

Diplomarbeit

**DO ANTIRETROVIRAL-DRUGS AFFECT THE  
CARDIOVASCULAR SYSTEM IN THE SOUTH  
AFRICAN POPULATION?**

**A Comparison of two Case Studies**

eingereicht von

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Graz, am 17. November 2017

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# Abstract

**Introduction:** The HI-virus is known to cause an acceleration of endothelial dysfunction and cardiovascular disease. However, the antiretroviral therapy for the virus also has adverse effects on the cardiovascular system. Even though Sub-Saharan Africa is one of the global hotspots of HIV and AIDS, only limited research has been carried out in this area.

**Aims/Objectives:** This thesis aims to explore differences in endothelial function in two HIV infected persons (one with and one without antiretroviral therapy) living in South Africa. Studying this is important as understanding the effects of ART per se on the cardiovascular system could lead to the development of guidelines for screening and treatment of HIV infected people, especially with respect to cardiovascular diseases.

**Material and Methods:** Analysed in this work were two cases of mixed race, HIV infected South African females: one on HAART and one who was HIV naïve (HIV positive but not on treatment). Both women were examined in terms of traditional cardiovascular risk factors (BMI, serum lipid level, blood pressure) and endothelial function. The endothelial function was measured by means of non-invasive flow-mediated dilatation (FMD) of the brachial artery.

**Results:** Both patients had been smoking and were pre-menopausal. The FMD measurement of the patient on HAART showed 6.14% dilation of the brachial artery, whereas the HIV naïve patient had a value of 12.56%. The patient on HAART was 18 years older, and had a BMI value of 29.5 kg/m<sup>3</sup>; her serum lipid results were: LDL 3.16 mmol/L, triglycerides 1.49 mmol/L and HDL 0.69 mmol/L. The HIV naïve patient had a BMI value of 48.3 kg/m<sup>3</sup>, and her serum lipid results were: LDL 2.64 mmol/L, Triglycerides 0.47 mmol/L and HDL 1.94 mmol/L.

**Discussion:** Despite the lower BMI value and a physically more active lifestyle, the patient on HAART showed signs of an accelerated vascular aging and had a significantly worse endothelial response (as seen in the lower FMD values) than the HIV naïve patient. As both of these patients were relatively matched in anthropometric measurements (except age) it appears that the differential FMD values seen in the two patients could have arisen due to the adverse effects of the antiretroviral therapy. However, what role age and the phase of the menstrual

cycle play in influencing the FMD results needs to be investigated in future studies and in larger cohorts of patients.

# Zusammenfassung

**Einführung:** Es ist bekannt, dass der HI-Virus die Entstehung von endothelialer Dysfunktion und Herz-Kreislauf-Erkrankungen beschleunigt. Jedoch hat die antiretrovirale Therapie (ART) gegen das Virus ebenfalls relevante unerwünschte Wirkungen auf das Herz-Kreislauf-System. Obwohl Subsahara-Afrika global zu den am meisten Betroffenen Regionen von HIV und AIDS gehört, wurde dort bisher nur wenig Forschung in diesem Bereich betrieben.

**Zielsetzung:** Diese Diplomarbeit widmet sich der Unterschiede in der endothelialen Funktion zweier, in Südafrika lebenden, HIV infizierter Personen (je eine mit und ohne antiretroviraler Therapie). Die Erforschung der Wirkungen der ART auf das Herz-Kreislauf-System könnte es ermöglichen Richtlinien für das Screening und die Therapie von Co-Morbidität HIV Infizierter zu entwickeln.

**Methoden:** Diese Arbeit analysiert zwei Fallberichte von HIV infizierten, südafrikanischen Frauen gemischter Ethnizität, je eine mit HAART (highly active, antiretroviral therapy) und eine Frau ohne Therapie. Beide Frauen wurden in Bezug auf konventionelle Risikofaktoren für Herz-Kreislauf-Erkrankungen (BMI, Serum-Lipidwerte, Blutdruck) und ihrer endothelialen Funktion untersucht. Die endotheliale Funktion wurde mithilfe der nicht-invasiven flow mediated dilatation (FMD) Methode an der Arteria brachialis (Oberarmarterie) gemessen.

**Ergebnisse:** Beide Patientinnen waren prämenopausal und Raucherinnen. In der FMD Messung hatte die Frau mit HAART einen Wert von 6,14% Vasodilatation der Arteria Brachialis, wogegen die HIV naive Patientin einen Wert von 12,56% aufwies. Die Patientin mit der Therapie ist 18 Jahre älter und hatte zum Zeitpunkt der Untersuchung einen BMI Wert von 29,5 kg/m<sup>3</sup>, ihre Serumlipidwerte waren: LDL 3,16 mmol/L, Triglyceride 1,49 mmol/L und HDL 0,69 mmol/L. Die HIV naive Frau hatte einen BMI Wert von 48,3 kg/m<sup>3</sup> und ihre Serumlipidwerte waren: LDL 2,64 mmol/L, Triglyceride 0,47 mmol/L und HDL 1,94 mmol/L.

**Diskussion:** Trotz des niedrigeren BMI Wertes und des aktiveren Lebensstils der Patientin mit HAART zeigte diese Zeichen von beschleunigter Gefäßalterung und signifikant schlechterer endothelialer Funktion (bezogen auf niedrigeren FMD Werten) als die HIV naive Patientin. Es zeigten sich, trotz der ähnlichen anthropometrischen Werte (außer dem Alter) der Patientinnen, sehr

unterschiedliche FMD Werte welche durch die unerwünschten Effekte der Antiretroviralen Therapie entstanden sein könnten. Der Einfluss des Alters und der Phase des Menstruationszyklus bei Frauen auf die FMD Messergebnisse müssten in zukünftigen Studien in größeren Kohorten untersucht werden.

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## List of Abbreviations

<b>3TC</b>	Lamivudine
<b>ABC</b>	Abacavir
<b>ABCA-1</b>	Adenosine-triphosphate binding cassette-1
<b>ACE</b>	Angiotensin- converting enzyme
<b>ACh</b>	Acetylcholine
<b>ACS</b>	Acute coronary syndrome
<b>AIDS</b>	Acquired immunodeficiency syndrome
<b>ART</b>	Antiretroviral therapy
<b>ARV</b>	Antiretroviral
<b>AUC</b>	Area under the curve
<b>AZT</b>	Zidovudine
<b>BMI</b>	Body mass index
<b>BP</b>	Blood pressure
<b>CCR5</b>	C-C chemokine receptor 5
<b>CD4</b>	Cluster of differentiation 4
<b>cDNA</b>	Complementary deoxyribonucleic acid
<b>CHD</b>	Coronary heart disease
<b>CVD</b>	Cardiovascular disease
<b>CVS</b>	Cardiovascular system
<b>CXCR4</b>	C-X-C chemokine receptor type 4
<b>d4T</b>	Stavudine
<b>DBP</b>	Diastolic blood pressure
<b>ddl</b>	Didanosine
<b>DNA</b>	Deoxyribonucleic acid
<b>EDTA</b>	Ethylenediaminetetraacetic acid
<b>EFV</b>	Efavirenz
<b>eGFR</b>	Estimated glomerular filtration rate
<b>ELISA</b>	Enzyme linked immunosorbent assay

<b>FDC</b>	Fixed dose combination
<b>FMD</b>	Flow- mediated dilatation
<b>FTC</b>	Emtricitabine
<b>GGT</b>	Gamma-glutamyl transferase
<b>gp 41</b>	Glycoprotein 41
<b>gp 120</b>	Glycoprotein 120
<b>HAART</b>	Highly active antiretroviral therapy
<b>Hba1c</b>	Glycated haemoglobin
<b>HDL</b>	High- density lipoprotein
<b>HIV</b>	Human immunodeficiency virus
<b>HR1</b>	Histamine receptor 1
<b>HR2</b>	Histamine receptor 2
<b>IgG</b>	Immunoglobulin G
<b>kDa</b>	kilo dalton
<b>LDL</b>	Low- density lipoprotein
<b>LTR</b>	Long terminal repeat
<b>MCP-1</b>	Monocyte chemoattractant protein-1
<b>MI</b>	Myocardial infarction
<b>mRNA</b>	messenger ribonucleic acid
<b>NaF</b>	Sodium fluoride
<b>NNRTI</b>	Non-nucleoside reverse transcriptase inhibitor
<b>NO</b>	Nitric oxide
<b>NRTI</b>	Nucleoside reverse transcriptase inhibitor
<b>NVP</b>	Nevirapine
<b>p24</b>	Capsid protein 24
<b>PCR</b>	Polymerase chain reaction
<b>PI</b>	Protease inhibitor
<b>RNA</b>	Ribonucleic acid
<b>ROI</b>	Region of interest
<b>SBP</b>	Systolic blood pressure

<b>SNP</b>	Sodium nitroprusside
<b>T-20</b>	Enfuvirtide
<b>T.B.</b>	Tuberculosis
<b>TDF</b>	Tenofovir
<b>VCAM-1</b>	Vascular cell adhesion molecule-1
<b>WHO</b>	World health organization

# 1 Introduction

## *1.1 The Human Immunodeficiency Virus*

Since the discovery of the HI-Virus in the 1980s, the Acquired Immunodeficiency Syndrome (AIDS) has remained a major global health issue. The Human Immunodeficiency Virus (HIV) belongs to the group of lentiviruses within the family of retroviruses. There are two different strains which cause the disease in humans: HIV-1 and HIV-2. The HI-Virus 1 appears to be responsible for the pandemic while the HI-Virus 2 is most common in the western part of Africa. (1, 2)

### *1.1.1 Epidemiology*

Current literature shows that the HI-Virus was zoonotically transmitted from the Simian Immunodeficiency Virus in the early 20<sup>th</sup> century. There are known subtype groups M, N and O for the HIV 1 strain. Regarding the HIV 2 strain, eight different subtypes of this strain, from A to H, have been discovered so far. It appears that the HIV 1 subtypes causes the epidemic outbreak of the disease, while the HIV 2 subtypes are mostly found in western parts of Africa, where sooty mangabeys are domiciled. (2–4)

This thesis will specifically focus on single participants of the EndoAfrica study in South Africa.

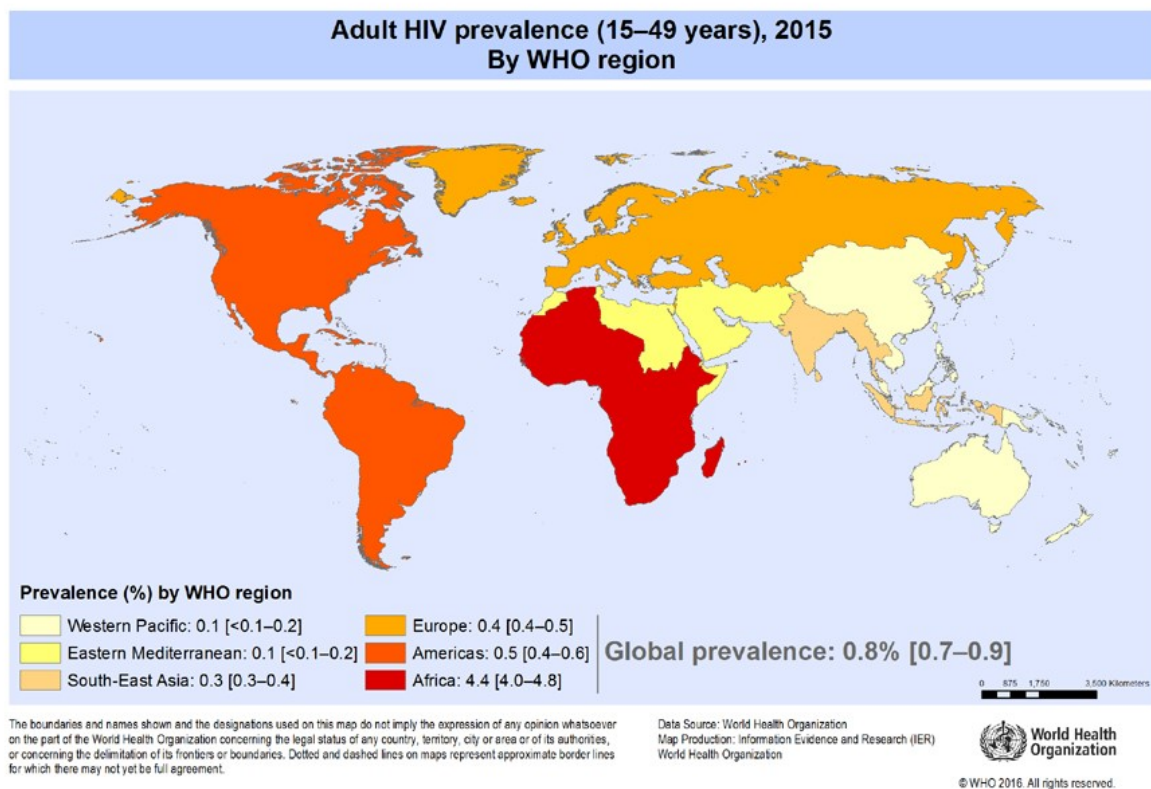


Figure 1: Worldwide distribution of HIV in 15-49 year old population (reproduced from <http://www.who.int/gho/hiv/en/>)

### 1.1.2 HIV in Sub-Saharan Africa

Sub-Saharan Africa is particularly affected by the HI-Virus. In 2015 an HIV rate for adults at the age of 15-49 years of approximately 20% was recorded in the country of South Africa. The same year there were 180,000 (150,000-220,000) deaths related to AIDS in South Africa. Especially young women have disproportional high rates of HIV infection in comparison to young men in these regions. (5)

### 1.1.3 Transmission

Sexual transmission prevails as the most common mode of transmission both in the developing world and industrial countries. Another major mode of transmission of HIV is mother-to-child during pregnancy, at the time of delivery, or postnatally via breastfeeding. Other modes of transmitting the virus are exposure to blood, needle sharing at injection drug use and, occasionally, transfusion. (6, 7)

### 1.1.4 Structure of HIV

The structure of the main particles are the same in HIV 1 and HIV 2. The RNA and the enzymes are surrounded by a HIV capsid out of polymers and core antigens with a diameter of 100nm and a membranous envelope out of lipoproteins with glycoproteins attached to it (1). The membrane itself is made of pairs (heterodimers) of trimers of the “gp 120” glycoprotein. Transmembranically, there is the gp 41 glycoprotein bound to gp 120 (8).

