Diplomarbeit

Effects of Different Types of Antiretroviral Therapy on Vasculature in HIV Patients: A Sub-Saharan Study

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Graz, am 25.01.2018

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Zusammenfassung

Hintergrund: Das Humane Immundefizienz-Virus (HIV) ist weltweit eine der Hauptursachen für Morbidität und Mortalität. Bis heute gibt es keine kurative Therapie zur Behandlung dieser Infektion. Seitdem jedoch die antiretrovirale Therapie (ART) verfügbar ist, hat sich die Lebenserwartung der HIV-PatientenInnen signifikant verlängert. Als Folge ist die HIV-Infektion heute eine chronische Erkrankung, was den Fokus von AIDS und AIDS-typischen, opportunistischen Erkrankungen zu möglichen Langzeitfolgen der ART lenkt. ART zeigt vor allem Nebenwirkungen auf das kardiovaskuläre System. Aktuell leben 70 % aller HIV-infizierten Menschen in Subsahara-Afrika, dennoch gibt es nur begrenzt Daten zu den Langzeitfolgen der ART in dieser Region.

Ziele: Diese Diplomarbeit hat das Ziel, die kardiovaskulären Effekte verschiedener Antiretroviraler Therapieregime, die derzeit in Subsahara-Afrika verwendet werden, zu untersuchen. Im Speziellen werden die Effekte der First Line und Second Line ART auf Serumlipidspiegel und die vaskuläre Funktion analysiert.

Methodik: Zwei HIV Patientinnen, unter First Line und Second Line ART, wurden aus der Kohorte des derzeit durchgeführten *EndoAfrica*-Projekts ausgewählt und werden als Fallstudien präsentiert. Patientin A erhielt First Line ART (Efavirenz 600mg, Emtricitabine 200mg, Tenofovir 300mg), während Patientin B mit Second Line ART (Lapanavir 200mg, Ritonavir 50mg, Zidovudine 300mg, Lamivudine 50mg) therapiert wurde. Neben den Parametern für die klassischen kardiovaskulären Risikofaktoren (Blutdruck, Body mass index (BMI), Serumlipide, Blutglukose) wurde die Flussvermittelte Vasodilatation (FMD) als Methode der Früherkennung von Endothelialer Dysfunktion, einer Vorstufe von Atherosklerose, evaluiert.

Ergebnisse: Beide Patientinnen hatten Blutdruckwerte, BMI, C-reaktives Protein und Blutglukose im Normbereich. Die Serumlipidlevel wiesen Unterschiede auf, Patientin A hatte normale Werte, während Patientin B ein Gesamtcholesterin von 5,21 mmol/L (Zielwert < 4,90 mmol/L) und ein LDL-Cholesterin von 3,49 mmol/L (Zielwert < 3,00 mmol/L) aufwies. Patientin A hatte eine FMD von 8,65 % in der Arteria brachialis, wohingegen Patientin B 18,30 % zeigte.

Diskussion: Die unterschiedlichen Serumlipidlevel könnten mit unterschiedlichen ART Regimes assoziiert werden, da Protease Inhibitoren (Teil der Second Line ART) auch in anderen Studien gezeigt haben, dass sie Dyslipidämien verursachen. Beide FMD-Ergebnisse liegen im Normbereich, folglich scheint die vaskuläre Funktion nicht von dem Typ der Antiretroviralen Therapie beeinflusst zu werden. Diese Ergebnisse sollten mit Vorsicht interpretiert werden, da nur zwei PatientenInnen untersucht wurden. Zukünftige Studien sollten die komplexe Interaktion von anderen Faktoren wie Rauchen, physischer Aktivität und Alter auf das kardiovaskuläre System in HIV-PatientenInnen unter verschiedenen ART-Typen berücksichtigen. Ebenso sollten Aspekte wie Viruslast, Dauer der ART und andere Komorbiditäten in die zukünftige Forschung eingeschlossen werden.

Abstract

Background: The human immunodeficiency virus (HIV) is one of the leading causes of morbidity and mortality worldwide. Up to date there is no curative therapy available to treat this infection. However, since antiretroviral treatment became available, HIV patients are living longer. Thus, the HIV infection has developed into a chronic disease, which shifts the focus from typical AIDS and AIDS-associated opportunistic diseases to possible long-term complications of antiretroviral therapy (ART). This is particularly so as ART has been shown to affect the cardiovascular system. Seventy percent of all HIV-infected persons currently live in Sub-Saharan Africa. Despite this, only limited data about the long-term effects of ART in this region are available.

Aims and objectives: This diploma thesis aims to investigate the cardiovascular effects of different antiretroviral regimens currently used in Sub-Saharan Africa. It specifically examines the effects of first- and second-line ART on lipid parameters and vascular function.

Methods: Two HIV patients on first- and second-line ART were selected from the cohort of the ongoing *EndoAfrica* study and are presented as case studies. Patient A was on first-line ART (Efavirenz 600mg, Emtricitabine 200mg, Tenofovir 300mg) and Patient B on second-line ART (Lapanavir 200mg, Ritonavir 50mg, Zidovudine 300mg, Lamivudine 50mg). Cardiovascular risk factors (blood pressure, body mass index, (BMI), serum lipids, blood glucose) as well as early markers of endothelial dysfunction and atherosclerosis (flow mediated dilatation, FMD) were evaluated.

Results: Both patients had normal ranges of blood pressure, blood glucose, C-reactive protein and BMI. The serum lipid levels presented differences: Patient A had normal values whereas patient B showed a total cholesterol of 5.21 mmol/L (target value < 4.90 mmol/L) and a LDL cholesterol of 3.49 mmol/L (target value < 3.00 mmol/L). Patient A had an FMD of 8.65% in the Arteria brachialis, whereas Patient B had an FMD of 18.30%.

Discussion: The different serum lipid levels could be associated with the variable ART regimens because protease inhibitors (part of second-line ART) have been shown to cause dyslipidemia in other studies. Both FMD results are within the normal range; therefore, vascular function does not seem to be affected by ART

type. These results should be interpreted with caution, as they have been obtained from only two patients on ART. Future studies should also investigate the complex interaction of other factors such as smoking, physical activity and age on the cardiovascular system in HIV patients on different types of ART. Aspects such as viral load, period of ART duration as well as any other co-morbidities should be included in future research.

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Abbreviations

Aa.	Arteriae
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
BMI	Body mass index
BP	Blood pressure
cART	Combination antiretroviral therapy
CCR5	C-C- chemokine receptor 5
CD4	Cluster of differentiation 4
CDC	Center for disease control
CVD	Cardiovascular disease
CXCR4	C-X-C chemokine receptor 4
CYP	Cytochrome p450
CYP3A4	Cytochrome p450 3A4
Dbase	Baseline dilatation
Dmax	Maximum dilatation
DNA	Deoxyribonucleic acid
Drecovery	Recovery dilatation
eGFR	estimated glomerular filtration rate
FDA	Food and Drug Administration
FMD	Flow mediated dilatation
GFR	Glomerular filtration rate
GGT	Gamma glutamyl transferase
GI	Gastrointestinal
Gp	Glycoprotein
HAART	Highly active antiretroviral therapy
HbA1c	Hemoglobin A1c
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
Hs CRP	Highly sensitive C-reactive protein

HSV	Herpes simplex virus
INSTI	Integrase inhibitor
LDL	Low-density protein
LTNP	Long-term nonprogressors
LTR	Long-term repeats
MSM	Men who have sex with men
MTCT	Mother-to-child transmission
NCD	Non-communicable disease
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NO	Nitric oxide
NRTI	Nucleoside reverse transcriptase inhibitor
PCP	Pneumocystis-carinii pneumonia
PEP	Post-exposure prophylaxis
PI	Protease inhibitor
PrEP	Pre-exposure prophylaxis
R	Rand
RNA	Ribonucleic acid
SIV	Simian immunodeficiency virus
START	Strategic timing of antiretroviral treatment
TB	Tuberculosis
TLR	Toll-like receptor
US	United States
VDRL	Venereal disease research laboratory
WHO	World Health Organization

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1 Introduction

1.1 HIV and AIDS

The human immunodeficiency virus (HIV) attacks, above all, the CD4 cells, which are important cells of the immune system. With no treatment, the decline of these cells leads to Acquired Immune Deficiency Syndrome (AIDS), which is characterized by immunodeficiency (1). Since the 1980s, HIV has developed into a pandemic, which to date has cost nearly 40 million lifes. Today, AIDS is number six among the top 10 causes of death worldwide and one of the leading causes of morbidity and mortality, especially in Sub-Saharan Africa (SSA) (2). Since the combination antiretroviral therapy (cART) became available in 1996, HIV/AIDS-related deaths have been significantly reduced (3).

1.1.1 Epidemiology

HIV incidence

The number of HIV new infections was highest in 1997 with 3.3 million new infections worldwide. Between 1997 and 2005 the HIV incidence was declining rapidly (3). In 2016, 1.8 million people were new infected with HIV (4). The sinking number of new HIV infections can be traced to dropping rates in heterosexual transmission. In contrast, in populations where the way of transmission is mostly between men who have sex with men (MSM), the rates are stable. The number of new infections in children decreased by 38% from 2009 to 2012 due to better access to antiretroviral therapy (ART) for pregnant HIV-positive women (62% in 2012) to prevent mother-to-child transmission (MTCT) (5). The number of HIV new infections is still highest in SSA, with 75.4% of all new infections worldwide in 2015, even though it holds only 12% of the worldwide population (3, 6). In 2015, 1.3 million people were new infected with HIV in SSA (7).

HIV mortality

Since 2004, HIV mortality has dropped by 5.5%, from a steady rate of 1.8 million deaths in 2005 to 1.2 million deaths in 2015, especially in low- and middle-income countries (3).

HIV prevalence

In the last 30 years, the number of people living with HIV increased from 2.4 million in 1985 to 28 million in 2000, reaching 38.8 million in 2015 (3). In 2015, approximately 25.5 million people were living with HIV in SSA. The number of people living with HIV has never been higher. This can be explained by the increasing availability of treatment possibilities (8).

Figure 1 illustrates the worldwide prevalence of HIV among adults in 2016.

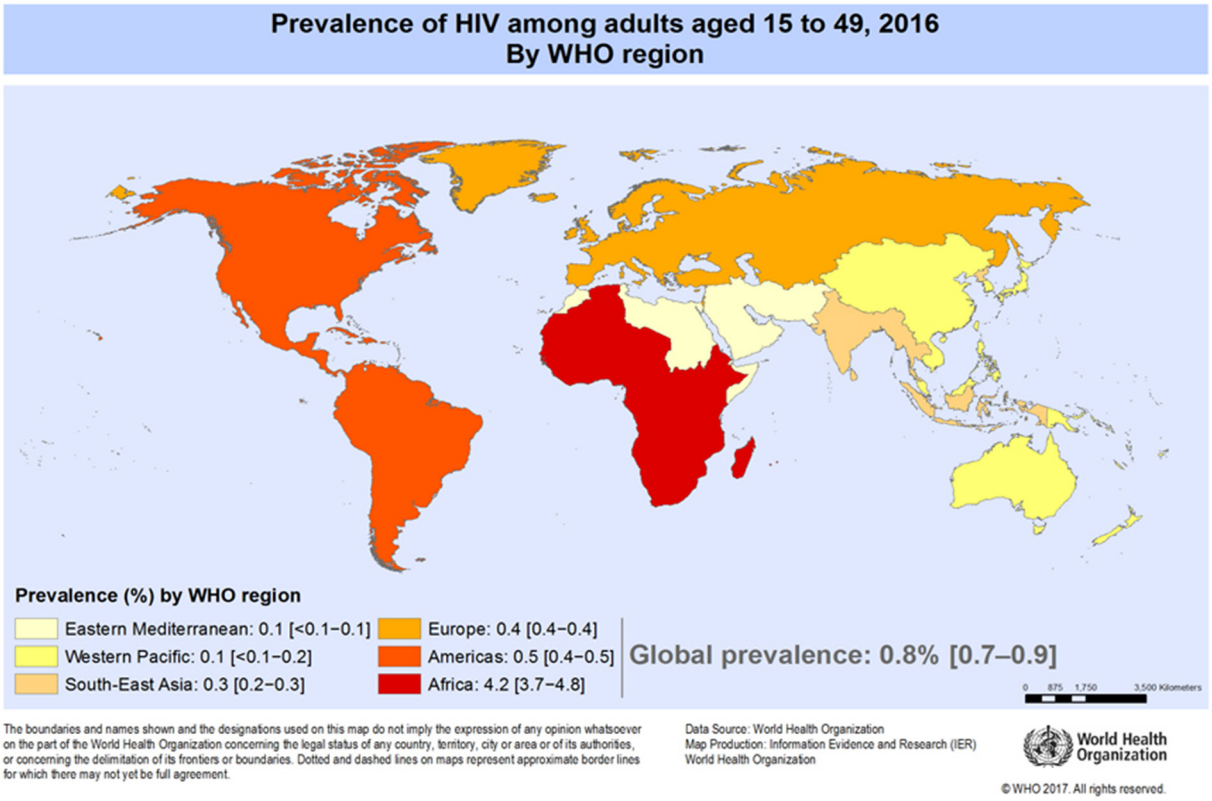


Figure 1: Prevalence of HIV in percent in adults (15-49 years), 2016
(reproduced from: http://www.who.int/gho/hiv/hiv_013.jpg?ua=1, access 07.09.2017)

HIV and antiretroviral therapy

Today's treatment of HIV is ART, which has been available since the mid-1990s. Approximately 12 million life years were added to the world between 1996 and 2008 because ART has been more easily accessible worldwide (9).

Antiretroviral coverage has grown every year. In 2010, only 7.7 million people had access to antiretroviral treatment; in 2015, it was 17.1 million, and by the year 2016, 19.5 million people had access to antiretroviral treatment. In 2016, 53% of all HIV-positive patients received ART. Figure 2 highlights that, globally, 60% of all HIV-positive women and 47% of all HIV-infected men have access to ART (4). In SSA, almost 12 million people receive ART (10).

