Diplomarbeit

Effects of HIV/AIDS and Antiretroviral Therapy on the Cardiovascular System

A review of current literature

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Graz, am 6. Mai 2015 ***Jennifer Mellin eh***

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Abstract

Background: HIV/AIDS and cardiovascular diseases are both among the top 10 causes of death worldwide. While HAART (highly active antiretroviral therapy) has significantly decreased the mortality and morbidity among HIV-infected individuals, the immunodeficiency virus and antiretroviral therapy have lately both been associated with an increased risk of cardiovascular diseases. It is questionable whether the pathogenesis follows the same pathway as in the general population or if additional HIV and ART-specific factors further accelerate the development of endothelial dysfunction and atherosclerosis.

Objectives: The aim of this diploma thesis is to compare available information about the effects of HIV/AIDS and antiretroviral therapy on the cardiovascular system from secondary literature such as textbooks to the current primary literature found in medical databases and science journals.

Methodology: Standard English and German textbooks on "Physiology", "Pathology" "Microbiology" and "Endothelial Biomedicine" were used as secondary literature (complemented by current guidelines where applicable). A systematic literature search of medical databases with specific inclusion and exclusion criteria provided the relevant primary literature for the review.

Results: Of 600 article titles that were evaluated, 85 full text articles and their references were reviewed for this diploma thesis.

Discussion: The etiology of cardiovascular diseases in HIV-infected individuals with or without antiretroviral therapy is most likely multifactorial. The involved mechanisms remain mostly unclear. The effects of HIV/ART and ART on vascular function, traditional cardiovascular risk factors as well as "emerging" risk factors seem to play an important role. Many studies from developed countries have associated HIV and ART with an increased degree of endothelial dysfunction and early atherosclerotic changes in comparison to healthy controls. Unfortunately studies from Africa, the most affected region of this world, are very scarce. The impact of geographical and ethnical differences therefore remains to be explored.

Zusammenfassung (German Abstract)

Hintergrund: HIV/AIDS und kardiovaskuläre Erkrankungen gehören weltweit zu den 10 häufigsten Todesursachen. Die Einführung von hochaktiver antiretroviraler Therapie (highly active antiretroviral therapy = HAART) hat die Morbidität und Mortalität in der HIV Population signifikant reduziert. Allerdings wird HIV/AIDS und antiretrovirale Therapie (ART) nun mit einem erhöhten Risiko an kardiovaskulären Erkrankungen in Verbindung gebracht. Es stellt sich die Frage, ob die Pathogenese in HIV-infizierten Patienten der Pathogenese der Allgemeinbevölkerung entspricht oder ob HIV und die antiretrovirale Therapie als zusätzliche Faktoren die Entstehung von endothelialer Dysfunktion und Atherosklerose beeinflussen.

Ziel: Sekundärliteratur wie Lehrbücher soll mit Primärliteratur (zum Beispiel Artikel aus medizinischen Fachzeitschriften) zum Thema der Effekte von HIV/AIDS und antiretroviraler Therapie auf das kardiovaskuläre System verglichen und diskutiert werden.

Methoden: Als Sekundärliteratur wurden englische und deutsche Standardlehrbücher in den Fachbereichen „Physiologie", „Pathologie" „Mikrobiologie" und „Endothelialer Biomedizin" verwendet. Zusätzlich wurden aktuelle Richtlinien in die Einführung eingearbeitet. Für die Erfassung der relevanten Primärliteratur wurde eine systematische Literaturrecherche mit expliziten Ein- und Ausschlusskriterien mithilfe mehrerer medizinischer Datenbanken durchgeführt.

Ergebnisse: Von über 600 gesichteten Titeln der systematischen Literatursuche wurden schlussendlich 85 relevante Artikel ausgewählt, deren Volltext analysiert wurde.

Diskussion: Die Ätiologie der kardiovaskulären Erkrankungen in der HIV Population, derzeit therapiert oder untherapiert, ist wahrscheinlich multifaktoriell. Die involvierten Pathomechanismen sind immer noch weithin ungeklärt. Allerdings scheinen die Effekte von HIV/AIDS und ART auf die vaskuläre Funktion, auf traditionelle Risikofaktoren sowie auf den Immunstatus (chronische Aktivierung und Inflammation) eine wichtige Rolle zu spielen. Viele Studien, die zum größten Teil in Industrieländern durchgeführt wurden, assoziieren HIV und ART mit einem erhöhten Grad an endothelialer Dysfunktion und frühen atherosklerotischen Veränderungen. Leider sind Ergebnisse von Studien aus Entwicklungsregionen wie Afrika, die am meisten an den Folgen von HIV leiden, sehr rar. Daher lassen sich kaum Aussagen über geografische und ethnische Unterschiede treffen.

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Abbreviations

Ab	Antibody
AE	Adverse effects
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
AP	Angina pectoris
ART	Antiretroviral therapy
AVR	Arteriolar to venular ratio
CAD	Coronary artery disease
CDC	Centers of Disease Control and Prevention
cDNA	Complementary deoxyribonucleic acid
CFU-EC	Colony forming unit – endothelial cell
CHD	Coronary heart disease
CRAE	Central retinal arteriolar equivalent
CRF	Circulating recombination form
CRVE	Central retinal venular equivalent
CVD	Cardiovascular disease
EC	Endothelial cell
ECFC	Endothelial colony-forming cell
ED	Endothelial dysfunction
EKG	Electrocardiogram
ELISA	Enzyme linked immunosorbent assay
EMP	Endothelial microparticle
EPC	Endothelial progenitor cell
FACS	Fluorescence-activated cell sorter
FMD	Flow mediated vasodilation
GM-CSF	Granulocyte macrophage colony-stimulating factor
HAART	Highly active antiretroviral therapy
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High density lipoprotein
HIV	Human immunodeficiency virus

hs-CRP	High sensitivity C-reactive protein
ICAM-1	Intercellular cell adhesion molecule 1
IDU	Intravenous drug users
IHD	Ischemic heart disease
IL	Interleukin
IMT	Intima-media thickness
INF-γ	Interferon gamma
INSTI	Integrase strand transfer inhibitor
LDL	Low density lipoprotein
MCP-1	Monocyte chemoattractant protein 1
MetS	Metabolic syndrome
mo-LDL	Mildly-oxidized low density lipoprotein
MSM	Men who have sex with men
NAT	Nucleic acid testing
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NO	Nitric oxide
NRTI	Nucleoside reverse transcriptase inhibitors
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PEP	Post-exposure prophylaxis
PET	Positron emission tomography
PI	Protease inhibitor
PWV	Pulse wave velocity
TC	Total cholesterol
TDR	Transmitted drug resistance
TG	Triglyceride
VCAM-1	Vascular cell adhesion molecule 1
VSMC	Vascular smooth muscle cells
WHO	World Health Organization