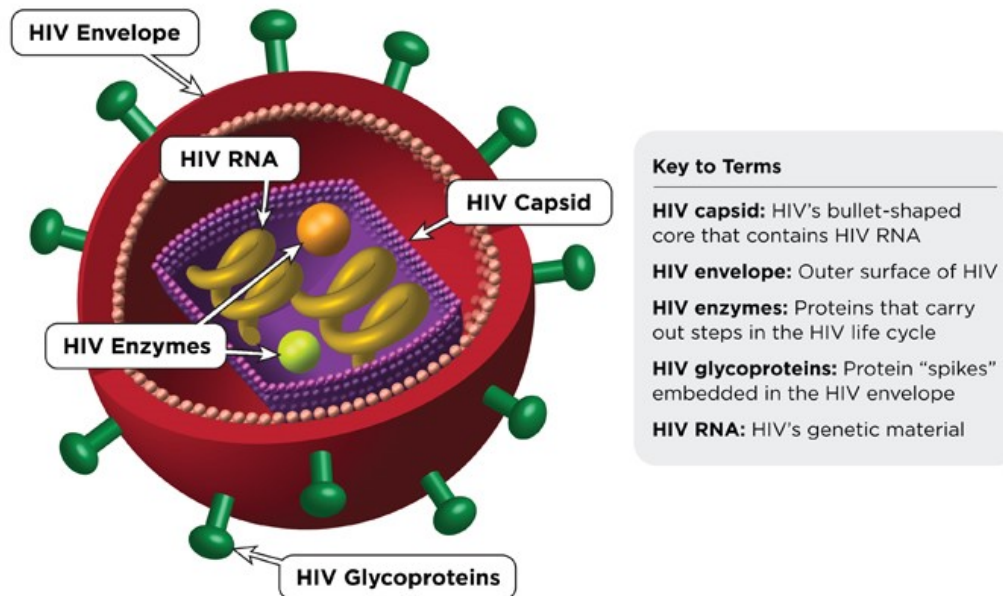


Figure 2: HIV structure (reproduced from: (9))

### 1.1.5 Genetics

Both strains of the HI-virus basically have the same genomic structure. The three main exons of the virus' gene are called “gag”, “pol” and “env” and encode very specific proteins for the structure of the core (gag), enzymes which are important for replication (pol) and glycoproteins for the envelope of the virus (env) (8). At both ends of the genetic information there are two LTR (long terminal repeat) introns at the 3' and the 5' end of the genome.(10)

There are also some other actors which are briefly presented in Figure 3.

## The HIV genome

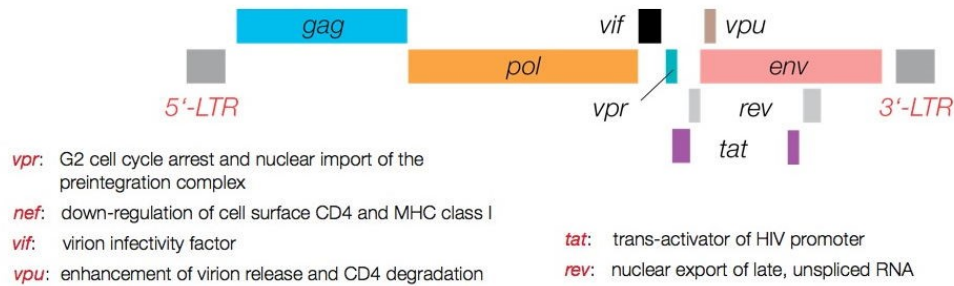


Figure 3: ("hiv [https://aidsinfo.nih.gov/education-materials/fact-sheets/19/73/the-hiv-life-cycle\\_3dpic.htm](https://aidsinfo.nih.gov/education-materials/fact-sheets/19/73/the-hiv-life-cycle_3dpic.htm)," n.d.)

### 1.1.6 Replication

After an infection there is a long period of clinical latency during which the virus replicates and possibly affects the central nervous system. For a better overview, it makes sense to describe the cycle of the virus in six steps.

#### 1) Binding and entry:

The glycoprotein complex of the envelope consisting of gp 120 and gp 41 is crucial for the recognition and the entry of the virus into the target cell. The gp 41 has a specific peptide at its amino terminus that is mainly responsible for the fusion of the virus' and target cell's membranes. By contrast, gp 120 binds a 58 kDa monomeric glycoprotein of the CD4 (Cluster of Differentiation 4) complex, which is expressed on the cell surface of T-cell-precursors, monocytes/macrophages, eosinophils, dendritic cells and microglial cells. The CD4 complex itself normally serves to recognize a foreign antigen. Gp120 binding CD4 induces a structural change of the virus envelope, which enables gp 120 to bind also chemokine receptors. There are several chemokine ligands that HIV can bind. The most important are CCR5 (C-C chemokine receptor 5) and CXCR4 (C-X-C chemokine receptor type 4) which are expressed on many cells, especially those of the secondary immune system. This double binding of gp 120 to CD4 as well as to a chemokine receptor makes the attachment more stable and facilitates the gp 41 to penetrate the cell membrane. Repetitive sequences of HR1 and HR2 (histamine receptor 1 and 2 ) lead to a collapse of the extracellular stock of gp 41. That process brings the membranes closer together until they fuse and thus allows the entry of the viral capsid.

## 2) Uncoating:

Once having entered the cell, the virus core uncoats and releases the viral RNA together with enzymes into the cytoplasm of the cell.

## 3) Reverse transcription

The reverse transcriptase writes the viral RNA into a double helix DNA molecule

## 4) Integration

Through the enzyme integrase the proviral DNA is transferred into the cell nucleus and integrated into the host cell's DNA.

## 5) Virus protein synthesis

After transcription of the integrated virus DNA into a messenger RNA the cell starts to synthesize HIV proteins to build more HIV particles. The transcribed RNA has its own promoter (tat gene) and some other genes to enhance the synthesis of viral proteins in the cell. (Figure 3)

## 6) Budding

The two newly written RNA strands stick together with the replication enzymes, whilst the core proteins configure the virus capsid. Depending on the type of the host cell the budding occurs in different locations. In T-lymphocytes, as shown in figure 4, budding happens at the cell surface. By contrast, in monocytes and macrophages this process takes place within the intracellular vacuoles.

(8)

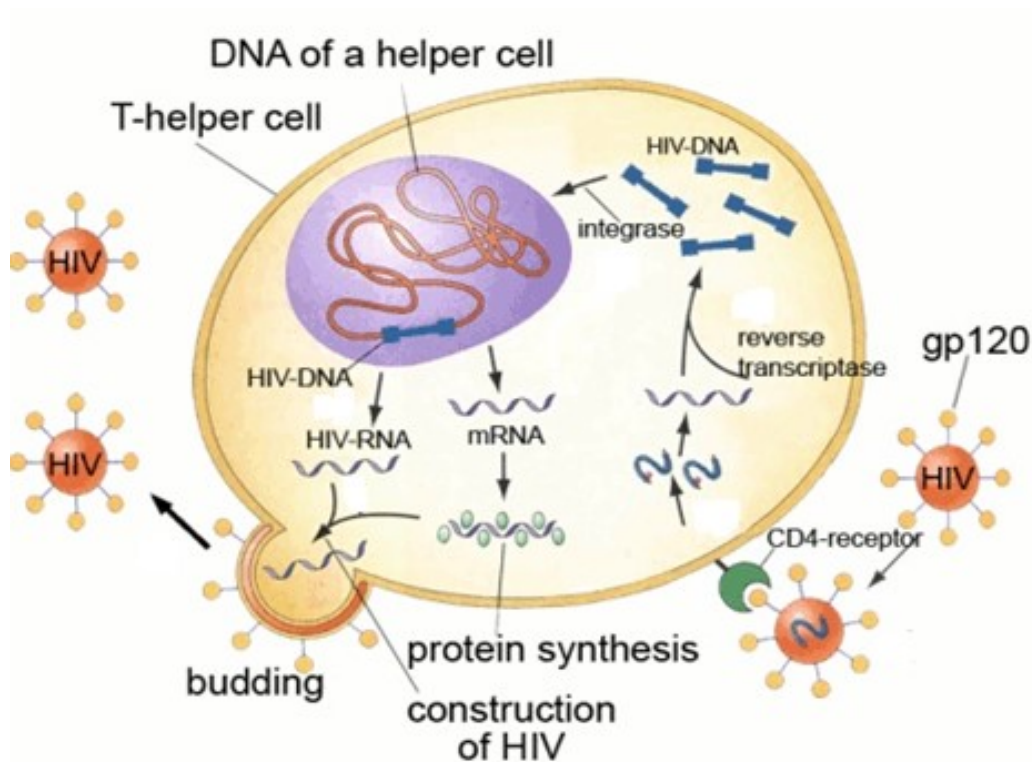


Figure 4: replication cycle of the HI-virus in a T-helper cell (reproduced from <http://photobucket.com/gallery/user/rajesh190888/media/cGF0aDovcGhhcm1hdHV0b3ltYXJ0LTE0MzAtMI96cHNhNzhkMTM0NC5wbmc=/?ref=>)

### 1.1.7 Progress and symptoms

The infection is classified into four clinical stages

#### 1) Acute infection

About 30% of recently infected patients develop mononucleosis-like symptoms, such as fever, swelling of the lymph nodes, splenomegaly, tonsillitis, dermatitis, myalgia and diarrhoea within the first six weeks of infection. HIV antibodies are usually detectable within 3 months of transmission. This stage mostly corresponds to the acute immune response of the infiltration of the virus.

#### 2) Latent persistency

After the acute phase the virus goes into latency and can remain asymptomatic for many years as a sign of immunological management of the virus, however, the virus continuously replicates during these years.

Non treated patients in this stage are highly contagious.

#### 3) AIDS related complex

The AIDS related complex is a pre-AIDS stage and has milder symptoms than the terminal disease. Many patients develop a lymphadenopathy syndrome with a persistent swelling of the lymph nodes at least in two different, non-inguinal locations and occasionally a seborrheic eczema as a first sign of the AIDS disease. Furthermore there will appear sporadic AIDS-related opportunistic infections.

#### 4) AIDS

In the end-stage of the AIDS disease, the CD4+ cell count drops so low that the immune system is no longer able to fight infections. Patients have multiple opportunistic infections and can develop malignant AIDS-related diseases, such as Kaposi's sarcoma and lymphoma. Besides, symptoms such as night sweat and weight loss continuously weaken the patient (11).

Figure 5 shows the correlation of the HIV viremia and the CD4+ cell count in different phases of infection. The progress clearly shows in the high viral load of the acute and the AIDS symptomatic phases: the loss of the immune compatibility increasingly manifests itself in the terminal state of infection.