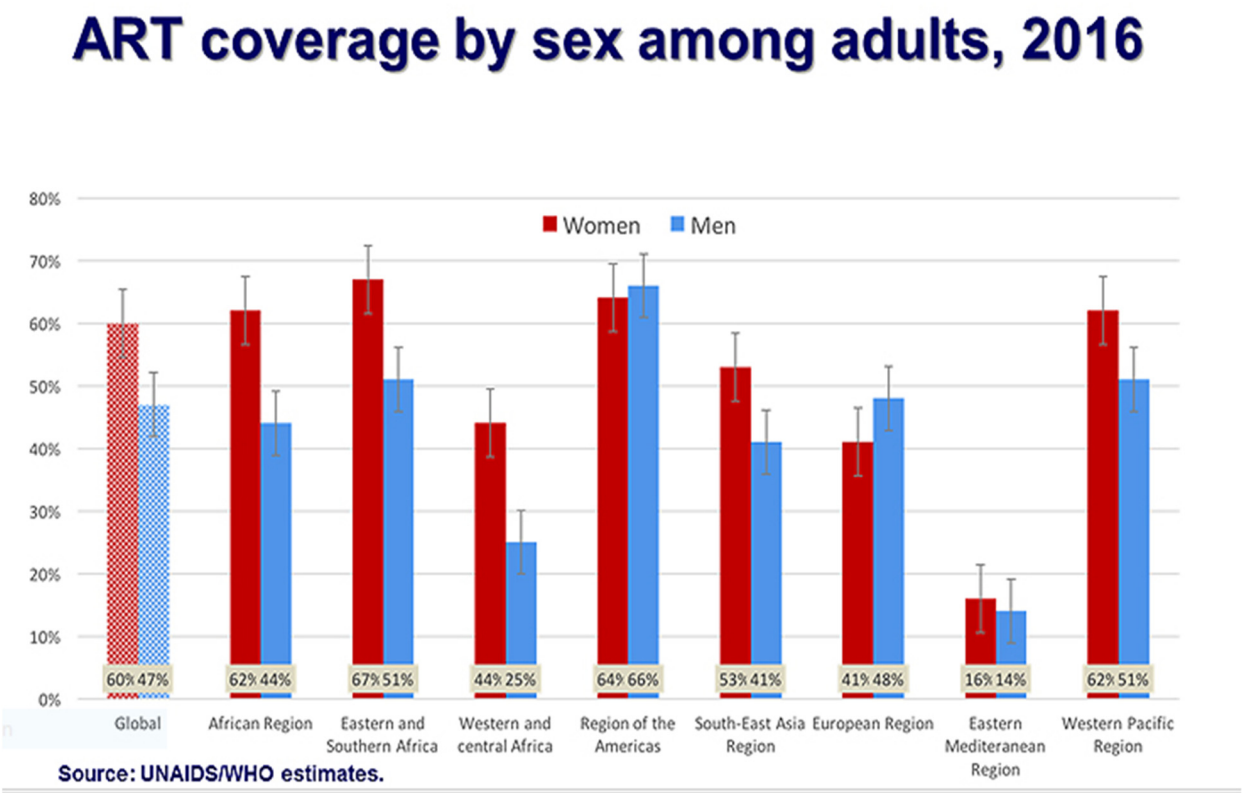


Figure 2: ART coverage by sex among adults (2016)
(reproduced from: http://www.who.int/gho/hiv/epidemic_response/ART/en/, access 07.09.17)

1.1.2 Characteristics of the human immunodeficiency virus

The human immunodeficiency virus belongs to the family of retroviruses and to the species of lentiviruses. *Retrovirus* is an RNA virus, an obligate parasite that targets a host cell. Once inside the cytoplasm, different viral enzymes work together to finally integrate the virus' RNA into the cell's DNA so that the viral RNA can be transcripted and translated along with the host cell's DNA. *Lentiviruses* (Latin for "slow") are a genus of retroviruses that cause chronic diseases, typically with a long incubation period. The genome of the lentivirus is integrated into the DNA of the host, which causes persistent infection. The HI virus is characterized by genetic diversity, which involves great rates of mutation and recombination during the viral replication process. Lentiviruses only affect several mammalian hosts, e.g. humans, primates, felines, cattle, goats and sheep (11, 12).

1.1.3 The HI virus' structure

The HI virus is a single-stranded positive-sense RNA virus whose genome is compounded of 9,749 nucleotides that encode nine viral proteins. Figure 3 presents the gene products of HIV-1 and their corresponding genomic sequence. Three major genes named Gag, Pol, and Env contain the information for the major structural proteins and essential enzymes. The Gag gene encodes viral core proteins, whereas the Pol gene holds the information for enzymes that are necessary for viral replication. The Env gene encodes the protein that forms the viral surface, which is called glycoprotein (gp) 160. The genome additionally contains genes that encode regulatory proteins such as Tat and Rev which can activate viral transcription and control the splicing process.

Four other accessory proteins, namely, Vif, Vpr, Vpu and Nef, are not required for replication in certain tissues. The viral genome is enclosed by long-term repeats (LTRs) that are necessary for viral transcription, reverse transcription and integration, and hence for the viral replication process (12, 13).

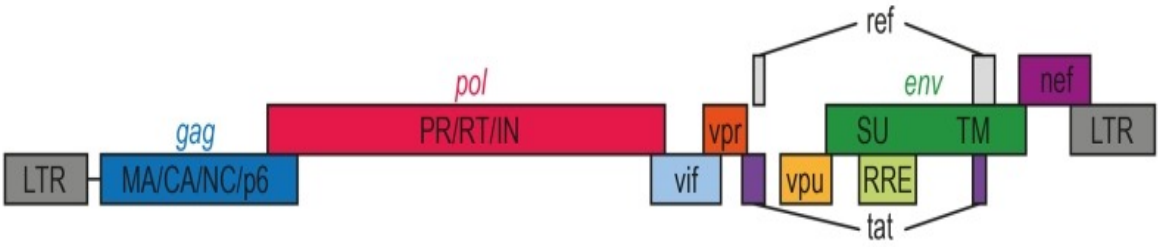


Figure 3: The HIV-1 gene products encoded by the HIV-1 genomic sequence
(reproduced from: https://www.ncbi.nlm.nih.gov/pubmed/28357381, access 08.09.17)

1.1.4 Classification

Two different kinds of HIV can be distinguished: HIV-1 and HIV-2, which can be further divided into more subtypes regarding there genetic composition (12). HIV-1 comprises four different lineages, groups M, N, O, and P, each of them the result of an independent cross-species transmission event. Group M (m for main) causes the global HIV pandemic. It can be found in every continent and in every country. Group O is less prevalent than M (1% of the global HIV infections) and is limited to West Africa. Groups N and P are even less prevalent than group O. Only a few cases have been reported; these only in Cameroon (11).

1.1.5 Differences between HIV-1 and HIV-2

HIV-1 and HIV-2 only share 30-60% of their genetic material, but they present a similar organization of structure and genetics. They resemble also in their transmission ways and affected cells. The different types originated as a result of different cross-species transmission events. HIV-2 mainly affects people in West Africa and Southern India, whereas HIV-1 can be found all around the globe. The viral load in people who have been infected with HIV-1 tends to be higher than in patients with HIV-2. This could explain the lower transmission rates of HIV-2.
Not many people infected with HIV-2 progress to AIDS; this is called long-term no progressors (LTNP). If they do develop AIDS, it has the same clinical symptoms than in people infected with HIV-1. While HIV-1 continues to be a global health problem, HIV-2 rates are declining in West Africa (11, 14).
This Diploma thesis will only deal with HIV-1, since it is the most endemic group in SSA.

1.1.6 The HIV/AIDS pandemic

Origins

HIV-1 originated from the chimpanzee simian immunodeficiency virus (SIV) in central Africa and is responsible for the HIV pandemic worldwide (15, 16). It originated approximately 100 years ago and has since diversified into many different subtypes, subsubtypes and recombinant forms (17). In comparison, HIV-2 evolved from another primate lentivirus that resulted from a cross-species transmission of SIV from sooty mangabeys to humans in Western Africa (11, 18). It is unknown how humans acquired HIV; however, since the biology of this virus is well known, transmission had to be possible through cutaneous or mucous membrane exposure to ape blood or body fluids which were infected. Most likely, these exposures occurred in relation to bush hunting (11).

AIDS was first recognized in the early 1980s when increasing numbers of young homosexual men fell ill with uncommon opportunistic infections and rare forms of cancer (11), such as Pneumocystis carinii pneumonia and Kaposi's sarcoma (19, 20). In 1983, the virus that causes AIDS was discovered and was given different names. Today, it is known as the human immunodeficiency virus (HIV) (19).

Spreading around the world

In a few decades, HIV spread around the world so that today it can be found in every country on the globe. A few characteristics of the virus made its circulation around the world quite easy. First, HIV evolves one million times faster than mammalian DNA. The viral generation time is short and the reverse transcriptase is prone to errors (11). During HIV replication, it is also possible that within one host cell infected with more than one HIV subtype (through co.-infection or superinfection) a recombination of two viruses takes place. As a consequence, the viral diversity is higher (13).

Current challenges

New HIV- subtypes are still evolving, which makes therapeutic intervention challenging. It is possible for new recombinant viruses to arise through infection with two or more genetically different viruses.

Superinfections in which there is a time difference between infections with two distinct subtypes have been described, but this is less frequent than coinfections. There is therefore strong evidence that clinical progression to AIDS could be faster in patients with a dual infection. This highlights the importance of encouraging safer sex in individuals who are already HIV-positive.

The different subtypes and genetic diversity explains the difficulty in vaccine development and are the reason why, to date, it is not possible to cure HIV infection (5, 21, 22).

1.1.7 Transmission

Transmission of HIV requires contact with a body fluid from an HIV-positive person. The body fluid has to contain infectious viruses (virions), HIV-infected cells, or both. The HI virus can appear in every body fluid, but it is known that transmission occurs most notably through blood, semen, vaginal and rectal fluids, and breast milk. Tears, urine, and saliva can also contain virions, but the concentration is so low that transmission through these fluids is extremely rare and it has not been confirmed that the transmission occurs at all.

The main route of HIV transmission is unprotected sexual contact, followed by needle sharing, commonly by drug addicts, and MTCT (vertical transmission).

The easiest way of transmission is blood transfusions, followed by vertical transmission, receptive anal intercourse, needle sharing, percutaneous needle stick, insertive anal intercourse, receptive penile-vaginal intercourse, and insertive penile-vaginal intercourse.

The transmission risk for oral sex is quite low, possibly because of the thicker epithelial layer of the oropharynx and the number of CD4-lymphocytes and HIV-specific antibodies, which are more concentrated in the mouth and oropharynx area compared to the cervico-vaginal area and the penis.

The transmission odds for anal intercourse are 18 times higher than for vaginal intercourse. This is due to differences between the tissues involved. The rectal mucosa has a higher number of lymphoid follicles, which are specialized in antigen uptake. Furthermore, immune cells such as CD4 memory T cells, macrophages, and dendritic cells appear in close proximity, which could simplify HIV replication. In addition, the single-layered epithelium of the rectum could be more prone to abrasions than the vaginal mucosa (13, 23-25).

Risk factors for transmission

The major factor that raises the risk of transmission is a high viral load. Other factors are the number of HIV particles in genital secretions (which is independent from HIV plasma concentrations) and the stage of infection. In the acute phase, approximately 4 weeks after infection, the transmission risk is 30-300 times higher than in the post-acute phase, because at this stage many people do not know of their infection and therefore do not receive antiretroviral treatment or are sensitized for their sexual behavior. Other sexually transmitted diseases such as genital ulcers of any kind, herpes simplex type 2 (HSV2) infection and bacterial vaginosis increase transmission risk (5, 26, 27).

Any factor that increases the opportunity for HIV to reach immune cells increases the risk of HIV transmission, for example, the presence of foreskin in men and the use of contraceptives that thin the epithelial layer of the vagina and increase the risk for cervical ectopy or presence of blood during sexual intercourse in women (13). Behavioral risk factors are numerous sexual partners and concurrent partnerships. Non-injectional drug use, for example alcohol, is associated with increased sexual risk behavior, while injectional drug use increases the risk for HIV infection by sharing needles.

In contrast, male circumcision can be linked with a reduced risk of sexually transmitted HIV (5). The high-risk groups for HIV infection are therefore MSM, injectional drug users and commercial sex workers (5).

Prevention of HIV transmission

Use of antiretroviral therapy, pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP), male condom use and medical male circumcision are factors that can lower the risk of HIV transmission (13). In MTCT, elucidation about breast feeding is an important factor, as well as ART treatment during pregnancy, access to prenatal care, HIV testing and MTCT interventions (28).

1.1.8 Pathogenesis

Infection

The transmission of one single virion is enough for infection with HIV-1 to occur. This virion infects a single cell close to the entry site. Mucosa represents the main point of transmission. An obligatory factor for transmission is the presence of immune cells such as CD4 T cells, sub epithelial dendritic cells, macrophages, and monocytes, which can be reached after the virus passes the mucosal epithelium (13).

Infiltration of cells

After infection, HIV's main targets are activated CD4+ T-lymphocytes. These cells support other leucocytes in immunobiologic processes, e.g. in maturation of the B-lymphocytes to plasma and memory cells or in activating cytotoxic T-lymphocytes. The virus enters the cell after gp120, the external glycoprotein from the virus, attaches to the CD4+ receptors. Interaction between chemokine co-receptors CCR5 or CXCR4 results in irreversible conformational changes. Within minutes, pores are formed so that the viral core can enter the cytoplasm. Other cells that bear the above mentioned receptors can also be the target of infection. These are resting CD4+ T cells, monocytes, macrophages, and dendritic cells. Resting CD4+ T cells (memory cells) form long-living reservoirs for HIV. These cells are the reason why it is not possible to fully eradiate HIV (29-31).

Integration into the genome

After the core diverges, the viral genome is reverse transcripted into DNA by the reverse transcriptase of the HI virus. The integrase is now able to insert the viral genome into the cell genome. With this step, the cell is irreversibly transformed into a potential virus-producing cell (21, 32).

Assembly and release

Until the HI virus is ready to infect other cells, the different components of the virus have to be assembled, packaged, and released from the cell. The production of viral particles can be host-driven as well as virus-driven. The completed viral proteins are transported to the cell membrane and make use of a special vesicular sorting pathway to leave the cell. Through cleavage of the Gag-Pol poly protein, the viral protease produces infective virions that can infect new cells. Since cytoplasmic molecules and cell surface components are absorbed by the new viral particles, virions carry characteristics of the cells in which they were produced (21, 33).

Spreading in vivo

The HI virus can infect new cells through two different pathways. The first is cell-free spread. Here the virus is replicated inside an infected cell and the new produced viruses are released into the extracellular room, where they can infect other CD4 cells.

The second possibility is cell-to-cell spread. Here, a T-helper cell transmits the virus directly into another T-helper cell by creating a virological synapse (34, 35).

Eclipse phase

1-2 weeks after infection

In the first days after infection, HIV replication increases, as do the inflammatory cytokines and chemokines. This is remarkable, since the initial response in other infectious chronic diseases such as hepatitis C or B is rather weak. The HI virus replicates freely and disperses from the source of infection to the organs and tissues, which provide cells for infection and replication.

Blood: no viremia, no immune response

Symptoms: none (36)

Without antiretroviral therapy, the HIV infection advances in three stages: *Acute infection*, *chronic infection*, and *AIDS*.

Acute infection

2-4 weeks after infection

Blood: high viremia (up to 107 or more copies of RNA/ml blood) and high numbers of infected CD4 cells, induction of inflammatory cytokines and chemokines.

The number of CD4 cells decreases, followed by a recovery to almost normal concentrations that then gradually decrease again. Most patients present antibodies after three to four weeks. The time between primary infection and seroconversion is called the “window period”, because a definite diagnosis is not possible and HIV antigen/antibody tests may be negative (37).