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1 Introduction

1.1 Human Immunodeficiency Virus

The human immunodeficiency virus is a human T-cell lymphotropic retrovirus of the lentivirus subgroup that induces immunosuppression and causes the acquired immunodeficiency syndrome (AIDS). The lentivirus subgroup causes "slow" infections, meaning there is a latent clinical phase between infection and outbreak of disease. There is currently no cure for HIV – it is a life-long and chronic infection (Tortora et al., 2010).
There are two types of human immunodeficiency viruses – HIV-1 and HIV-2 – that can both cause AIDS. While HIV-1 is found worldwide, the less severe and virulent HIV-2 is mainly prevalent in West Africa.[1] HIV-1 can be divided into 4 groups (M, N, O, P): M standing for "major" and being predominant with 90% of all HIV infections today (Levinson, 2010). Group M is further classified into clades (A to I and numerous "circulating recombinant forms" (CRFs)). The CRFs are combinations of clades that are numbered consecutively, for example CRF01_AE or CRF02_AG. To date, the HIV databases document a list of 72 detected CRFs (HIV databases, 2015). While clade B is mainly found in Europe and in the Americas, clade C is most common in Sub-Saharan Africa. The genomes of the various clades differ by up to 30% (Tortora et al., 2010; Mandell et al., 2010).

1.1.1 History

Retrospectively, HIV-1 was first diagnosed in a serum specimen from Central Africa (De Cock et al., 2011). HIV isn't an endogenous human virus; it was probably transferred from HIV-1 infected chimpanzees to humans sometime in the 1930s. Urbanization in Central Africa allowed the virus to spread and at an unknown time the virus was transported to the Western Hemisphere (De Cock et al., 2011; Sepkowitz, 2001).
An article about five young and healthy homosexual men with unusual infections (pneumocystis pneumonia) was published in the CDC *Morbidity and Mortality Weekly Report* on June 5th of 1981 – this was the first mention of what became to be known as

[1] Unless otherwise noted HIV refers to HIV-1 infection in adults.

AIDS. The first affected groups were mainly men who had had sex with men (MSM), intravenous drug users (IDUs), Haitians and hemophiliacs (4H risk groups) (De Cock et al., 2011; Sepkowitz, 2001).

While there were still doubts about whether the cause of this syndrome was viral, the CDC implemented a national register for Kaposi Sarcoma and Opportunistic Infections to keep track of the number of affected individuals. The isolation of the actual virus wasn't successful until May of 1983. Luc Montagnier and Robert Charles Gallo received the Nobel Prize for Medicine for this accomplishment in 2008 (De Cock et al., 2011; Mandell et al., 2010). It took approximately 2 more years for the first serological testing to become widely available (the implementation of the polymerase chain reaction (PCR) in 1994, however, has since revolutionized the detection of HIV RNA in the plasma) (Mandell et al., 2010).

The story of HIV and AIDS epidemic changed in 1987 when the FDA approved the first antiretroviral drug – zidovudine (azidothymidine) – followed by other drug agents (the combination of various drugs in the 1990s became known as highly active antiretroviral therapy (HAART)) (Sepkowitz, 2001).

Today HIV and AIDS can still be considered a pandemic that is going to challenge scientists and global health workers for many years to come (De Cock et al., 2011; Sepkowitz, 2001).

1.1.2 Epidemiology

Since the recognized beginning of the epidemic in the 1980s, almost 78 million people have been infected with HIV and 35 million have died from the consequences. In 2013, there were 35 million people infected with HIV worldwide, with an incidence (rate of annual new infections) of 2.1 million and a mortality rate of 1.5 million per year. The figure below shows the worldwide geographical differences in prevalence in adults aged 15 to 49 years. 71% of all people living with HIV/AIDS live in Sub-Saharan Africa. The number of infections in South America and Southeast Asia is also growing alarmingly (World Health Organization, 2013). The great socioeconomic impact that HIV/AIDS carries in many countries has to be considered as well. The virus most often affects young adults (Murray et al., 2013).