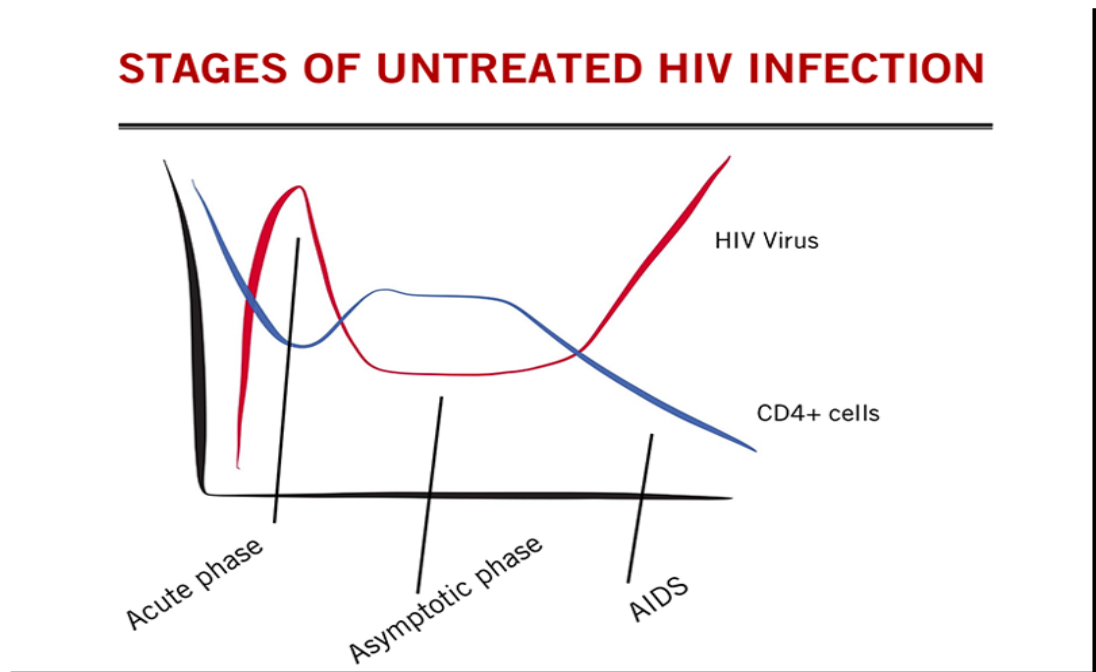


Figure 5 The level of viremia and CD4+ cell count in different phases of HIV infection. (reproduced from <http://www.canfar.com/news/stages-hiv-infection>)

As already discussed, HIV primarily leads to a retroviral syndrome as a reaction of the immune system to the infiltration of the virus. When the disease becomes

symptomatic the patients will have an accumulation of unspecific infections, reoccurrences of zoster eczema and swelling of the lymph nodes. Further there are some clearly AIDS related illnesses like fungal infections, reactivated tuberculosis, wasting syndrome and a lack of white blood cells as a sign of the proceeding failure of the immune system.

Table 1 shows a brief overview of the most important opportunistic and typical illnesses that are AIDS-related, as well as the last stage malignancies of the AIDS disease.

*Table 1: WHO Clinical Staging of HIV/AIDS for Adults and Adolescents (reproduced from <https://aidsetc.org/guide/hiv-classification-cdc-and-who-staging-systems>)*

Clinical Stage	Clinical Conditions or Symptoms
<b>Primary HIV Infection</b>	<ul style="list-style-type: none"> <li>• Asymptomatic</li> <li>• Acute retroviral syndrome</li> </ul>
<b>Clinical Stage 1</b>	<ul style="list-style-type: none"> <li>• Asymptomatic</li> <li>• Persistent generalized lymphadenopathy</li> </ul>
<b>Clinical Stage 2</b>	<ul style="list-style-type: none"> <li>• Moderate unexplained weight loss (&lt;10% of presumed or measured body weight)</li> <li>• Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis)</li> <li>• Herpes zoster</li> <li>• Angular cheilitis</li> <li>• Recurrent oral ulceration</li> <li>• Papular pruritic eruptions</li> <li>• Seborrheic dermatitis</li> <li>• Fungal nail infections</li> </ul>

Clinical Stage	Clinical Conditions or Symptoms
<b>Clinical Stage 3</b>	<ul style="list-style-type: none"> <li>• Unexplained severe weight loss (&gt;10% of presumed or measured body weight)</li> <li>• Unexplained chronic diarrhea for &gt;1 month</li> <li>• Unexplained persistent fever for &gt;1 month (&gt;37.6°C, intermittent or constant)</li> <li>• Persistent oral candidiasis (thrush)</li> <li>• Oral hairy leukoplakia</li> <li>• Pulmonary tuberculosis (current)</li> <li>• Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia)</li> <li>• Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis</li> <li>• Unexplained anemia (hemoglobin &lt;8 g/dL)</li> <li>• Neutropenia (neutrophils &lt;500 cells/<math>\mu</math>L)</li> <li>• Chronic thrombocytopenia (platelets &lt;50,000 cells/<math>\mu</math>L)</li> </ul>
<b>Clinical Stage 4</b>	<ul style="list-style-type: none"> <li>• HIV wasting syndrome, as defined by the CDC (see Table 1, above)</li> <li>• <i>Pneumocystis</i> pneumonia</li> <li>• Recurrent severe bacterial pneumonia</li> <li>• Chronic herpes simplex infection (orolabial, genital, or anorectal site for &gt;1 month or visceral herpes at any site)</li> <li>• Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs)</li> <li>• Extrapulmonary tuberculosis</li> <li>• Kaposi sarcoma</li> </ul>

## Clinical Stage

## Clinical Conditions or Symptoms

- Cytomegalovirus infection (retinitis or infection of other organs)
- Central nervous system toxoplasmosis
- HIV encephalopathy
- Cryptococcosis, extrapulmonary (including meningitis)
- Disseminated nontuberculosis mycobacteria infection
- Progressive multifocal leukoencephalopathy
- Candida of the trachea, bronchi, or lungs
- Chronic cryptosporidiosis (with diarrhea)
- Chronic isosporiasis
- Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis)
- Recurrent nontyphoidal *Salmonella* bacteremia
- Lymphoma (cerebral or B-cell non-Hodgkin)
- Invasive cervical carcinoma
- Atypical disseminated leishmaniasis
- Symptomatic HIV-associated nephropathy
- Symptomatic HIV-associated cardiomyopathy
- Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

### 1.1.8 Diagnostics

There are several tests to verify and quantify both the HI-virus and the functionality of the immune system. It is obligate to have the person's agreement for an HIV test for it can determine their whole further life.

#### Rapid test

Rapid assays are suitable to detect an HIV infection within 30 minutes.

#### HIV-serology

The serology is tested via an enzyme linked immunosorbent assay (ELISA), which is a test to detect either IgG (immunoglobulin G) antibodies in the blood or the HIV's capsid protein p24. If positive, the infection has to be verified with the more specific western blot test. The results of these tests are only of use if the infection took place at least 6 weeks before.

#### Detection of viral nucleic acid

With a test called, polymerase chain reaction (PCR) it is possible to amplify and prove the presence of proviral DNA in the host cell. There is also a reverse technique used to detect viral RNA. In this test, the laboratorian adds reverse transcriptase to produce cDNA (complementary DNA), which is detectable. This measurement is much more sensitive than serology and can be performed before the six weeks of false negative results in serology.

The PCR is also used to determine the **viral load**, which is a crucial value to quantify the HIV level.

#### CD4+ cell count

The CD4+ cell count is a very important value for the surveillance of HIV infected people and is related to the functionality of the immune system under HIV influence.

The CD4+ cell count is tested via the flow cytometry. (11, 12)

### 1.1.9 Antiretroviral Therapy

Strategies for treating the virus are prohibition of the viral entrance into the CD4+ cell and the inhibition of its specific enzymes: the reverse transcriptase, protease and the integrase. (14)

#### Substance Classes

The nucleoside reverse transcriptase inhibitors (NRTI): have a chemical modification at the glucoside terminal of a nucleoside which impairs the transcribed proteins if integrated.

Substances: Tenofovir (**TDF**), Emtricitabine (**FTC**), Zidovudine (**AZT**), Stavudine (**d4T**), Abacavir (**ABC**), Lamivudine (**3TC**), Didanosine (**ddl**)

Non-nucleoside transcriptase inhibitors (NNRTI): These drugs are different from nucleosides. They inhibit the reverse transcriptase by binding at the catalytic centre of the enzyme. There is a rapid development of resistances to these substances.

Substances: Efavirenz (**EFV**), Etravirine , Nevirapine (**NVP**), Rilpivirine

Protease inhibitor (PI): The PIs split the HIV protease between two amino acids. This enzyme is important for the replication of the virus. Therefore it leads to a specific inhibition of the viral augmentation.

Substances: Atazanavir, Darunavir, Fosamprenavir. Indinavir, Lopinavir, Nelfinavir, Ritonavir, Saquinavir, Tipranavir

Integrase Inhibitors: By inhibiting the viral integrase, this drug prohibits the integration of the viral DNA into the human genome.

Substances: Dolutegravir, Elvitegravir, Raltegravir

Fusion/Entry inhibitors: The entry inhibitors bind at the gp 41 at the viral capsule and inhibit the fusion between the virus and the CD4+ cells. Therefore the virus cannot enter and infect the host cell.

Substances: Enfuvirtide (**T-20**), Maraviroc

(12, 13)

Figure 6 shows an illustration of the targets for the antiretroviral therapy.

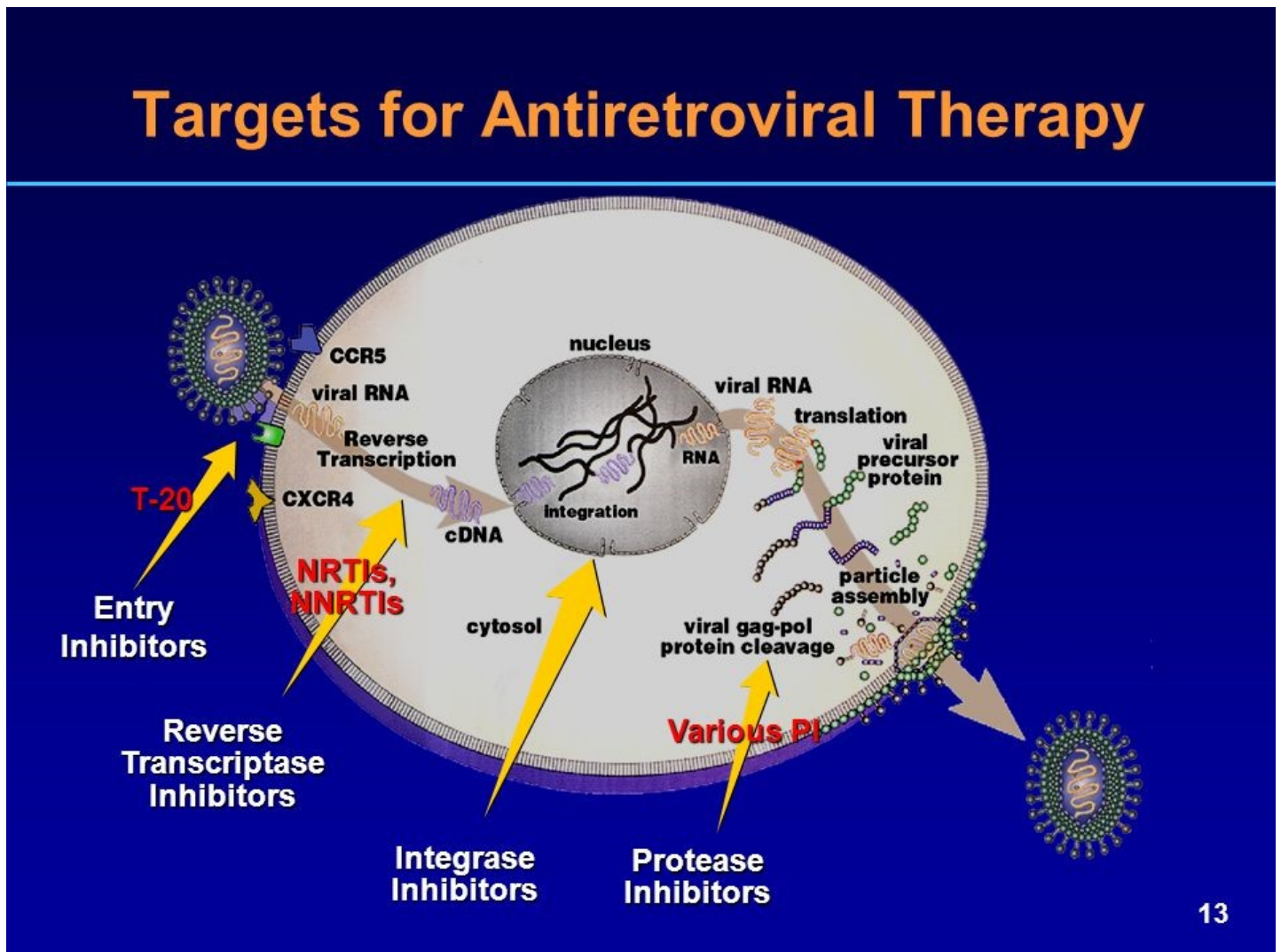
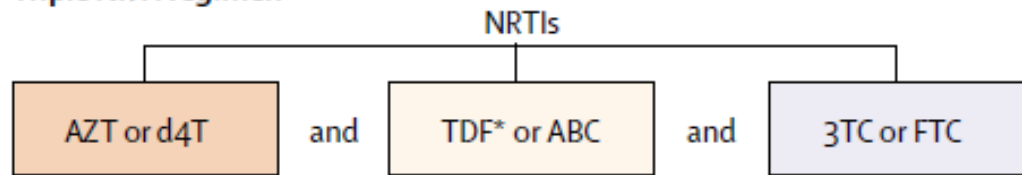


