DIPLOMARBEIT

HOW DOES HIV AFFECT THE VASCULAR RESPONSES?

CASE STUDIES WITHIN THE FRAMEWORK OF THE ENDOAFRICA PROJECT.

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ABSTRACT

Background: Cardiovascular disease and HIV/AIDS have been reported to be leading causes of death worldwide in the last decade. HIV is known to influence and accelerate the progression of endothelial dysfunction and cardiovascular disease through multiple pathological pathways. Through the constant improvement of HIV treatment, which now delays the onset of AIDS, the research focus has shifted from the prevention of opportunistic infections to the understanding of mechanisms that contribute to the associated co-morbidity of HIV, especially cardiovascular diseases. Although sub-Saharan Africa is the region most affected by HIV/AIDS, only limited research has been done in this region, this is especially the case for research on the effects of HIV on the cardiovascular system.

Objectives: Endothelial dysfunction has been found to be an early precursor of cardiovascular diseases. However, the extend in which endothelial dysfunction occurs in patients infected with HIV, especially those living in southern Africa, is currently unknown.

Methodology: This diploma thesis analyses two case reports of mixed race South African women, one infected with HIV and one acting as a healthy control. Examined were differences in cardiovascular and endothelial function in the patient and the healthy control. In addition to the traditional cardiovascular risk factors (blood pressure, BMI, serum lipid levels, fasting blood glucose), flow-mediated dilatation was used as a non-invasive measurement of endothelial function.

Results: Both subjects had normal blood pressure, BMI and fasting glucose levels. In the HIV infected patient the FMD measurement values were 3% brachial artery dilatation, whereas in the healthy control the values were 12%. The HIV infected test subject displayed low HDL levels, with otherwise normal lipid levels. Serum lipid levels were normal in the healthy control.

Discussion: The subject with HIV shows an impaired flow-mediated dilatation response. The low HDL and the poor FMD in the HIV patient suggest that the endothelium of the patient is already injured and that she experiences endothelial dysfunction, whereas the healthy control shows no signs of endothelial injury. These unique results from the sub-Saharan region con-

firm the previous observation that HIV affects the endothelium through multiple pathological pathways and leads to cardiovascular diseases.

ZUSAMMENFASSUNG

Hintergrund: Sowohl kardiovaskuläre Erkrankungen, als auch HIV/AIDS waren in der letzten Dekade unter den häufigsten Todesursachen weltweit. Es ist bekannt, dass HIV die Entstehung und das Fortschreiten von endothelialer Dysfunktion und kardiovaskulären Erkrankungen über verschiedene Wege begünstigt. Durch die Entwicklung neuer Behandlungsmethoden und die somit spätere Entstehung von AIDS, hat sich der Fokus der Forschung in den letzten Jahren von der Behandlung opportunistischer Infektionen auf die Untersuchung der zugrunde liegenden Mechanismen zwischen HIV und bestimmten Begleiterkrankungen, im besonderen kardiovaskulären Erkrankungen, gerichtet. Obwohl das südliche Afrika die am meisten betroffene Region ist, wurde die Auswirkung HIVs auf das kardiovaskulär System in dieser Region noch nicht ausreichend erforscht.

Ziel: Endotheliale Dysfunktion ist ein frühes Anzeichen für kardiovaskuläre Erkrankungen. Allerdings ist nicht bekannt in welchem Ausmaß endotheliale Dysfunktion in HIV infizierten Patienten im südlichen Afrika vorkommt.

Methoden: Diese Diplomarbeit analysiert zwei Fallberichte südafrikanischer Frauen, von denen eine an HIV erkrankt ist und die andere als gesunde Kontrollperson dient. Untersucht wurden mögliche Unterschiede im kardiovaskulär System und bei der endothelialen Funktion. Zusätzlich zu den klassischen kardiovaskulären Risikofaktoren (Blutdruck, BMI, Serumlipidspiegel, nüchtern Blutzucker) wurde die flussmediierte Dilatation (FMD) als nicht invasive Messung der endothelialen Funktion genutzt.

Ergebnisse: Beide Probandinnen hatten einen normalen Blutdruck, BMI und nüchtern Blutzucker. Die HIV infizierte Probandin hatte einen FMD Wert von 3%, die gesunde Probandin einen Wert von 12%. Die HIV infizierte Probandin hatte bis auf einen niedrigen HDL Wert, normale Serumlipidspiegel. Bei der gesunden Probandin gab es keine Auffälligkeiten beim Serumlipid.

Diskussion: Die HIV infizierte Patientin zeigt eine verminderte Reaktion auf die flussmediierte Dilatation. Der niedrige HDL und der schlechte FMD Wert der HIV Patientin sind Zeichen einer endothelialen Schädigung und einer bereits bestehenden endothelialen Dysfunktion, wohingegen die gesunde Probandin keine Zeichen für eine Schädigung des Endo-

thels hat. Diese Ergebnisse aus dem südlichen Afrika bestätigen vorausgegangene Beobachtungen, dass HIV über unterschiedliche Wege das Endothelium schädigt und die Entstehung kardiovaskulärer Krankheiten begünstigt.

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ACRONYMS

AIDS	Acquired immunodeficiency syndrome
ART	Anti-retroviral therapy
BMI	Body mass index
BP	Blood pressure
CCR5	C-C chemokine receptor type 5
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
cGMP	Cyclic guanosine monophosphate
cGTP	Guanosine-5'-triphosphate
CRP	C-reactive protein
CXCR4	C-X-C chemokine receptor type 4
DNA	Deoxyribonucleic acid
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
ESCRT	Endosomal sorting complexes required for transport
FMD	Flow-mediated dilation
gp120	Glycoprotein GP120
gp41	Glycoprotein 41

HDL High-density lipoprotein

HIV Human immunodeficiency virus

HLA Human leukocyte antigen

IL-1 Interleukin-1

INSTI Integrase strand transfer inhibitor

KS Kaposi's sarcoma

LDL Low-density lipoprotein

MAP Mean arterial pressure

MHC Major histocompatibility complex

mRNA Messenger RNA

NF-κB Nuclear factor kappa-light-chain-enhancer of activated B cells

NNRTI Non-nucleoside reverse transcriptase inhibitor

NO Nitric oxide

NRTI Nucleoside and nucleotide reverse transcriptase inhibitor

PCP Pneumocystis pneumonia

PI Protease inhibitor

PWV Pulse wave velocity

RNA Ribonucleic acid

ROS Reactive oxygen species

SIV Simian immunodeficiency virus

tRNA Transfer RNA

VLDL Very-low-density lipoprotein

vWF Von Willebrand factor

WHO World Health Organization

1 INTRODUCTION

1.1 HIV

The human immunodeficiency virus (HIV) is a lymphotropic lentivirus of the retrovirus family, which causes HIV infection and acquired immune deficiency syndrome (AIDS). HIV infects CD4 cells, in particular CD4 helper T cells, but also macrophages and dendritic cells. After an infection and a period of latency the immune system weakens and the body becomes predisposed for opportunistic infections.[1]

There is currently no cure for an HIV infection, but since its discovery in the early 1980s treatment has evolved greatly. With the correct antiretroviral treatment the life expectancy was prolonged many years and the infection has become manageable.
Since its discovery HIV has become a pandemic and although HIV is found on every continent, Sub-Saharan Africa is the most affected region with 66% of all HIV patients living there.[2]

1.1.1 *Discovery and History*

In the beginning of the 1980s the spread of a previously unknown disease was discovered in the United States. The symptoms included Pneumocystis pneumonia (PCP) and Kaposi's sarcoma (KS) a rare form of skin cancer. PCP as well as KS were usually found in severely immunodeficient patients, but were now being observed in otherwise healthy, young males. Due to the fact that most of the patients were homosexuals the term GRID 'gay-related immune deficiency' was formed.[3, 4]

A task-force was set up by the Centers for Disease Control and Prevention (CDC), which soon discovered that most of the patients were not only homosexuals but also intravenous

heroin users, Haitians or hemophiliacs the term ‘4H’ disease was coined. When it became obvious that all parts of the population could be infected by the new disease, the term ‘acquired immune deficiency syndrome’ or AIDS was formed.[5]

In 1983 HIV (type I) was first isolated from a tissue sample of an AIDS patient by a group of scientists led by Françoise Barré-Sinoussi and Luc Montagnier. The findings were confirmed in 1984 by Robert Gallo, who isolated the same virus from an AIDS patient. The virus was later (re)named HIV - ‘Human immunodeficiency virus’.[6]

In 2005 a group of scientists succeeded in linking HIV to the simian immunodeficiency virus (SIV). Different SIVs are found in the chimpanzee and gorilla populations of Western Africa. It is assumed that between 1900 and 1930 SIV was transmitted from a chimpanzee to a human, in which it first mutated and was then transmitted to other humans. This was the origin of the O-group of HIV-1. There have been more instances where SIV was transmitted from apes to humans (HIV-2, different groups of HIV-1).[7]

Through the newly colonized Belgian Congo HIV was able to spread to the bigger cities in the region around Kinshasa (formerly Leopoldville) and from there on to the rest of Africa and the world. The oldest recorded case where HIV was found, was a tissue sample from a Congolese man that was taken in 1959.[8, 9]

1.1.2 *Classification*

There are two different types of HIV, HIV type 1 and HIV type 2. HIV-1 can further be classified into the groups M (major), N (new), O (outlier) and P. The M-group of HIV-1 is the most common one with more than 90% of all HIV infections. It can further be divided into sub-types A to K. Whereby different sub-types are more common in certain regions of the world. Sub-type A is most common in West Africa, sub-type B in Europe and America and sub-type C in Southern and Eastern Africa and India. HIV-2 is subdivided into 8 groups (A to H), in which A and B are epidemic and mostly confined to West Africa.[10]

1.1.3 *Epidemiology*

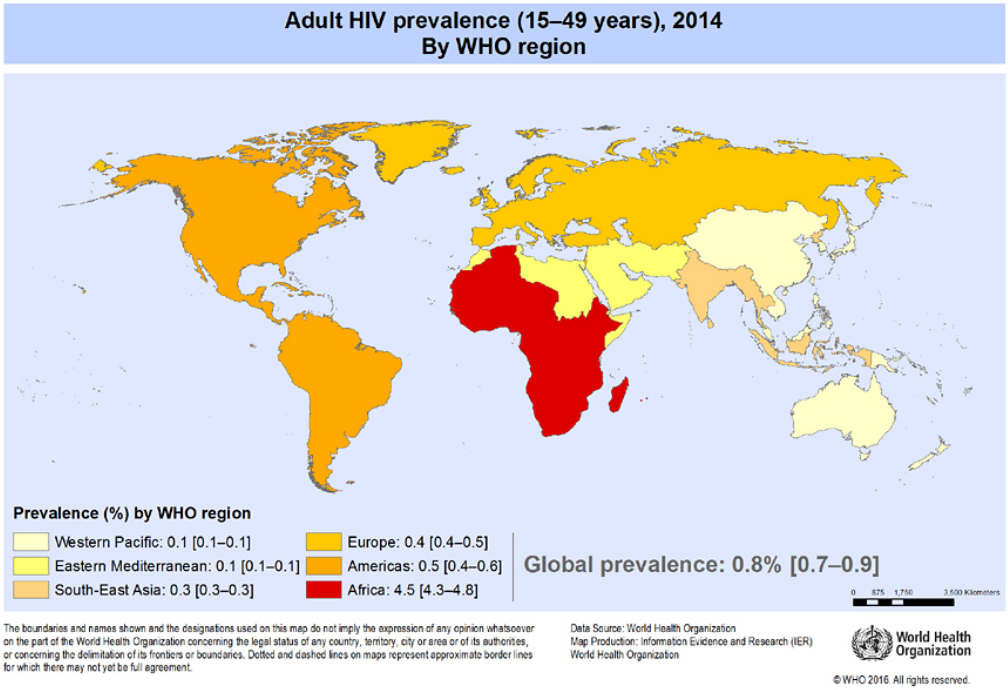

Figure 1: HIV prevalence in adults (15-49 years) in 2014 reproduced from: `http://www.who.int/gho/hiv/hiv_013.jpg?ua=1`