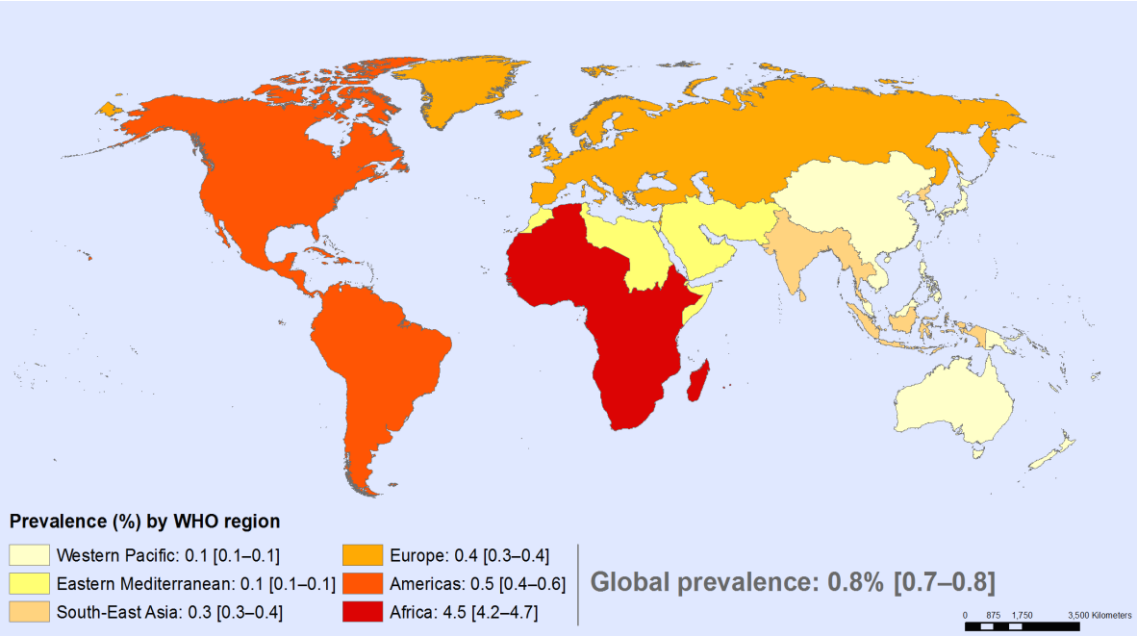

Figure 1 Geographical differences in the prevalence of HIV infection in adults (15-49 years) by WHO region in percent, 2013 (reproduced from: http://www.who.int/gho/hiv/hiv_013.jpg?ua=1**)**

1.1.3 Classifications

Both the CDC and the WHO have implemented staging schemes that have been altered multiple times over the past 30 years. Both have in common that the HIV infection must be proven by laboratory results. CDC now classifies in 3 stages based on the CD4+ T-cell count. Stage 3 is defined as AIDS with a cell count below 200 cells/mm^3 or the presence of an AIDS defining condition. The WHO on the other hand uses a 4-stage system combining CD4+ cell count for the grade of immunodeficiency and clinical conditions typical for every stage. The latter is predominantly applied in developing countries (Mandell et al., 2010).

The classification is still based on the most advanced stage; there are no recommendations for reclassification currently in place although a lot of HIV-infected individuals respond well to HAART and their condition changes for the better (Mandell et al., 2010).

The table below shows a comparison of CDC and WHO staging. Detailed lists of the defining clinical conditions (for CDC and WHO) can be found in the appendix.

Table 1 Comparison of WHO and CDC staging systems (modified from: (Mandell et al., 2010))

WHO Stage	CD4+ cell count [cells/mm^3]	CDC Stage	CD4+ cell count [cells/mm^3]
Stage 1	≥ 500	Stage 1	≥ 500
Stage 2	350 – 499	Stage 2	200 - 499
Stage 3 (= advanced HIV disease)	200 - 349		
Stage 4 (= AIDS)	< 200	Stage 3 (= AIDS)	< 200

1.1.4 Structure and Genome

As mentioned above the human immunodeficiency virus is a retrovirus of the lentivirus subtype that infects CD4 positive cells (mainly T helper cells and monocytes/macrophages). HIV is a roughly spherical, enveloped virus containing a bar-shaped nucleocapsid. The envelope derives from budding off from the host plasma membrane and contains specific glycoproteins – gp120 and gp41. The viral genome, two separate positive single strands of RNA, is found in the nucleocapsid; it is more complex than in any other retroviridae. It contains the typical retroviral genes *gag*, *pol* and *env* that encode structural proteins plus 6 further regulatory genes. Genes, encoded proteins and their functions can be seen in the table below (Levinson, 2010).

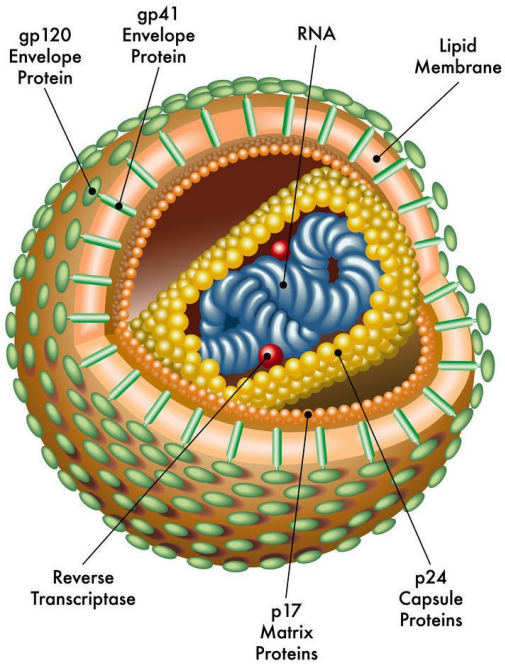

Figure 2 Structure of HIV (reproduced from: http://imgkid.com/hiv-aids-pictures-virus.shtml)

Table 2: Genes and proteins of HIV (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-dividing cells
vpu	vpu	Enhancement of viral release from the cell

1.1.5 The Replication Cycle

The initial and most important step in the replication cycle is the binding of the virion to the host cell. The envelope glycoprotein gp120 adheres to the CD4 protein on the host cell surface and additionally to specific chemokine co-receptors. HIV strains that use CCR5 chemokine receptors as a co-receptor are called M-tropic strains. They are usually found early in infection and affect monocytes/macrophages as well as dendritic cells, memory cells and CD4+ cells. A mutation in gp120 causes a switch from M- to T-tropism (usually found later in the progression of infection). These strains use CXCR4 as a co-receptor to the CD4 receptor. Dual-tropic strains can bind to both receptors. The glycoprotein gp41 finally facilitates the fusion of virion and host cell after which the RNA genome, the reverse transcriptase and the nucleocapsid enter the host cytoplasm (Murray et al., 2013).
There the reverse transcriptase, a RNA-dependent DNA polymerase, first produces a complementary DNA strand (cDNA), then degrades the RNA and finally creates a sense

DNA from the antisense cDNA. The reverse transcriptase is prone to errors (without proofreading activity), which explains the heterogeneity of HIV strains in one individual. The now double-stranded cDNA is transported into the nucleus where it can remain as a provirus or where it is integrated into the host DNA by the viral integrase. When the cell is activated, the host RNA polymerase transcribes the viral DNA into a full length mRNA. The translated large polyproteins are not yet functional. The immature virion cumulates in the cytoplasm and the viral protease cleaves the polyproteins when the virion buds off from the host cell. The result is a mature and infectious virion ready to infect other cells (Murray et al., 2013).