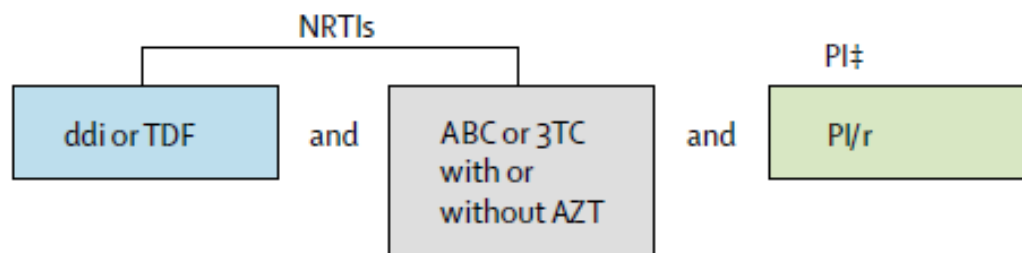
Figure 6 (reproduced from: [http://images.slideplayer.com/25/8122672/slides/slide\\_13.jpg](http://images.slideplayer.com/25/8122672/slides/slide_13.jpg))

Due to its high mutation rate the HI-virus builds up resistances over long term ART. To prevent this, the virus is treated with a combination of antiretroviral drugs (13). The treatment is predetermined in fixed dose combinations (FDC). There are different regimen used for HIV treatment. The treatment has to be monitored and adapted if necessary according to cost, availability, adverse effects and viral resistance. Figure 7 shows the proposal of the World Health Organization (WHO) to develop a public antiretroviral therapy scheme (15).

### Triple NRTI regimen



### Second-line regimen if standard first-line used



### Second-line regimen if triple NRTI used

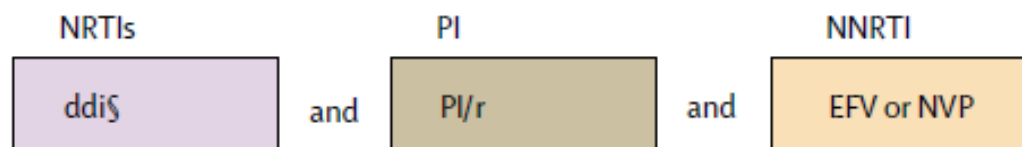
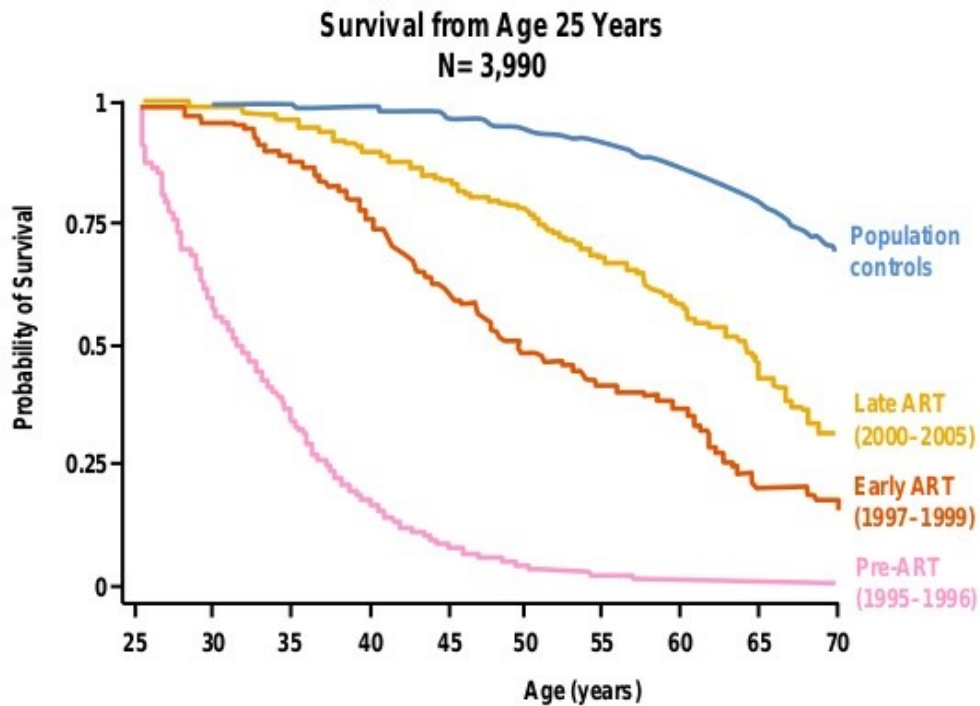


Figure 7: WHO recommendation for HIV treatment (reproduced from: (15))

By **lowering** the **viral load**, the ART aims to **sustain a functional immune system**, **reduce** the **HIV related morbidity** and **avert** its **transmission**. (16)

Despite their numerous adverse effects, the benefits of ART outweigh them. Figure 8 shows the improved survival of HIV infected patients.

## Improved Survival in ART Era



Adapted from Lohse N, et al. Ann Intern Med 2007;146:87-95

Figure 8 (reproduced from: <https://image.slidesharecdn.com/aidsclinicalrounds032114gianellaweibel-140409154522-phpapp02/95/inflammation-persists-even-during-hiv-therapy-4-638.jpg?cb=1397067347>)

Adverse effects: In addition to nausea, headache, diarrhoea, fever and rash as mild symptoms the ART is also related to some severe adverse effects. These are neuropathy, myopathy, pancreatitis, psychosis and myelosuppression. In the long term, the drugs, especially the PIs, often elicit lipodystrophy and metabolic syndromes. The antiretroviral drugs can also impair the renal and liver function for they are mainly metabolized by those organs (14, 15, 17).

Figure 9 shows an overview of drug class related adverse effects of antiretroviral (ARV) drugs.

# ARV Drug Class Adverse Effects

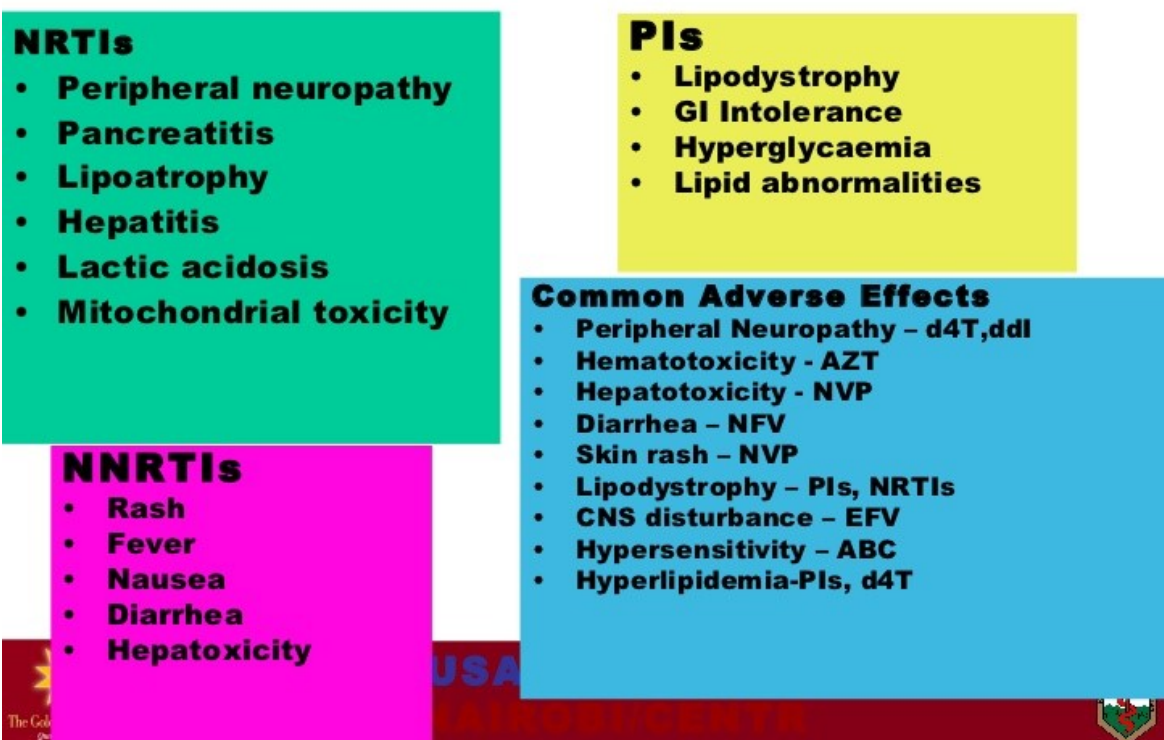


Figure 9 (reproduced from: <https://image.slidesharecdn.com/02-01adultartclassificationactionandsideeffectsgsn-110430042814-phpapp01/95/0201-adult-art-classificationaction-a-nd-side-effects-gsn-14-728.jpg?cb=1304137922>)

In some patients the toxicity of the ARV drugs is a limiting factor for therapy. There are life threatening adverse effects, such as hypersensitive reaction, systemic hepatotoxic symptoms or severe cutaneous reactions, which require a stop of the antiretroviral therapy and the restart of an alternative treatment after the symptoms have diminished. Milder effects can usually be handled by replacement of the drug to which they are related (18).

Besides these adverse and cumulative effects, the effectiveness, mostly related to viral resistance, or costs of the treatment often present reasons to choose one regimen over another.

## 1.2 *The Cardiovascular System*

### 1.2.1 *The Blood*

Blood constitutes 6-8% of a humans' body weight. It roughly consists of 40% of cells which can be divided into Thrombocytes (Platelets), Erythrocytes (Red Blood Cells) and Leucocytes (White Blood Cells) which can be subdivided into granulocytes, lymphocytes and monocytes. The other 60% of the blood volume are plasma, which is defined as a mixture of water, proteins, electrolytes, vitamins, gases, nutrients and metabolites.

Blood can be seen as a liquid organ and is a very important part of the human body. There are multiple **functions fulfilled by blood**, such as: 1.)

**transportation** of oxygen, glucose, amino acids, fatty acids, and vitamins which is crucial for the nutrition of the tissues, 2.) **body temperature regulation**, 3.) **signal transmission** via hormones and 4.) carrier and effector of the **immune system**.

(19)

### 1.2.2 *The Heart*

The human heart is divided into a left and a right heart, which can be imagined as two pumps in series connection. Both have an Atrium and a Ventricle, but two different circulations. The right heart pumps the oxygen-poor blood into the pulmonary circulation via the pulmonic arteries to refill its oxygen storage; then the blood goes into the left heart via the pulmonary veins. There, it is pumped through the aorta into the wider body circulation to provide the organism with oxygen rich blood. Via the vena cava inferior and superior the oxygen-poor ("used") blood gets back into the right atrium.(19)

### 1.2.3 *The Vascular System*

The blood is pumped through the body by a complex system of arteries, veins and capillaries. The lung circulation and the systemic circulation are serially connected.

#### 1.2.4 The vessels

The blood vessels can altogether be conceived of as a big hollow organ with a homogenous structure of its walls. It is made out of three layers – the **tunica intima**, the **tunica media** and the **tunica externa** (often called **adventitia**).

The intima consists of only one layer of cells - the endothelium. These cells build a barrier against loss of plasma, electrolytes and other blood components. In addition the endothelial cells also produce various vasoactive substances and therefore regulate the blood stream via constriction and dilation. Due to the pulsatile blood flow the veins also have valves in their intimal layer to prevent the blood from being poured against the direction of flow by gravity. The media is made out of smooth muscle cells, elastic and collagen fibres. It supplies the muscular strength to constrict the artery or maintain the blood pressure to ensure the blood supply of every region of the body. The externa, or adventitia mainly consists of connective tissue and embeds the vessel in the surrounding tissue. Especially in bigger arteries and veins the external layer is also important to nourish the vessel via its own little vessels (*vasa vasorum*) (20).

Figure 10 shows the general anatomy of the arterial wall.