No region of the world has been spared by the HIV pandemic. Until now 39 million people have died of AIDS and 78 million have been infected worldwide. In 2014 36.9 million people were living with HIV globally, two million became newly infected with HIV and 1.2 million died from AIDS-related illnesses. In March 2015 15 million people had access to antiretroviral therapy.[2]

In 2014 there were 25.8 million people living with HIV in Sub-Saharan Africa of which two million are children, 1.4 million became newly infected with HIV and 790000 died of AIDS-related diseases. In this region 10.7 million were accessing antiretroviral therapy, which is 41% of all infected.[2]

1.1.4 *Structure and genome*

HIV is a lentivirus of the retroviridae family. HIV particles are roughly spherical and 100 nm to 120 nm in diameter, which is big for a virus, but still much smaller than an erythrocyte (7500 nm). The virus is covered with a lipid membrane double layer which originates in the lipid layer of the host cell. Spikes consisting of the glycoproteins gp120 and gp41 are built into this envelope. These spikes are of importance for the virus during the process of penetrating the host cells, as described later.[11, 12]

Table 1: HIV - Gen to protein modified from Levinson, 2010

Gene	**Encoded Proteins**	**Protein function**
Typical retroviral genes		
gag	p24, p7	Nucleocapsid, (important serologic markers of infection)
	p17	Matrix
pol	Reverse transcriptase	Transcription of RNA genome into DNA; ribonuclease H activity for degradation of RNA before synthesis of double-stranded proviral DNA
	Protease	Cleaves precursor polypeptides
	Integrase	Integration of viral DNA into host cell DNA
env	gp120	Attachment to CD4 protein; high mutation rate
	gp41	Fusion to host cell
Regulatory genes for replication		
tat	tat	Activation of transcription of viral genes
rev	rev	Transport of late mRNAs from nucleus to cytoplasm
"Accessory" regulatory genes		
nef	nef	Decrease of CD4 and MHC class I proteins on surface of infected cells,inducing death of uninfected cytotoxic T cells
vif	vif	Enhancement of infectivity by inhibiting APOBEC3G action
vpr	vpr	Transport of viral core from cytoplasm into nucleus in non-dividingcells
vpu	vpu	Enhancement of viral release from the cell

Inside the lipid membrane and its adjoining matrix lies the capsid, which contains the viral genome in two single strain molecules of RNA, reverse transcriptase and integrase. The viral genome consist of 9.2 kb base pairs which contain nine genes gag, pol, env, tat, ref, nef, vif, vpr and vpu. Sometimes a tenth gene tev, a fusion of tat, env and ref can be found. The genes

gag, pol and env code for structural proteins of the virus. The other genes code for regulatory proteins that influence, among other things, the virus's ability to infect cells and replicate.[11, 12]

1.1.5 *Tropism and Replication cycle*

The primary receptor for the virus is the CD4 antigen, which is a surface protein of macrophages and helper T lymphocytes. The first step toward infection of a cell is when the gp120 envelope glycoprotein of the HI-virus binds to a CD4 receptor of one of these cells. After this initial step a change in conformation of gp120 allows it to interact with a chemokine co-receptor, either CCR5 or CXCR4. After this the gp41 initiates the fusion of the virus particle and the cell. Differences in the gp120 glycoprotein determine which co-receptor is used.[1, 11]

During this process, the capsid which holds the genome of the virus, is released into the cytoplasm of the host cell. Here, the reverse transcriptase comes into action. First it frees the viral single stranded RNA from the attached matrix proteins and afterwards converts it through multiple complicated steps into a complementary DNA molecule.[13]

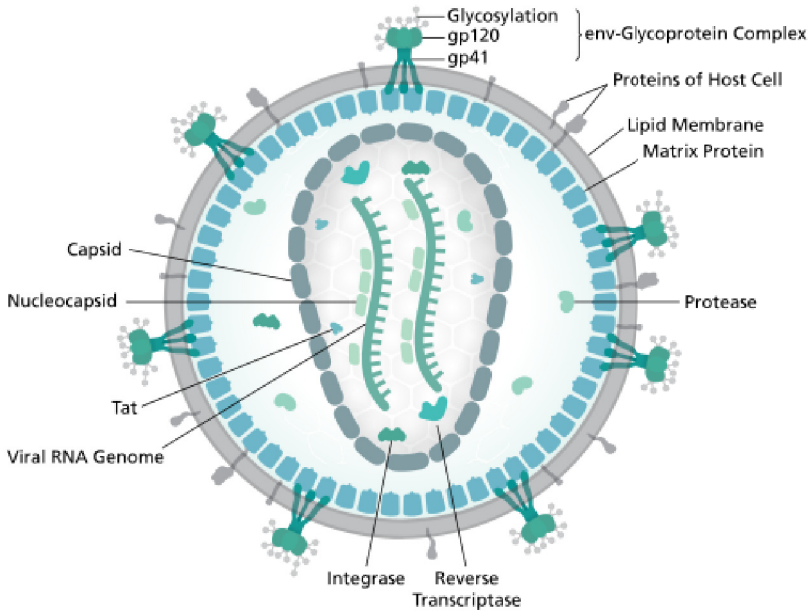

Figure 2: HI-Virus reproduced from `https://upload.wikimedia.org/wikipedia/commons/thumb/c/c4/HIV-virion-structure_en.svg/512px-HIV-virion-structure_en.svg.png`

Due to the fact, that reverse transcriptase has no proofreading ability, the mutation rate during the replication process is high and therefore leads to the ability of the HI-virus to de-

velop drug resistances and escape the immune response. The newly formed double stranded viral DNA is then transported into the cell nucleus and inserted into one of the host's chromosomes by the viral enzyme integrase. At this point the cell becomes a permanent carrier of the viral genome.[14]

Only one percent of the cells infected with HIV actively express viral RNA, the rest of the infected cells are latent. Because these latently infected cells don't express viral proteins the immune system can not recognize and neutralize them. Some of these "sleeper cells" have a very long life expectancy (memory T cells) and therefore a lifelong treatment with antiviral drugs is needed.[11]

A transcription factor is needed to activate the dormant viral DNA. The most common transcription factor is NF-κB which is normally found in the cytoplasm, but is transported to the nucleus when the cell is activated. NF-κB then activates the HIV transcription.[11]

The viral DNA is transcribed into mRNA and then spliced. Only the doubly spliced (2-kb) RNA is able to exit the nucleus and to be translated into the proteins tat and rev or nef. The regulator of expression of virion proteins (rev) is able to enter the nucleus via nuclear pores and is then able to bind to viral mRNA (unspliced 9-kb and single spliced 4-kb) and export the RNA to the cytoplasm where it is translated into proteins. The single spliced RNA codes for the proteins vif, vpr, vpu or env. The unspliced RNA codes for the proteins gag and gag-pol.[11]

The activator of transcription (tat) highly increases the amount of transcription of the viral DNA and therefore the amount of viral RNA produced by the cell. Both of these proteins tat and ref are essential for the replication of the virus, because they increase the transcription and regulate the transport of viral mRNA.[11]

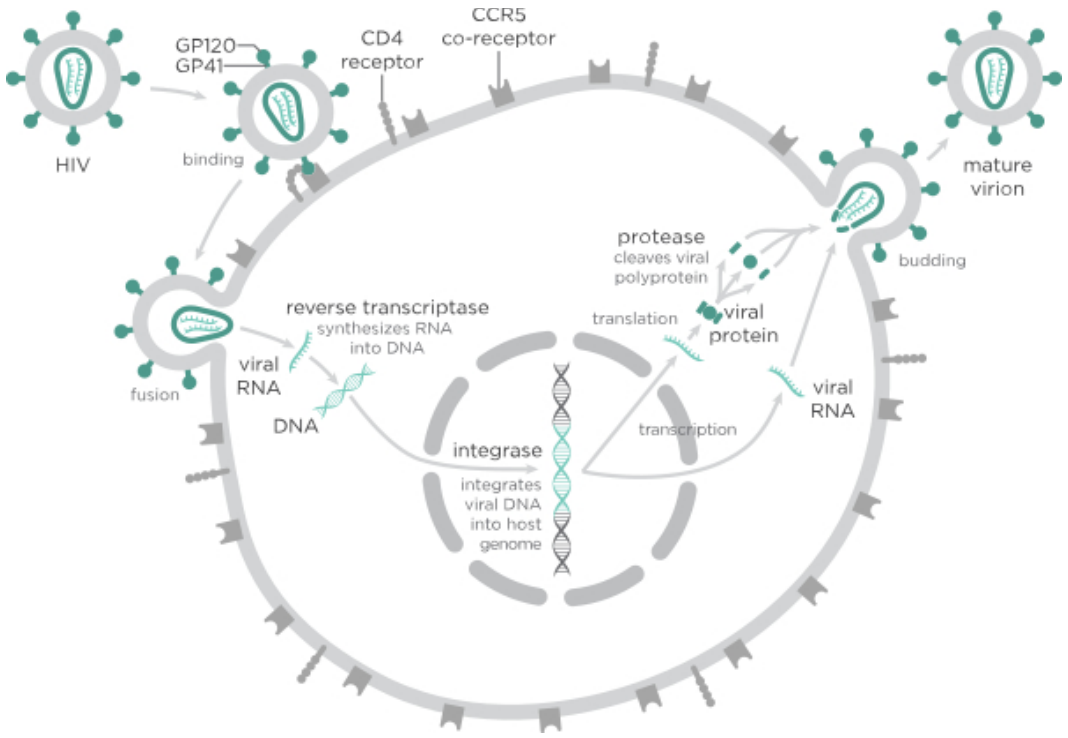

Figure 3: HIV replication reproduced from: https://upload.wikimedia.org/wikipedia/commons/5/51/HIV-replication-cycle.svg

1.1.6 *Assembly and Release*

To become an infectious virus the different HIV components have to be assembled, packaged and finally released from the cell via budding to then undergo the process of maturation.