The various steps of the replication cycle coincide with the possibility of antiretroviral agents to interfere (Murray et al., 2013).

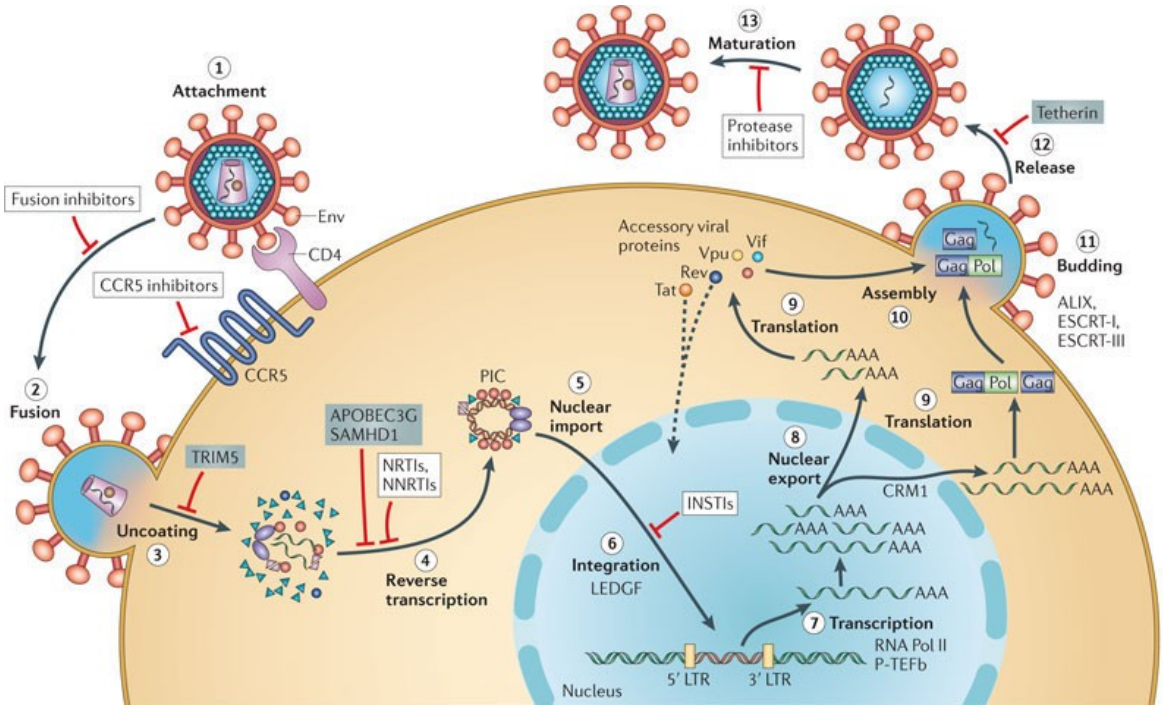

Figure 3 HIV replication cycle with interference points of antiretroviral drug agents (reproduced from: http://www.nature.com/nrmicro/journal/v10/n4/fig_tab/nrmicro2747_F1.html)

1.1.6 Transmission

While HIV is transmitted in the same pattern as Hepatitis B, the risk of infection, however, is much lower and requires a much higher viral load, which can mainly be found in two body fluids – blood and semen. HIV can be transmitted during sexual intercourse (homo- or heterosexual), through parenteral exposure to infected blood or blood products, which

includes IDUs, or vertically from mother to child. The latter can either take place in utero, at birth or through breast-feeding (Levinson, 2010). While the vertical transmission from mother to child has greatly decreased in the developed world (e.g. < 2% in the United States), about 420,000 infants worldwide are still infected by their mothers yearly (dependent of the mother's viral load/HIV stage). Before ART was widely implemented numbers of vertical transmission varied from 13% to 40%, with a risk of transmission during breastfeeding of up to 30% (Mandell et al., 2010).

These transmission pathways differ greatly geographically. The infection originally spread mostly among MSMs and IDUs who are still mainly affected in Europe and the Americas. However, heterosexual transmission is predominant worldwide today (85%), especially in Africa (Tortora et al., 2010).

1.1.7 Clinical Stages

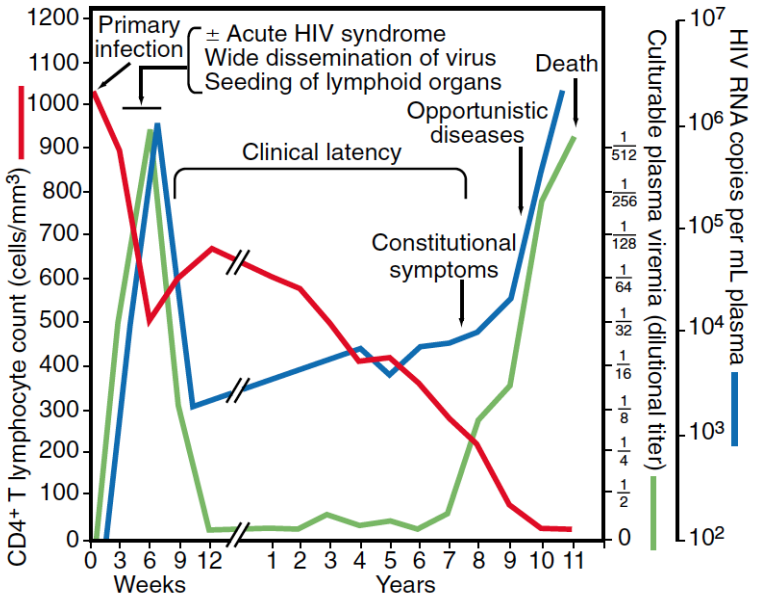

Figure 4 Natural course of HIV infection in untreated patients (reproduced from: (Mandell et al., 2010))

After sexual transmission (which is most common) the initial infection occurs in the mucosa-associated lymphatic tissue of the genital tract after which the local CD4 positive cells are infected. HIV RNA can first be detected in the blood by nucleic acid testing (NAT) about 10 to 14 days after infection (diagnostic "window") with high levels that drop 8 to 12 weeks post infection (still well detectable levels without treatment). During this first and acute period the lymphatic organs are seeded and become the main site of viral

replication. Three to six weeks after infection, symptoms described as "mononucleosis-like" can occur (including fever, lethargy, sore throat, generalized lymphadenopathy and a maculopapular rash). These can last about 2 to 3 weeks. The initially high viral load coincides with a peak of antigen levels (p24) and a primary drop in CD4+ T-cells (Jawetz et al., 2007). In most cases seroconversion is completed in about 4 weeks, but it can take up to 6 months and more (Levinson, 2010).