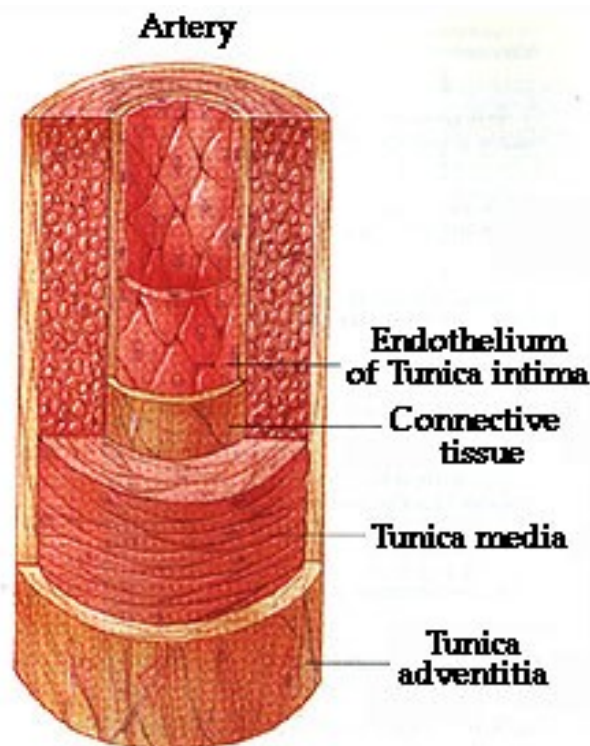


Figure 10: (reproduced from: [http://www.physioweb.org/circulation/blood\\_vessels.html](http://www.physioweb.org/circulation/blood_vessels.html))

### 1.2.5 *The Endothelium*

As mentioned above the endothelium is in charge of many regulatory mechanisms. Therefore it is necessary to provide a brief overview of all the tasks of this cell to be able to understand the consequences of a damaged endothelium.

Tasks of the endothelium:

- Formation of a semipermeable membrane as a barrier for non-regulated transfer of molecules
- Regulation of the vascular tone via NO (nitric oxide) and prostacyclin
- Influences in the blood pressure via the angiotensin-converting-enzyme (ACE)
- The endothelium is both pro-thrombotic as well as anti-thrombotic to prevent bleeding on the one hand and obstruction on the other hand
- Inflammatory response through the secretion of many chemokines
- Growth factors for new blood vessels

Additionally to avoid clotting and turbulences it is important that the endothelium cell has a smooth surface.(23, 24)

### 1.2.6 *Vascular assessment*

To predict and further prevent cardiovascular events multiple methods for the assessment of the vasculature have been developed.

Intra- arterial infusion of vasoactive agents via a coronary angiography is considered as the most precise method for assessing endothelial function. By using agonists and antagonists of nitric oxide (NO) the examiner is enabled to determine basal and, specifically, stimulated NO release. This procedure however is invasive and troublesome for the patient. This is why non-invasive procedures for measurement are used more regularly. (21)

### 1.2.6.1 Non-invasive endothelial assessment

The worldwide mostly used method to measure endothelial function is the **flow mediated dilation (FMD)**. This method measures the arterial diameter of the brachial artery with an ultrasound device before, during and after a short hyperaemic period to quantify the vascular response. This period is created by inflation of a blood pressure cuff above the systolic blood pressure at the forearm. Reduced shear stress and the release of nitric oxide (NO) elicit an endothelial-dependent dilation of the arteries. The arterial diameter change is assessed as the percentage of the baseline parameter in resting and the maximal dilation (22).

Figure 11 shows the basic principle of the FMD measurement.

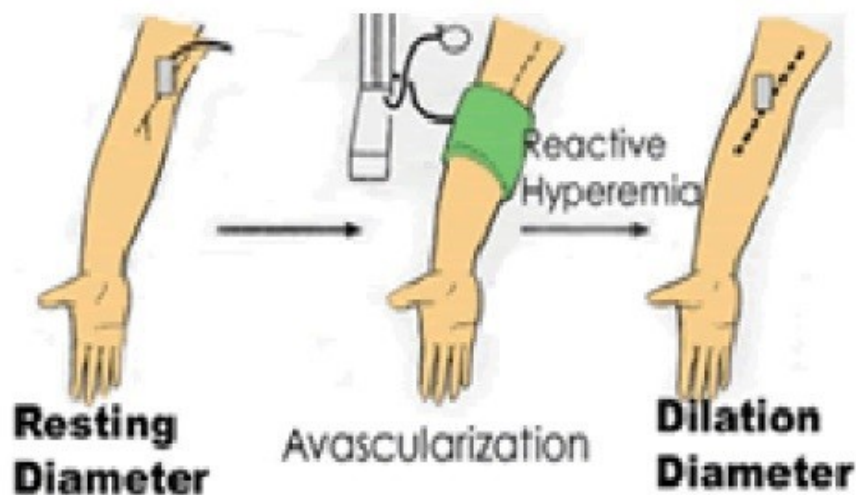


Figure 11 (reproduced from: <http://spo.escardio.org/eslides/view.aspx?eevtid=40&fp=1939>)

Figure 12 shows the area where the measurement takes place and the data it yields:

**A-** The image of the artery in the ultrasound device. The turquoise square is set as the region of interest (ROI) and the purple lines mark the intimal layer of the arterial wall to measure its diameter. **B-** The illustration of the measurement in a graph. It clearly demonstrates the peak of the maximal dilation and the recovery of the arterial wall.

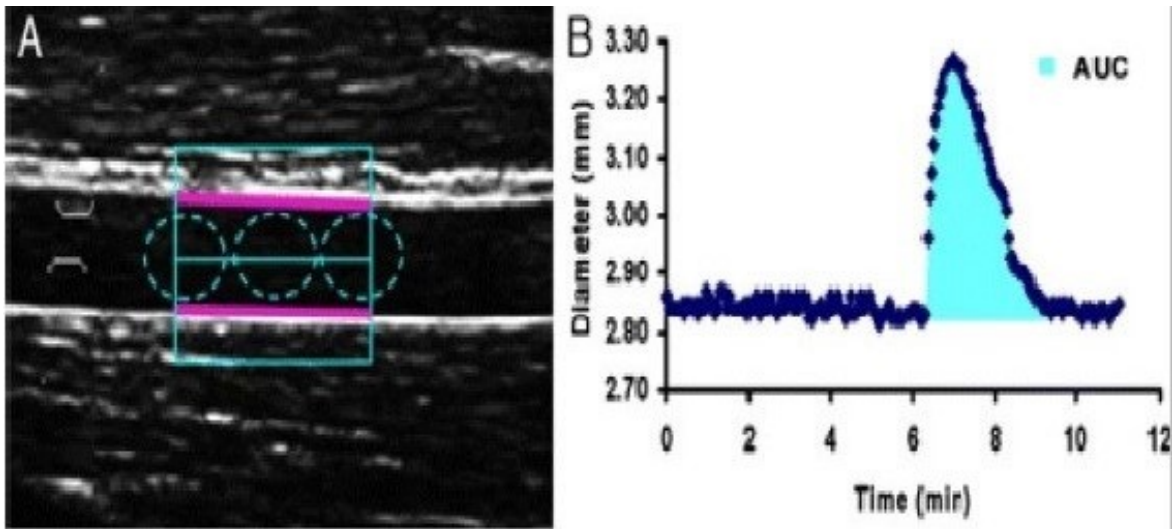


Figure 12 (reproduced from:<http://spo.escardio.org/eslides/view.aspx?eevtid=40&fp=1939>)

### 1.3 Pathophysiology

#### 1.3.1 Endothelial Dysfunction

It has been shown that the endothelial damage can be measured far ahead of the first macroscopic signs of a cardiovascular disease. In physiological terms the endothelium balances its tasks depending on its surrounding conditions (23).

Damage in the endothelium leads to higher rates of atherosclerosis, plaque rupture, thrombosis and vasospasm. These factors are associated with a higher rate of cardiovascular events, such as myocardial infarction, stroke and pulmonary embolism. They can also lead to chronic forms of these diseases such as, in most cases, coronary heart disease and atherosclerosis of the aorta, renal arteries and the carotids. Therefore patients with endothelial dysfunction more frequently suffer from attacks of acute coronary syndrome and ischemic attacks of the brain (24,25).

Figure 13 shows the difference of reaction in endothelial dysfunction.

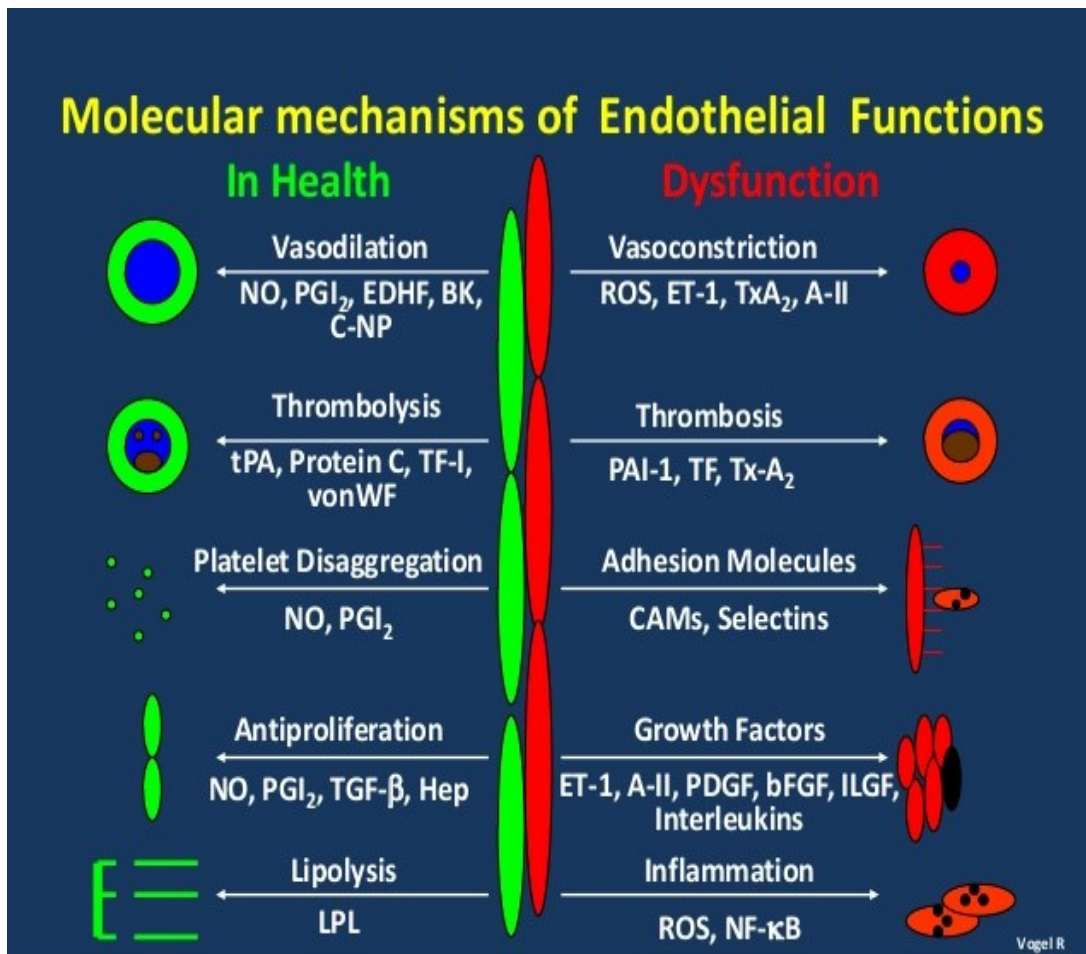


Figure 13: (reproduced from <https://www.slideshare.net/heartsense/endothelial-dysfunction-in-indian-scenario>)

### 1.3.2 Atherosclerosis

Atherosclerosis is a thickening and hardening of the arterial wall in a complex pathologic process which leads to plaque formation. It is the leading cause of coronary heart disease, cerebrovascular disease and other conditions. Main risk factors of this disease are a high fat and cholesterol diet, obesity, smoking, diabetes mellitus, hypertension, cardiovascular diseases in the family history, male sex and high age (24).

#### Pathogenesis

Pathogenesis is triggered by the so-called Virchow's triads which was first described in 1856 by Rudolf Virchow. This triads defines three factors that are responsible for the change of the arterial wall – 1. Damage of the vessel wall (especially the endothelium), 2. A disturbance in the blood flow and 3. Increased viscosity of the blood. The pathologic development of the lesions progresses from

endothelial injury to fatty streaks up to complex fibrotic plaques. The lesions start with a damage of the endothelial cell, which as a response causes an inflammation of the cell and raises its permeability. Many cytokines are released to attract immune cells. Then monocytes will migrate by the vascular cell adhesion molecule-1 (VCAM-1) into the intima and differentiate into macrophages. The Macrophages release more chemokines, phagocytic enzymes, growth factors and oxygen radicals that oxidize the present low-density lipoprotein (LDL). The oxidized LDL will injure the endothelium and the growth factors will lead to a smooth muscle cell proliferation into the intima. Due to its threat the macrophages then absorb the oxidized LDL, which turns them via deposits into foam cells until the cell goes into apoptosis. Thereupon the inflammation is triggered again and recruits more monocytes/macrophages.

