suggesting that the persistence of LLV is relevant for prognosis.

Another measure, which captures both the amplitude and the persistence of viremia, is VCY. VCY has been associated both with increased mortality [189] and incidence of SNAE [204] among PWH, but some methodological aspects should be considered

when interpreting these studies. Most previous studies have calculated VCY on the linear scale, but it has been suggested that VCY on the logarithmic scale is more predictive of mortality [187]. Besides, levels of VCY can generally not be compared between studies since this measure is highly dependent on sampling frequency [255]. For these reasons, we used time-updated viremia category rather than VCY as a measure of viremia exposure in paper II. In paper III, however, we analyzed viremia during ART both as VCY and as time-updated viremia category (giving further support to the observation that viremia during ART was not associated with cancer).

The association between LLV and the composite endpoint SNAE in paper II is, to the best of my knowledge, a novel finding. Compared with previous studies [146, 194], we had longer follow-up and used a wider definition of SNAE, including conditions such as venous thromboembolic disease and pulmonary arterial hypertension, which have increased incidences among PWH [95, 256]. Salinas et al. reported an increased risk of acute myocardial infarction for time-updated viremia of 200–999 c/mL [183], and myocardial infarction has also been associated with high baseline VL and VCY [257]. Although our study size did not allow subanalysis of specific types of SNAE, this association between viremia and myocardial infarction is one possible mechanism behind our results.

For people with LLV during ART, it is reassuring that we observed no indication of increased cancer risk (despite a lenient definition of LLV, <1000 c/mL). Previous observational studies have found associations between non-suppressed viremia and AIDS-defining [196–202] and infection-related non-AIDS-defining cancer [198, 201, 203–205], including both patients with and without ART. Of note, only two of these studies differentiated between pre-ART VL and viremia after ART initiation, and both did not include pre-ART VL in the final model [196, 202]. Lee et al. found an association between VL at six months after ART initiation and 10-year cancer risk, but when adjusting for baseline variables (including pre-ART VL), the cancer risk was similar across viremia categories, like in paper III [258]. In addition to being associated with increased risk of infection-related cancer in paper III, high pre-ART VL has been reported as an independent risk factor of mortality (even after adjustment for CD4 cell counts) in long-term follow-up of ART recipients in partly the same cohort as the present investigation [83]. The link between pre-ART VL and cancer is further supported by the START trial, which showed reduced risk of infection-related cancer in individuals who received immediate compared with deferred ART, and this risk reduction was partially mediated by HIV RNA levels [116]. Furthermore, two large observational trials support an association between early ART and lower cancer risk. The North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) showed 30% lower cancer risk (59% lower for virus-related cancer) for

people with early compared with deferred ART initiation [259]. A recent analysis of the D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) study provides further evidence of an effect of immediate ART for prevention of cancer, although the estimated benefit was small in these real-world data [260]. Lastly, the significance of pre-ART viremia and VLs during ART could be different for different types of cancer; for example, recent viremia exposure has been found to be more predictive for non-Hodgkin lymphoma, whereas viremia 4.5–8.5 years in the past was more predictive for anal cancer [261, 262]. Due to a low number of outcomes in each category, our data do not exclude the possibility that there could be a relationship between viremia during ART and some specific types of cancer.

In the SMART trial, as well as another trial on structured treatment interruptions, levels of HIV replication were associated with IL-6, D-dimer, and the adhesion molecule VCAM-1 [229, 263]. Furthermore, there was a strong association between baseline levels of IL-6, D-dimer and CRP and all-cause mortality in both arms of the SMART study [229]. Suppression of HIV viremia was also the only variable correlated with improvement of endothelial dysfunction as measured by flow-mediated dilation in a study on people initiating ART [264]. Moreover, *in vitro* evidence suggests that HIV proteins can have a role in the development of atherosclerotic disease; transgenic mice expressing the HIV proteins Env, Tat, Nef, Rev, Vif, Vpr, and Vpu develop increased arterial stiffness, impaired flow-mediated dilation, and increased carotid intima-media thickness [265]. This leads to the hypothesis that low-level HIV replication could be associated with inflammation and/or endothelial dysfunction, and that this could be one mechanism behind the observed increased risk of all-cause mortality and SNAE for people with LLV. Yet, several other explanations are possible. Unlike ART interruptions, LLV is not always associated with ongoing HIV replication. Monotypic LLV, without signs of viral evolution, has been associated with higher frequency of CXCR4-using variants and a distinct cytokine profile [135]. Patients with LLV could thus represent a group who started ART at a more advanced disease stage, and who therefore have worse prognosis. This is also supported by the observation in paper I–III that people with LLV had lower pre-ART CD4 cell counts than those with viral suppression (although we adjusted for pre-ART CD4 cell counts in the models). LLV could also be due to inadequate antiretroviral drug levels, often related to insufficient adherence; of note, suboptimal adherence has been associated with inflammatory biomarkers and immune activation even in patients with persistent viral suppression [266]. There could also be a risk of reverse causality if people with non-AIDS-morbidity undergo more frequent VL monitoring (increasing the probability of detection of LLV) or the concurrent illness causes a temporary increase in HIV VL. We