One to three months after initial infection, the immune system kicks in with a rise in CD8+ positive cells (cytotoxic T-cells), a rebound of the CD4+ cell count and consecutively lower viral RNA levels. This phase is considered the clinical latent period where the virus persists and quietly replicates in macrophages and T helper cells in the lymph nodes. Untreated, this period can last 7 to 10 years followed by disease progression. Antiretroviral drugs have considerably prolonged this phase (Jawetz et al., 2007).

The number of CD4+ cells is reduced through HIV induced apoptosis, increased permeability and syncytia formation. The table below shows the usual CD4+ cell functions that include the activation of CD8+ cells and other immune-competent cells. The loss of CD4+ cells has a major impact on the immune system; consecutively lowering counts mean a progression in the direction of advanced HIV disease and AIDS. The probability of opportunistic infections and malignancies increases exponentially without the proper therapy and chemoprophylaxis. Death usually occurs 2 years after progression to AIDS if the immunodeficiency remains untreated (Murray et al., 2013).

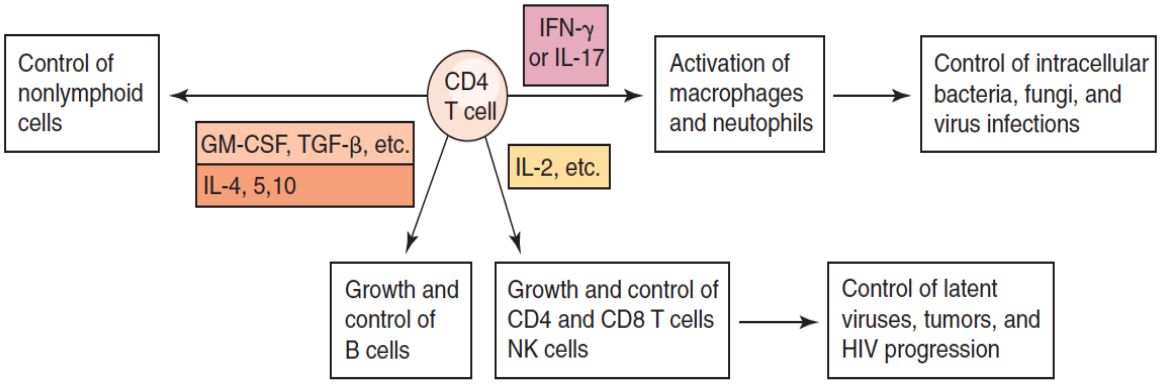

Figure 5 CD4+ T cell function (Abbreviations: GM-CSF = granulocyte macrophage colony-stimulating factor, IFN-γ = interferon gamma, IL-4, 5, 10, 17 = interleukins 4, 5, 10, 17, TGF-β = transforming growth factor beta (reproduced from: (Murray et al., 2013))

1.1.8 Testing and Diagnosis

Early diagnosis of HIV infection is essential because unidentified HIV carriers transmit the virus unknowingly. Assays based on nucleic acid testing (NAT) yield the earliest positive results at 10 to 14 days post infection. This time period is called the diagnostic window (which signifies the time from infection to the first positive test result) whereby the duration of this window is dependent on the testing method (see Figure 6). PCR is the most commonly applied NAT assay today. Principally, specific HIV regions are amplified during PCR, which makes a detection of HIV RNA possible at very low plasma levels. PCR includes the following steps: after RNA extraction from the plasma, a cDNA is created with the help of an exogenous reverse transcriptase. Millions of copies are transcribed by DNA polymerases in multiple cycles using this cDNA as a template. Fluorescence-labeled probes finally allow real-time detection of the PCR products. Quantification standards are used to specify the viral load of the plasma sample. The viral load is an important marker in the monitoring of HIV infection and the efficacy of antiretroviral therapy (Mandell et al., 2010; Murray et al., 2013).

Combined antibody/antigen tests are commonly used for HIV testing and can detect HIV infection within 20 to 30 days after infection. Assays that test only antibodies shouldn't be applied until 4 to 10 weeks after infection (when seroconversion is very likely) (Brennan, 2011). Usually ELISA (enzyme linked immunosorbent assay) is used as a screening test for detecting antibodies and/or antigens. It is a very sensitive method and the potential false-positive results are invalidated in the confirmatory Western blot. The ELISA produces a qualitative result of enzyme reactivity. Rapid tests that offer results in 15 to 30 minutes are also based on the principle of ELISA (only antibody detection) (Mandell et al., 2010)

The Western blot is commonly used as the confirmatory test. The viral proteins are separated by electrophoresis and then blotted onto filters with immobilized antibodies, which capture the viral proteins. Added anti-human serum produces enzyme substrates that are displayed as specified bands for positive results (Mandell et al., 2010; Tortora et al., 2010).

The European AIDS Clinical Society (EACS) advises that genotypic resistance testing be performed additionally at the time of HIV diagnosis or at the latest before initiation of therapy to identify "transmitted drug resistance (TDR)" (EACS, 2014).