Figure 14 shows a simplified overview of the progress of the role of the macrophages in the development of atherosclerosis (24, 26).

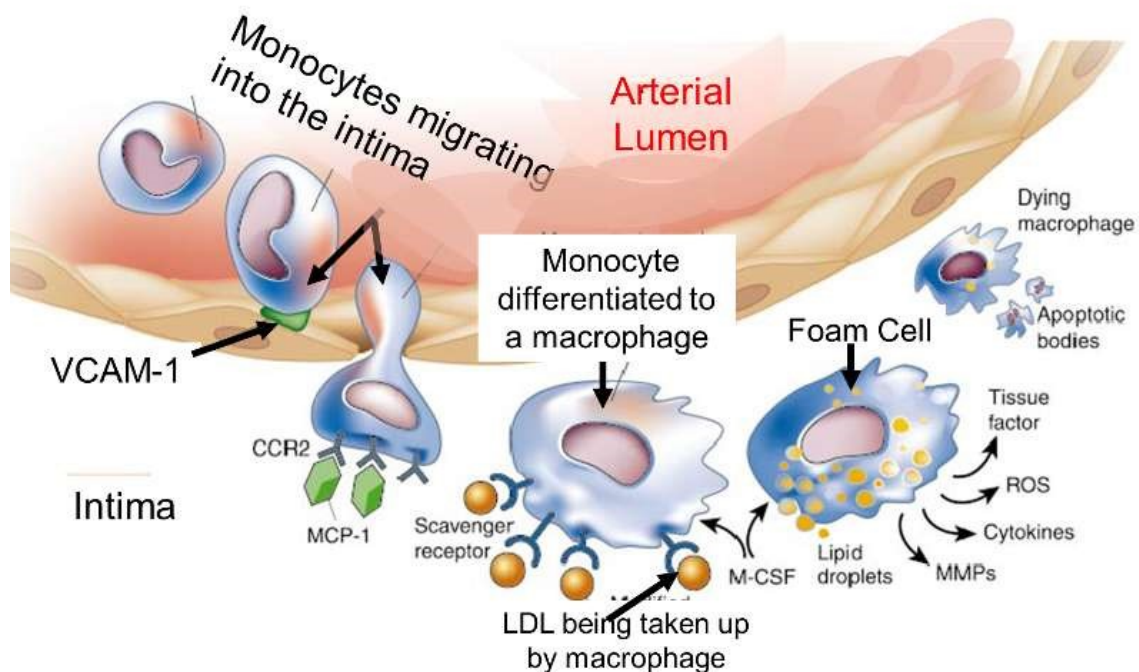


Figure 14: (reproduced from [http://sphweb.bumc.bu.edu/otlt/mph-modules/ph/ph709\\_heart/ph709\\_heart3.html](http://sphweb.bumc.bu.edu/otlt/mph-modules/ph/ph709_heart/ph709_heart3.html))

The manifestation of atherosclerosis in the coronary arteries is specifically defined as **coronary heart disease (CHD)**. Advanced CHD is often accompanied by an insufficient blood supply of the heart which leads to acute and chronic heart insufficiency, cardiac arrhythmia and myocardial infarctions. Clinically the critical shortage of myocardial supply exhibits as an **acute coronary syndrome (ACS)**. These complications of the coronary heart disease are emergencies and have to be treated urgently, otherwise they can be lethal (13).

### *1.3.3 Arterial hypertension*

Hypertension is defined as a sustained systolic blood pressure of 140 mmHg or higher or a diastolic pressure of 90mmHg or higher. It is one of the main cardiovascular risk factors and mostly correlated with endothelial damage. Even pre-hypertensive elevated blood pressure can affect the life expectancy of people concerned. High BP leads to:

- Excessive workload on the heart – early heart failure, coronary artery disease, congestive heart disease and heart attack
- Rupture of major blood vessels in the brain or obstruction of cerebral blood vessels = cerebral ischemia, stroke
- It damages the kidneys and leads to kidney failure

Environmental as well as genetic factors seem to be the cause of hypertension. Table 2 shows the classification of hypertension according to blood pressure values.

Table 2: (reproduced from <https://www.stockgumshoe.com/2013/06/microblog-is-there-a-common-theme-in-alternative-medical-therapies/>)

### Classification of blood pressure for adults

BLOOD PRESSURE CLASSIFICATION	SBP MMHg	DBP MMHg
NORMAL	<120	and <80
PREHYPERTENSION	120–139	or 80–89
STAGE 1 HYPERTENSION	140–159	or 90–99
STAGE 2 HYPERTENSION	≥160	or ≥100

*SBP, systolic blood pressure; DBP, diastolic blood pressure*

Both values of the blood pressure generally rise with advancing age, but due to the stiffness of the arteries the diastolic pressure falls and the difference between systolic and diastolic pressure increases. This can lead to both insufficiency and damage of the vasculature.

The form that is most harmful for the cardiovascular system is the non-dipper hypertension. As a term indicates, the blood pressure under this condition does not dip at night as it usually does and remains high. This causes even more damage to the endothelium due to the permanent exposure (21, 24, 25).

#### 1.4 HIV and Cardiovascular disease

There is an increased prevalence of cardiovascular diseases in HIV positive patients. The coronary heart disease is the main manifestation of cardiovascular disease in HIV patients. It often becomes noticeable by an acute coronary syndrome (ACS), often as a manifestation of a myocardial infarction (MI). HIV positive patients with coronary heart disease tend to be younger and have more and more severe ACS attacks than the general population. (29)

It is observed in HIV+ population that they exhibit a riskier lifestyle related to conventional risk factors, such as smoking, drug abuse, obesity, hypertension and dyslipidaemia. (30)

Current literature however shows that the virus infection itself accelerates the development of cardiovascular diseases. There are several mechanisms with which the virus contributes to atherosclerosis. The virus provokes a long lasting inflammation which leads to DNA methylation and alters the genes into a more proatherogenic constitution. The atherosclerotic plaques seem different too relating to their constitution and stability. They tend to be more inflammatory and susceptible to rupture and thromboembolism (23, 24). HIV also seems to infect the smooth muscle cells in vitro and in vivo and rises the release of monocyte chemoattractant protein-1 (MCP-1), which lures more monocytes to increase the inflammation and the development of foam cells. Furthermore the monocytes themselves are also hosts for the HI-virus. It mars the adenosine-triphosphate binding cassette-1 (ABCA-1) transporter of the macrophages, which impairs the reverse cholesterol transport. There is a direct effect on the HDL metabolism as well, thus HIV triggers the transfer of apolipoprotein B lipoproteins in HDL. These lipoproteins are important components of the LDL cholesterol and are known as proatherogenic (31).

Nevertheless it has been shown that there is no association between the viral load and the risk for cardiovascular disease (CVD) (31).

### *1.5 HAART and Cardiovascular disease*

The medications for HIV treatment have some relevant adverse effects on the cardiovascular system. Especially the first generation of NNRTIs and the PIs are known to increase the total cholesterol level and triglycerides, whereas they reduce the HDL level. Further the HAART has shown to deregulate the blood pressure regulating system and to provoke metabolic disorders and hypertension. (24, 25) Furthermore it has been shown that ART is related to insulin resistance, hyperlipidaemia and lipodystrophy as accumulation of central fat.

The pathophysiologic mechanism is not yet fully understood. These side effects are milder in the second generation ARTs, but not eliminated. (31, 32)

Especially the treatment with protease inhibitors and NRTIs are associated with an increased occurrence of cardiovascular diseases. Current studies have shown that there is an increased rate of myocardial infarctions with ritonavir, lopinavir, fosamprenavir, abacavir and didanosine. This risk appears not to accumulate over time and fades several months after ending the drug treatment. Nevertheless discontinuation of antiretroviral drugs has been confirmed to raise the risk of CVD by declining the HDL level (31).

It is assumed that a mitochondrial toxicity of the NRTIs causes several adverse effects. These drugs seem to lead to an inhibition of mitochondrial DNA polymerase and therefore impair the patients' metabolism. (17)

Figure 15 shows an overview of the interaction of traditional risk factors, HIV infection and the HAART on the cardiovascular system.

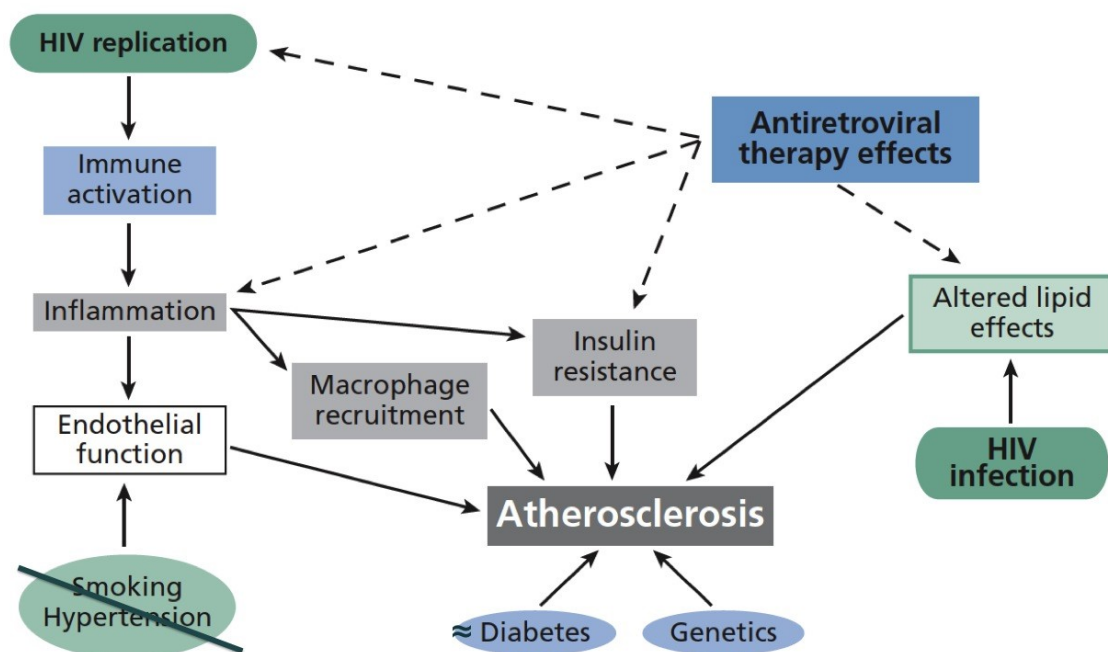


Figure 15: reproduced from (31)

### *1.5.1 Prevention and therapy of CVD in HIV infected patients*

Most of the interventions to treat the general population are similar. The first line therapy is usually smoking cessation, lifestyle alterations and treatment of hypertension and dyslipidaemia, since there is a high prevalence of traditional risk factors in HIV seropositive patients. There are also the same indications for revascularization therapy, but as already mentioned, the HIV positive population shows higher rates of recurrent ischemic events.

The numerous drug-drug interactions of the antiretroviral medications also need to be taken into consideration. These influence both hypertension and dyslipidaemia treatment. Therefore the medication combination should be elected wisely concerning the patients HAART. To evaluate the patients risk of further cardiovascular events, there are more specific risk scores for HIV positive individuals required, such as the D:A:D: equation. (30)

## 2 Aims and Objectives

After decades of knowing the HI-virus it is still an issue of global importance. Since the introduction of antiretroviral drugs there has been a tremendous reduction in mortality and morbidity of HIV infected persons. However, it has been shown that HIV seropositive patients tend to have more cardiovascular events during long term care (2).

Endothelial dysfunction has shown to be a predictor in the progress of cardiovascular diseases in the general population as well as in HIV infected subjects. Therefore there a vascular assessment was used to show how the endothelium reacts under a hypoxic trigger.

Sub-Saharan Africa is the global hotspot for the HIV and AIDS. Nevertheless there are only but a few studies specifically focussing on that region.

This thesis is written within the framework of the EndoAfrica project, which is currently carried out to provide the missing data for Sub-Saharan Africa.

The aim of this thesis is to discuss whether antiretroviral therapy is harmful, potentially harmful, neutral, or even a protection for the endothelial cells in the population of South Africa.

Due to ethical constraints, this thesis only presents two cases/ patients: one with HIV and another one on HIV and ART. I will compare these two cases and elaborate on the question of whether the antiretroviral drugs have worsened the endothelial function of the respective patients.

The EndoAfrica study will produce important results for a better understanding of the influence of ART and HIV on the cardiovascular system and thus help achieve better long term care for people with HIV in Africa.