Symptoms: flu-like symptoms such as fever and sore throat are often (but not always) present, lymphadenopathy, rash, myalgia/arthralgia, diarrhea, weight loss, and headache are also possible. The combination of symptoms is also known as Acute Retroviral Syndrome. None of these symptoms are specific to the HIV infection, which makes early diagnosis challenging (38).

Chronic infection/clinical latency

1-20 years after infection

The viral load decreases to a stable number, a so-called “set point”, which is maintained by the innate and adaptive immune response. The innate immune response especially mediated by natural killer cells is responsible for controlling the viral load. Due to the formation of viral escape mutants, the impact of natural killer cells is restricted.

Blood: constant or slowly increasing viremia (1-105 copies/ml), called the “set point”. CD4 cell levels are nearly normal or gradually falling (around 1,000 cells/µl).

Symptoms: usually none (13, 39-41)

Acquired Immune Deficiency Syndrome (AIDS)

Usually 5-10 years after infection (with great individual differences); the time of survival without treatment is around three years (42).

Blood: CD4 cells are usually under 200 cells per µl the median CD4 cell count at the time of the outbreak of opportunistic illnesses is 67 cells per µl (43).
Symptoms: various opportunistic illnesses are possible (Table 1)

AIDS is characterized by a severe and irreversible acquired defect in cell-mediated immunity that predisposes the host to severe opportunistic infections and/or unusual neoplasms such as Kaposi's sarcoma (44, 45).
AIDS is defined as a CD4 cell count under 200 cells per µl or the presence of any AIDS-defining condition, regardless of the CD4 cell count (46). AIDS-defining conditions are opportunistic illnesses that typically occur in immunocompromized patients. The opportunistic illnesses are mostly infectious but can also be certain malignancies or conditions with no other clear alternative etiology, such as encephalopathy or wasting syndrome. Table 1 provides an overview of AIDS-defining diseases.
Once the CD4 cell count is below 200 cells per µl and if the patient receives no adequate antiretroviral treatment, the median time for developing an AIDS-defining condition is 12 to 18 months. If the CD4 cell count falls under 50 cells per µl, it can be referred to as "advanced HIV infection". Most patients who die of AIDS-related illnesses have CD4 cell counts in this range (46).
Figure 4 presents the clinical stages of HIV infection.

Bacterial infections, multiple or recurrent*
Candidiasis of bronchi, trachea, or lungs
Candidiasis of esophagus
Cervical cancer, invasive$^{\varphi}$
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes), onset at age >1 month
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy (HIV related)
Herpes simplex: chronic ulcers (>1 month's duration) or bronchitis, pneumonitis, or esophagitis (onset at age > 1 month)
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (> 1 month's duration)
Kaposi sarcoma
Lymphoma, Burkitt (or equivalent term)
Lymphoma immunoblastic (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium complex (MAC) or Mycobacterium kansasii, disseminated or extrapulmonary
Mycobacterium tuberculosis of any site, pulmonary$^{\varphi}$, disseminated or extrapulmonary
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis jirovecii (previously known as "Pneumocystis carinii") pneumonia
Pneumonia, recurrent$^{\varphi}$
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain, onset at age > 1 month
Wasting syndrome attributed to HIV

Table 1: AIDS-defining conditions
(reproduced from: https://www.ncbi.nlm.nih.gov/pubmed/24717910, access 27.11.17)
*only among children aged < 6 years
$^{\varphi}$only among children aged > 6 years, adolescents, and adults

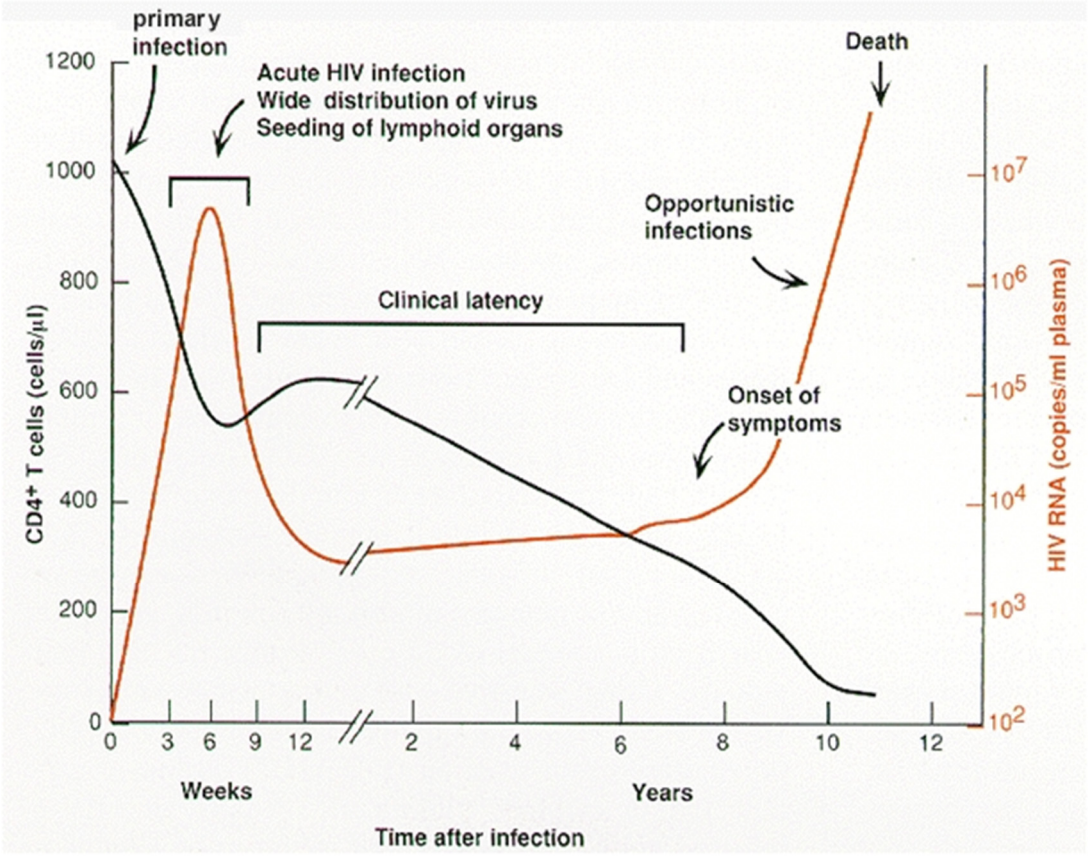


Figure 4: clinical stages of HIV infection
(reproduced from: https://www.ncbi.nlm.nih.gov/books/NBK19374/figure/A6274/?report=objectonly, access 07.09.17)

1.2 Antiretroviral therapy (ART)

ART became available for treatment against HIV in the mid-1990s. It consists of a combination therapy of diverse drugs that interfere with different parts of the life cycle of the HI virus. ART is always a lifelong treatment because viral replication can be suppressed but, to date, it is not possible to cure the disease or fully eradicate the virus. This is due to the presence of latently infected rested memory CD4 cells, which contain the viral DNA but do not express the viral RNA or viral proteins; they therefore are beyond the reach of ART and cannot be reached by the immune system either. For asymptomatic HIV positives, it is a great challenge to adhere to the therapy. It is important, however, that the therapy is continued throughout life because even short breaks result in a viral rebound.
In recent years, the regimens have become less toxic and more effective; they have a lower pill burden and can be dosed less frequently than the original drugs. Due to ART, HIV has changed from a fatal to a chronic disease (13, 47-49).

1.2.1 Initiation of ART

The newest guidelines regarding initiation of antiretroviral treatment from the world health organization (WHO) are from 2015. It is recommended that ART should be initiated in all adults regardless of WHO clinical stage and at any CD4 cell count. This is new compared to the guidelines from 2013, which recommended ART only for patients who reached a CD4 cell count of less than 350 cells/mm³ and with severe or advanced HIV clinical disease.
The reasons for that change are recent reviews and cohort analyses that support the assumption that untreated HIV infection could be associated with non-AIDS-defining diseases such as cardiovascular, kidney and liver disease, cancers, and neurocognitive disorders. By initiating ART at an early stage, the risk of such diseases can be reduced and the survival rate would improve. In addition, with early ART, HIV transmission can be significantly reduced (50). Nevertheless, the long-term side effects of ART are still largely unknown and long-term toxicity needs to be followed carefully (51).

There are two up-to-date studies that support early ART initiation. The Temprano trial included 2,056 patients from the Ivory Coast and could show that early ART initiation with CD4 cell counts above 500cells/mm³ reduces the risk of death by nearly 44%.

The Strategic Timing of Antiretroviral Treatment (START) trial which worked with 4,685 patients in various countries, revealed that the risk of death in those who were treated early was 57% lower compared to the group not treated until the CD4 cell counts were < 350 cells/mm³. In both studies, one important overall benefit was the reduced rate of tuberculosis after starting ART at an early stage. Cancer rates were also lower in the intermediate ART group, but cardiovascular diseases were similar between the two groups. It should be noted that, very often, it is not possible to start ART at an early stage because HIV infection is only diagnosed in advanced stages (52, 53).

1.2.2 Effects of ART

Antiretroviral drugs have the potential to reduce HIV-associated morbidity and mortality. The life expectancy of people living with HIV has increased and can be as long as in HIV-negative individuals, especially in patients with a normal CD4 cell count and undetectable viral load (8, 48, 54-56).

After starting antiretroviral therapy, the viral load decreases and within three months is not detectable in the plasma. In contrast, the CD4 cell levels are variable. In a study published by Tuboi et al., after six months on antiretroviral therapy 56.1% of the HIV infected patients included in the study, had both a successful virological response and a CD4 cell recovery. 19 % presented only a viral load response without a CD4 cell response, and 14.8% had a CD4 cell response without a virological response (5, 57).

In contrast to complete responders, virologic-only responders were older and had a higher baseline CD4 count as well as a lower baseline plasma viral load. Immunologic-only responders were younger, had a lower baseline CD4 count and had a higher baseline plasma viral load (57).

Individuals who present a reduced CD4 cell recovery in spite of viral load suppression have an increased risk of serious non-AIDS events.

The success of the treatment can be measured through the plasma viral load, which ideally should be constantly undetectable (< 50 copies/ml); consequently, it should lead to a reconstruction of the immune system (58).
Additionally, with ART HIV transmission can be prevented (59, 60).
Antiretroviral therapy, however, is associated with other complications that may occur in HIV-positive patients on ART, which are summarized as “non-AIDS conditions” or “serious non-AIDS events” such as cardiovascular disease, cancer, and kidney disease (among others). These long-term side effects of ART are relatively unknown and provide new challenges for the successful treatment of HIV patients (49, 56).

1.2.3 The treatment cascade of care

In order to maximize the treatment benefits of ART, it is necessary for the HIV-positive individual to pass through different steps in the cascade of care. First, HIV testing is necessary; then, the individual has to access care and start the right treatment. It is furthermore mandatory for patients to stay in care and adhere to therapy. In the US, approximately 28 out of 100 patients successfully managed all of these steps. In resource-limited countries such as SSA, the number of patients is probably much lower, especially because HIV testing still causes problems (49, 61).

1.2.4 Forms of ART

Because of their specificity, the enzymes integrase, reverse transcriptase and HIV protease as well as specific adhesion proteins of the HI virus work as excellent points of attack for antiretroviral drugs. The different drugs are graded into six groups depending on their mechanism of action:
Non-nucleoside reverse transcriptase inhibitors, nucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, entry inhibitors and integrase inhibitors (48, 62). Table 2 provides an overview of FDA-approved antiretroviral treatment options.

Drug Class	CCR5 Antagonist	Fusion Inhibitor	NRTI	NNRTI	INSTI	PI
FDA Approved Drugs	Maraviroc	Enfuvirtide	Zidovudine Didanosine Zalcitabine Stavudine Lamivudine Abacavir Tenofovir Emtricitabine	Nevirapine Delavirdine Efavirenz Etravirine rlipivirine	Raltegravir Elvitegarvir[1] Dolutegravir	Saquinavir Indinavir Ritonavir Nelfinavir Amprenavir Lopinavir[2] Fosamprenavir Atazanavir Tipranavir Darunavir

Table 2: US FDA-approved antiretroviral drugs
(reproduced from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4143801/, access: 22.11.17)

1.2.4.1 Reverse transcriptase inhibitors

The first drugs that became available for HIV treatment were reverse transcriptase inhibitors, in 1987. These drugs interfere with one HIV-specific enzyme: the reverse transcriptase. This enzyme copies the virus' RNA in proviral DNA. Without the proviral DNA, the HI virus cannot be integrated in the host cell's DNA and quickly becomes destroyed by cellular enzymes. There are two classes of reverse transcriptase inhibitors: Nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (62).

Nucleoside reverse transcriptase inhibitors (NRTIs)

These drugs were the first to become available for treatment in the US and they remain important. They operate through the competitive inhibition of the reverse transcriptase and DNA chain termination. This means that the drug itself takes the function of a nucleoside and binds to the active side of reverse transcriptase. It is integrated in the growing DNA chain, but because it has the wrong chemical composition, the chain cannot be continued and there is a chain termination of the HIV proviral DNA chain.

Currently licensed are *Zidovudine, Lamivudine, Abacavir, Emtricitabine, Stavudine*, *Didanosine, Zalcitabine* and *Tenofovi*r (63).

Side effects

The main reason for side effects with the use of NRTIs can be explained by the mitochondrial damage that these drugs provoke through their integration into the mitochondrial DNA by means of the DNA polymerase. This is clinically manifested as lipoatrophy, neuropathy, myopathy, pancreatitis, hepatic steatosis, and rarely, lactic acidosis. Most frequent are headaches; severe is bone marrow depression, which manifests clinically in anemia or leucopenia (64).

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

They are a group of different substances that do not bind to the active side of the reverse transcriptase but to a different part, so that the active side changes and enzyme activity decreases. These agents are very specific, so they inhibit only the HIV-1 reverse transcriptase and not the one for HIV-2.

The licensed drugs are *Nevirapine, Efavirenz, Etravirine, Rilpivirine* and *Delavirdine* (63).

Side effects

Non-nucleoside reverse transcriptase inhibitors are less toxic than NRTIs, but they are never used for monotherapy because they quickly create resistant strains with a loss of antiretroviral effect. Common side effects are rash and hepatitis. Skin changes such as the Stevens-Johnson and Lyell syndromes can occur, especially with Nevirapine (64). Drug interactions are also common, because NNRTIs are metabolized by Cytochrome P450 and inducers of this enzyme.

1.2.4.2 Protease inhibitors (PIs)

This class of drugs bind competitively to the viral protease. This enzyme normally has the function of cutting a large structural core protein that can only leave the infected cell and infect other cells after the cutting process. The inhibition of the viral protease leads to the production of immature virus particles that are unable to infect other cells. They remain an important class of drugs because of their high barrier to resistance (48).