The assembly process takes place near the cell's plasma membrane on the inside of this membrane all necessary particles (two copies of the complete viral RNA, tRNA, gag polyprotein, envelope protein and the viral enzymes reverse transcriptase, integrase and protease) are gathered.[14]

The gag polyprotein coordinates the necessary steps of the assembly. These steps include the binding to the plasma membrane, protein-protein interactions to form a spherical protein envelope out of 2500 gag molecules, and the packaging of the genomic RNA. [15]

During "budding" the virus uses the cell's own endosomal sorting complexes requiered for transport (ESCRT) mechanism, which normally regulates cellular abscission and multiple other cellular functions. The second assembly step takes part during budding or shortly after,

the gag polyprotein is cleaved by the viral protease and through this modification the capsid becomes conical and the virus becomes infectious.[14]

1.1.7 *Spread in vivo*

There are two different pathways in which HIV can infect new cells and spread through the body. The first way is the cell-free spread. Hereby the virus is replicated inside an infected cell and is then released into the blood or extracellular fluid, where it can infect other CD4+ cells.
The second way is the cell-to-cell spread. An HIV infected T helper cell can transmit the virus to another T helper cell via a direct link by forming a virological synapse.[16]

1.1.8 *Transmission*

HIV is transmitted through contact with infected body fluids. Blood, sperm, vaginal secret but also breast milk and liquor cerebrospinalis have been found to be infectious. Possible entry points of the virus are lesions on mucous membranes such as the vaginal or anal mucous membrane, but also the membranes itself. The most common way of infection is through unprotected vaginal or anal intercourse.[1, 14]

The risk of sexual transmission is dependent on multiple factors, not only the viral infectiousness and the viral load but also the type of sexual contact and the current state of the immune system play an important role.[1, 14]

Genital ulcer and venereal disease are known to increase the risk of infection up to fivefold and are therefore an important cofactor for an infection. HIV infected pregnant women can also transmit the disease to their child. This type of transmission is called "vertical transmission". It occurs during pregnancy, delivery and afterwards through breastfeeding. The rate of infection is dependent on the viral load of the mother. Up to 50% of children are infected in untreated mothers, whereas the infection rate drops to 1% or less if mother and child are treated with antiretroviral drugs. [1, 14]

Table 2: HIV transmission risk recreated from: www.uptodate.com/contents/image?imageKey=ID/60145&source=graphics_search&rank=0&search=hiv+transmission

Exposure route		**Risk per 10,000 exposures to an infected source**
Blood-borne exposure	Blood transfusion	9000 (9/10)
	Needle-sharing injection drug use	67 (1/150)
	Percutaneous needle stick	23 (1/435)
	Mucous membrane exposure to blood (eg, splash to eye)	10 (1/1,000)
Sexual exposure	Receptive anal intercourse	138 (1/72)
	Insertive anal intercourse	11 (1/900)
	Receptive penile-vaginal intercourse	8 (1/1250)
	Insertive penile-vaginal intercourse	4 (1/2500)
	Receptive or insertive penile-oral intercourse	0-4
Other	Biting, spitting, throwing body fluids (including semen and saliva), sharing sex toys	Negligible

1.1.9 *Classification*

To classify the clinical stages of HIV infections two systems are used. Both were developed in the 1980s and had to be adapted several times. The WHO "disease staging system for HIV infection and disease" and the CDC "classification system for HIV infection". Both systems use the CD4+ T cell count and clinical symptoms and diseases. Although these systems have some differences, it is still possible to compare them.

Table 3: HIV infection stages from CDC and WHO

CD4+ cell count / mm³	WHO stage	CDC stage
>500	stage 1	stage 1
350 - 499	stage 2	stage 2
200 - 349	stage 3	stage 2
<200	stage 4 (AIDS)	stage 3 (AIDS)

Both systems also include a list of AIDS defining diseases, as soon as these diseases are developing, the patient is directly classified as stage 3 or 4, even if the CD4 cell count is higher than 200 cells/mm³.[1, 17, 18]

1.1.10 *Clinical Stages*

In HIV infected patients three stages can be seen. The first stage is the acute infection, which occurs about three weeks after the contraction of the virus. It presents like an influenza like illness, with fever, malaise, headache and weight loss, symptoms are often misinterpreted and can sometimes be completely absent.[12]

This primary stage lasts about four weeks. During this time the virus spreads through the body and many copies of the virus can be found in the blood. In this clinical stage the patient is highly infectious. Towards the end of the acute infection there is a strong immune response and the virus level in the blood decrease again. After this primary stage the infection enters the clinical latency. The latency period can last form two to twenty years, but is normally around eight years. In the beginning there are commonly no physiological symptoms, but there can be psychological problems if the patient knows of the infection. During the latency the amount of CD4 cells drops constantly and towards the end of the latency phase symptoms such as fever, weight loss and diarrhea are characteristic.[12]

After the latency phase untreated patients progress to AIDS, the "acquired immunodeficiency syndrome". As described above, the CD4 cell count drops below 200/mm³ and/or the patient develops one or more AIDS defining diseases, such as pneumocystis carinii pneumonia, tuberculosis or kaposi's sarcoma. Also the patient develops viremia, as the copies of the virus in the blood increase drastically.[12]

1.1.11 *Diagnosis*

There are currently different options to diagnose an HIV infection, but to confirm an infection there has to be a positive result of at least two different testing methods.

The screening test for HIV that is routinely used is the enzyme-linked immunosorbent assay (ELISA) method. This test detects antibodies against HIV in a serum sample. Modern screening tests are 99% specific, but to verify a positive result a western blot test is always

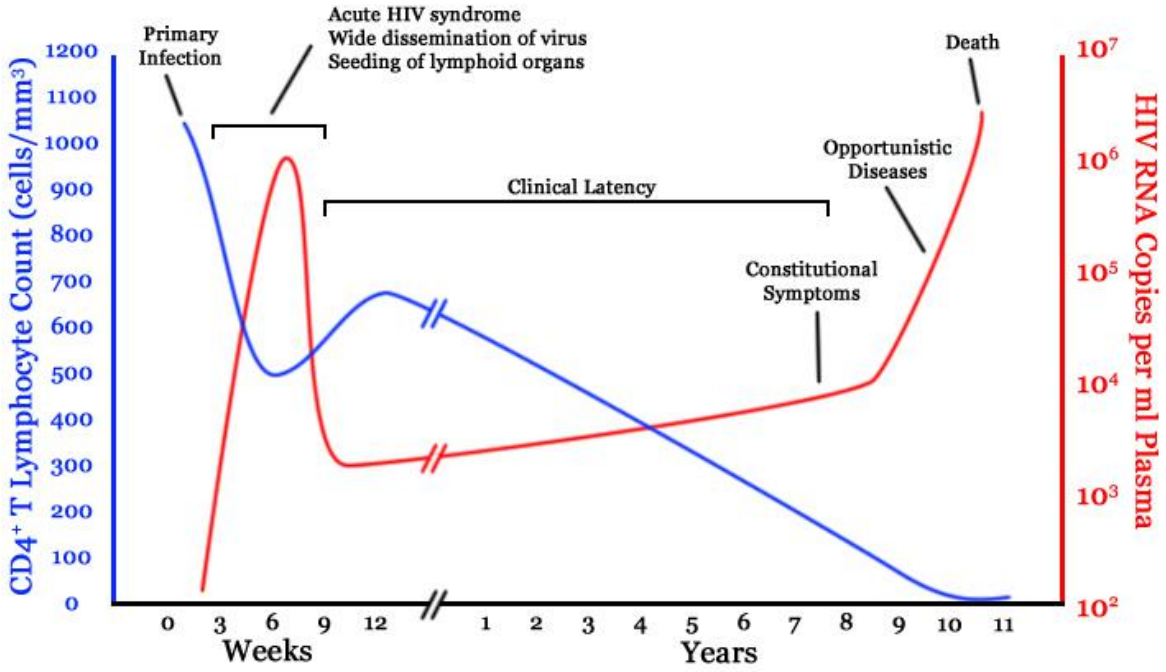

Figure 4: HIV Phases reproduced from: `https://upload.wikimedia.org/wikipedia/commons/thumb/0/0e/Hiv-timecourse_copy.svg/2000px-Hiv-timecourse_copy.svg.png`

required. The western blot method is able to detect antibodies against different HIV proteins and can also differentiate between HIV-1 and HIV-2.[19]

Both tests have a diagnostic window, which means that they are not able to detect an infection between the transmission and the seroconversion when the body's immune response produces the first antibodies. This serological window is test specific, third generation antibody test can detect an infection about 25 days after the transmission.[19]

1.1.12 *Prevention*

There are three ways of HIV transmission sexual intercourse, exposure to infected blood and perinatal transmission. For all these kinds of transmission a different prevention method is needed.

The risk of transmission during sexual intercourse is greatly dependent on the type of intercourse. While receptive anal intercourse has the greatest risk of infection with one trans-

mission for 72 sex acts, oral intercourse has the lowest risk with zero to four transmissions per 10000 sex acts (see table 2).

To prevent transmission condoms have proven to be up to 95% effective. Especially if the HIV status of one partner is unknown or they engage in high risk behavior, condoms should be used.[20]

To prevent an HIV positive patient from spreading the virus the next best method, after the consequent use of condoms, is an effective antiretroviral therapy (ART) to reduce the viral load in the patient and reduce the risk of spreading the infection. Due to the high viremia during the acute infection and the late-stage disease (AIDS) it is important to diagnose the infection as early as possible to prevent the spread of the virus during the acute infection stage. If the viremia is below 200 copies/ml the risk of infecting a sexual partner is very low. Studies have shown, that for heterosexual males circumcision can reduce the risk of contracting HIV by up to 60%.[21–23]

This could be caused by the high density of Langerhans cells and macrophages in the foreskin, which are target cells for HIV. For women the risk of contracting HIV is not reduced if the partner is circumcised.[24]

To prevent perinatal transmission the infected mothers should receive antiretroviral therapy to reduce the viral load. After birth the child should receive antiretroviral postexposure prophylaxis for six weeks.[25]

For patients that have been exposed to HIV mucosal or parenteral, it is possible to reduce the risk of infection during the first 72 hours with postexposure treatment with ART. In addition to these medical and biological prevention methods counseling of at-risk populations and behavioral changes in high-risk populations play a big role in preventing the spread of HIV.[26]

1.1.13 *Treatment*

The treatment of HIV is a complex and important topic on its own and will only be discussed briefly in this thesis. After a patient has been diagnosed with HIV, the urgent question, when to start treatment, has to be discussed. At the moment the Department of Health and Human Services in the United States and the International Antiviral Society both recommend to start treatment as soon as possible. This recommendation is even more important for pregnant women, to prevent the transmission to the unborn child and for patients with an acute symptomatic HIV infection. These patients have shown to have a much faster progression to AIDS and it is assumed that they will have the biggest advantage from an early treatment.[27, 28]

Another big advantage of an early treatment, during the first six month of an infection is the decreased risk of transmission. During the beginning of the infection the viral load is much higher than during the latency phase and this high viral load is associated with high risk of transmission.[29]

Some models show that up to 50% of new infections are caused by acutely infected patients, though this percentage depends on the studied population. If patients with acute infections are treated earlier, preferably directly after diagnosing the infection, the viral load could be lowered in these individuals and the risk of transmitting HIV to an uninfected sexual partner decreased.[29–31]

When it is not possible to treat an individual diagnosed with HIV right away, either because of health care limitations in resource poor countries or because of individual reasons, the best way to proceed is monitoring the CD-4 cell count of the patient. If the CD-4 cell count drops below 350 cells/mm^3 it is highly recommended to start the treatment right away.[32]

A standard regimen of antiretroviral therapy (ART) consist of two different nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs) and either one integrase strand transfer inhibitor (INSTI), one protease inhibitor (PI) or one non-nucleoside reverse transcriptase inhibitor (NNRTI). For all these classifications there are a multitude of different substances

to choose from, which will not be discussed here.[32]

To choose the right regimen of drugs it is advised to test the virus for drug resistances and to adapt the drugs to these resistances as it has been shown that up to 18% of treatment naive patients are infected by a virus with at least one major drug resistance.[33]

The patient's CD-4 count, comorbidities and personal choices (especially for a one pill a day regimen) should be regarded while choosing the right drugs. Also if the patient is positive for the HLA-B*5701 allele, Abacavir should be avoided, due to a hypersensitivity associated with this allele.[34]

1.2 THE CARDIOVASCULAR SYSTEM

1.2.1 *The Heart*

The heart is the central organ of the cardiovascular system. It is a muscular organ, which works as a force and suction pump to maintain the blood flow throughout the body. The heart is located between the sternum and the spine in the mediastinum. Anatomically it can be divided into the “right heart”, which supplies blood to the pulmonary circulation and the “left heart”, which supplies blood to the systemic circulation. Both, the “right heart” and the “left heart” contain an atrium and a ventricle.