Lastly, the CD4+ T-cell count plays an important role in the decision-making process of starting antiretroviral therapy and chemoprophylaxis against opportunistic infections. Leukocytes are differentiated using a fluorescence-activated cell sorter, a specialized flow cytometer (Murray et al., 2013)

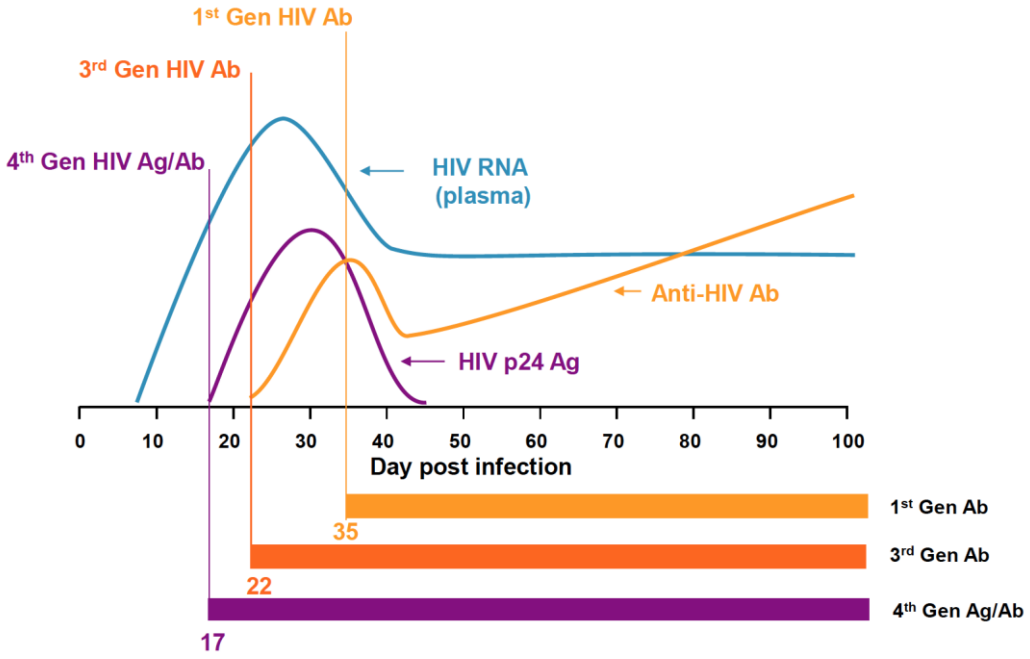

Figure 6 Comparison of diagnostic windows of combined Ag/Ab tests versus sole Ab tests (Abbreviations: Ab = antibody, Ag = antigen) (reproduced from: (Brennan, 2011))

1.1.9 Prevention

There is currently no vaccine available. All preventive measures concentrate on the avoidance of transmission. First and most importantly health education should inform people of high-risk behavior that can lead to HIV infection (Levinson, 2010).

Since the introduction of effective antiretroviral therapy, the "danger" of HIV has become less apparent and less people practice safe sex resulting in a rising incidence of HIV infections. The use of condoms is the only way of preventing HIV transmission during sexual intercourse. This would also limit the number of co-infections with other sexually transmitted diseases (Levinson, 2010).

Special programs against needle sharing for intravenous drug users have been launched. The use of sterile and disposable needles is widely promoted especially in urban settings. Regulations for testing blood products by PCR have greatly decreased the risk of infection through blood transfusions (implemented in the EU in 1999). The CDC also published

recommendations for preventative measures in health care settings. All body fluids and patients should be considered contaminated. Dependent on the procedure, gloves and additional protective gear should be worn. Algorithms for the behavior after needle-stick injuries should be implemented including potential post-exposure chemoprophylaxis (PEP) (Levinson, 2010).

The vertical infection of infants was significantly reduced by the following preventative measures: testing and counseling of pregnant women, providing antiretroviral therapy to reduce the viral load of the mothers-to-be, performing elective Cesarean sections and advising against breastfeeding. Although the latter can only be recommended in countries where high quality substitutes for breast milk are easily available (Levinson, 2010; Mandell et al., 2010).

1.1.10 Antiretroviral Therapy

The approval of antiretroviral drugs has changed the HIV epidemic substantially. Although it doesn't present a cure for HIV infection, it turns HIV into a manageable chronic disease. ART has definitely decreased mortality and increased the quality of life of affected individuals. Therapy results in a restitution of the immune system with a reduction of the viral load (even beneath detectable plasma levels), which also shows positive effects on the occurrence of opportunistic infections and malignancies. Sadly many agents and regimens are still very costly and therefore insufficiently available in developing countries (Levinson, 2010).

There are several classes of drugs that interfere at various points in the replication cycle (also see Figure 3 in the chapter "Replication Cycle"). While non-nucleoside and nucleoside reverse transcriptase inhibitors (NNRTIs and NRTIs) and protease inhibitors (PIs) are quite "old" drug classes, entry inhibitors and integrase strand transfer inhibitors (INSTIs) are fairly "new". Entry inhibitors bind to envelope glycoproteins gp41 and gp120, which prevents the fusion of the virion with the plasma membrane of the host cell (Levinson, 2010). INSTIs block the viral integrase so that the strand transfer of the cDNA into the nucleus and the integration into the host DNA do not take place. NRTIs terminate the DNA synthesis during the reverse transcription. The structure of these drugs prevents the elongation of the proviral DNA strand and therefore causes chain termination. NRTIs also show effects on the host's cellular DNA polymerases accounting for the various adverse drug effects described in the table below. NNRTIs bind to the enzyme and cause a

change in confirmation resulting in a reduced activity of the reverse transcriptase. Because of a low threshold for resistance, NNRTIs are usually used early in therapy. Protease inhibitors bind to and inhibit the viral protease that is in charge of cleaving polyproteins to make them functional. The viral particles that are still released remain uninfectious. All protease inhibitors are known to show lipodystrophy as an adverse drug side effect (Mandell et al., 2010).