The licensed drugs are *Amprenavir, Fosamprenavir, Atazanavir, Darunavir, Indinavir, Lopinavir with Ritonavir, Nelfinavir, Ritonavir, Saquinavir* and *Tipranavir* (63).

Side effects

Protease inhibitors block the metabolism of Cytochrome P450. Insulin resistance, hyperlipidemia, and truncal fat accumulation (lipohypertrophy/lipodystrophy) are common side effects with PI therapy. An increased cardiovascular risk is often described. Ritonavir is badly tolerated in patients, but it is used in low concentrations as a “booster” to reduce the first pass metabolism (via the inhibition of Cytochrome P450 3A4 (CYP3A4)) of other PIs so that the bioavailability of these drugs improves and they can be dosed less frequently and in lower doses (48, 65-67).

1.2.4.3 Integrase inhibitors (INSTIs)

It is necessary for the HI virus to integrate its RNA in the host DNA so that the production of viruses can begin. The viral enzyme integrase, which is blocked by integrase inhibitors, is responsible for this step.
A licensed drug is *Raltegravir* (63).

Side effects

Typical side effects are diarrhea, nausea, fever, and headaches. There is no data about the long-term effects of this drug class, as they are relatively new drugs (68).

1.2.4.4 Chemokine receptor antagonists

Chemokine receptors CCR5 and CXCR4 facilitate the entry of HIV into the cell (69). Chemokine receptor antagonists can block the interaction of the HIV gp120 and the CCR5 receptor of the cell. It is important to know that HIV can use both the CCR5 and the CXCR4 for cell entry but chemokine receptor antagonists only block CCR5. Before prescribing this class of drugs, it is therefore necessary to perform a viral tropism testing to confirm that the patient’s virus only uses the CCR5 co-receptor.
A licensed drug is *Maraviroc* (48, 70).

Side effects

Possible orthostatic hypotonia; in general, only a few side effects are known.

1.2.4.5 Fusion inhibitors

This class of drugs interrupts the entry of the HI virus by preventing the viral protein gp41 from binding to the CD4 receptor so that the viral envelope cannot fuse with the cell membrane. This drug needs to be administered through subcutaneous injections twice daily, so it is reserved for patients with multiple drug resistance.
A licensed drug is *Enfurvitide* (71).

Side effects

Local reactions at the site of the subcutaneous injection are typical (Enfurvitide would be destroyed in the GI tract so it has to be administered through subcutaneous injections). Skin infections, pneumonia, pancreatitis, and central nervous interferences are possible side effects (64).

Table 4 provides an overview of the major types of toxicity of the most common antiretroviral drugs.

ARV drug	Major types of toxicity
Abacavir	Hypersensitivity reaction
Atazanavir	Electrocardiographic abnormalities (PR and QRS interval prolongation) Indirect hyperbilirubinemia (clinical jaundice) Nephrolithiasis
Zidovudine	Severe anemia, neutropenia Lactic acidosis or severe hepatomegaly with steatosis Lipoatrophy Lipodystrophy Myopathy
Dolutegravir	Hepatotoxicity Hypersensitivity reactions

Darunavir	Hepatotoxicity Severe skin and hypersensitivity reactions
Efavirenz	Persistent central nervous system toxicity (such as dizziness, insomnia, abnormal dreams) or mental symptoms (anxiety, depression, mental confusion) Convulsions Hepatotoxicity Severe skin and hypersensitive reactions Gynecomastia
Etravirine	Severe skin and hypersensitivity reactions
Lopinavir	Electrocardiographic abnormalities (PR and QRS interval prolongation, torsades de pointes) Hepatotoxicity Pancreatitis Dyslipidemia Diarrhea
Nevirapine	Hepatotoxicity Severe skin rash and hypersensitivity reaction, including Stevens-Johnson syndrome
Raltegravir	Rhabdomyolysis Myopathy Myalgia Hepatitis and hepatic failure Severe skin rash and hypersensitivity reaction
Tenofovir	Chronic kidney disease Acute kidney injury and Fanconi syndrome Decreases in bone mineral density Lactic acidosis or severe hepatomegaly with steatosis

Table 3: The most common antiretroviral drugs and their major types of toxicity
(reproduced from: www.who.int/hiv/pub/arv/chapter4.pdf, access: 09.09.17)

1.2.5 First-line regimens

First-line regimens are used for the treatment of treatment-naïve HIV-positive patients. First-line regimens include three different antiretroviral drugs. It is necessary to combine different drugs to effectively suppress viral replication and to prevent viral resistance (48). They usually consist of one NNRTI or one PI plus dual NRTIs (63).

Possible combinations are:

- 1 NNRTI+ 2 NRTI
- 1 PI+ 2 NRTI

This regimen is also known as highly active antiretroviral therapy (HAART) or combination antiretroviral therapy (cART). Fusion inhibitors or CCR5 antagonists are not recommended for initial ART (48, 71).

1.2.6 Second-line regimens

A switch to second-line regimens is recommended when clinical, immunological or virological failure is recognized. They consist of one Ritonavir-boosted protease inhibitor plus dual NRTIs:

1 PI+ 2 NRTIs (72).

1.2.7 Monitoring

The treatment response can be verified by measuring the HIV RNA (viral load) or the CD4 cells (73). The viral load should be persistent below the level of detection (HIV RNA ≤ 20 to 75 copies/ml). Treatment failure is defined as a confirmed viral load > 200 copies/ml. Higher peaks (usually < 400 copies/ml) are not uncommon and do not count as treatment failure (74).

After starting ART, viral suppression is reached within 8 to 24 weeks. Figure 5 illustrates the typical decrease of the viral load after starting ART. The CD4 cell count measures the immunologic response to ART.

An adequate response is a 50-150 cells/mm³ increase in the first year (75).

In low-income countries and especially in SSA, the CD4 cell count is used for monitoring where viral load monitoring is unavailable. Since CD4 cell counts can be inaccurate, they may lead to unnecessary switches to second-line therapy or the continuation of ineffective first-line regimens, which can lead to increasing numbers of resistant mutations (76).

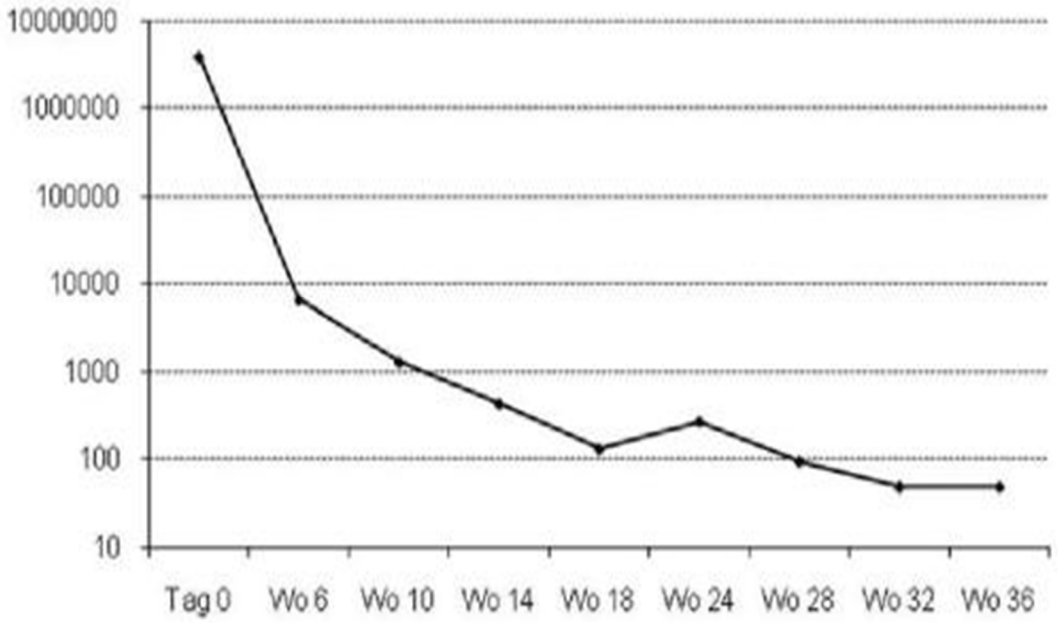


Figure 5: Typical decrease of the viral load after starting ART
(reproduced from: https://hivbuch.de/tag/viruslast/, access 20.09.17)

1.3 Cardiovascular Diseases

Cardiovascular disease (CVD) is a generic term for diseases that affect the heart and the blood vessels. The most common manifestations are coronary heart disease, cerebrovascular disease, peripheral artery disease, and deep vein thrombosis, which can lead to pulmonary embolism.
There are certain risk factors that promote the development of these diseases, such as hypertension, diabetes, hyperlipidemia, tobacco use, age, sex, physical inactivity and obesity. They all have in common that they lead to atherosclerosis, which can lead to CVDs such as stroke or myocardial infarction (77-80).

1.3.1 Global health burden

Cardiovascular diseases are the number one cause of death worldwide. In 2015, 17.7 million people died of CVDs, i.e., 31% of all deaths globally (81). In resource-limited countries the prevalence of cardiovascular diseases is especially high and still rising. More than 75% of the 17.7 million people who die annually of CVDs and diabetes currently come from resource-limited countries (82).

1.3.2 Atherosclerosis

1.3.2.1 Basics

Structure of Arteries

There are two types of arteries: elastic arteries, usually the big arteries close to the heart (such as the truncus pulmonalis and aorta), and muscular arteries such as the Aa. Brachiales, femorales and faciales, as well as the small arteries with no name. Histologically, arteries are built of three different layers:

The tunica interna

The tunica interna contains the endothelium and a subendothelial layer, mostly an extracellular matrix with just a few cells. The endothelium has one layer and contains polygonal, plain cells, which sit on a basal lamina. It controls the passage of blood and prevents contact between blood cells and the extracellular matrix. The surface of the endothelium is covered with a glycocalyx that prevents the adhesion of blood cells. On the surface, it contains many different proteins, proteoglycans, and coagulation factors. It is therefore also responsible for preventing blood coagulation in the intact endothelium and supports coagulation if required.

It is also responsible for vascular tone. It can secrete substances that affect the contraction of smooth muscle cells of the tunica media: Nitric oxide (NO), a lipophilic gas with a half time of about six seconds, dilates the vessel, whereas endothelin constricts it.

The subendothelial layer varies with age. In children, the layer is very thin so that it appears that the intima adjoins the internal elastic lamina. With aging, smooth muscle cells can migrate from the media. They cause a thickening of the intima. This layer is where atherosclerotic changes take place (83).

The tunica media

The tunica media is the biggest layer of the vessel wall and contains smooth muscle cells and extracellular matrix (83).

The tunica externa

The tunica externa or adventitia contains connective tissue that fixes the vessel in the surrounding area. It contains fibroblasts, proteoglycans, elastic fibers, and collagen fibers. Nutritional vessels such as small blood and lymphatic vessels and nerves can be found in this part. They supply the outer layers while the inner layer is nourished from the lumen of the artery through diffusion (83).

1.3.2.2 Pathogenesis of atherosclerosis

Endothelial dysfunction

Under physiological circumstances, the endothelium is in a balanced state. This means it can produce substances that provoke vasoconstriction (endothelin) as well as substances that cause vasodilatation (NO). It also prevents blood clotting and the adhesion of blood cells and increases permeability (84).

Traditional cardiovascular risk factors such as hypertension, dyslipidemia, smoking, and diabetes provoke changes in the endothelium so that it cannot sustain its regular functions. One of these changes is that the production of NO is reduced. That means that the blood vessels tend to vasoconstriction. Another change is the increased permeability of the endothelial layer, which causes leukocyte adhesion. This unbalanced state is called endothelial dysfunction, which can be seen as a precursor of atherosclerosis (85).

As displayed in Figure 6, all cardiovascular risk factors leading to endothelial dysfunction result in an increased risk of atherosclerosis. A central part of the loss of function of endothelial cells is the restricted production of NO. This leads to modified vasoreagibility, and relaxation is disturbed. This increases the adhesion of leucocytes and can entail thromboembolic complications as a result of the mismatch between homeostasis and fibrinolysis (86).

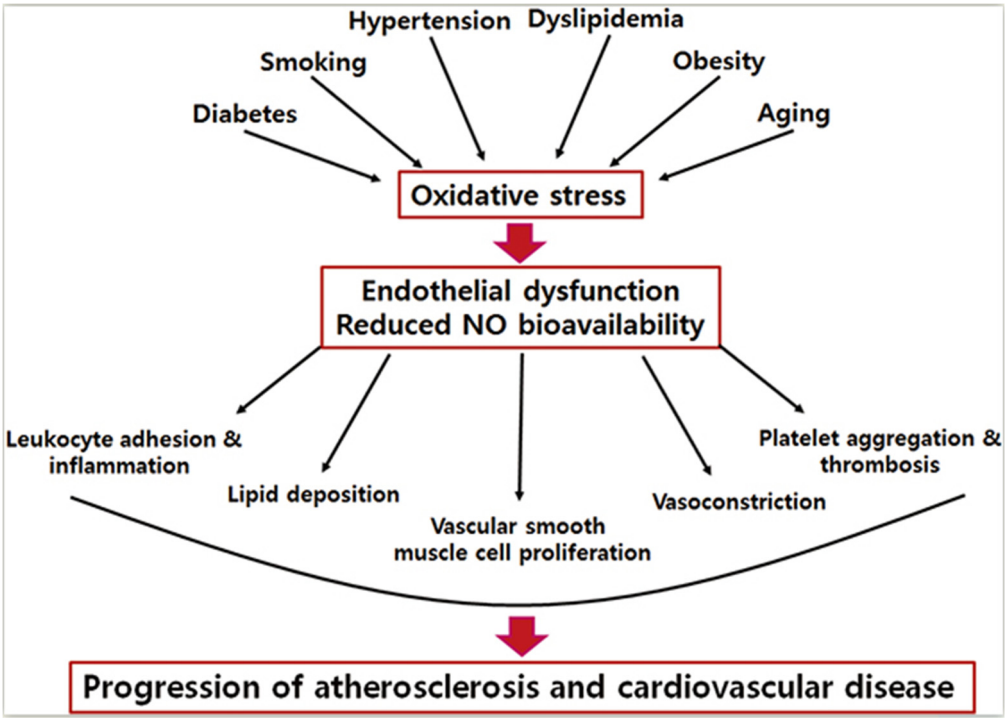


Figure 6: Risk factors of endothelial dysfunction and how they lead to atherosclerosis (reproduced from: https://synapse.koreamed.org/DOIx.php?id=10.3346/jkms.2015.30.9.1213&vmode=PUBREADER#!po=1.47059, access 27.11.2017)

Definition of arteriosclerosis

Arteriosclerosis can be described as the thickening, hardening, and loss of elasticity in blood vessels. It is possible to distinguish three different types:

Arteriolosclerosis: Only the small arteries (arterioles) are affected.

Atherosclerosis: Big- and medium-sized arteries are affected. This is the most common form.

Monckeberg's Arteriosclerosis: A disease of the elderly, with minor clinical relevance (77).