The heart is protected by the pericardium, a sac which encases the heart and attaches it to the mediastinum. The walls of the heart are built from three different layers, epicardium, myocardium and endocardium. The supply of oxygen and nutrients to the heart and the removal of metabolic wastes is managed by the coronary circulation, which consists of coronary arteries and cardiac veins.[35, 36]

1.2.2 *The Blood*

Blood has many different functions in the body. First it provides oxygen and nutrients to all cells of the body and secondly it removes metabolic waste and carbon dioxide from these cells. Furthermore, it is responsible for the distribution of hormones and the protection of the body against invading organisms.[36]

An average adult has roughly 5 liters of blood, which is about 7% of the body weight. The blood itself is composed of 55% plasma and the remaining 45% are composed of different kinds of cells.[37]

Most of these cells are "red blood cells" or erythrocytes, which are able to take up oxygen in the capillaries of the lung and release it in other tissues in exchange for carbon dioxide, which is transported back to the lungs.[36]

The second class of blood cells are “white blood cells” or leucocytes. These cells are part of the immune system and can be further divided into granulocytes, lymphocytes and mono-

cytes. During an inflammation those cells are able to migrate into the inflamed tissue. A third kind of cell in the blood are thrombocytes, which are the smallest blood cells and play a vital role in hemostasis.[36]

The plasma itself consists to 95% of water and contains electrolytes, glucose, hormones, dissolved proteins and also a part of the carbon dioxide, that is expelled through the lungs.[36]

1.2.3 *The Vasculature*

The vascular system is basically a network of tubes, consisting of two types, arteries and veins, in a multitude of different sizes and functions.[38]

Arteries transport the blood away from the heart and towards every last corner of the body. Veins bring the blood back to the heart to start the cycle over again. In between those main vessels is a third kind of tube the capillaries. Because arteries and veins are too thick to establish sufficient transport of nutrients and oxygen into the tissue, capillaries, whose walls are thin enough for this task, are switched in between those two to assure the supply of all tissues.[38]

Arteries can be divided into two groups, systemic arteries, which transport blood away from the heart to the body, and pulmonary arteries, which carry deoxygenated blood from the heart towards the lung for re-oxygenation.[38]

Histologically arteries consist of three different layers. The outermost layer is the tunica externa, which is composed of collagen and anchors the artery in the surrounding tissue. The tunica externa in arteries is also supported by an elastic membrane, which is not found in veins.[39]

The middle layer is the tunica media, which consists of smooth muscle cells and elastic tissue. Small arteries contain the least elastic tissue and only one to four layers of plain muscle fibers, which are arranged into lamellæ. Larger arteries, like the carotid and iliac artery, contain more elastic fibers, which also form lamellæ.[40]

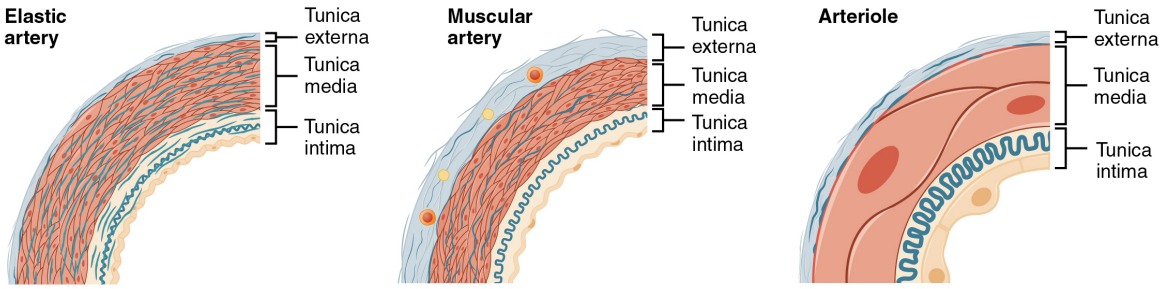

Figure 5: Cross section of an artery reproduced from: https://cnx.org/resources/f0a964f91ecd3ef9cf0f0323f66d5d266f9d71b9/2103_Muscular_and_Elastic_Artery_Arteriole.jpg

The largest arteries, like the aorta and the brachiocephalic artery, contain the most elastic tissue, which gives them the ability to function as a pressure reservoir. During the heart's ejection period the pressure causes them to stretch and recoil, once the ventricles are relaxing, forcing the blood towards the smaller arteries.[39, 40]

On the lumen of the artery the tunica intima is located. This inner layer consist of three parts. The endothelium is in direct contact with the blood and consists of only one layer of endothelial cells. The subendothelial layer consists of connective tissue and the outermost layer is the elastic or fenestrated layer. This layer varies in thickness dependent on the size of the blood vessel. It consists of elastic fibers, which form a network and can have a membranous character.[39]

Veins are composed of the same three layers as arteries, but the thickness of each layer is different due to the different function of veins. The tunica intima and the tunica media of veins are much thinner compared to arteries of the same size. The tunica externa consists of elastic fibers and collagen and is the strongest layer in veins.[39]

Veins are distensible and can adapt to volume and pressure. Many veins contain valves, especially in the limbs, a feature, which does not exist in arteries. These valves, formed by the tunica interna, prevent the backflow of blood and help in the venous return of blood to the heart.[40]

To ensure the sufficient exchange of nutrients and oxygen, all tissues, with a few exceptions, contain capillaries. Those that have high metabolic requirements, such as the brain or kidneys have a more extensive capillary network than tissues with lower metabolic requirements, such as ligaments and tendons. To ease the exchange of substances, capillaries have no tunica media or externa. The capillary walls consist of only one layer of endothelial cells and a basement membrane. On the outside of the capillary pericytes wrap around the endothelial cells.[38]

There are three different types of capillaries, which all fulfill different functions in different kinds of tissues.[38, 39]

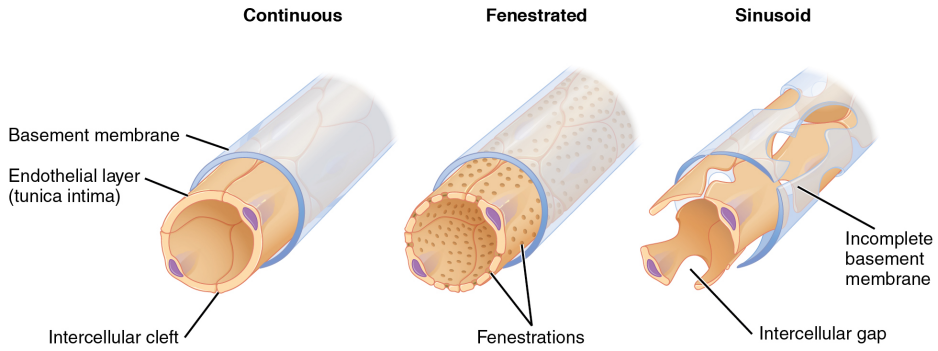

Figure 6: Types of capillaries reproduced from: `https://upload.wikimedia.org/wikipedia/commons/8/8d/2104_Three_Major_Capillary_Types.jpg`

- Continuous capillaries - in which the endothelial cells form a continuous layer with only intercellular clefts in between. This type of capillary is the most common one.
- Fenestrated capillaries - in which the endothelial layer has small fenestrations (pores or holes) that ease the exchange of substances from the blood to the tissue. This type of capillary is found in the intestines and the kidneys.
- Discontinuous capillaries (sinusoids) - in which the endothelial cells form large fenestrations and the basement membrane is discontinuous or absent. This type of capillary is found in the bone marrow and the spleen.

1.2.4 *Functions*

1.2.4.1 *Circulation*

All circulation of blood in the body begins and ends in the heart. It is the pump for both circulatory systems in the body, the pulmonary circulation and the systemic circulation.

The cycle starts with the pulmonary circulation. From the right atrium of the heart the blood is moved into the right ventricle during the diastolic phase of the cardiac cycle. During the systolic phase it is pumped through the pulmonary artery towards the lung capillaries, where oxygen and carbon dioxide are exchanged. Through the pulmonary veins the now oxygenated blood is brought to the left atrium of the heart and the systemic circulation begins. During the diastolic phase the blood enters the left ventricle of the heart and is expelled through the aortic valve into the aorta with the next systole.[36, 40]

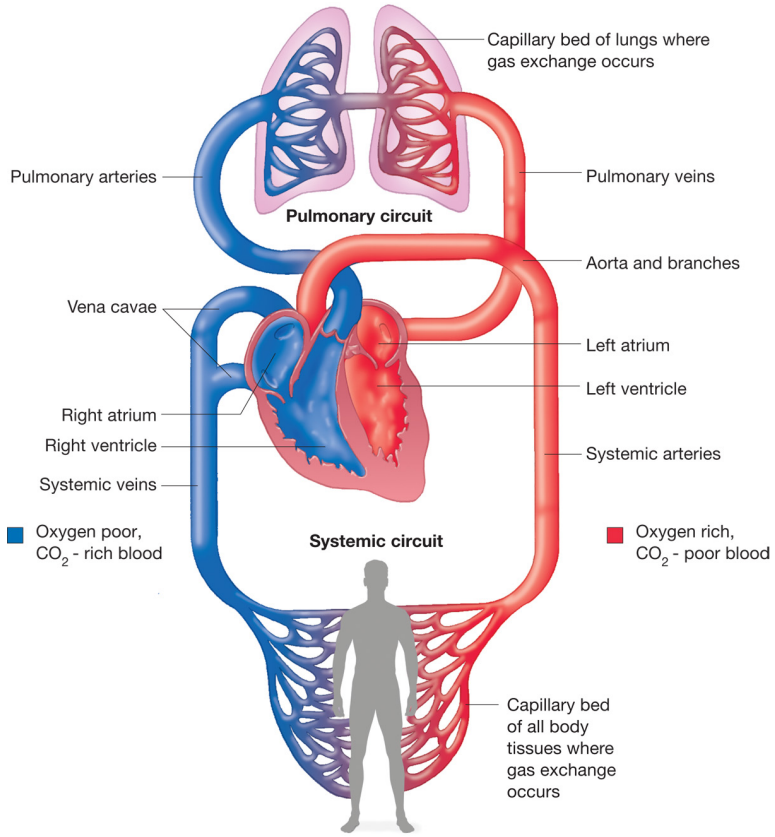

Figure 7: Systemic and pulmonary circulation reproduced from `https://s-media-cache-ak0.pinimg.com/736x/d3/5a/1f/d35a1f4d25707c070663d081927dcde2.jpg`

From the aorta onwards it is distributed through narrowing arteries to every last corner of the body, where the blood finally enters the capillaries and oxygen and nutrients can be exchanged.[36, 40]

After passing through the capillaries the blood flows through a low pressure system of veins, constantly increasing in size, until it enters the upper or lower vena cava and is brought back to the right atrium of the heart, where the pulmonary circulation can start again.[36, 40]

1.2.4.2 *Blood flow*

The overall blood flow in the circulatory system can be determined by the cardiac output of circa 5 liters per minute for a healthy adult. Because the circulatory system is a closed system, the same amount of blood has to flow back to the heart at the same time. [41]

To determine the blood flow in one specific vessel, Ohm's law is used:

$$F = \frac{\Delta P}{R}$$

In which F is the blood flow in liter per second, Δ P is the pressure difference in mmHg and R is the resistance of the vessel in mmHg x min/l. The greater the pressure difference or the smaller the resistance the greater the volume of blood that flows through the vessel.[41]

The aorta for example has a big pressure difference and a small resistance, therefore there is a large volume of blood going through this vessel each minute. A small artery on the other hand has a much lower pressure gradient and at the same time a bigger resistance, due to the smaller diameter, thus the blood flow is much lower.[41]

To calculate the velocity of the blood in a given vessel, the following formula can be used:

$$v = \frac{F}{A}$$

In which v is the velocity in meter per second, F is the blood flow in liter per second and A is the diameter of the vessel in square meter. We can see that the velocity is dependent on the amount of blood flow and on the size of the vessel at the same time. [41]

The smaller the vessel, the faster the blood has to flow to reach the same volume in the same amount of time, compared to a bigger vessel.