further explored the possible mechanisms behind the observed increased mortality and morbidity in people with LLV in paper IV and V, discussed below.

Discussion and comparison of paper IV and V

Some important differences between paper IV and V should be noted. The demographics of PWH differ markedly between Sweden and South Africa, which is reflected by a high proportion of men in paper IV and a predominance of women in paper V. The Swedish epidemic is largely concentrated to certain population groups, whereas the South African epidemic is more generalized throughout the society. The South African epidemic is dominated by HIV subtype C (while B is most common in Sweden), which has been linked to increased risk of virologic failure and occurrence of certain resistance mutations [267]. All participants in paper V received an EFV-based regimen, but paper IV included people treated with a range of different regimens. Interestingly, the proportion of EFV-based ART in paper IV was higher among cases with LLV (47%) than among controls with suppression (29%), although the difference was not statistically significant. Furthermore, there could be differences in lifestyle factors and genetic predisposition. Perhaps most importantly, viremia classification was based on a single VL in paper V, whereas at least three VLs in the LLV range were required in paper IV. The median VL for cases with LLV was also higher in paper IV.

The observed elevation in levels of GDF-15 and D-dimer among people with LLV in paper IV could have clinical implications. GDF-15 is expressed in low amounts in many tissues under physiological circumstances, and its production is upregulated in cardiovascular as well as non-cardiovascular tissue in different pathological conditions (reviewed in [268]). In the general population, higher GDF-15 levels are associated with all-cause mortality, fatal and non-fatal CVD, cancer mortality, and incident heart failure [106, 269]. PWH have higher levels than uninfected controls, and GDF-15 levels in PWH have been associated with all-cause mortality, as well as with pulmonary hypertension [108]. In one study (a cohort from San Francisco, California, with 56% white and 32% black participants), a “cardiac phenotype” with elevated GDF-15, ST2, and NT-proBNP was associated with increased risk of pulmonary hypertension and all-cause mortality. White race or ethnicity was independently associated with having a cardiac phenotype in this material [109]. Higher D-dimer levels were associated with all-cause mortality as well as SNAE (CVD, end-stage renal disease, decompensated liver disease, and non-AIDS cancer) in an analysis of the control arms of three large clinical trials on PWH [270]. Should the associations between LLV and GDF-15 and D-dimer be confirmed in other materials, this could suggest that people with LLV have elevated CVD risk.

While cases with persistent viremia in paper IV had elevated GDF-15 compared with controls, no such trend was observed for cases with a single detectable VL in paper V. Apart from differences in the study populations, the amplitude or persistence of LLV is one possible explanation for these discordant results. Interestingly, overall GDF-15 levels were markedly higher in paper IV compared with paper V (median, 3169 pg/mL for cases and 1455 pg/mL for controls in paper IV versus 670 pg/mL for the total cohort in paper V). For comparison, Swedish apparently healthy individuals (median age, 63.5 years) had median GDF-15 levels of 897 pg/mL (IQR, 656–1154) [271]. We have no data on D-dimer levels in the EndoAfrica-NWU study. Still, analysis of a range of cardiovascular markers and measures suggests no association between detectable viremia and worse cardiovascular function or subclinical atherosclerosis in this cohort.