Risk factors

Table 4 lists the risk factors that lead to endothelial dysfunction and atherosclerosis. They can be divided into unswayable risk factors and influenceable ones. The influenceable risk factors are further divided into primary and secondary risk factors, which differ in their potential risk range (87, 88).

Primary risk factors	Secondary risk factors	Unswayable risk factors
Increased LDL/VLDL	Physical inactivity	Male gender
Decreased HDL	Stress	Genetic abnormalities
Diabetes mellitus	Hyperuricemia	Age
Smoking	Adipositas	Family history of atherosclerosis
Elevated CRP		
Hypertonia		

Table 4: Risk factors for endothelial dysfunction

Pathophysiology

Atherosclerosis is caused by endothelial dysfunction, vascular inflammation, and the accumulation of lipids within the intima of the vessel wall. Figure 7 provides an impression of the most common processes that contribute to the development of an atherosclerotic plaque (89).

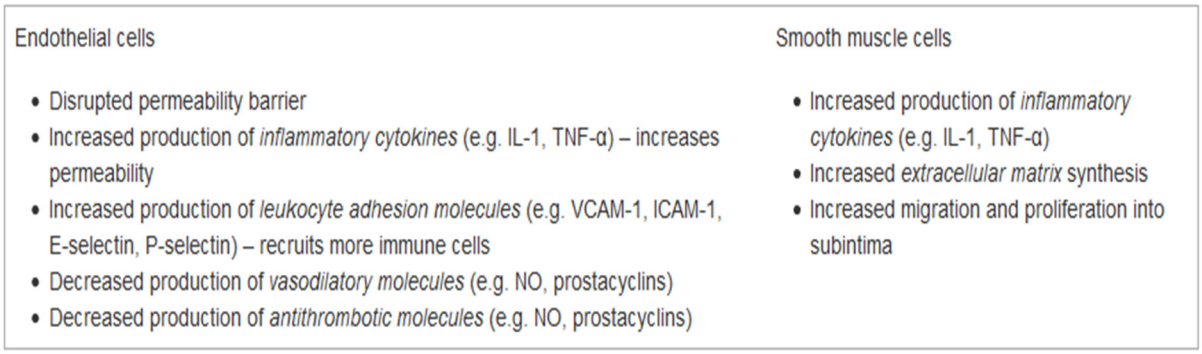


Figure 7: Consequences of inflammation in vascular cells
(reproduced from: http://www.pathophys.org/atherosclerosis/, access 22.09.17)

Consequences

The consequences of the development of atherosclerotic plaques are calcification, rupture, embolization, and aneurysm, which often lead to myocardial infarction, peripheral artery disease, angina pectoris and stroke (90, 91).

1.3.3 Evaluation of cardiovascular function

Flow-mediated dilatation

Blood vessels can self-regulate tone and adjust the blood flow to changes in the local environment. Most blood vessels respond on an increased blood flow with dilatation. This phenomenon is called flow-mediated dilatation (FMD). A principal mediator of FMD is endothelium-derived NO (92).

The FMD is the gold standard for non-invasive endothelial dysfunction measurement. This technique allows measuring an endothelium-dependent function. The stimulus provokes the endothelium to release NO, which is responsible for vasodilatation; this can be illustrated via ultrasound. It is an appealing technique because it is non-invasive and can be easily used for repeated measurements (92).

1.4 HIV/ART and Cardiovascular Diseases

1.4.1 Cardiovascular diseases in the HIV-positive population

When HIV first began to spread around the world in the 1980s, people died of opportunistic infections such as tuberculosis, Kaposi's sarcoma or Pneumocystis carinii pneumonia (93). Much research was therefore dedicated to these complications. Since the mid-1990s, ART has been a treatment possibility; hence, the life expectancy of people living with HIV has increased significantly (9). From 2003 to 2008, the worldwide coverage of ART expanded from 7% to 42%. Today, 19.5 million people receive ART (94); therefore, increasing numbers of HIV-positive people grow old. With the increase in the life years of these patients, the chronic complications of HIV infections have become more important (95). In summary, the HIV-positive population 30 years after the discovery of HIV has to deal with new complications. In the current treatment era, chronic, non-infectious diseases pose a clinical challenge, especially cardiovascular diseases (96-99).

Since many of these diseases are connected to aging in the general population, the term "premature aging" or "accelerated aging" is used for the spectrum of chronic complications of HIV (49).

Substance abuse, polypharmacy, chronic inflammation, hypercoagulation, and multimorbidity are common in the HIV-positive population. These factors are all linked to the manifestations of aging that usually appear in the general population later in life (49, 100, 101).

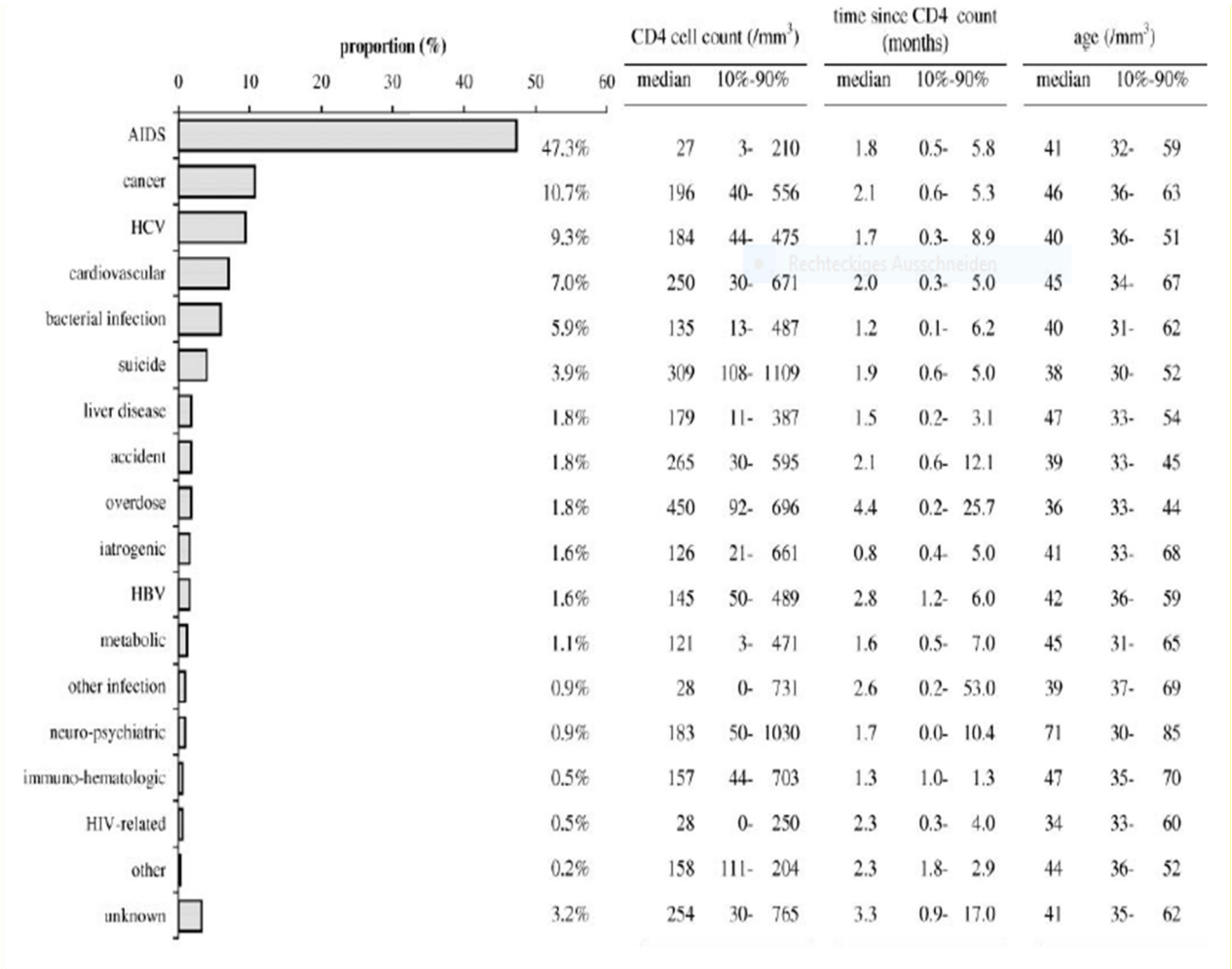
1.4.2 Differences in the treatment of HIV positives and negatives

Cardiovascular diseases are the main cause of death in developed countries. Many guidelines therefore exist, but these guidelines are for the general population and cannot be applied to HIV-positive patients. In many cases, cardiovascular interventions used for the general population have proven to be of minor success for HIV-positive groups. The cardiovascular risk calculators used for the general population are furthermore not well validated for the HIV-infected population; it has even been discussed to include HIV infection as in independent risk factor for CVD.

Hence, it is important to dedicate research to the chronic disease complications of HIV and furthermore to take care to translate the results into clinical practices and guidelines especially designed for HIV-positive patients.
Additionally, typical cardiovascular risk-reducing drugs such as aspirin are prescribed at significantly lower rates in HIV-infected patients. They are also treated at lower rates for diabetes and hypertension. It is not clear if this is due to a lack of awareness that the risk for CVDs is higher in this group. The current recommendation, however, is to treat CVD risk factors in HIV-positive patients at least as aggressively as in the general population (96, 102).

1.4.3 Causes of death in the era of antiretroviral therapy

Since antiretroviral therapy became available, both the mortality and the incidence of AIDS-defining diseases have dropped in the HIV-positive population (103). As a result, HIV has become a chronic infection (49). In the era of ART, HIV-positive patients die of other diseases. Lewden et al. have examined the causes of death of HIV-infected people in France. Of 64,000 HIV-infected patients, 964 died in the time of observation. 456 (47%) of these patients died of AIDS-defining illnesses and 477 patients (50%) died of a non-AIDS-related illness. Most frequent non-AIDS-related causes are non-AIDS-related cancer (10.7%), hepatitis C infection (9.3%), CVD (7%), bacterial infections (5.9 %) and suicide (3.9%). Among the 67 cardiovascular deaths, 22 died of coronary heart disease, two of a cerebrovascular event, nine died of heart failure, six of pulmonary hypertension, four of venous thrombosis or pulmonary embolism, four of valvular disease with endocarditis, two of pericardial disease, one of arrhythmia, one of aortic aneurysm and six with no precision. In 10 patients (1%), antiretroviral treatment was under suspicion of being the underlying cause of death. In conclusion, the most frequent non-AIDS-related deaths are cancer, viral hepatitis and cardiovascular disease (104).
Figure 8 provides an overview of the most frequent non-AIDS-related causes of death in the era of ART.



		CD4 cell count (/mm³)		time since CD4 count (months)		age (/mm³)	
		median	10%-90%	median	10%-90%	median	10%-90%
AIDS	47.3%	27	3- 210	1.8	0.5- 5.8	41	32- 59
cancer	10.7%	196	40- 556	2.1	0.6- 5.3	46	36- 63
HCV	9.3%	184	44- 475	1.7	0.3- 8.9	40	36- 51
cardiovascular	7.0%	250	30- 671	2.0	0.3- 5.0	45	34- 67
bacterial infection	5.9%	135	13- 487	1.2	0.1- 6.2	40	31- 62
suicide	3.9%	309	108- 1109	1.9	0.6- 5.0	38	30- 52
liver disease	1.8%	179	11- 387	1.5	0.2- 3.1	47	33- 54
accident	1.8%	265	30- 595	2.1	0.6- 12.1	39	33- 45
overdose	1.8%	450	92- 696	4.4	0.2- 25.7	36	33- 44
iatrogenic	1.6%	126	21- 661	0.8	0.4- 5.0	41	33- 68
HBV	1.6%	145	50- 489	2.8	1.2- 6.0	42	36- 59
metabolic	1.1%	121	3- 471	1.6	0.5- 7.0	45	31- 65
other infection	0.9%	28	0- 731	2.6	0.2- 53.0	39	37- 69
neuro-psychiatric	0.9%	183	50- 1030	1.7	0.0- 10.4	71	30- 85
immuno-hematologic	0.5%	157	44- 703	1.3	1.0- 1.3	47	35- 70
HIV-related	0.5%	28	0- 250	2.3	0.3- 4.0	34	33- 60
other	0.2%	158	111- 204	2.3	1.8- 2.9	44	36- 52
unknown	3.2%	254	30- 765	3.3	0.9- 17.0	41	35- 62

Figure 8: The most frequent non-AIDS-related causes of death in the era of ART
(reproduced from: https://www-1ncbi-1nlm-1nih-1gov-1pubmed.han.medunigraz.at/pubmed/15561752, access 10.09.17)

1.4.4 Cardiovascular diseases in the HIV-positive population

HIV-positive patients have an increased risk of cardiovascular disease compared to the general HIV-negative population, with a higher risk of cardiovascular events and an increased mortality rate (49, 105, 106). This can be caused either by the virus itself or due to antiretroviral treatment (95).

The risk among HIV-infected patients for cardiovascular disease is 1.5 to 2.0 fold compared to the general population (107).

The data collection on adverse effects of anti-HIV drugs (D:A:D) study has indicated that CVDs are responsible for 11% of deaths among the HIV-infected population (108).

Data from the EuroSIDA study indicate that cardiovascular events account for one-third of non-AIDS-defining diseases (109).
Multiple studies have demonstrated elevated rates for acute myocardial infarction as well as coronary heart disease. The risk of stroke and cerebrovascular endpoints is higher, as is the risk of sudden cardiac death.
The underlying mechanism is probably a complex interaction between mechanistic pathways of cardiovascular disease and effects related to immunologic long-term sequelae of HIV infection (96). So far, there is no great knowledge of how the HI virus promotes these diseases and what is the role played by ART (9).

1.4.5 The HI virus itself as a reason for elevated risk of CVD

There are different reasons why HIV infection is associated with an increased cardiovascular risk. On the one hand, HIV strengthens the traditional risk factors for CVDs such as insulin resistance, higher blood lipid profiles, and changes in the distribution of body fat. More people with HIV are also smokers (9, 49, 110).
On the other hand, the HI virus itself could be the reason for increased cardiovascular risk by bringing chronic inflammation into the body. This is triggered by the direct effect of HIV on toll-like receptors (TLR7 and TLR8), which can be found on plasmacytoid dendritic cells, followed by the production of interferon-alpha as well as microbial translocation with lipopolysaccharide, followed by the production of proinfammatory cytokines (interleukin 6 and tumor necrosis factor alpha).
It has been proven that immune activation can also be found in patients who receive ART whose CD4 cell counts are unobtrusive. This inflammation is associated with mortality, CVD, cancer, neurological disease, and liver disease.
With acute HIV infection, the number of CD4 cells in the gastrointestinal tract decreases. This permits microbial translocation through the gut into the circulation and is a trigger for chronic immune activation and inflammation.
Even though patients may receive ART so that the HI virus is supressed by ART, replication is possible at a low level. This could also be a driver for ongoing inflammation. Finally, viral coinfections that are common in the HIV-positive population, especially cytomegalovirus, hepatitis B and C, could contribute to the chronic inflammation and through that to advanced cardiovascular risk (102). More

studies are needed to find out if patients would benefit from the reduced residual inflammation that could be gained with certain drugs or possibly even with physical activity (111, 112).