As HIV displays a high mutation rate, it is recommended to prescribe a combination of antiretroviral agents. Monotherapy enhances the risk of generating resistance against the applied drug agent. The usual regimens known as "highly active antiretroviral therapy (HAART)" include two nucleoside reverse transcriptase inhibitors plus a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor. Today integrase strand transfer inhibitors are also used in first line therapy. The exact combination of antiretroviral drug agents is dependent on the available drug agents (great differences between regions of high and low income) (Levinson, 2010). The EACS recommends starting therapy at CD4+ cell count levels of 350 cells/mm^3 and lower. Earlier initiation (meaning at higher CD4+ cell count levels) should be discussed in the following cases: the HIV-infected individual wants to start treatment, the HIV infection is symptomatic, other HIV-associated diseases such as HIV-associated neurocognitive impairment or Hodgkin's lymphoma become apparent or if the HIV-infected individual also has chronic viral hepatitis (EACS, 2014).

A good adherence and compliance are essential for a successful treatment of HIV. When ART is stopped or not administered correctly, the minimizing affects on viral replication halt and viremia reappears with falling CD4+ cell counts and progression of disease (Levinson, 2010). Other reasons for virological failure (with viral loads greater 50 copies/mL after 6 months on ART) could be the development of drug resistance or drug-drug/food-interactions (EACS, 2014).

Table 3 List of antiretroviral drugs with frequent and/or potentially life-threatening adverse side effects (Abbreviations: GFR = glomerular filtration rate, IHD = ischemic heart disease) (modified from: (Levinson, 2010; EACS, 2014)**)**

Drug Class	**Drug Name**	**Adverse Effects or *Details***
Entry Inhibitors		
Fusion inhibitor	Enfuvirtide	- *Binds to gp41* AE: injection site reactions
Co-receptor antagonist	Maraviroc	- *Blocks CCR5 receptor* AE: IHD
Reverse transcriptase inhibitors		
NRTIs	Abacavir	AE: severe multi-organ hypersensitivity reaction, ischemic heart disease
	Didanosine	AE: pancreatitis, hyperlactatemia, hepatic steatosis
	Emtricitabine	- *Derivative of lamivudine*
	Lamivudine	- *Well tolerated* - *Also used for HBV treatment*
	Stavudine	AE: pancreatitis, hepatic steatosis, peripheral neuropathy, dyslipidemia/lipoatrophy, hyperlactatemia
	Azidothymidine (Zidovudine)	AE: rhabdomyolysis, hyperlactatemia, lipoatrophy
	Tenofovir	- *Actually a nucleotide* AE: renal toxicity (Fanconi syndrome), fracture risk ↑
NNRTIs	Efavirenz	AE: dizziness/sleep disorders, dyslipidemia, low vitamin D
	Etravirine	
	Rilpivirine	
	Nevirapine	AE: severe multi-organ hypersensitivity reaction, rash
Integrase inhibitors		
	Raltegravir	AE: rhabdomyolysis
	Dolutegravir	AE: decreased estimated GFR, severe multi-organ hypersensitivity reaction
	Elvitegravir/ Cobicistat	AE: nausea/diarrhea, hyperbilirubinemia, decreased estimated GFR
Protease inhibitors		
	Atazanavir	AE: nausea/diarrhea, dyslipidemia
	Darunavir	AE: nausea/diarrhea, dyslipidemia
	Fosamprenavir	AE: nausea/diarrhea, dyslipidemia, IHD
	Indinavir	AE: nausea/diarrhea, dyslipidemia, IHD, dry skin, nephrolithiasis, increased abdominal fat
	Lopinavir/Ritonavir	AE: nausea/diarrhea, dyslipidemia, IHD
	Saquinavir	AE: dyslipidemia
	Tipranavir	AE: dyslipidemia, hepatitis, intracranial hemorrhage

1.1.10.1 Immune Reconstitution Syndrome

Co-infections such as HBV, HCV, Cryptococcus neoformans and toxoplasmosis should be treated before the initiation of HAART. The increasing numbers of CD4+ cells due to therapy enhance the body's abilities to launch an inflammatory response. This can cause exacerbation of symptoms of the co-infections. This is known as immune reconstitution syndrome (Levinson, 2010).

1.2 Cardiovascular System

The cardiovascular system is the "transport system" of the body. The heart as the pump and the vessels as the complex tube system make sure that the blood reaches every last "corner" of tissue. The blood transports the oxygen, nutrients, water and other substances such as hormones to the organs and collects the waste and carbon dioxide at the same time (Faller and Schünke, 2008).

1.2.1 The Blood

While the heart and the vascular system pose as the transport system, the blood is the actual transport medium. It is a complex composition of plasma and cells (erythrocytes, leukocytes and thrombocytes, which are mostly produced in the bone marrow). It makes up for about 8% of our body mass (Faller and Schünke, 2008).

Erythrocytes are responsible for the gas transport and exchange of oxygen and carbon dioxide in the lungs and the tissue. Leukocytes are part of the immune system and can be further divided into granulocytes, lymphocytes and monocytes. They migrate from the blood into the connective tissue as part of an inflammatory response. The plasma is the transport medium for nutrients, metabolic products, electrolytes, vitamins and soluble proteins. Platelets are part of the coagulation system and adhere to injuries of the vascular wall and start a coagulation cascade together with other soluble factors from the plasma (Silbernagl and Despopoulos, 2007).

1.2.2 The Heart

The heart is a muscular organ that is enveloped by the pericardium and located in the mediastinum between the spine and the sternum. It has about the size of the person's fist (usually about 300 to 350 grams). It is divided into a "left heart", which supplies the system circulation and the "right" heart, which supplies the pulmonary circulation. Each "side" is composed of an atrium and a ventricle. The walls of the heart can be divided into three layers: the endocardium, the myocardium (the actual heart muscle) and the epicardium (Faller and Schünke, 2008).

As any other tissue, the heart muscle requires oxygen and nutrients. These are supplied via the right and left coronary artery that feed distinctive parts of the heart. A blockage in the arteries can cause severe symptoms and restrictions in the physiological function of the heart (see myocardial infarction) (Silbernagl and Despopoulos, 2007).

1.2.3 The Vascular System

1.2.3.1 Components of the Vascular System

The vascular system is basically made up by a very complicated system of elastic tubes. Size, structure and number of the vessels vary and are adapted to the functions they fulfill. Very generally speaking, the arteries distribute the blood to the microcirculation where the diffusion and filtration takes place. The veins, however, collect the blood from the tissue and bring it back to the heart (Boron and Boulpaep, 2005).