Levels of CRP were similar in people with LLV and viral suppression in both paper IV and V. This corroborates previous studies, which observed no association between CRP and LLV [173, 174, 177]. IL-6, which has been linked to LLV in two previous studies [174, 175] and induces CRP production, was not associated with viremia category in paper V. We considered IL-6 in a pilot study for paper IV but had to exclude it due to a large proportion of undetectable values (probably related to degradation during storage [272]). Of note, median CRP levels were higher in the South African cohort than the Swedish: 2.6 mg/L in paper V versus 0.9 mg/L among cases and 1.3 mg/L among controls in paper IV. This could reflect a higher CVD risk [273], but could also be related to other concomitant diseases. Lastly, while CRP is a well-established biomarker of inflammation and CVD risk, the causal relationship between inflammation markers and CVD remains controversial. Mendelian randomization studies (that use genetic polymorphisms as instrumental variables for modifiable risk factors and thereby are less likely to be influenced by reverse causation and confounding) suggest that IL-6, but not CRP, has a causal role in the development of coronary heart disease in the general population [274, 275]. To my knowledge, this has not yet been investigated specifically for PWH.

Paper V is, to the best of my knowledge, the first biomarker study on people with LLV living in sub-Saharan Africa. Furthermore, few studies have compared cardiovascular measures between people with LLV and viral suppression. Some previous data indicate a positive relationship between HIV VL and carotid intima-media thickness (reflecting atherosclerosis) and, less consistently, pulse wave velocity (reflecting arterial stiffness) [276–280], although traditional risk factors likely are more important [279, 280]. These studies have all included some individuals with non-suppressed viremia, which (depending on how the viremia variable is modelled) could be highly influential for the relationship between viremia and the outcome. For people with VL <1000 c/mL, one study reported higher pulse wave velocity in those with detectable (80–999 c/mL) than

in those with undetectable (<80 c/mL) viremia (although not statistically significant in multivariable analysis) [281], and another study reported higher carotid artery intima-media thickness in patients with detectable residual viremia of <20 c/mL compared with those with undetectable viremia [282]. Nevertheless, our study is larger than these previous reports, and we observed no indication of worse cardiovascular profile in people with low-level detectable VL.

Detectable viremia during ART: a spectrum of related conditions

In this and other works, different patterns of detectable viremia during ART are classified into separate categories such as viral suppression, residual viremia, blips/intermittent viremia, LLV, and virologic failure. While this nomenclature is useful for researchers and clinicians when discussing the impact of different types of viremia, it should be noted that these phenomena might not represent distinct biological entities. Rather, some patients with LLV could have ongoing viral replication with risk of emerging drug resistance—a situation similar to virologic failure. LLV and blips could also arise from clonal expansion of the viral reservoir without ongoing replication and infection of new cells—as is the case for residual viremia. Since blips could be as short-lived as two days [125], it is impossible to differentiate between blips and more prolonged LLV in clinical materials with months between VL samples. Lastly, the distinction between residual viremia and viral suppression is largely dependent on the sensitivity of the assay, as the majority of ART recipients have detectable viremia with single-copy assays [153].

Notably, the choice of plasma VL as a biomarker is partly based on the convenience of obtaining plasma specimens, compared with other tissues. The cell and tissue sources of residual viremia during ART have not been identified (reviewed in [154]), although follicular helper T cells are considered likely candidates [283]. The gut is a possible tissue source of viremia since it has been estimated to harbor 83–95% of all infected cells in the body of a person with HIV [284]. One study found discordantly elevated concentrations of HIV RNA in cerebrospinal fluid in 7/40 (18%) of patients with LLV (median plasma VL, 92 c/mL; IQR, 59–179), compared with 0/43 patients with durable viral suppression in plasma. Drug resistance mutations to antiretrovirals taken at the time of sampling were observed in five of these cerebrospinal fluid samples. The authors provided two possible interpretations: 1) ongoing replication in the central nervous system as a source of plasma LLV, or 2) suboptimal viral control for another

reason (such as more advanced disease at ART initiation) as a mutual cause of plasma LLV and detectable virus in cerebrospinal fluid [285]. LLV has also been associated with cerebrospinal fluid viral escape in other studies [286, 287]. Consequently, it is possible that analysis of other sample specimens, such as lymph nodes, gut-associated lymphoid tissue, or cerebrospinal fluid, could be more informative in certain situations, compared with peripheral blood as studied in this thesis.