Figure 9 illustrates the path of immune activation and chronic inflammation in HIV positives.

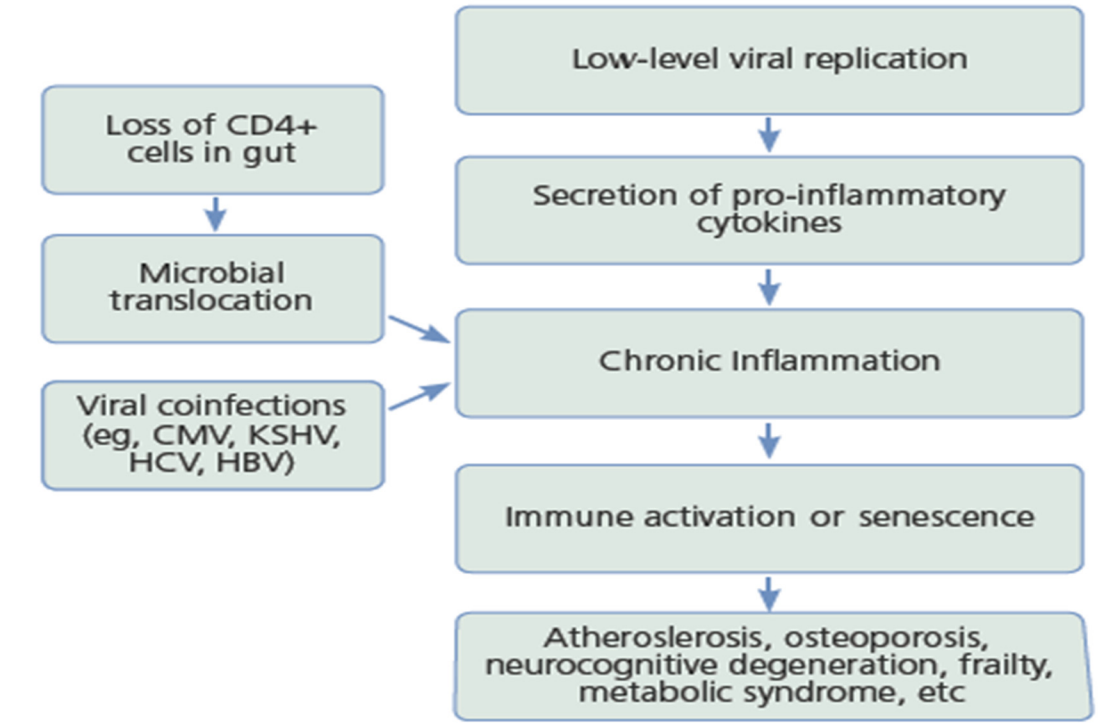


Figure 9: How HIV could lead to a higher cardiovascular risk
(reproduced from: https://www-1ncbi-1nlm-1nih-1gov-1pubmed.han.medunigraz.at/pubmed/28208120, access 10.09.17)

1.4.6 ART and cardiovascular complications

Since the discovery of the HI virus and its treatment possibilities are relatively young, not much is known about the long-term effects of ART.

Antiretroviral therapy reduces AIDS-related morbidity and mortality; in contrast, non-AIDS-defining illnesses such as cardiovascular diseases and neoplastic, liver, and lung complications increase in HIV-positive people treated with ART (9, 113). When used for many years, antiretroviral drugs can lead to metabolic changes (peripheral lipoatrophy, central lipoaccumulation), insulin resistance, diabetes mellitus, and hyperlipidemia (49).

Not all available antiretroviral drugs have the same cardiovascular risk profile. In particular, PIs are a class of drugs that are under consideration for having adverse effects of the cardiovascular system.

Protease inhibitors are a central part of ART. These drugs' side effects include dyslipidemia, greater levels of plasma triglycerides and an adverse cholesterol profile. These effects could lead to insulin resistance, cardiac dysfunction, and myocardial infarction, but the molecular effects of PIs on the heart and vascular system are still poorly understood (114).

Reyskens et al. have suggested that the PI-mediated contractile dysfunction in the cardiac muscle is caused by altered calcium handling (114). Antiretroviral therapy could therefore be considered a risk factor for CVDs.

It should be noted, however, that ART itself is an effective treatment for CVDs, since it reduces the immune activation of HIV and, as described above, cardiovascular risk. Overall, the benefits of ART outweigh the risk of CVD (96). Nevertheless, ART should be chosen carefully, especially in people with an increased cardiovascular risk. (105)

Treatment guidelines already recommend drugs based on their risk profile rather than on their antiretroviral efficacy, but there is still much that is unknown about the direct effects of ART on the body; further studies are needed to fill this gap (49).

1.4.7 Management of cardiovascular diseases in HIV patients in Africa

Most of the data about cardiovascular diseases in HIV-positive patients have been collected in western countries, so not much is known about the population living in countries with the highest rate of HIV/AIDS, i.e., the population in SSA (49, 82). It is likely, however, that the data present differences because several factors are different in SSA compared to western countries.

First of all, the HIV subtypes circulating in SSA are different from the subtypes that are common in western countries (115). Subtype C is most frequent in South Africa, whereas subtype B is most common in the western world. It remains unclear, however, if there is an actual difference between the subtypes.

Second, the management of chronic diseases in SSA is very different from the management in developed countries. Even though ART has become more widely accessible in SSA in recent years, so that chronic complications have been coming into focus, the rising need for medicines for hypertension, hyperglycemia or hyperlipidemia to treat the chronic complications like metabolic syndrome or CVD have not kept up with the rising need. These medicines therefore lack demand (95). In developed countries, expanded routine screening for CVDs as well as screening for potential risk factors for CVDs is a standard procedure, but in SSA, where there is a shortage of health personnel and necessary expertise is lacking, such screening processes are skipped (82, 95). Since most CVDs are chronic diseases, it is important to ensure chronic care, but especially in SSA resources such as services for reproductive health, non-AIDS morbidity (e.g. CVDs), tuberculosis (TB) prevention, and antiretroviral management are lacking. It is still common to focus only on acute care so that treatment for risk factors and chronic long-term complications of HIV and ART is missing (49, 116-119).

One of the major causes of death in HIV patients is CVD. That is why cardiovascular risk reduction, as well as lifestyle modifications and carefully choosing the right antiretroviral drug for the underlying cardiovascular risk profile of each patient, has to be started in SSA.

Figure 10 summarizes the challenges that accompany chronic HIV infection.

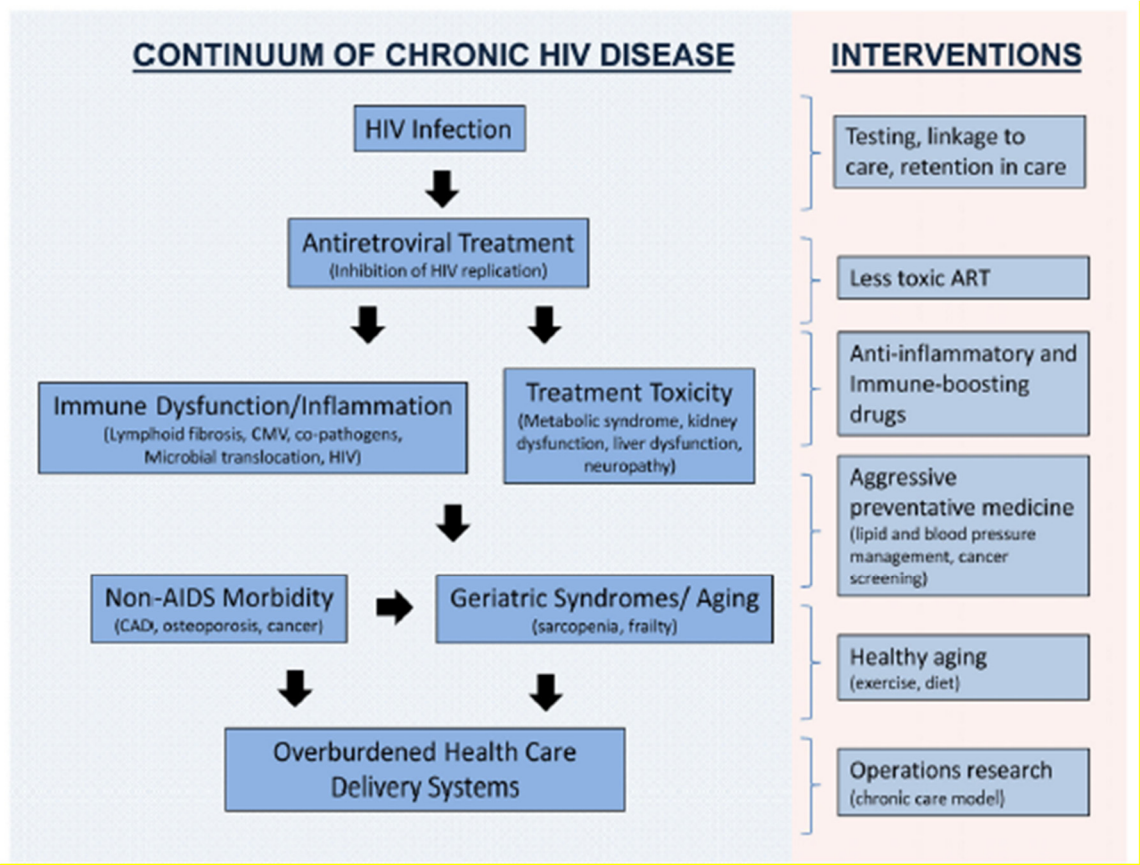


Figure 10: HIV infection as a chronic disease
(reproduced from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4058441/ access 21.11.2017)

2 Aims and objectives

The HI virus is one of the leading causes of morbidity and mortality worldwide. Since 1996 ART has provided an option to prolong the life of HIV-positive patients or even prevent the outbreak of AIDS. There is no cure to date; however, with lifelong antiretroviral treatment, HIV-infected patients have an almost normal life expectancy.

Thus, HIV infection is a chronic disease whose focus has shifted from the typical opportunistic AIDS diseases to the possible long-term complications of ART. Antiretroviral therapy is suspected to have effects on the cardiovascular system, on the one hand by strengthening traditional cardiovascular risk factors, and on the other though direct interactions with the cardiovascular system.

Even though SSA hosts 70% of all HIV-infected people and is the region most affected by the HIV epidemic in the world, there is only limited data about the long-term effects of ART in this area.

This diploma thesis aims to investigate the cardiovascular effects of different antiretroviral regimens currently used in SSA. It specifically examines the effects of first-line and second-line ART on lipid parameters and vascular function, with the aim of filling the gap in the long-term complications of ART in the region where HIV is by far the most threatening health hazard: SSA.

This thesis presents the complex interactions between ART and CVDs and the healthcare challenges caused by these complications, especially in the African countries most affected by the epidemic.

This has been achieved by analyzing data collected within a multinational epidemiological study called the *EndoAfrica* project, which is currently being conducted in Kenya, Côte d'Ivoire, and South Africa. This thesis presents two cases selected from a cohort of patients in South Africa.

3 Methods

This diploma thesis compares two different case studies on the effects of different ART regimens on the cardiovascular system. Two individual cases are presented, both collected in the context of the *EndoAfrica* project in South Africa.
The first case deals with an HIV-positive patient who currently receives first-line ART. The second case is about an HIV-infected patient who receives second-line ART. Both cases are analyzed and compared referring to risk factors and precursors of CVD and the possible link to antiretroviral drugs.

3.1 The EndoAfrica Project

The data presented in this diploma thesis is part of the *EndoAfrica* project. The *EndoAfrica* project is a multinational epidemiological study to investigate the prevalence of endothelial dysfunction as a precursor of CVD. This unique study is currently being carried out in three African countries: Kenya, Côte d'Ivoire, and South Africa. It aims to investigate endothelial dysfunction and the possible relation to HIV infection, ART, and air pollution exposure.
The prospective study follows different cohorts over three years, each cohort consisting of 250 patients. Cohort one includes HIV-infected but ART naïve patients, cohort two includes HIV-positive patients who already receive ART and cohort three consists of an HIV-negative control group. The investigations include endothelial function by determining FMD, screening for hypertension, diabetes, obesity, dyslipidemia, and smoking, as well as measuring air pollution exposure. The investigations are repeated after 12 and 24 months and are completed within 30 months. In addition, animal and in vitro investigations explore direct vascular and endothelial effects of first-line ART regimens by investigating vascular function (rat aortic ring relaxation) and endothelial cell function (cultured rat aortic endothelial cells). The unique results of the *EndoAfrica* project provide new data regarding the effects of HIV infection and ART on vasculature in SSA populations. Furthermore,

the overall prevalence of endothelial dysfunction in the population of SSA is determined.

The measurements for the data presented in this thesis took place at the University of Stellenbosch and in the local community health center in Worcester, both located in the Western Cape Province of South Africa. The patients were recruited by experienced and qualified nurses at community healthcare centers or HIV clinics in Cape Town and the local community health center in Worcester. All participants were tested for HIV antibodies and CD4 cell count at the time of recruitment, then assigned to their adequate cohort. It is important to know that according to South African ART guidelines, all newly diagnosed HIV-positive patients should receive ART regardless of their CD4 count. Most participants start ART soon after being diagnosed and then swap from the HIV-positive ART naïve group to the HIV-positive on ART group.

The tests are repeated 12 and 24 months after recruitment. A clinical and laboratory investigation is additionally performed to screen for cardiovascular risk factors. Table 5 provides an overview of the evaluated parameters relevant for this thesis and their associated risk factor. Flow-mediated dilatation as a method to determine endothelial dysfunction is also evaluated. In addition, the C-reactive protein (CRP) as a marker for acute infection and creatinine and glomerular filtration rate (GFR) to evaluate kidney function are determined to rule out other diseases.

To ensure compartibility, the blood samples are only analyzed in one laboratory in South Africa, although the collection is done in multiple sites all over the country. General information about the patients, such as age, physical exercise, and past medical history were evaluated with a health questionnaire.

Evaluated parameters	Associated risk factor for CVDs
Blood pressure	Hypertension
BMI, waist, hip circumference	Obesity
Fasting blood glucose, random blood glucose	Diabetes mellitus
Total cholesterol, LDLc, HDLc, triglycerides	Dyslipidemia
Flow-mediated dilatation	Endothelial dysfunction

Table 5: Evaluated parameters and associated cardiovascular risk factors

3.2 Flow-Mediated Dilatation (FMD)

The FMD measurements are taken using the same equipment on all investigation sites. The machine used is an Esaote My LapTM Five Mobile Ultrasound System. The FMD measurement instruction is to be conducted by the same instructor, and only one person will perform the measurement on each side in order to avoid interpersonal-related inaccuracies.