If we look at the overall diameter of the cross-section area of all the blood vessels in the body by type of vessel, as seen beside the text, we note that the cross-section area of the big arteries is much smaller than the cross-section area of the capillaries. If we put these numbers into the formula above, we can see, that the blood velocity is the fastest in the aorta (0,3 m/s) and the slowest in the capillaries (0,0003 m/s).[38, 41]

Vessel	Cross-Section Area (cm^2)
Aorta	2.5
Small arteries	20
Arterioles	40
Capillaries	2500
Venules	250
Small veins	80
Venae cavae	8

The body has to be able to regulate the blood flow towards the tissue to ensure oxygen and nutrients in the tissue during times of high demand. The heart is able to increase the cardiac output by up to five times, but this is not enough to supply certain tissues, that may need up to 30 times more blood during high metabolic use.[38, 41]

The body is therefore able to regulate the blood flow directly towards the tissue. This is made possible within seconds to minutes by vasoconstriction and vasodilatation of arterioles and precapillary sphincters, this will be described in detail later.[41]

There are two types of blood flow in the vessel. The first is the laminar or streamline flow and the second, the turbulent flow. During laminar flow the blood moves at different speeds inside the vessel. The outer lamina moves the slowest, due to high friction near the vessel wall. Towards the middle of the vessel, the blood moves faster and the fastest flow takes place in the center. Laminar flow occurs, when the blood flows through a smooth, straight vessel at a continuous rate.[38, 41]

Turbulent flow occurs when the blood passes an obstruction in the vessel, a section with a rough surface or the vessel takes a sharp turn. Turbulent flow is characterized by blood flowing crosswise in the vessel and forming whorls. These whorls are called eddy currents. During turbulent flow the resistance is much higher than during laminar flow.[41]

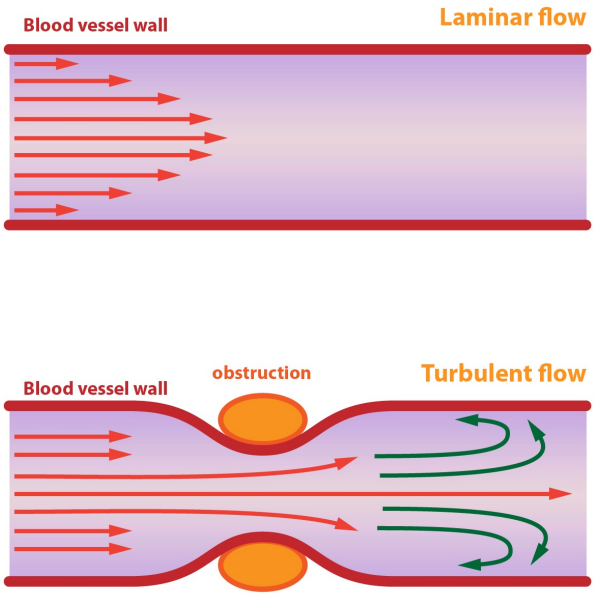

Figure 8: Laminar flow and Turbulent flow reproduced from `http://fblt.cz/wp-content/uploads/2013/12/Kapitola-10-02-ENG-03.jpg`

Eddy currents are more likely when the blood flows with a high velocity and the diameter of the vessel is large.[41]

1.2.4.3 *Blood pressure*

Blood pressure is the force that the blood exerts on the surrounding vessel. It is measured in millimeter of mercury (mmHg) or torr. The arterial blood pressure consist of two values, systolic blood pressure and diastolic blood pressure.

The systolic blood pressure, the highest pressure reached in the arteries, is measured during the ejection phase of the heart. The diastolic blood pressure is measured when the arterial blood pressure is at its lowest point during the systolic phase of the heart while the aortic valve is still closed. The optimal systolic blood pressure lies between 100 and 130 mmHg and the optimal diastolic blood pressure lies between 60 and 90 mmHg.

Sometimes it is important to know the average arterial pressure or mean arterial pressure (MAP). To calculate the MAP this formula is used:

$$MAP = \frac{1}{3}x(2P_d + P_s)$$

in which P_d is the diastolic pressure and P_s is the systolic pressure.

1.2.5 *The endothelium*

As mentioned before the endothelium is a monolayer of cells which lines all blood vessels in the human body. It is the barrier between organs and the blood and therefore has many different organ specific tasks and functions. This heterogeneity can be explained by looking at the requirements of the different organs. The endothelium of the brain for example consists of tight junctions and a continuous layer to maintain the blood-brain barrier. Whereas the kidneys require a fenestrated continuous layer to allow the filtration of blood. Consequently there are many organ-specific endothelial cell molecules that are just found in endothelium of specific organs. [41, 42]

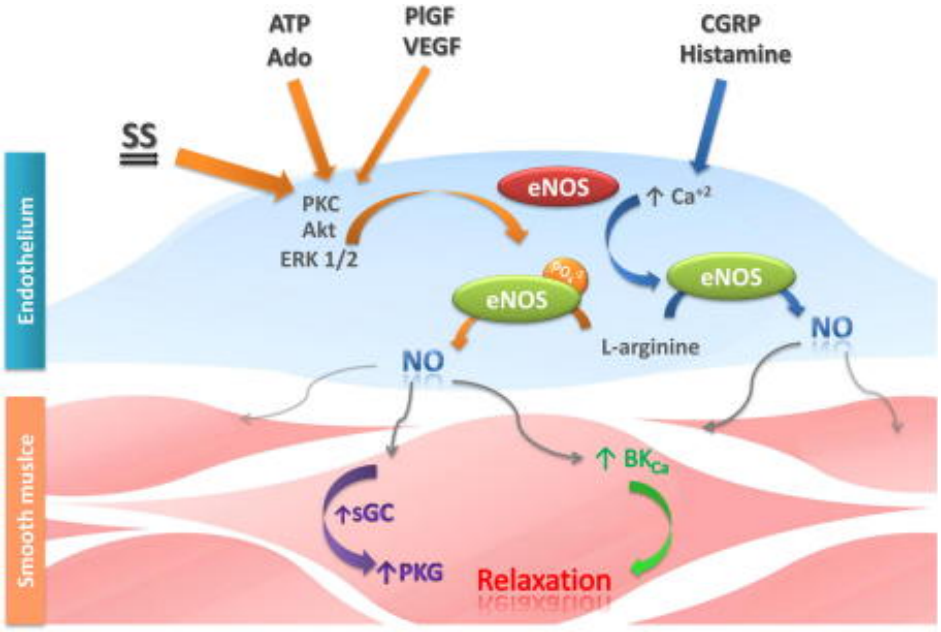

Figure 9: Endothelial functions and shear stress (SS) reproduced from: https://www.researchgate.net/profile/Paola_Casanello/publication/51530026/figure/fig2/AS:195684520206342@1423666233564/Nitric-oxide-dependent-vasodilation-in-the-placenta-Vasodilator-effects-of-NO-in.png

One of the important functions of the endothelium is the regulation of the vascular tone. Endothelial cells are able to synthesize several substances, which have an effect on the contraction or relaxation of the smooth muscle cells surrounding the blood vessels.[41, 42]

When blood flows through the arteries and arterioles the endothelial cells experience shear stress. This stress causes ion-channels in the luminal membrane to open, so that Ca^{2+} can enter the cell. Inside the cell Ca^{2+} activates endothelial derived nitric oxide synthase enzymes (eNOS), which are able to synthesize nitric oxide (NO) from arginine.[41, 42]

NO is a lipophilic gas with a very short half-life in the blood of about six seconds, which affects only the surrounding tissue by activating soluble guanylate cyclase in smooth muscle cells. This causes the conversion of guanosine-5'-triphosphate (cGTP) to cyclic guanosine monophosphate (cGMP), which causes the smooth muscle cell to relaxe and thereby vasodilatation.[41–43]

Another important function of the endothelium is the prevention of blood clotting. The endothelial cell has three different ways to achieve this:

- Contact activation of the intrinsic clotting system is prevented by the smooth surface of the endothelial cell.
- Platelets and clotting factors are repelled by the endothelial glycocalyx and thus clotting is prevented.
- Thrombomodulin, a protein which binds thrombin, is attached to the outer membrane of the endothelium. By removing thrombin the clotting process in the vessel is slowed down.

Through these processes the endothelium plays an important role in preventing blood clotting, but can be a weak point at the same time if the endothelial layer is damaged.[41, 42]

1.2.6 *Endothelial dysfunction*

Under physiological conditions the endothelial cell and the endothelium as a whole are in a state of balance. On one side the endothelial cell produces substances which relax the smooth muscle cells, on the other side it produces endothelin which activates the smooth muscle cell and causes constriction. As mentioned before the endothelial cell also prevents blood clotting with its non-adhesive and non-thrombogenic surface.[44, 45]

If the cell's surroundings change, for example by reduced oxygen levels, hypertension or contact with lipids or viruses, the endothelial cell becomes activated. During endothelial activation the cell increases the expression of certain factors and alters the expression of others. Chemokines produced by the activated cell can either increase or decrease vasoconstriction, either procoagulant or anticoagulant factors and a variety of other molecules can be produced. Under physiological conditions the cell can return from its activated state to the balanced steady-state.[44, 45]

When the endothelium is exposed to damaging factors, such as hypertension, hyperlipidemia, toxins, viruses, hyperhomocysteinemia and other influences, it can become chronically injured. In this state the normally regulating functions of the endothelial cell become impaired. The endothelial permeability is increased, and so are platelet aggregation and leucocyte adhesion. In addition, the cell decreases its production of NO which leads to vasoconstriction. This imbalanced state of the endothelium is called endothelial dysfunction. A central aspect of endothelial dysfunction is an impaired vasodilatation due to decrease in NO production.[44, 45]

1.2.7 *Pathophysiology*

Arteriosclerosis is described as the hardening, thickening and loss of elasticity of arterial walls. All sizes of arteries can be affected. There are three different types of arteriosclerosis:

1. Arteriolosclerosis – only the small arteries are affected.
2. Atherosclerosis – only the big and medium sized arteries are affected.
3. Monckeberg's arteriosclerosis – affects mainly the elderly and commonly only the arteries of the extremities.