Considering the different mechanisms behind LLV [135], the group referred to as LLV in this thesis may in reality be a heterogenous category including some individuals with ongoing viral replication (diverse LLV) and some without replication (monotypic). Since a relatively high proportion of participants in paper V reported treatment interruptions (15% of those with LLV or viral suppression), ongoing replication could be a more important mechanism in this setting. The group referred to as viral suppression included a substantial proportion (35% in paper II) of individuals with isolated elevated VLs, which could either represent short-lived blips or transient episodes of LLV. Thus, determination of type of LLV or improved viremia classification by more frequent VL sampling could yield further insights into the relationship between LLV and adverse clinical or virologic events. On the other hand, the definitions used in this thesis are reflective of the clinical reality, with VL sampling with 3–6 months interval. Despite the crudeness of our classifications, our results could therefore have a direct clinical interpretation.

External validity of study results

Paper I–III are not based on selected samples, but rather all participants in the geographical region (Malmö and Gothenburg for paper I, Sweden for paper II and III) during the specified years who met inclusion criteria. Since we analyzed a nationwide cohort, we believe our results could be transferable to other high-income settings, with some caveats. Risk factors for LLV, and consequently characteristics of people with LLV, could be different depending on the setting (insurance status could be relevant in some health care systems, for instance [288]) and local treatment recommendations. Several new antiretroviral drugs have been developed during the study period, which could affect the generalizability of our results to people starting ART today. For this reason, we have performed sensitivity analyses restricted to more recent time periods, with similar results. In paper III, exclusion of participants without pre-ART VL could result in selection bias. Still, these individuals were largely diagnosed before introduction of ART and VL monitoring, and we argue that exclusion of this group probably improves the generalizability of our results to current settings.

There are several factors that impact the external validity of paper IV and V. Paper IV included all participants with available plasma samples, and the characteristics of included individuals are similar to that of the complete Malmö and Gothenburg cohort in paper I. Only one participant in paper IV received an INSTI-based regimen, so our results may not be generalizable to people using these drugs. Due to differences related to the virus (such as subtype and resistance patterns), host (such as genetic disposition, concomitant diseases, and lifestyle factors), type of ART, and society (such as organization of health care and socioeconomic factors), it is difficult to transfer results from HIV studies in high-income settings to low- and middle-income countries (where the majority of all PWH live). The inclusion of paper V, set in South Africa, is thus a strength of this thesis. Importantly, the study population in this study was composed of a convenience sample, which limits generalizability to the general population of PWH (since certain subgroups are more likely to participate in a trial).

Limitations

In paper I–III, we report associations between viremia and different outcomes. Importantly, there are several threats to a causal interpretation of the relationship between viremia and these outcomes. Firstly, there could be residual confounding if unmeasured factors are associated with both the exposure (viremia) and the outcome. We controlled for several well-established factors but lack information on some potential confounders. It was recently explored whether the effect of smoking on cancer risk among PWH differed dependent on CD4 cell counts and VLs; this analysis found no evidence of a different relationship between smoking and cancer depending on CD4 cell counts or viral suppression [289]. We do not know if the distribution of smoking habits was similar across viremia categories in paper I–IV. Recording of smoking status was recently implemented in InfCare HIV, and in 2017, 20% of the cohort were smokers [83]; smoking could thus influence our results. Somewhat reassuringly, smoking does not seem to be associated with HIV VL [290], but this does not exclude the possibility of unmeasured confounding due to smoking, socioeconomic status, or other factors.