To ensure optimal FMD measurements, the process adheres as closely as possible to FMD guidelines. Influences that could affect the FMD should be eliminated. These are temperature, food, drugs, sympathetic stimuli, and others (92). The patient should therefore fast for 8 to 12 hours prior the measurements. Smoking is prohibited 4 to 6 hours before the measurements, and medication that is known to affect vascular tone should be paused, if possible. The investigation rooms are temperature-controlled and should be quiet. Substances that can affect the FMD, namely caffeine, high-fat foods and vitamin C, should not be consumed within 4 to 6 hours prior to the measurement, and patients should not exercise 6 hours prior to the measurements.

Procedure

The measurements are usually done in the morning. First, the patients have to report fasting time, when they smoked their last cigarette and females have to report the phase of their menstrual cycle. The participant is given 10 minutes time to rest before the FMD can begin. In this time, the brachial artery can regain its baseline diameter. The patient lies in a comfortable position with the left arm stretched out in order to take the measurements of the brachial artery, which is the best location for FMD. Figure 11 illustrates the setup for FMD measurements.

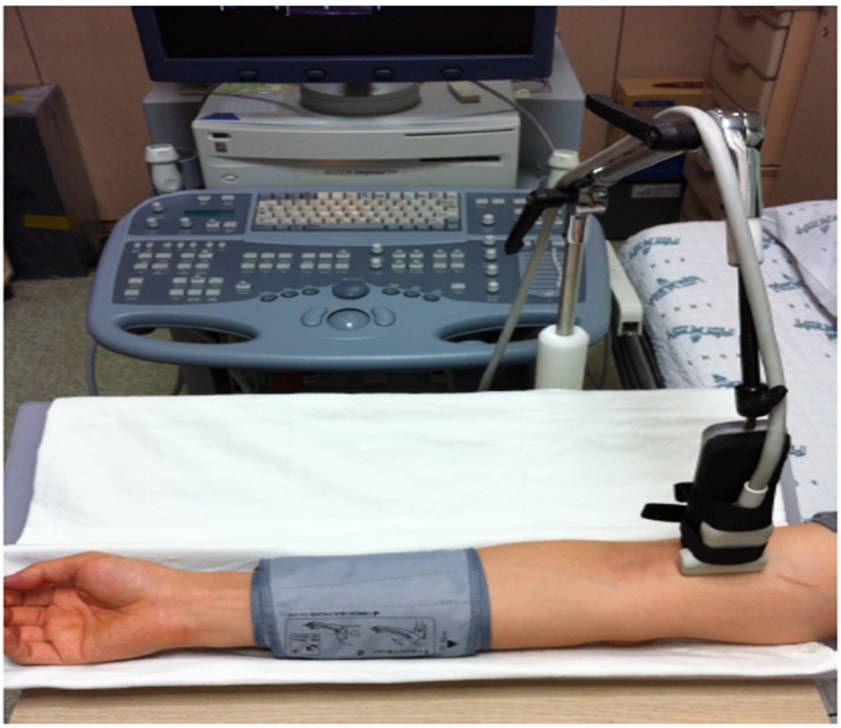


Figure 11: Setup for FMD measurements
(reproduced from:
https://openi.nlm.nih.gov/detailedresult.php?img=PMC3519716_1476-7120-10-39-1&req=4, access 25.09.17)

The left brachial artery is detected in B-mode above the antecubital fossa with an Esaote MyLap Five ultrasound and 12 MHz linear probe (Esaote, Italy). First, the image of the artery is shown in cross section because it is easier to find it that way; then, the probe is turned so the brachial artery is shown in a longitudinal plane. It is very important that there is a clear picture that includes the brachial artery over the whole screen. Doppler mode can help to find the artery.

For the evaluation of the FMD, part of the artery is chosen in which both anterior and posterior vessel walls are visible. It is important that the intima and the lumen of the vessel is clearly visible. To keep the exact position, anatomic landmarks such as veins or fasciae and/or a stereotactic holding device can be used. In this case, a single axis precision probe holder from SMT medical (Wurzburg, Germany) was used. The depth is increased to 3 cm and is switched to pulse wave mode. Next, the angle of insonation is set to +60°. The program used for recording is the FMD Studio and Cardiovascular Suite version 2.8.1 software (Quipu, Italy). It constantly controls the brachial artery diameter and shear rate measurements.

The measurement starts with recording the baseline FMD for one minute. In the next step, a blood pressure cuff is placed around the forearm of the left arm and is

inflated to values of 50 mmHg above the systolic blood pressure value of the patient measured earlier.

The cuff remains inflated for five minutes. This provokes ischemia and leads to vasodilatation of downstream blood vessels due to autoregulatory mechanisms (92). This phase is called the ischemic phase (120).

After five minutes the cuff is deflated, which leads to hyperemia as well as increased shear rate. This induces endothelium-dependent dilatation of the brachial artery. The same section of the brachial artery is then located again. Finally, there is a recovery phase, which is recorded for another three minutes. The maximum dilatation of the artery is reached 60 seconds after deflation of the cuff.

Figure 13 provides an impression of an ultrasound image of the brachial artery at baseline and after reactive hyperemia.

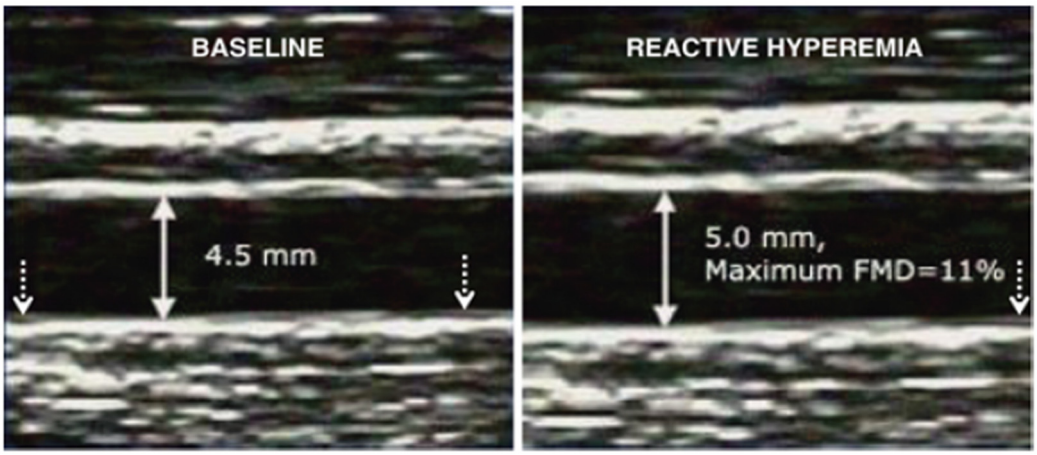


Figure 12: Ultrasound image of brachial artery before and after reactive hyperemia. Right: Normal reactive hyperemia induced flow-mediated dilation (FMD) of 11% from baseline (left). Solid arrows indicate media–adventitia interface ('m'-line). Dotted arrows indicate the blood-endothelial cell interface.
(reproduced from:
http://www.nature.com/ijir/journal/v22/n2/fig_tab/ijir200959f3.html?foxtrotcallback=true, access 25.09.17)

In the next step, the software calculates the baseline artery diameter, FMD in % (difference between maximum and baseline diameter expressed as %; (max. – baseline) / baseline = FMD), baseline shear rate and maximum shear rate.

4 Results

4.1 First Case

4.1.1 General information

The representative for the HIV-positive cohort on first-line ART (Efavirenz 600mg, Emtricitabine 200mg and Tenofovir 300mg) is a 40-year-old colored female (Patient A). At the time the measurements were taken, she was not menstruating. She was fasting since the evening before her appointment. She is a smoker and smoked the morning before the measurements were taken (between 6.30 and 7.30 am). She currently has no children younger than three months; she is not pregnant or breastfeeding.

Lifestyle

She does not drink alcohol and is not physically active. She smokes but it is not known how many cigarettes she smokes per day or how many pack years she has. She is a full-time employee and her household receives a government social grant. Her income per month lies between R1,000 to R4,999 (€69.40 to €347).

Medical background

She indicates never having suffered from any cardiovascular event, e.g. myocardial infarction, stroke, or others. She has never had TB. She currently does not have any kind of heart disease, no high cholesterol, no hypertension, no longer-lasting health problems, no pulmonary TB, and no diabetes.

Family history of cardiovascular diseases

Her mother never had an appreciable CVD or any risk factor for it (e.g., hypertension, hypercholesterolemia, diabetes, or others), although her father has high blood pressure. His cholesterol status is unknown.

Drug history

She currently takes vitamin C and a triple combination of antiretroviral drugs: Efavirenz (600mg), which is an NNRTI; Emtricitabine (200mg), an NRTI; and Tenofovir (300mg), another NRTI. This combination is a typical first-line regime.

4.1.2 Results

Anthropometry

Her height is 169 cm and her weight is 68.6 kg, which results in a BMI of 23.8 (weight in kg/height in m²). Her waist circumference is 93 cm and her hip circumference is 108 cm. This leads to a waist-to-hip ratio of 0.86 (waist circumference in cm/hip circumference in cm).

Blood pressure

The blood pressure was measured three times on the left arm to avoid false values due to the white coat effect. Her results were 1st: 133/77mmHg, heart rate 72 bpm, 2nd: 109/78 mmHg, heart rate 72 bpm and 3rd: 112/81 mmHg, heart rate 71 bpm.

Flow-mediated dilatation

Baseline dilatation (Dbase) was 3.61 mm, maximum dilatation (Dmax) was 3.92 mm, the recovery dilatation (Drecovery) was 3.78 mm. The outcome is a calculated FMD of 8.65% ((Max. – baseline)/ baseline=FMD).

Laboratory

Patient A has a creatinine of 58 µmol/L and an estimated glomerular filtration rate (eGFR) above 60ml/min/1.73m². Her random plasma glucose level is 5.1mmol/L. The inflammatory marker CRP is 1mg/L. Her total cholesterol is 4.15mmol/L, her HDL cholesterol is 1.42mmol/L and her LDL cholesterol 2,46mmol/L. Her blood triglyceride level is 0.59 mmol/L.

4.2 Second Case

4.2.1 General information

The representative for the HIV-positive group on second-line ART (Lopinavir 200mg, Ritonavir 50mg, Zidovudine 300mg and Lamivudine 50mg) is a 29-year-old colored female (Patient B). At the time the measurements were taken, she was not menstruating. She had been fasting since the evening before the measurements. She is a smoker and smoked the morning before the measurements (around 6.30 a.m.) She currently has no children younger than three months; she is not pregnant or breastfeeding.

Lifestyle

In the last 12 months, she did not consume any alcohol. She is mildly physically active more than three times a week. She is a smoker although it is not known how many cigarettes she smokes or how many pack years she has so far.

She has a degree of College/University/Other tertiary institution and is currently unemployed, looking for work. Her household receives a social grant. Her income per month lies between R1,000 and R4,999 (€69.40 to €347).

Medical background

She has had no cardiovascular events in her past (e.g., myocardial infarction, stroke, or others). She has TB, diagnosed on July 22, 2015, and is currently under treatment. She does not suffer from diabetes or hypertension and has no other known cardiovascular risk factors.

Family history of cardiovascular diseases

Neither her mother nor father have suffered a cardiovascular event.

Drug history

She takes vitamin C. Her antiretroviral medication consists of Lopanavir (200mg) and Ritonavir (50mg), which both belong to the PI class; furthermore, she takes

Zidovudine 300mg and Lamivudine 50mg, both of them belong to the NRTIs. She currently receives TB treatment.

4.2.2 Results

Anthropometry

She is 158 cm tall and her weight is 55.8 kg. Her calculated BMI is therefore 22.35 (weight in kg/height in m²). Her waist circumference is 90 cm and her hip circumference 99 cm. Her waist-to-hip ratio was calculated at 0.91 (waist circumference/hip circumference).

Blood pressure

Her blood pressure was measured three times on the left arm to avoid false values because of the white coat effect. 1st: 104/80 mmHg and heart rate 72 bpm; 2nd: 108/81 mmHg and heart rate 81 bpm; 3rd: 115/76 mmHg with a heart rate of 69 bpm.

Flow-mediated dilatation

Patient B has a Dbase of 3.49 mm, a Dmax of 4.12 mm and a Drecovery of 4.08 mm. The calculated FMD is therefore 18.30% ((Max. – baseline)/ baseline=FMD).

Laboratory

Patient B has a creatinine of 65 µmol/L and an eGFR above 60ml/min/1.73m². Her random plasma glucose level is 4.5mmol/L. The CRP is 1mg/L. Her total cholesterol is 5.21mmol/L, her HDL cholesterol is 1.11mmol/L and her LDL cholesterol 3,49mmol/L. Her blood triglyceride level is 1.33 mmol/L.

Table 6 provides an overview of the results of case one and case two.

	Patient A	Patient B
Age (years)	40	29
BMI	23.8	22.35
Waist circumference (cm)	93	90
Hip circumference (cm)	108	99
Waist-to-hip ratio	0.86	0.91
BP1 (mmHg) (heart rate bpm) **BP2 (mmHg) (heart rate bpm)** **BP3 (mmHg) (heart rate bpm)**	133/77 (72) 109/78 (72) 112/81 (71)	104/80 (72) 108/81 (81) 115/76 (69)
Baseline diameter (mm)	3.61	3.49
Maximum diameter (mm)	3.92	4.12
Recovery diameter (mm)	3.78	4.08
FMD (%)	8.65	18.3
Creatinine (µmol/L)	58	65
eGFR (ml/min/173m²)	>60	>60
Random glucose (mmol/L)	5.1	4.5
Total cholesterol (mmol/L)	4.15	5.21
HDL cholesterol (mmol/L)	1.42	1.11
LDL cholesterol (mmol/L)	2.46	3.49
Triglyceride (mmol/L)	0.59	1.33
CRP (mg/L)	1	1

Table 6: Overview cases A and B

5 Discussion

Flow-mediated dilatation

Patient A had a baseline dilatation of 3.61 mm. The maximum dilatation measured after 5 minutes of occlusion and 60 seconds after the cuff was released was 3.92 mm. The increase in lumen diameter was therefore 0.31 mm or an FMD of 8.05 %. Patient B had a baseline dilatation of 3.49 mm; her maximum dilatation was 4.12 mm. That makes a lumen increase of 0.73 mm or an FMD of 18.05 %. The endothelium can self-regulate tone and blood flow. A stimulus, for example shear stress, triggers a complex cascade of different reactions that leads to the production of NO. NO causes vasodilatation. The cuff on the forearm, during FMD measurement, interrupts the blood flow. When it is released, shear stress is induced by the increased blood flow. The endothelium responds by dilating the artery. The extent of dilatation can be used to distinguish a healthy endothelium from endothelial dysfunction.

By comparing the results, it stands out that Patient A has a lower FMD compared to Patient B. This means that the endothelium of Patient A is not able to react to a stimulus, in this case shear stress, as well as the endothelium of Patient B.