Of these three types atherosclerosis is the most common one and plays the biggest role in morbidity and mortality. In the western world in the age group from 30 to 65 years, 30% of deaths are related to atherosclerosis and up to 50% in people older than 65 years.[46]

Certain risk factors for the development of atherosclerosis have been found with statistical analyses. These risk factors are associated with an earlier onset of the disease and with a

faster progression. The risk factors can be grouped in primary risk factors and secondary risk factors and also in modifiable and non-modifiable risk factors. Primary risk factors include (among others):

- Increased low-density lipoprotein (LDL) / very-low-density lipoprotein (VLDL)
- Decreased high-density lipoprotein (HDL)
- Homocysteinemia
- Diabetes mellitus and adipositas
- Male gender
- Genetic abnormalities
- History of arteriosclerosis in close relatives
- Elevated serum C-reactive protein (CRP)
- Smoking
- Advanced age

Secondary risk factors include lack of physical activity, stress, adipositas and hyperuricemia among others.[46–48]

For the pathogenesis of atherosclerosis there are different theories. The most recent and most referred to is the “response to injury” hypothesis. In this model endothelial injury leads to a chronic inflammation. Modified lipoproteins and macrophages interact with the endothelium and the smooth muscle cells and cause the lesion to progress.[46–48]

During endothelial dysfunction and injury the endothelial layer becomes more permeable, leukocyte adhesion increases and the surface loses its non-thrombogenic qualities. In this state lipoproteins accumulate in the walls of the blood vessel, especially in the intima, where they aggregate and can become oxidized by free radicals. Cholesterol crystals form and trigger an inflammatory response.[46–48]

Monocytes emigrate from the bloodstream into the intima, where they turn into macrophages. Through scavenger receptors those macrophages then accumulate the oxidized LDL, which they can not completely break down. The macrophages fill up with lipids and turn into foam cells. Both cells release pro-inflammatory factors, such as interleukin-1 (IL-1), which recruit more monocytes from the bloodstream.[46–48]

Through platelet adhesion and activation and from activated macrophages growth factors and chemokines are released, which cause smooth muscle cells to emigrate from the media into the intima.[46–48]

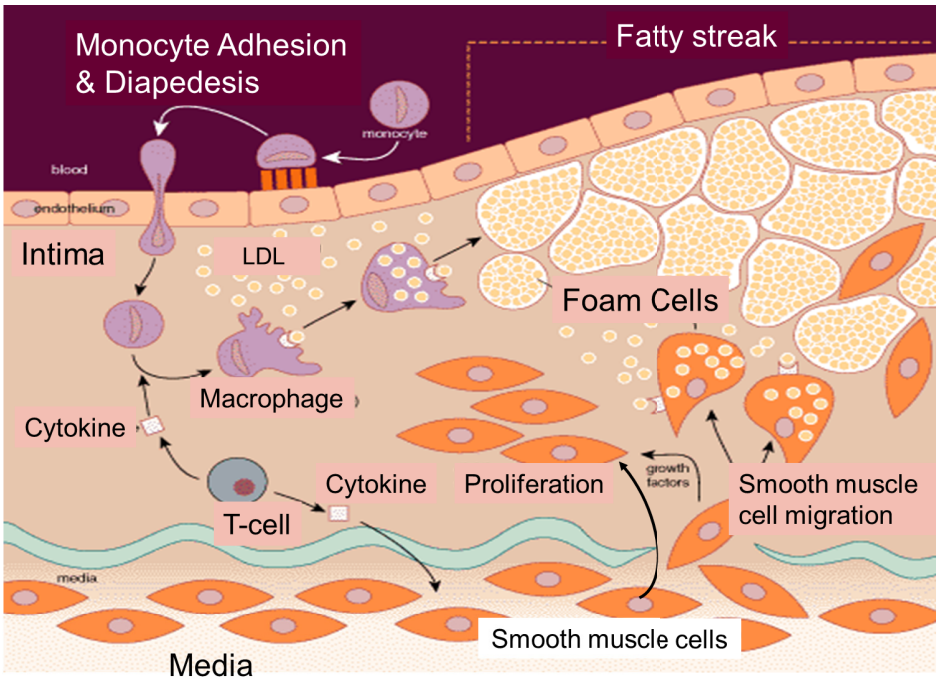

Figure 10: Development of Arteriosclerosis reproduced from: `http://sphweb.bumc.bu.edu/otlt/MPH-Modules/PH/PH709_Heart/FattyStreak2.png`

Those smooth muscle cells are able to engulf lipids, but can also proliferate and produce extracellular matrix (collagen). When foam cells die lipids and necrotic debris remain, which further increase the inflammatory response. In addition to this the proliferation and collagen production of the smooth muscle cells are responsible for the progressive growth of the atherosclerotic lesion.[46–48]

The constant inflammatory process, the remodeling and calcification of the plaque can lead to all kinds of cardiovascular problems for example stenosis of a vessel, thrombotic occlusion or an aneurysm.[46–48]

1.2.8 *HIV and Cardiovascular Disease*

Cardiovascular abnormalities occur in a wide variety in patients infected with HIV. Dependent on age, duration of the HIV infection, CD4 cell count and also duration of ART treatment those abnormalities can include opportunistic infections, myocardial and pericardial disease, left ventricular dysfunction and vascular heart disease. Infectious endocarditis is mostly found in HIV patients which are also intravenous drug users.[19]

Infectious myocarditis is most common in the late stage of HIV infection and is mostly caused by opportunistic infections including Toxoplasma gondii and also mycobacteria and fungi.[19]

Cardiomyopathy with left ventricular dysfunction is more common in HIV patients and can lead to congestive heart failure. Because of the ambiguous clinical symptoms, like fatigue and exertional dyspnea, congestive heart failure is often missed or misdiagnosed.[19]

Studies have shown that patients infected with HIV have an up to 50% higher risk for myocardial infarction, compared to uninfected patients with otherwise identical risk factors. Both an HIV infection and the antiretroviral therapy accelerate atherosclerosis and increase the risk for a myocardial infarction.[19, 49]

1.2.9 *Assessment of Vascular Function*

Vascular function consists of two parts the endothelial function and the function of the tunica media. The endothelial layer, as mentioned before, is responsible for the regulation of the vascular tone, hemostasis and the permeability of the vessel. The tunica media is mainly

responsible for the arterial elasticity.

To evaluate these functions there are different options, blood markers can be used to identify endothelial dysfunction, but there are also invasive and non-invasive diagnostic options.

1.2.9.1 *Non-invasive Diagnostics*

To evaluate the arterial elasticity or to diagnose arterial stiffness the pulse wave velocity (PWV) is used. In young healthy adults the big arteries are very elastic and are able to stretch when the heart pumps blood and the blood pressure is at its maximum. This elasticity results in a slower pulse wave velocity. With progressing age and atherosclerosis, the tunica media loses its elasticity and becomes more and more stiff, resulting in an increase in pulse wave velocity.[50]

To calculate the velocity the time the pulse wave needs to reach two different points with a known distance is measured. These points are normally the right common carotid artery and the right femoral artery. The velocity can be calculated based on the time difference the pulse wave needs to hit those points and the measured distance between them.[50]

To evaluate the endothelial function flow-mediated dilatation (FMD) can be used. One main function of the endothelium is the dilatation of the vessels. Shear stress and different substances in the blood trigger the release of NO, which relaxes the smooth muscle cells and dilates the vessels as described above.[51]

To measure the flow-mediated dilatation the baseline diameter of a superficial artery (commonly the brachial artery) is measured via ultrasound in a longitudinal plane. After this a cuff around the arm is inflated to 50 mmHg above the systolic blood pressure for five minutes to stop the blood flow in the artery. During this time local ischemia builds up and the pH in the tissue changes.[51]

After five minutes the cuff is released and the artery dilates because of the ischemia. This dilatation increases the risk of turbulent flow and induces shear stress, which activates the

endothelium, which therefore causes further vasodilatation.[51]

Shortly after the cuff is opened again the diameter of the now dilated vessel is measured and the two measurements can be compared. FMD is normally expressed as the increase in percent from the baseline diameter. FMD measurements are very dependent on the examiner and the technique, therefore only highly trained personnel should be used for these measurements.[51–54]

As an alternative to this non-invasive measurement of flow-mediated dilatation there is the possibility of triggering the endothelial release of NO by injecting certain substances directly into the artery. Acetylcholine and bradykinin are known to stimulate the release of NO from healthy endothelial cells and cause measurable vasodilatation. In arteries with damaged endothelium and endothelial dysfunction the same substances can cause an adverse effect vasoconstriction. One advantage of this technique is that it can also be used to test the epithelial function in coronary arteries during coronary angiography. Due to its invasiveness this method is not suitable for use in healthy individuals or for the general screening for endothelial dysfunction.[51–54]

1.2.9.2 *Plasma markers*

In order to examine the endothelial function and dysfunction by measuring markers in the blood, two criteria are important. Firstly, the factor has to be tissue specific, which means, only the endothelium is able to produce it, and secondly the factor has to be measurable in a laboratory setting. Regarding these two criteria, von Willebrand factor (vWF), thrombomodulin, E-selectin and endothelin can be used. Because of its very short biological half-life NO can not be used to determine the endothelial function.[55]

Von Willebrand factor is constitutively secreted into the bloodstream and has two functions during blood clotting. VWF is a carrier of the clotting factor VIII and it is able to promote platelet adhesion by binding to subendothelial collagen. Increased levels of vWF can be physiological in some conditions, but have been linked to a faster progression of car-

diovascular disease.[56]

E-selectin plays a vital role in the inflammation process. Its function is to recruit leukocytes to the point of injury. Soluble E-selectin is a marker for endothelial activation, but can not only be found in connection with cardiovascular disease, but also in connection with certain types of cancer.[43, 57]

Endothelin or specifically endothelin-1 is continuously produced by the endothelium and causes vasoconstriction. Physiologically it plays a role in sustaining the basal vascular tone, but increased plasma endothelin levels are linked to cardiovascular diseases, such as heart failure.[56]

2

AIMS AND OBJECTIVES

The human immunodeficiency virus has been known for over 30 years and has been around for a much longer period. From the beginning during the 1980s until the 1990s, when antiretroviral therapy was introduced, the research was mainly focused on slowing the progression of the disease towards AIDS and treating life threatening comorbidities.

The average time between infection and the progression to AIDS was only 7 years and slowly developing diseases were not in the research focus. After the introduction of ART the latency phase increased to over 30 years and much more research on the effects of HIV/AIDS on slowly progressing diseases has been done since.[19]

The pathophysiology of endothelial dysfunction and its progression to other cardiovascular diseases has been studied extensively in uninfected individuals. Many studies have also shown that HIV increases the risk and influences the progression of cardiovascular diseases, but the pathways and mechanisms have not yet been understood in detail.

Endothelial dysfunction has been found to be an early precursor of cardiovascular disease, however it has not yet been extensively studied as a precursor of cardiovascular disease in individuals with HIV, especially those infected in southern Africa.

The aim of this diploma thesis is to compare the cardiovascular functions, risk factors and endothelial function of those living with HIV to uninfected individuals living with the same risk factors and in the same environment. I will achieve this by analyzing data collected in South Africa during the ENDOAfrica project.

Although most of the people infected with HIV live in Africa, only limited research has been done on the influence of HIV on the cardiovascular system in this area. The ENDOAfrica project and this diploma thesis for a small part will seek to improve this lack of research.

3

METHODOLOGY

This diploma thesis is comparing two case studies on the effects of HIV/AIDS on the cardiovascular system. I will analyze two individual cases, both collected during the ENDOAfrica project in South Africa.

The introduction chapter delivers insights into the main subjects of this thesis, the human immunodeficiency virus and the cardiovascular system. For this chapter secondary literature as well as articles were used. Through the libraries of the Medical University of Graz and the University Medical Center Groningen I had access to these books, as well as through the library of the Institute of Physiology at the Medical University of Graz.

The first case report is of an HIV patient that is currently not on antiretroviral therapy and the second case report is of an uninfected individual acting as a healthy control, both case reports were collected in South Africa. After analyzing both cases I will compare them in regard to risk factors and precursors of cardiovascular disease.

The ENDOAfrica project is a prospective cohort study currently being done in South Africa, Kenya and Côte d'Ivoire. The study follows three cohorts for a time period of three years. The first cohort consists of HIV infected individuals which are currently not on ART. The second cohort consists of individuals infected with HIV and currently on first-line ART and the last cohort consists of uninfected individuals acting as a healthy control group.

The cohorts consist of 250 people each, all participants will be tested for HIV antibodies and CD4 cell count at the time of recruitment and after 12 and 24 months. In addition to this a clinical investigation will be done at those points. The clinical investigation includes:

- Medical history and current medication
- Clinical examination

- Screening for traditional cardiovascular risk factors:
  - Blood pressure for hypertension
  - Body mass index and waist circumference for obesity
  - Urine dipstix (glucosuria) and fasting blood glucose for diabetes mellitus
  - Fasting total cholesterol, LDL-cholesterol, triglycerides, and HDL for dyslipidemia
- Blood investigations: hemoglobin, urea and creatinine (renal function)
- Serum collection and storage
- non-invasive endothelial function measurement: Flow-mediated dilatation (FMD)
- non-invasive endothelial function measurement: Retina Camera imaging
- non-invasive personal air pollution exposure measurements

To ensure the comparability of the measurements the blood samples will be analyzed in only one laboratory in each country, although there are multiple collection sites all over these countries.