Another limitation is the possibility of measurement error, both for the exposure (as discussed, several different assays have been used during the study period and there is considerable intra- and interassay variability in the LLV range [68]) and the outcomes. The data from the Cause of Death Register in paper II is one example of probable measurement error of the outcome, since causes of death from register data may not be completely reliable for PWH (explaining why the number of HIV-related deaths exceeds

the number of AIDS cases in paper II) [221]. Lastly, all these papers reported hazard ratios (HR), which could be problematic. HRs are difficult to interpret, since they are weighted averages of all HRs during follow-up, and attempts to determine time-specific HRs could result in selection bias. Furthermore, HR is a non-collapsible parameter, meaning that the estimate depends on which covariates that are included, even in the absence of confounding. It has thus been suggested that other measures than HR (based on the survival function rather than the hazard) could be preferred for survival analyses [291]. For these reasons, our findings do not provide evidence of a causal relationship between LLV and virologic failure, all-cause mortality, or SNAE, nor between pre-ART VL and infection-related cancer. Still, for practical reasons, it is unlikely that randomized trials will ever shed light on the question of LLV and long-term clinical outcomes. Therefore, we argue that the type of register studies included in this thesis could yield valuable information that, when interpreted together with results from other cohorts, could guide future research and inform treatment recommendations.

In paper IV, we had limited volume of biobank samples, so we were unable to reanalyze results that were outside the limit of quantification. We tried to address this using a log-rank test, which decreased the statistical significance of the difference in GDF-15 levels. Moreover, cases and controls were not perfectly matched; the median year of LLV samples was 2008 for cases and 2011 for controls, which could influence the results in case of a storage effect for some analytes. Lastly, and importantly, we had nine outcome measures, which could increase the risk of type I errors (erroneously rejecting the null hypothesis), especially for results with a P value close to 0.05. Hence, our results should be interpreted as preliminary and warrant confirmation in other materials. The fact that the difference in GDF-15 levels could not be corroborated in paper V decreases the likelihood that our result in paper IV represents a true finding, although we argue that this could also be explained by differences in cohort characteristics and study design.

The most important limitations of paper V are related to its cross-sectional design. Viremia classification was based on a single sample (precluding distinction between blips and LLV), and we cannot exclude the possibility that current viremia affects future cardiovascular function or atherosclerosis. It is biologically probable that some time is needed for the hypothetical effect of viremia to have an impact on the cardiovascular measures; another study design with longer follow-up time or more VL history is needed to further analyze this.

What are the implications of these findings?

More research is needed to further guide the clinical management of people with LLV. This thesis does not include any studies on interventions that may be beneficial for this group. Still, our results have some clinical implications.

The finding of increased risk of virologic failure for people with LLV of 200–999 c/mL, together with previous data, highlights the importance of active management of such patients. This includes intensified adherence counselling, consideration of drug–drug and drug–food interactions, and resistance testing. Based on data from clinical trials on treatment intensification with RAL or DTG for people with residual viremia [206, 207, 292], it is unlikely that adding more antiretroviral drugs would have an impact on persistent viremia of <50 c/mL (although significant decrease in CD8 T cell activation has been reported following treatment intensification in one study [293]). For patients with higher levels of LLV, it is possible that genotype-guided regimen modification could be beneficial [209, 210], although there is no consensus for LLV of <200 c/mL. The approach by Taramasso et al. [212], who divided patients with LLV into 1) inadequate ART in light of resistance testing, 2) inadequate drug exposure related to adherence or interactions, and 3) unexplained LLV, could prove useful for clinical practice in the future. Even in the absence of resistance testing, there is a theoretical advantage of using regimens with high resistance barrier for these patients.

We also observed higher risks of the following endpoints for people with LLV: all-cause mortality, SNAE (for LLV of 200–999 c/mL) and higher levels of GDF-15 and D-dimer (despite the limitations discussed above). It is unknown whether ART modification or any other intervention could impact this association, or if LLV simply is a marker of worse prognosis. We found no signature of increased inflammation or immune activation in either paper IV or V, providing evidence against the proposed mechanism between LLV, inflammation, and atherosclerotic disease [171]. Still, awaiting more data on the association between LLV and adverse outcomes, it is important to address modifiable risk factors for this patient group. As for all PWH, smoking cessation and treatment of hypertension, diabetes mellitus, and dyslipidemia should be a priority (the comorbidities section of the EACS guidelines could be used for this purpose [55]).

Our results give further support to early initiation of ART (which is now recommended for everyone) as the main HIV-specific intervention to prevent cancer.