### 3 Materials and Methods

The case studies that are presented here are part of a larger ongoing EndoAfrica project (32). Briefly, the project is a prospective, multicentre study involving co-operations between institutions in the Ivory Coast, Kenya, South Africa, Belgium and Austria. Its objective is to collect data related to cardiovascular risk in HIV positive people, with and without treatment. The study design is a cohort study with two groups of HIV positive individuals, either with or without ART and one HIV negative control group. The participants were selected using strict inclusion and exclusion criteria. Inclusion criteria are: The participants have to be 18 years or above, must not be pregnant and mothers must be at least 3 months postpartum to be eligible for the study. Once the patients have been chosen as subjects for the study, they are classified into one of the cohorts depending on their HIV status and on whether if they are on anti-retroviral therapy. Following this, the patients are assessed for traditional cardiovascular risk factors and biomarkers of cardiovascular disease and will be followed up 18 months after determination of the baseline. This is intended to yield comparable data on the progression/regression of the cardiovascular disease in these participants. The examination consists of retinal imaging, flow mediated dilation (FMD) at the forearm, urine and blood chemistry including creatinine, glucose level, HbA1c, GGT, blood CD4+ cell count, viral load of HIV and C - reactive protein. For reasons of convenience this thesis just deals **with the FMD and the Lab results** and only with **two compared case studies** from a clinic in Worcester, a small town located about one and a half hours from Cape Town. The case studies are based on the general data sheet, which is completed whenever a patient is recruited and contains information on many main influences that could appear. (32)

#### Overview of the two case studies

Both of the cases reported here involved a common assessment protocol. This is summarized in the following sections.

### *3.1 Examination*

At the beginning the weight and height of the patients was recorded and the doctor filled out a general data sheet for both participant.

The participants were asked to fill out a form which includes factors like age, sex, ethnicity, smoking- and drinking habits, physical activity and menstruation status (for women).

Next the doctor also measured the blood pressure and the heart rate three times and documented these parameters.

Then medical history was documented, including former heart attacks, T.B., stroke, present high blood pressure (hypertension), heart diseases, high cholesterol, and any other long lasting health problems. There was also an extra field for diabetes where the participants were asked about type, onset and management of the condition.

The family history was also documented regarding heart diseases, stroke diabetes, high blood pressure and high cholesterol. The patient was also asked about family planning e.g. if there was a baby younger than 3 months, current pregnancies, breast feeding or plan to have a baby.

Another big issue was the patients' drug history. There was an extra field for Beta blockers, statins, aspirin, Calcium channel blockers, ACE inhibitors and anti-inflammatory drugs because they could affect the CVS.

Last but not least the patients were asked about their social status which means employment and income of the household and if they received social grant.

#### *3.1.1 Blood collection and measurements*

To determine the lab parameters a phlebotomy was performed by the nurse. There are several tubes, but for this thesis only the results of the EDTA, the NaF and the serum tube were needed

### 3.2 Flow mediated Vasodilation:

The Flow-Mediated Vasodilation measurement was used for the evaluation of the endothelial reaction under hypoxic conditions (33). Usually the endothelium sets free endothelium-derived nitric oxide (NO), which leads to vascular dilatation. The measurement itself is technically challenging and takes several months of training. The measurement needs a blood pressure cuff, an ultrasound device and a fixation for the ultrasound probe. In these studies the cuff is attached at the forearm and the ultrasound probe is fixed at the upper arm of the participants.

First the artery is observed in a steady position for one minute then the BP cuff is inflated for five minutes. After the deflation the recordings last for another four minutes. (33)

#### 3.2.1 Equipment:

The following devices were needed for a FMD measurement:

An **ultrasound system** equipped with 2-dimensional imaging *with* colour and spectral Doppler, a **probe holder device**



Figure 16: Ultrasound system with ultrasound probe (left) and an ultrasound probe holder device (right)

A **blood pressure cuff** and a **MacBook** with the “Cardiovascular Suite” software

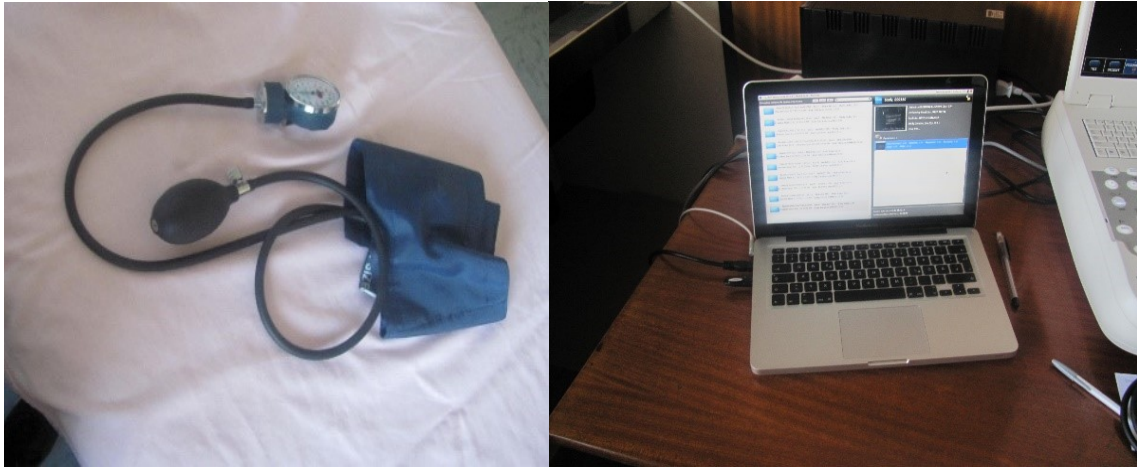


Figure 17: Blood pressure cuff (left) and the used MacBook (right)

### 3.2.2 Procedure:

There are numerous factors which will influence the results of the measurement such as oral intake, alcohol and smoking within the last 4 hours also temperature, or menstruation of a female participant.

The patient was advised to lie down on the examination bed and the blood pressure cuff was placed at the forearm of the straight arm, which had been positioned on the probe holder platform. Then the brachial artery was detected with the ultrasound probe until it was represented as a continuous line on the screen. Therefore the colour Doppler setting of the ultrasound was used to facilitate locating the artery, as is shown in figure 18.

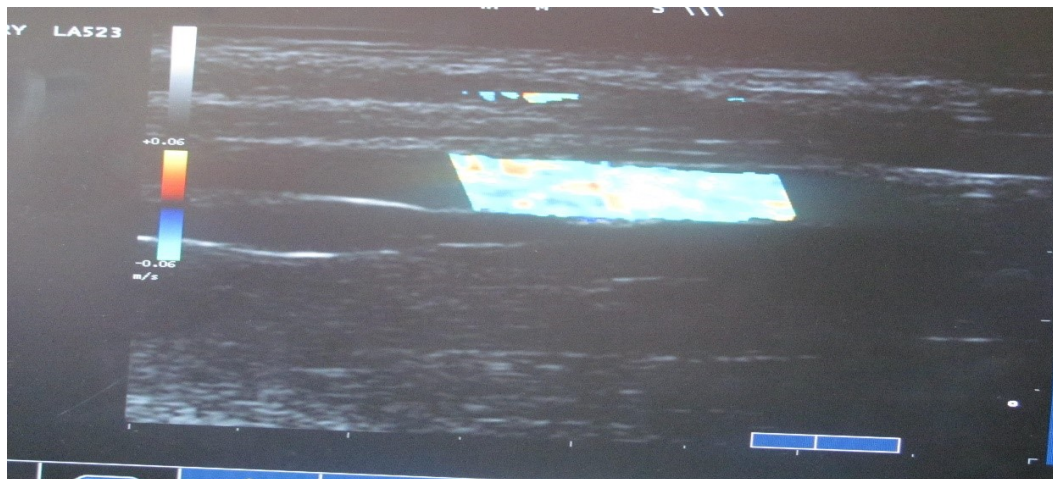


Figure 18: Colour doppler image of the brachial artery

After the fixation of the probe the operating area was set as shown in Figure 19.



Figure 19: Prepared setting of the patients' arm

The ultrasound device was plugged into the MacBook to read the data into the “**Cardiovascular Suite**” program, which measured the diameter of the artery over time.

The **settings** of the **ultrasound device** were normalized as followed for the described cases:

- Musculo skeletal application
- Depth 3 cm
- Steer: maximum right.
- Frequency 6.6. MHz
- PRF (Pulse repetition frequency): 6.7
- Pulse wave mode,
- SV size 1, angle +60,

When the settings were set and the image was clear, as shown in figure 20, the program “Cardiovascular Suite” was started on the MacBook.

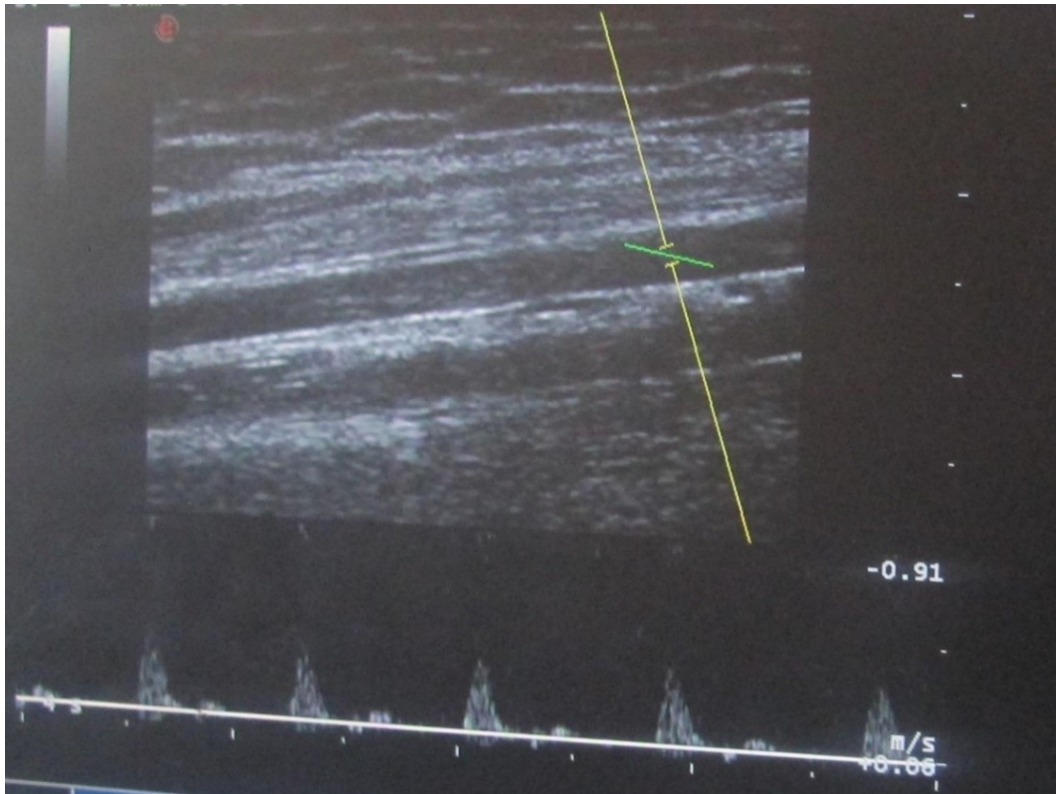


Figure 20: Pulse wave image of the brachial artery

**“Cardiovascular Suite” settings:**

The recording time field of the program was completed as follows:

- Baseline: 60 seconds
- Ischemia: 300 seconds
- Vasodilation: 120 seconds

In this protocol the shear rate detection was turned on.

**Calibration of the x and y axis:**

For these measurements the x- and y axis were calibrated by using a 4 seconds example of the Doppler flow image of the artery as described in the manual of the Cardiovascular Suite. The region of interest (ROI) was set on the artery where the measurement took place.

Then a new study was launched and the data sheet was filled out with the identification code for each participant.

Before starting the measurement it was important to ensure that the participant had rested for at least 5-10 minutes to prevent any additional stimulus of the sympathetic system. After a minute the software reminded the examiner to inflate the blood pressure cuff over the systolic pressure for creating a hypoxia. This pressure was maintained for 5 minutes when the cardiovascular suite software signalled again to deflate the cuff. Approximately 30 seconds after deflation the artery reached the point of maximum dilation which is an important value of the measurement (32,33).

## 4 Results

This thesis will compare and discuss two cases from different cohorts in terms of their endothelial function and their cardiovascular risk, one with untreated HIV and one with HIV under antiretroviral therapy. In the following, the two observed cases are presented.

### 4.1 Case One

The first case was a 28-year-old female of mixed race infected with HIV. She did not receive any kind of anti-retroviral treatment. Her viral load was 66.867 copies/mm<sup>3</sup> and her CD4+ cell count was 310 cells/mm<sup>3</sup>. She was a smoker, drank alcohol on weekends and was physically minimally active. The highest level of graduation was the High school matrix, she was full time employed and her household received a social grant.

On the date of the examination the participant was menstruating, had fasted in the morning and had smoked 3-4 hours before the examination. The patient was not on any medication that could potentially affect the cardiovascular system.

Her waist to hip ratio was 0.9 and the Body mass index (BMI) was 48.3 kg/m<sup>2</sup>. The blood pressure values were 127/95; 127/91 and 116/90 with a heart rate of 98; 98 and 92 beats per minute.