The FMD measurements will be taken by the exact same equipment on all sites. The machine used is a Esaote MyLab™Five Mobile Ultrasound System. In addition, only one person will take the measurements on each site and all personnel taking FMD measurements will be trained by the same instructor. These measures are taken, to ensure comparable results in flow-mediated dilatation.

To ensure optimal FMD measurements the whole process adheres closely to FMD guidelines. Firstly, all influences and stimuli which could influence the flow-mediated reactivity are eliminated or, if this is not possible, at least brought to the same level.

The test subject should be fasting 8 to 12 hours before the measurements and not smoke 4 to 6 hours before. Medication that is known to be vasoactive should be withheld prior to the measurements, if possible. The examination rooms are temperature-controlled and have the

same temperature during all measurements. [58]

Before the measurements begin the test subject is given some time to rest, so that the brachial artery can reach its baseline diameter.

The patient can sit or lie in a comfortable position with one arm stretched out to take the measurements. The best location for the measurements is the brachial artery. The ultrasonic probe is positioned above the antecubital fossa to image the artery in a longitudinal plane.

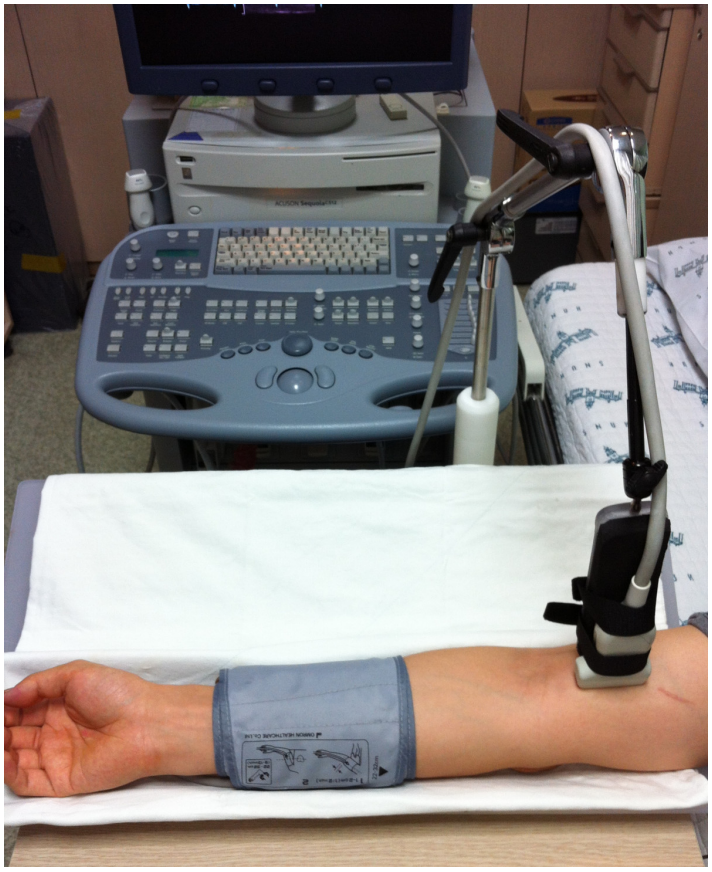

Figure 11: Demonstration of FMD measurement reproduced from: https://static-content.springer.com/image/art%3A10.1186%2F1476-7120-10-39/MediaObjects/12947_2012_Article_439_Fig1_HTML.jpg

Although FMD measurements of the radial artery or the axillary artery are possible, the brachial artery should be used to eliminate variations. In addition, arteries with a baseline diameter of less than 2,5 mm are more difficult to measure.[59]

The blood pressure cuff can be placed around the forearm or above the transducer position. Both placement options provide similar results, but the upper cuff placement is more chal-

lenging for the examiner to handle, due to the collapse of the artery and soft tissue shifting. Therefore the lower cuff placement is recommended.[59]

For the FMD measurement a segment of the artery is chosen, where the anterior and the posterior vessel walls are visible. The border between the intima and the lumen of the vessel should be clearly distinguishable to ensure reliable measurements.[59]

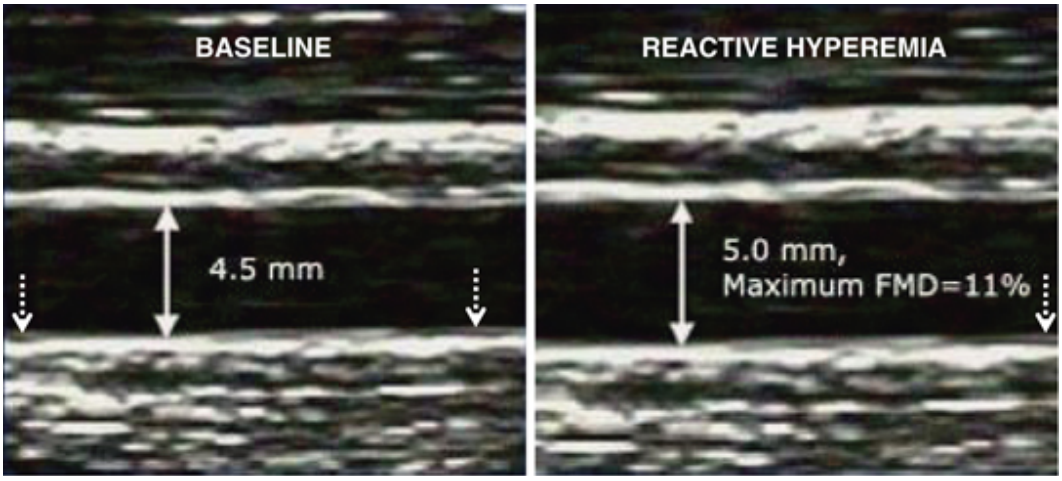

Figure 12: Ultrasound image of brachial artery before and after reactive hyperemia reproduced from: http://www.nature.com/ijir/journal/v22/n2/fig_tab/ijir200959f3.html

The artery must be imaged in a longitudinal plane and the angle of the ultrasonic transducer should be vertical to the artery. These specifications are set in place to make sure the true diameter of the artery is measured.[59]

Anatomic landmarks such as veins and fasciae can be used to maintain an exact position on the artery, in addition to this a stereotactic probe-holding device can be used to hold the ultrasonic transducer in place and the position of the transducer can be marked on the skin with ultrasound gel or a marker.[58, 59]

After a section of the brachial artery has been chosen for the measurements a picture is taken to determine the baseline diameter of the artery. It is important to time the shot of the image with the cardiac cycle of the test subject. All measurements should be done at the same point in the cardiac cycle, favorably during the end-diastolic phase.[58]

After an image has been taken the examiner has to identify the borders between the lumen and the intima of the vessel wall, these points can be marked and measured with the ultrasonic device. To achieve the least variability the average baseline diameter is used by measuring the distance at different points of the segment.[58, 59]

After the baseline diameter has been determined the blood pressure cuff is inflated to 50 mmHg above the systolic blood pressure of the test subject. After 5 minutes the cuff is released and the same section of the brachial artery is located again.

The maximum increase of the artery is reached 60 seconds after the release of the cuff. The image to determine the maximum diameter should be taken at this moment, again timed with the cardiac cycle.[58, 59]

4

RESULTS

4.1 FIRST CASE

The first case is of a 36 year old female of mixed race. She is infected with HIV and does not receive antiretroviral therapy at the time of the examination. Her CD-4 count is 293 cells/mm³ and her viral load 15260 copies/mm³. She smokes, but does not drink alcohol.

On the date of the examination she has a BMI of 20.2 and a waist-to-hip ratio of 0.84. Her systolic blood pressure measurements are 109, 116 and 109 mmHg. And her diastolic blood pressure is 71, 76 and 74 mmHg correspondingly. Her heart rates during the blood pressure measurements are 72, 68 and 70 beats per minute.

The blood-serum analysis provided the following results: her total cholesterol is 2.5 mmol/l, her triglycerides are 1.1 mmol/l, her HDL cholesterol is 0.9 mmol/l and her LDL cholesterol is 1.1 mmol/l.

The creatinine in her blood is 42 µmol/L. Her estimated glomerular filtration rate is above 60 ml/min/1.73m². The fasting glucose level in her blood is 4.0 mmol/l. The inflammatory marker C-reactive protein is 6.5 mg/l.

The Flow-mediated dilatation was measured during the examination. The baseline diameter is 2.62 mm and the maximum diameter after the cuff release is 2.7 mm. The percentage of flow-mediated dilatation is 3%.

Table 4: Results

	Subject 1 (HIV positive)	***Subject 2 (HIV negative)***
BMI	20.2	25.6
waist-to-hip ratio	0.84	0.84
BP1 *mmHg* (**heart rate** *bpm*)	109/71 (72)	110/75 (59)
BP2 *mmHg* (**heart rate** *bpm*)	116/76 (68)	107/79 (59)
BP3 *mmHg* (**heart rate** *bpm*)	109/74 (70)	118/82 (63)
Total cholesterol *mmol/l*	2.5	3.69
Triglycerides *mmol/l*	1.1	0.6
total HDL *mmol/l*	0.9	1.47
total LDL *mmol/l*	1.1	1.94
Creatinine *µmol/l*	42	72
eGFR *ml/min/1,73m²*	60	60
fasting Glucose *mmol/l*	4	4.5
CRP *mg/l*	6.5	5
baseline diameter *mm*	2.62	4.10
maximum diameter *mm*	2.70	4.60
FMD *%*	3.12	12.18

4.2 SECOND CASE

The second case is of a 47 year old female of mixed race living in the Western cape. She is not infected with HIV and serves as a healthy control in the ongoing study. She is a non-smoker, does not drink alcohol and is not physically active.

On the date of the examination she has a BMI of 25.6 and her waist-to-hip ratio is 0.84. Her blood pressure was taken three times with similar results during each measurement. The systolic blood pressure is 110, 107 and 118 mmHg. Her diastolic blood pressure is 75, 79 and 82 mmHg. Her heart rate is 59, 59 and 63 beats per minute during those measurements.

The blood test results of the subject were analysed in the lab. Her total cholesterol is 3.69 mmol/l. Her triglycerides are 0.60 mmol/l. Her HDL cholesterol is 1.47 mmol/l. Her LDL cholesterol is 1.94 mmol/l.

Her blood level for creatinine is 72 µmol/L. Her estimated glomerular filtration rate is above 60 ml/min/1.73m². The fasting glucose level in her blood is 4.5 mmol/l. The inflammatory marker C-reactive protein is 5 mg/l.

The Flow-mediated dilatation was measured during the examination. The baseline diameter is 4.10 mm and the maximum diameter after the cuff release is 4.60 mm. The percentage of flow-mediated dilatation is 12%.

5

DISCUSSION

In this case study of two subjects, it was observed that the subject with HIV shows an impaired flow-mediated dilatation response. The low HDL and the poor FMD in the HIV patient suggest that the endothelium of the patient is already injured and she experiences endothelial dysfunction, whereas the healthy control shows no signs of endothelial injury. These unique results from sub-Saharan Africa confirm the previous observation that HIV affects the endothelium through multiple pathological pathways and leads to cardiovascular diseases.

To compare the results of the physical examination, the laboratory values and the FMD measurements it is important to first look at similarities and differences in the physical characteristics of the two test subjects. Both subjects are female and in the same age group, born roughly 10 years apart from each other. Both are of the same ethnicity, although it does not arise from the data, if they are from the same region in South Africa as well. Both women do not drink alcohol.