Conclusions

- For people starting ART in Sweden between 1996 and 2017, LLV was a common phenomenon.
- In line with previous research, people with LLV of 200–999 c/mL had increased risk of future virologic failure, compared with those with viral suppression (<50 c/mL).
- LLV of 50–999 c/mL was associated with increased all-cause mortality compared with viral suppression. People with LLV of 50–199 c/mL also had significantly higher mortality. A subanalysis suggested that the relationship with mortality was restricted to persistent LLV (defined as $\geq 25\%$ of measurements).
- We found no indication of an increased risk of AIDS for people with LLV.
- LLV of 200–999 c/mL was associated with increased risk of SNAE compared with viral suppression, and the most common type of SNAE was CVD.
- While we observed an association between high pre-ART VL and incident invasive cancer (driven by increased risk of infection-related cancer), degree of viremia during ART had no statistically significant association with cancer.
- People with LLV had similar levels of markers of inflammation and immune activation as controls with viral suppression. South African cases with an elevated VL of 50–999 c/mL had similar cardiovascular profile as controls with suppression, suggesting that LLV is not linked to CVD in this setting.
- An analysis of 34 Swedish cases with LLV (at least three VLs of 50–999 c/mL) suggested elevated GDF-15 and D-dimer compared with controls with persistent viral suppression. In 95 South African cases with a single VL in the range 50–999 c/mL, however, GDF-15 levels were similar to that in controls with <50 c/mL.

Future perspectives

Our results add to mounting data that LLV is associated with worse prognosis in ART recipients. Moreover, our studies do not indicate that this would be mediated by inflammation and immune activation. The association between LLV of 50–200 c/mL and the risk of future virologic failure remains unclear, as does the impact of intermittent LLV/blips. We recently initiated a project using data from the EuResist Integrated Database, which contains VL, CD4 cell counts, ART data, and HIV genotype data from >100 000 PWH. The aim is to further delineate the effects of these patterns of viremia on the risk of virologic failure. Additionally, since this database includes a large number of genomic sequences, this survey is also expected to provide more data on the importance of subtype and drug resistance mutations in relation to LLV.

The analysis from paper V is one of the first to compare cardiovascular measures between people with LLV and those with viral suppression during ART. Similar data but with longer VL history, to enable better characterization of LLV and blips, are much anticipated.

Prevention of CVD is an important part of HIV care, and prediction models can be used to identify individuals at high risk. A specific prediction model for PWH has been developed based on the D:A:D study [294, 295]. Only the most recent VL (<50 c/mL, yes/no) was considered when constructing the risk equation, and HIV RNA was excluded due to non-significance. Since we found increased risk of SNAE among people with LLV, and an association between cumulative viremia and myocardial infarction has previously been observed [183, 257], it is possible that some measure of viremia history could improve the predictive capacity of this and similar models.

A few interesting studies have been published on the different mechanisms of LLV [134, 135]. Still, studies on the different types of LLV have hitherto been relatively small (reflecting extensive laboratory work to characterize the type of LLV) and the clinical impact of monotypic versus diverse LLV remains unknown. Bull et al. reported a characteristic pattern of analytes such as CRP and soluble CD163 in association with type of LLV [135]; should these results be confirmed, perhaps these—or other markers—could be used as a proxy for LLV type? That would enable analysis of the relationship between monotypic versus diverse LLV and clinical and virologic endpoints, respectively,

in larger materials. As patients with LLV likely constitute a heterogeneous group, it would also be interesting to study which factors (related to the host, virus, or treatment) that predict adverse virologic and clinical outcome for these individuals.

Globally, DTG-based ART is currently rolled out to replace EFV-based therapy as the preferred first-line ART regimen. The relevance of LLV during NNRTI-based treatment could thus decrease in the future, and the importance of LLV during INSTI-based ART will be a topic of future research. Still, it is estimated that 47% of all first-line regimens were NNRTI-based in 2020, and in 2024, it is estimated that 1.7 million people (7% of the estimated number of individuals in low- and middle-income countries with access to generic ART) will still receive these regimens [296]. Moreover, while DTG resistance is rare when used in combination treatment, extensive NRTI-resistance could result in functional DTG monotherapy. Surveillance of the role of LLV in this context should be a priority.

Finally, much of the management of PWH in high-income settings during the modern ART era has shifted towards improving tolerability and minimizing drug–drug interactions. As a result, new treatment strategies, such as two-drug regimens, have been introduced. The frequency and implications of detectable HIV viremia during these regimens remains to be determined.

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