In the blood chemistry the total cholesterol with 4.80 mmol/L and the LDL cholesterol with 2.64 mmol/L were fairly elevated. The HDL cholesterol with 1.94 mmol/L and the triglycerides with 0.47 mmol/L were within a good range.

The creatinine in her blood was 74 µmol/L and within normal range and the eGFR was >60 ml/min/1.73 m<sup>2</sup>. Her HbA1c in percentage was 5.3%, fasting glucose 4.7 mmol/L and the C - reactive protein 4 mg/L.

The flow mediated vasodilation measurement was carried out. Her baseline diameter was 4.10 mm and her maximum diameter was 4.62 mm. The percentage of the FMD was 12.56 %.

## 4.2 Case Two

The second case was a 46 year old female of mixed race infected with HIV and on first line ART. Her viral load was 14.190 copies/mm<sup>3</sup> and her CD4+ cell count was 225 cells/mm<sup>3</sup>. She was a smoker and drank alcohol on weekends. The patient was physically, moderately active more than three times a week. Her highest level of education was primary school and she was unemployed and looking for a job at the time of the examination. She lived with her family who received social grant.

On the date of examination the patient had fasted before the visit. Her waist to hip ratio was 0.87 and the BMI 29.5 kg/m<sup>2</sup>. The blood pressure values were 150/101; 151/103 and 139/95 with a heart rate of 77; 72 and 71 beats per minute.

In the blood chemistry the total cholesterol was 4.53 mmol/L and the LDL was 3.16 mmol/L. The HDL cholesterol was 0.69 mmol/L and the triglycerides were 1.49 mmol/L.

The creatinine was 57 µmol/L, the eGFR was >60 mL/min/1.73 m<sup>2</sup>. Her HbA1c in percentage was 5.7%, fasting glucose was 4.5 mmol/L and the C reactive protein was 12 mg/L.

In the immunology the patient had a viral load of 14.190 copies/mm<sup>3</sup> and her CD4+ cell count was 225 cells/mm<sup>3</sup>.

The Flow mediated vasodilation was carried out in line with the examination. Her baseline diameter was 3.30mm, maximum dilation was 3.50mm. The percentage of the FMD was 6.14%.

## Comparison of the two cases

Table 3 below shows the anthropometric, biochemical parameters and the FMD values of both cases.

<i>Table 3</i>		
	<b>CASE ONE</b>	<b>CASE TWO</b>
<b>COHORT</b>	HIV	HIV + HAART
<b>AGE</b>	28y	46
<b>BMI</b>	48.3 kg/m <sup>2</sup>	29.5 kg/m <sup>2</sup>
<u>HIV IMMUNOLOGY</u>		
<b>VIRAL LOAD</b>	66,867 copies/mm <sup>3</sup>	14,190 copies/mm <sup>3</sup>
<b>CD4+ CELL COUNT</b>	310 cells/mm <sup>3</sup>	225 cells/mm <sup>3</sup>
<u>LIPIDS</u>		
<b>TOTAL CHOLESTEROL</b>	4.80 mmol/L	4.53 mmol/L
<b>LDL</b>	2.64 mmol/L	3.16 mmol/L
<b>HDL</b>	1.94 mmol/L	0.69 mmol/L
<b>TRIGLYCERIDES</b>	0.47 mmol/L	1.49 mmol/L
<u>BLOOD PRESSURE/HEART RATE</u>		
1st	127/95mmHg	150/101mmHg
2nd	127/91mmHg	151/103mmHg
3rd	116/90mmHg	139/95mmHg
<u>FMD</u>		
<b>DBASE</b>	4.10 mm	3.30 mm
<b>DMAX.</b>	4.62 mm	3.50 mm
<b>FMD%</b>	12.56 %	6.14 %

Table 4 presents the standard treatment for antiretroviral drugs in South Africa. Patient two is under first line therapy namely Efavirenz + Emtricitabine + Tenofovir as a combination of two NRTIs and one NNRTI.

Table 4

TREATMENT REGIMEN IN SOUTH AFRICA

**1<sup>ST</sup> LINE THERAPY:**

EFAVIRENZ (600mg) + EMTRICITABINE (200mg) + TENOFOVIR (300mg)

**2<sup>ND</sup> LINE THERAPY:**

ZIDOVUDINE (300mg) + LAMIVUDINE (300mg)

OR LOPINAVIR (400mg) + RITORNAVIR (100mg)

## 5 Discussion

Our data show that there is an impact of HAART on the endothelial function, as seen in the poor FMD response of 6.14% in the patient on the HAART. In comparison, the patient without treatment showed a much higher FMD value (12.56%). The baseline vascular diameter in-patient on HAART was 30 mm smaller as compared to the patient without HAART (4.10 mm). These results suggest a significant endothelial damage in patients on HAART.

Both patients are females, from the same ethnicity and have a similar socio-economic status. Even though the data are only from two case studies in females, it would be interesting to examine males in the future. Literature shows that healthy men until the age of 70 have a higher baseline vessel diameter, but lower FMD percentages than healthy women (34). The authors of this study speculate that there is a higher rate of subclinical atherosclerosis in men that seems to affect the endothelial response.

Ethnic differences have also been reported to play a role in microvascular function. Caucasians seem to have a higher baseline diameter and a higher maximum response than people of mixed ancestry, who in turn have a higher response than the black population. The percentage of the microvascular response was the highest in the mixed ancestry population and the lowest in Caucasians (35,36). Current studies have provided evidence that there are ethnic differences in arterial stiffness and vascular responses to the release of acetylcholine (Ach) and sodium nitroprusside (SNP) (35,36). In the two case studies presented in this work, no such influences are expected as the test subjects had the same ethnic background and sex was similar in both subjects.

There was an age difference of 18 years between the two test subjects. Aging has been reported to be linked with impaired endothelial-dependent dilation (27), which is suggestive of endothelial dysfunction and is an independent factor of cardiovascular risk. It is assumed that the aging related endothelial impairment is strongly linked to a decreased NO production (37), which could affect the extent of vascular dilation. These data are derived mainly from healthy women who are

older than 50 years (37,38). As HIV infected persons show worsening of traditional risk factors of cardiovascular diseases, and the patient of antiretroviral therapy was only 46 years old, her poor FMD could have arisen due to accelerated vascular aging due to HAART (27).

Smoking is known to be a risk factor for cardiovascular diseases. Since both of the patients are smokers, this most certainly affects their endothelial function (25). The patient on HAART is older than the HIV naïve patient, which means it is very likely that she had been smoking for a longer time than the other patient at the date of examination. In this respect, cigarette smoking would have had a bigger influence on the FMD result of HIV patient on HAART than in the HIV naïve patient.

A high oestrogen level is known as a predictive factor for CVD. Therefore post-menopausal women have a higher cardiovascular risk. It has shown that pre-menopausal women have a better lipid profile than postmenopausal women (39). Both the women included in our study are pre-menopausal. Nevertheless, menstruation in premenopausal women has been reported to influence the blood flow and could therefore have an impact on the results of the FMD measurement. (40). In our study, the HIV naïve patient was menstruating during the measurements, which could have affected the results. Therefore, in future females should be studied in similar phases of the menstrual cycle.

Compared to the patient on HAART - who claimed to exercise regularly at a moderate level - the HIV naïve woman is physically minimally active. Unsurprisingly, the HIV naïve patient had a higher BMI value of 48.3 kg/m, compared to the patient on HAART with a BMI of 29.5 kg/m<sup>2</sup>. However, both women are overweight. Indeed based on the WHO criteria (41), the patient on HAART can even be classified as pre-obese while the HIV naïve patient as obese class 3. As outlined above, obesity is a risk factor for atherosclerosis and cardiovascular disease; therefore this needs to be taken into account in both patients.

In the patient without treatment the viral load was three times as high as in the patient on HAART. Lower viral load in the HIV patient taking HAART could be due

to the ingestion of antiretroviral medication. Although the CD4+ cell count is very low in both patients, it is noticeably lower in the patient on HAART. This is paradoxical as antiretroviral drug therapy should lead to an increase in the number of CD4+ cells. One reason for the poor CD4+ cell count could be a discontinuation of the treatment arising from a lack of compliance or reduction in efficacy of the HAART therapy due to possible concomitant effects of any other drugs this patient may be taking (34). Another possible reason for the lower CD4 counts may be a late initiation of the treatment, as prior to 2015 HAART in South Africa was only started with a CD4+ cell count of <200 cells/mm<sup>3</sup> or upon a stage 4 AIDS-defining illness (43,44). Therefore, it is possible that the longer untreated influence of the virus could have led to an impaired recovery of CD4+ cells. Furthermore, the longer untreated period could have damaged the endothelium even prior to the commencement of HAART and thus affected the results of this study.

Both patients have relatively high total cholesterol levels, but with a different distribution of other lipid parameters. The HIV naïve patient had LDL levels of 2.64 mmol/L, while the patient on HAART had levels of 3.16 mmol/L. The triglycerides levels in the HIV naïve patient and the patient with HAART were 0.47 mmol/L and 1.49 mmol/L, respectively. The protective lipid HDL levels were higher (1.94 mmol/L) in the HIV naïve patient as compared to patient on HAART (0.69 mmol/L). Taken together, these findings suggest that the HIV naïve patient has a better lipid profile in relation to the cardiovascular risk. For instance, higher blood lipid levels and the specific distribution of low HDL and high LDL are known to cause atherosclerosis, which is associated with a thickening of the intimal layer, a loss of elasticity and a smaller lumen of the vessels. (24, 26) Therefore, a far progressed atherosclerotic status raises the risk of thromboembolic events and increases the shear stress, which is known to be associated with endothelial damage (24, 26).

The systolic blood pressure values of the HIV naïve patient are 127, 127 and 116 mmHg and can be considered to be in the normal to pre-hypertensive range. The diastolic blood pressure values, however, of 95, 91 and 90 mmHg are in hypertension class 1 (45) range. In the patient on HAART the systolic and diastolic blood pressure values (150/101, 151/103 and 139/95 mmHg) are even higher and can be classified into hypertension grade 1 to 2 (45). As mentioned above, the

higher blood pressure of the HIV patient on HAART predisposes her to a higher cardiovascular risk, and this can be associated with endothelial dysfunction, which, in turn, can influence the FMD measurement (31).

The patient on HAART is on first-line regimen of South Africa's antiretroviral programme. This consists of a combination therapy of the NNRTI Efavirenz, and the two NRTIs Emtricitabine and Tenofovir. The NRTI class of drugs are especially known to cause, because of their mitochondrial toxicity, several adverse effects on the cardiovascular system. These include lipoatrophy as well as increases in lipid and lactate levels (17,18). Emtricitabine can specifically cause effects such as hypertriglyceridemia and neutropenia and fatty degeneration of the liver. Efavirenz causes an increase in the transaminases; it therefore seems to affect the liver (17,18). In summary, these effects could have had an impact on the blood lipid parameters, blood pressure and, therefore, the FMD result of the patient on HAART (31).

### *5.1 Conclusion*

The comparison of these two cases suggests that HAART has an impact on the endothelial function in mixed race HIV infected women in South Africa. The possible effects on the blood pressure and the blood fat values of the antiretroviral drugs could have led to the poor FMD result of the patient on HAART as compared to the HIV naïve woman. Nevertheless, there are several other factors, such as age difference and the phase of the menstrual cycle that could also have influenced the results. The influence of these factors on flow mediated dilatation should be investigated in further studies.

### *5.2 Limitations*

There are some limitations of the studies reported in this thesis. Firstly, due to the limited number of samples presented here, it is indeed difficult to generalize these data to HIV naïve patients and those receiving HAART. However, the strength of this study is that despite presenting only two patients with HIV, clear differences can be seen between naïve patients and those receiving HAART. The pilot

evidence generated can serve as a basis for planning a full-blown comprehensive study involving more patients.

Another shortcoming is that there is no follow-up data that would show the progress of the endothelial dysfunction in these patients. Cross-sectional data do not yield not so much information as data taken in the same persons over time. Longitudinal data collection in patients allows assessment of the time course of the changes in the vascular endothelium (that is, in HIV patients' with and without HAART therapy).

Despite the limitations of this work, it should be mentioned here that this thesis is written within the framework of the *EndoAfrica* study. This currently on-going study involves measurements in over 450 HIV patients, with and without HAART. The EndoAfrica project will definitely yield new knowledge related to the effects of HIV on the cardiovascular system and on how HAART modulates this risk in Sub-Saharan populations (32). Thus, in the future there might be better screening for cardiovascular risk stratification in HIV infected patients, including the question of how HAART could act as an independent risk factor for cardiovascular diseases. Such knowledge would allow doctors to intervene at an early stage of the disease and reduce the burden of cardiovascular diseases in these particularly vulnerable populations.

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