The key difference between the two subjects is that subject one is infected with HIV and subject two is not. There is no information on how long subject one has been infected. When the examination took place she was not receiving antiretroviral treatment. Another difference between both subjects is, that subject one is a smoker. Although it is not clear how many pack years she has or how much she smokes at the moment. This is important to note, as smoking is known to influence the development of cardiovascular disease, more on this will follow later in the discussion. [60]

The first subject has a BMI of 20.2 and a waist-to-hip ratio of 0.84. She has normal weight, according to WHO guidelines on BMI which ranges from 18.5 to 25. The second subject has a BMI of 25.6 and a waist-to-hip ratio of 0.84. A BMI value between 25 and 30 is a sign of overweight according to WHO guidelines. A waist-to-hip ratio of 0.84 is not considered obese by the WHO for women all ratios above 0.84 are a sign of obesity, but are considered

overweight by other organizations. A borderline overweight BMI is a predicament, as the BMI itself is not an exact measurement, but can deviate from the real percentage of body fat for different types of body shapes. Also it has been found that the relation between BMI and percentage of body fat can vary for different ethnicities. [61, 62] A BMI measurement of over 25, is considered a risk factor for endothelial dysfunction. But because most BMI guidelines originate in the western world and there are no available studies on the relation of BMI to percentage of body fat in the mixed race population of South Africa, it has to be carefully considered if a BMI of 25.6 can be named as a risk factor for endothelial dysfunction for subject two.[63]

During the physical examination the blood pressure and heart rate of both subjects were measured three times as listed above. For both subjects all three measurements were in the optimal range recommended by the clinical guidelines.[64] Blood pressure is an important measurement, as hypertension is the most important risk factor for cardiovascular disease. Hypertension and endothelial dysfunction are associated and are known to influence each other. Through local and systemic inflammation and excessive oxidative stress hypertension can cause endothlial dysfunction. [65, 66]

During the laboratory test, different lipid levels were examined. The total Cholesterol of subject one (2.5 mmol/L) is below the margin of 5.2 mmol/L and therefore in the best category of three. Her total HDL (0.9 mmol/L) is below 1 mmol/L and according to clinical guidelines in the worst category of three. Her total LDL (1.1 mmol/L) is below 1.8 mmol/L, which is the desirable for people who already have a heart disease or diabetes. Her Triglycerides (1.1 mmol/L) are in the desirable margin below 1.7 mmol/L. In conclusion the lipid levels of subject one can be considered normal with the exception of the total HDL which is too low. [67]

The serum lipid levels of subject two can be regarded as normal according to clinical guidelines. Her total Cholesterol (3.69 mmol/L) is below 5.2 mmol/L which is considered desirable. Her total HDL (1.47 mmol/L) is in the medium range, but very close to the optimal level of 1.5 mmol/L and above. Also the total LDL (1.94 mmol/L) is in the optimal range for people at risk for heart disease, which should be under 2.8 mmol/L. And last her

Triglycerides (0.6 mmol/L) are in the best possible category below 1.7 mmol/L. [67]

After comparing the serum lipid levels of both subjects to clinical guidelines it is clear that both subjects have normal lipid levels. All levels, except the total HDL of the first subject, were considered healthy by clinical guidelines. To explain the low total HDL of the second subject three factors have to be considered. First, it is not clear if she is physically active. Physical activity has an improving impact on HDL levels, as shown in studies. [68] The second factor for a low total HDL level could be, that subject two is a smoker. Studies have shown, that current smokers have lower total HDL levels than former smokers or non-smokers. [69] The third factor for a low total HDL, the HIV infection, has to be considered. HIV infection, a CD-4 cell count below 350 cells/mm³ and no antiretroviral therapy have all been linked to low total HDL blood levels. [70] For the low HDL level a combination of these three factors is the most likely explanation. The protective effects of HDL on the cardiovascular system will be explained below.

The other blood parameters creatinine, estimated glomerular filtration rate (eGFR), fasting glucose and C-reactive protein (CRP) are all normal for both test subjects. Creatinine and eGFR are examined to determine the kidney function of the test subjects. Subject one has a creatinine concentration of 42 µmol/l which is below the normal range, but has no negative effect on the health of the kidney or the test subject in general. Subject two has a creatinine level of 72 µmol/l which is in the normal range of 49-90 µmol/l for the testing laboratory. [71] The eGFR is not exactly measured, only if the filtration rate is above 60 ml/min/1.73m³, which is true for both test subjects. If the eGFR is below 60 ml/min/1.73m³, it would suggest a significantly decreased kidney function and should be investigated further. [71] The fasting glucose levels are measured to determine if the subjects have diabetes mellitus, but both subjects have normal levels. Both have levels below 5.6 mmol/l, subject one has 4.0 mmol/l and subject two has 4.5 mmol/l. [71] The CRP is measured to rule out an active bacterial inflammation in the body. Both subjects have levels of CRP below 10 mg/l. Subject one has a level of 6.5 mg/l and subject two has a level of 5.0 mg/l, which rule out an acute inflammatory process. [71]

As described before arteries have the ability to self-regulate tone and blood flow. This autoregulation is dependent on the endothelium. If physical stimuli such as shear stress are detected by the endothelium a complicated cascade of reactions is set in motion which results in the generation of nitric oxide. NO itself is the main mediator for dilatation of the blood vessel. During flow-mediated dilatation an increase in blood flow and therefore shear stress is used to trigger the autoregulation of the blood vessel causing the artery to dilate. This dilatation is measured to assess the health of the endothelium and detect endothelial dysfunction.

Subject one has a baseline artery diameter of 2.62 mm. The maximum diameter measured after five minutes of occlusion and circa 60 seconds after cuff release is 2.70 mm. This means an increase in lumen diameter of 0.08 mm or a FMD of 3%. Subject two has a baseline artery diameter of 4.10 mm. The maximum diameter is 4.60 mm. The increase in lumen after the cuff was released is 0.5 mm, which gives a FMD of 12%. It can clearly be seen, that subject one has a much lower FMD in comparison to subject two. This shows, that the endothelium lining her brachial artery is not able to respond to a stimulus, in this case shear stress, in the same amount as subject two's endothelium can. At some point in the process of self-regulating the vasotonus the endothelium is not able to produce enough NO to trigger the relaxation of smooth muscle cells and therefore the dilatation of the vessel. During FMD the diameter of a healthy brachial artery should increase between 5% and 15%.[72] According to another study the increase after FMD using the pressure cuff on the lower arm should be above 6% in healthy brachial arteries.[73] Subject two is on the upper end of this margin with a FMD of 12%, well above a 6% increase in artery diameter. This leads to the conclusion that her endothelium functions well and there is no indication to assume that she suffers from endothelial dysfunction. Subject one on the other hand has a FMD of 3%, well below the lower limit of 5%. Her brachial artery is not able to increase its diameter in an adequate way after experiencing shear stress. This leads to the conclusion that her endothelium is damaged and she suffers from endothelial dysfunction.

FMD varies from person to person and is influenced by a variety of different factors. In these two cases three main differences can be named. First subject one has a lower high-

density lipoprotein level. Secondly she is a smoker and thirdly she is infected with HIV. All these factors are known to influence and damage the endothelium.

Isolated low HDL has been linked to a lower flow-mediated dilatation in healthy adults, which had no other risk factors compared to a control group, which had higher HDL but otherwise shared the same characteristics.[74, 75] HDL has three protective properties. Firstly it is anti-inflammatory it prevents the expression of adhesion molecules by the endothelial cell, thus preventing the immigration of immune cells. Secondly it has an anti-oxidant effect, by preventing LDL from becoming oxidized. And lastly it regulates platelet adhesion to the endothelium.[76, 77] All these protective effects are minimized with low serum levels of HDL.

Smoking has been known to damage the endothelium and impair flow-mediated dilatation. Cigarette smoke causes endothelial dysfunction through different pathological effects. Free radicals and reactive oxygen species (ROS) in cigarette smoke react with nitric oxide to form peroxynitrate. This causes a shortage of nitric oxide bioavailability, one of the main components of endothelial dysfunction. NO is responsible for the relaxation of the smooth muscle cells and the dilatation of the blood vessel. In addition cigarette smoke decreases the function of endothelial nitric oxidase synthase, adding to the lack of NO bioavailability. Aldehydes in cigarette smoke activate NADPH oxidase, which produces ROS, responsible for multiple functions in the healthy endothelial cell, such as proliferation and differentiation. Under the influence of cigarette smoke NADPH oxidase produces abnormally high levels of ROS, which can lead to endothelial dysfunction. Subject one is a smoker, and although it is not recorded how many pack years she has and how long before the FMD measurement her last cigarette was, it has to be assumed that this habit influences her endothelium and is one of the reasons for her low flow-mediated dilatation.[78, 79]

HIV is known to effect the endothelium in complex ways, directly by infecting endothelial cells or indirectly through chronic inflammation and dyslipidemia. Endothelial cells can be infected and activated by HIV and infected individuals have a lower count of circulating endothelial progenitor cells, which are required for the repair of damaged endothelium.[80, 81] In addition to this, cytocines secreted by infected monocytes and the HIV proteins gp120 and tat, can cause the activation of endothelial cells. Gp120 is also thought to stimulate the proliferation of smooth muscle cells, which is one of the processes that cause the development of atherosclerotic plaques (see figure 10). Through these processes and through the

state of chronic inflammation and through plasma lipid changes, HIV influences the endothelium and causes endothelial dysfunction.[82] The low FMD measurements of subject one are the result of a combination of these three factors, low HDL, cigarette smoking and the HIV infection, whereby the low HDL is also partly caused by the other two factors.

The results obtained from this diploma thesis are in agreement with the known association between HIV and cardiovascular disease. Although only two individual cases were analyzed, the data shows the effect an HIV infection has on the cardiovascular system, especially on the endothelium. This outcome is congruent with preliminary data from the ENDOAfrica project, which is currently ongoing.

5.1 LIMITATIONS

A shortcoming of this work is, that only one set of data from each test subject was used. Nevertheless, a difference of endothelial function between the HIV infected patient and the healthy control was observed. Furthermore this thesis is written within the broader context of the ENDOAfrica study, which will compare the differences in endothelial dysfunction in a whole population of HIV patients to a population of healthy controls, by collecting data of more test subjects. As mentioned before there will be 250 people in each cohort, which should be enough to reach statistically significant outcomes.

Another shortcoming is, that there is no follow-up data. The data in this thesis only depicts the state of the vasculature at one point in time, but is not able to describe the process of slowly developing endothelial dysfunction. Also there is no information on how long the first subject has been infected with HIV and therefore how much time the virus had to influence the endothelium. Again the ENDOAfrica study will collect more data over a period of time. All participants will have the same tests and examinations after 12 and 24 months. With this data it will be possible to identify a progression of endothelial dysfunction in connection with ongoing HIV infection.

5.2 CONCLUSION

These unique results from an HIV positive patient from sub-Saharan Africa, who shows signs of endothelial dysfunction, confirm the previous observation that HIV affects the endothelium through multiple pathological pathways and leads to cardiovascular disease.

This thesis shows how important a comprehensive treatment is to combat HIV infection and its complications. It is not enough to treat only the virus itself and complications that arise in the later stages of the disease, but it is equally important to check the patient's cardiovascular status frequently once the infection is diagnosed and to intervene once cardiovascular dysfunction arises.

The ENDOAfrica project will certainly have a broad influence on both theory and practice. Among other things it will deepen the understanding on how HIV affects the endothelium and the cardiovascular system in general. In addition to this it will give an insight into how the antiretroviral therapy influences the development of endothelial dysfunction. The fact that the ENDOAfrica project studies the effects of this disease in the region most affected by it is particularly important, as 66% of all individuals infected by HIV live in Sub-Saharan Africa.

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