It should be considered, however, that the results of both patients are within the normal range. Healthy arteries present an increase in diameter above 6 %, if the cuff is used on the forearm (121).

Since both patients are in normal range of FMD, this cannot be referred to as endothelial dysfunction.

BMI and waist-to-hip ratio

Patient A has a BMI of 23.8, compared to Patient B's BMI of 22.35. A BMI between 18.5 and 25 is considered normal, whereas a BMI between 25 and 30 is considered overweight. A BMI of more than 25 is a risk factor for endothelial dysfunction (122). In this case, both patients are of normal weight.

The waist-to-hip ratio is considered normal in women until a value of 0.85 (123). Both patients have a higher value: Patient A has 0.86 and Patient B 0.91. According to the WHO guidelines, values over 0.85 in women are considered as obese, which

can lead to a higher cardiovascular risk. The waist-to-hip ratio responds to the fact that certain body structures are associated with higher cardiovascular risks; abdominal fat is most linked to cardiovascular events (124, 125).

Blood pressure

Blood pressure and heart rate were measured three times in both patients. The measurements took place in an optimal range, as recommended by clinical guidelines (126).

Patient A began with a blood pressure of 133/77 mmHg, which in the second measurement decreased to 109/78 mmHg. In the third measurement, the results were 112/81 mmHg. Patient B had stable values of around 110/80 mmHg. Except for Patient A's first value, all measurements were within the range of optimal blood pressure according to WHO guidelines, i.e., a blood pressure under 120/80 mmHg. Blood pressure measurements are very important, since hypertension is the main risk factor for CVD. Hypertension and endothelial dysfunction influence each other, so that through local inflammation and oxidative stress, hypertension can cause endothelial dysfunction (127).

Laboratory results

The laboratory test presented normal values for kidney function parameters in both patients. Both have an eGFR above 60 mL/min/1.73m² (128). Patient A presented a creatinine of 58 µmol/l and Patient B one of 65 µmol/l. Values between 53 and 97 µmol/l are considered normal in women aged between 18 and 60 years (129).

The blood chemistry furthermore revealed normal values for blood glucose levels in both patients. The critical value is 11.1 mmol/L for the random plasma glucose, and both patients had considerably lower levels (Patient A 5.1 mmol/L and Patient B 4.5mmol/L). It can be concluded that neither patient suffers from diabetes mellitus because the criteria are not met (patient with diabetic symptoms and random plasma glucose ≥11.1mmol/L) (130).

The CRP was determined as a marker for acute bacterial inflammation. A value of under 10mg/L is considered normal. Both patients had a value of 1mg/L, which rules out an acute inflammatory process.

The blood lipid levels were furthermore analyzed in the laboratory. Patient A had a total cholesterol of 4.15 mmol/L, which is within the treatment target of under 4.9

mmol/L. Her triglycerides reached 0.59 mmol/L, which is considered normal aswell, since it is under the margin of 1.7 mmol/L. Her HDL cholesterol was 1.42 mmol/L; here, levels above 1.2 mmol/L are desirable for women. Her LDL cholesterol is 2.46 mmol/L, which is again within the treatment target of under 3 mmol/L.
In comparison, Patient B had a total cholesterol of 5.21 mmol/L, which according to ESC clinical guidelines is too high.
Her triglycerides were 1.33 mmol/L, which is considered normal. Her HDL cholesterol was 1.11, which is within the normal range but considerably lower than in Patient A. Her LDL cholesterol, at 3.49 mmol/L, was too high.
In summary, Patient A's lipid levels were all within normal range, whereas the total cholesterol and the LDL cholesterol of Patient B were elevated and her HDL cholesterol, although in normal range, was lower than desirable. Both patients had normal triglyceride levels (131, 132).

The factors that could influence the patients' FMD and serum lipids are discussed in detail below.

Antiretroviral therapy

Both FMD results were within the normal range; therefore, it seems that they are not affected by different types of ART.
Patient A receives a typical first-line regime, consisting of Evavirenz 600mg (an NNRTI) and two NRTIs (Emtricitabine 200mg and Tenovofir 300mg) (133). Patient B takes a second-line regime, in which a switch from NRTI/NNRTI-based first-line regimens to PI-based ones is common (134); therefore, she receives the NRTI Zidovodine 300mg and Lamivudine 50mg, additionally, the PI Lopinavir 200mg with Ritonavir 50mg as a boosting drug.
Antiretroviral therapy is suspected to cause endothelial dysfunction. In particular, NRTIs and PIs are under scrutiny. The SMART trail has suspected that NRTIs lead to a higher risk of myocardial infection. Abacavir was especially found to generate a higher risk profile as soon as the drug was taken. Other studies have suggested that PIs cause slowly worsening atherosclerosis over long-term exposure to these drugs (65, 135-138).

Other investigators have found that viral load, CD4 cell count, cardiovascular risk factors, drug injection and alcoholism have a much stronger correlation with impaired endothelial function than ART (139, 140).
Torriani et al. have demonstrated that endothelial function improved significantly after starting ART in HIV-positive patients. This could be similar for all three of the ART regimens included in the study (NRTI (Lamivudine+Stavudine, Sidovudine or Tenofovir) +Efavirenz; NRTI+PI (Lopinavir/Ritonavir); Efavirenz+PI) (141).
The SMART study supports this finding. It has determined that the overall mortality and the risk for CVDs in HIV-positive people is higher if they do not take ART or if they discontinue it (142). Torriani et al. furthermore demonstrated that regardless of their positive effect on FMD, nearly all the tested drugs have negative effects on lipids.

The serum lipids presented differences between the two patients. Patient B receives a combination of Zidovudine plus Lamivudine with Ritonavir. This could explain the higher serum lipid levels of Patient B compared to Patient A, who receives Tenofovir and Emtricitabine.
Patient B's lower HDL cholesterol level could also be caused by a second-line ART regime with PIs Lopinavir and Ritonavir, typically associated with hypercholesterinemia, high LDL cholesterol and low HDL cholesterol (143).
It must be considered, however, that other factors influence HDL cholesterol as well. Physical activity is known to improve HDL cholesterol levels, smoking is responsible for lower HDL cholesterol levels and finally, the HIV infection itself influences serum HDL levels. The lower CD4 cell concentrations usually observed in advanced stages of HIV infection are linked with lower HDL blood levels and higher LDL serum levels because the LDL clearance is reduced (144-146). In different studies, the PIs that are mainly used in second-line regimens are being suspected for causing hyperlipidemia (147, 148).
Figure 14 provides an overview of the most-used antiretroviral drugs and their effect on lipid profiles.
In a study conducted by Shafran et al., HIV-negative healthy volunteers took Ritonavir in the boosted form concentration (100mg). This led to a 26% increase in triglycerides and a 16% increase in LDL cholesterol after only two weeks of therapy

(149). This, however, is not true for all PIs. Atazanavir and a few of the newer PIs have only a limited effect on serum lipid levels (150, 151).

In the study by Gallant et al., NRTI Tenofovir plus Emtricitabine were compared to NRTI Zidovudine plus Lamivudine (in both regimens, Efavirenz was added). This demonstrated that Tenofovir plus Emtricitabine causes smaller increases in total cholesterol and LDL cholesterol than Zidovudine (152).

Currently Recommended First-Line Agents are in Bold

Antiretroviral	Total Cholesterol	LDL-C	HDL-C	Triglycerides
PIs (boosted)				
Lopinavir	↑↑	↑↑	↔/↓	↑↑↑
Atazanavir	↑	↔/↑	↔/↓	↔
Fosamprenavir	↑	↑	↔/↓	↑↑
Saquinavir	↑↑	↑↑	↔/↓	↑
Darunavir	↑	↑	↔/↓	↑
Tipranavir	↑↑	↑↑	↔/↓	↑↑↑
NNRTIs				
Efavirenz	↑	↑	↑	↑
Nevirapine	↑	↑	↑↑	↔/↑
NRTIs				↔/↑
Tenofovir	↔/↑	↔/↑	↔/↑	
Abacavir	↔/↑	↑	↑	↑
Lamivudine	↔	↔	↔	↔
Zidovudine	↑	↑	↑	↑↑
Stavudine	↑↑	↑↑	↑	↑↑
CCR5 Inhibitors				
Maraviroc	↔	↔	↔/↑	↔
Integrase Inhibitors				
Raltegravir	↔/↑	↔/↑	↔/↑	↔

Figure 13: Antiretroviral drugs and their effect on serum lipid levels
(reproduced from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3106351/, access 04.12.2017)

Age

Patient A is 40, therefore 11 years older than Patient B. Advanced age is a major risk factor for endothelial dysfunction and hence for cardiovascular diseases. This is due to the reduced bioavailability of NO due to oxidative stress, which increases with age (153-155). The younger age of Patient B could therefore contribute to her normal FMD results, regardless of her therapy with PIs.

Smoking

Both patients are smokers, although it is not clear from the data how many pack years they have. Both smoked the morning before the measurements were taken. Smoking is an important factor because it is known to impair endothelial function (156, 157).

Smoking represents the most important preventable risk factor for atherosclerosis. Cigarette smoke induces a variety of complex cascades that lead to endothelial dysfunction. The formation of foam cells is triggered, which contributes to the formation of atherosclerotic plaques. In addition, it has a negative effect on serum lipids. Smoking furthermore activates the immune system, which correlates with inflammation in the vessel walls. It has negative effects on the coagulation system: Platelets are activated, the coagulation cascade is stimulated and fibrinolysis is reduced so that these processes could lead to atherothrombosis (156, 158). It is not clear how many pack years the two patients have; however, it would be important to evaluate this because it certainly has an effect on the FMD results.

The HIV infection itself

As described above, the HI virus itself is a risk factor for CVD. Not only does it strengthen traditional risk factors such as hypertension, diabetes, and dyslipidemia, even in people treated successfully with ART, a low level of viral replication is possible. This is a trigger for immune activation and inflammation, which leads to endothelial dysfunction and therefore to CVDs (159).

It would be interesting to know the period of HIV infection and the viral load the patients had when starting effective antiretroviral treatment. Unfortunately, this is not apparent from the data.

Coinfections

In addition to ART treatment, Patient B receives treatment against TB. TB infection in HIV-positive patients is common. HIV is the strongest risk factor for infection with mycobacterium tuberculosis; it provokes a progression to the active disease. Unfortunately, there is no data about the TB coinfection, the duration, and outbreak of the active disease.

In one study, CMV/HIV coinfections were associated with a higher risk of non-AIDS events, including CVDs, independently of other prognostic factors. Coinfections may

have a strong impact on FMD and therefore on the development of CVDs in HIV-positive patients (159-161).

Physical activity

In comparison to Patient A, who is not physically active, Patient B is mildly physically active three times a week. Abbott et al. have determined that physical activity correlated with better FMD results in children (162). Siasos et al. were able to demonstrate the correlation in middle-aged and elderly persons. The study revealed that there is no difference between the physically active elderly person and the physically inactive middle-aged person, meaning that with physical activity it is possible to fight the physiological aging process of the endothelium (163). The better FMD results of Patient B could be explained with the physical activity of Patient B.

Other factors

It is also possible that other factors that were not evaluated, such as mental stress or hyperhomocysteinemia, contributed to the different cardiovascular risk profiles (164, 165). Hormones could also influence NO production and therefore endothelial function (166).

5.1 Limitations

Since this case study refers to only one set of data for each category, no statistically significant results can be presented; however, both subjects are in the same age group, both are smokers, and both are HIV positive. To this researcher's knowledge, there is no such study that compares different ART regimens in the SSA population, which makes the results particularly interesting. This case study was furthermore conducted within the context of the *EndoAfrica* project. The number of participants in the actual study is much bigger, so it will be possible to achieve statistically significant results.

Due to national data protection, no follow-up data can be presented. It is therefore not possible to display and compare the development of endothelial dysfunction over time. It would be interesting to see the potential long-term complications of ART. This is also different in the *EndoAfrica* project, where the test subjects are

examined at the beginning, after 12 months, and after 24 months. This leads to excellent data about the long-term side effects of different antiretroviral drugs and their effect on the cardiovascular system over time.

Due to national data protection rules, some factors that could influence the present results were not available for description in this diploma thesis. **There are no details about the time of HIV infection, the duration of ART, or the number of pack years of the patients.** Since these factors could influence the FMD, more studies are needed to draw exact conclusions as to which of these factors are responsible for the different results obtained in this thesis.

5.2 Future Directions and Perspectives

Antiretroviral therapy has prolonged and improved the quality of life of HIV-infected patients. Nevertheless, it is a lifelong treatment associated with high costs for the health system and long-term effects on the body that are still poorly understood. For patients, adhering to the treatment poses a challenge, especially because many do not feel any symptoms of the HIV infection. Researchers have recently begun looking for a way to eliminate the virus from the body and therefore cure the disease, making ART dispensable. To date, however, it is not possible to eradicate the HI virus and until this is possible, ART is the best treatment option for HIV-positive patients (49, 167).

Antiretroviral treatment has changed the field of HIV infection from the threat of AIDS to chronic, long-term complications that can be associated with the virus itself or with the side effects of ART.

As a chronic illness, HIV requires lifelong treatment and screening for the typical comorbidities of this disease. Especially in SSA, a chronic care model has yet to be fully established. This has become increasingly important and should include effective antiretroviral treatment as well as services for reproductive health and non-AIDS morbidity, such as CVDs, TB prevention and treatment options (49). In the future, more expertise will be required to manage CVDs and other chronic complications.

Antiretroviral therapy should be carefully chosen and patients should receive ART regimens with regard to their cardiovascular risk profile. Screening for chronic, long-term complications of HIV should be established in SSA.
HIV-positive people should be screened for hyperlipidemia at diagnosis, annually, and at the beginning of ART. Their individual risk should be evaluated with different scores (e.g. Framingham or Joint British Societies score).
Future research should address how ART influences the cardiovascular system and which regimens have the best effect-side-effect profile, especially in countries most affected by the HIV epidemic: In SSA where more than two-thirds of the worlds' HIV-infected population lives. In the long term, a cure for HIV would resolve health care problems and long-term side effects, which makes research in this field highly important.

5.3 Conclusion

The different serum lipid levels of the two patients could be associated to the variable ART regimens. Patient B receives second-line therapy consisting of 2 NRTI and a boosted PI; she presents elevated levels of LDL cholesterol and total cholesterol.
These findings support other studies that have determined that, in particular, the PIs Lopanavir and Ritonavir cause dyslipidemia.
Both FMD results are within the normal range; therefore, vascular function does not seem to be affected by ART type. These results should be interpreted with caution, as they have been obtained from only two patients on ART. It remains important, however, to choose antiretroviral drugs carefully to avoid unfavorable combinations in people with higher cardiovascular risk. Future studies should also investigate the complex interaction of other factors such as smoking, physical activity, and age on the cardiovascular system in HIV patients on different types of ART. Aspects such as viral load, periods of ART duration as well as any other comorbidities should be included in future research.

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