The different types of blood vessels have a principal structure of three layers; these vary in thickness. The innermost and thinnest layer is the tunica intima, which consists of a single layer of squamous endothelial cells surrounded by sub-endothelial tissue and an elastic membrane. The tunica media containing smooth muscles cells and elastic fibers follows the tunica intima. On the outside, the vessels are enveloped by the tunica adventitia, which is mainly built from connective tissue (Faller and Schünke, 2008).

The arteries are the blood vessels transporting blood away from the heart. They have an extensive muscular layer containing elastic tissue regulating vascular resistance and blood pressure via vasodilation and vasoconstriction. They can be divided into a muscular and an elastic type. The elastic type (aorta and heart near arteries) is responsible for the "bag pipe" function, which supplies the microcirculation with a continuous flow rather than the pulsatile blood flow from the heart. Arteries are part of the high-pressure system. The

smallest branches of arteries are called arterioles. They are responsible for the highest resistance in the circulation, which is controlled by autonomic nerve fibers. The smallest vessels are called capillaries. They are very thin walled (single endothelial layer plus basal lamina), which is essential for the bi-directional exchange of nutrients/waste and oxygen/carbon dioxide in the organ tissue. Because of the sheer amount of capillaries, they have the biggest total cross-sectional area although their single diameter is merely about 3µm (Boron and Boulpaep, 2005; Costanzo, 2011). The velocity in the capillaries is only a 1/1000th of the 33cm/s in the aorta (at rest) (Guyton and Hall, 2006). The venules are the connecting parts between capillaries and bigger veins. Veins are lastly formed by merged venules. They generally have thinner walls with less distinct muscular layers. Venous valves limit venous regurgitation. In comparison to the arteries veins are under low pressure but contain about 75% of the blood in the system (Faller and Schünke, 2008).

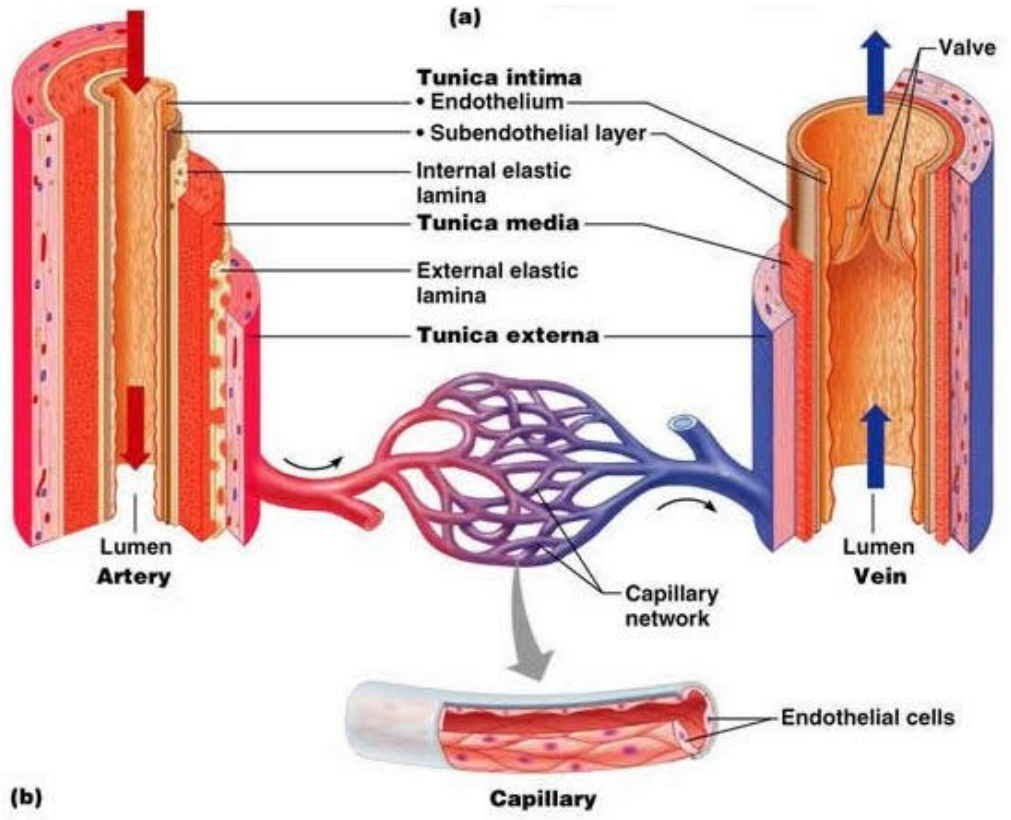

Figure 7 Comparison of the arterial, venous and capillary vascular wall structure
(reproduced from: http://classes.midlandstech.edu/carterp/Courses/bio211/chap19/chap19.html)

Endothelium

As mentioned above the endothelium builds the innermost vascular wall. It is a specialized kind of epithelium and a heterogeneous cell type that is required to adapt to site-specific

differences of organ tissues. Generally, three types of endothelium can be distinguished - continuous, fenestrated and discontinuous or sinusoidal endothelium (Aird, 2007).

The endothelium isn't a mere layer of tissue though; it should rather be thought of as a complex and active endocrine, autocrine and paracrine organ that regulates many processes in homeostasis. The integrity of the endothelium is essential for the regular vascular function (Aird, 2007).

The endothelium serves as a mechanical barrier but also forms the semipermeable membrane across which nutrients and metabolic substances are transported between the blood and the tissue. It also expresses many receptors and adhesion molecules on the surface. The secretion of numerous mediators influences the vascular tone, coagulation, leukocyte migration, angiogenesis and innate immunity. The maintenance of an anti-thrombotic and non-adhesive surface is very important for a laminar blood flow (Aird, 2007).

The figure below aims to show the extent of the endothelial role in homeostasis. These complex cascades can run out of hand when the endothelium is damaged resulting in endothelium activation or even dysfunction (influencing the underlying pathophysiology).

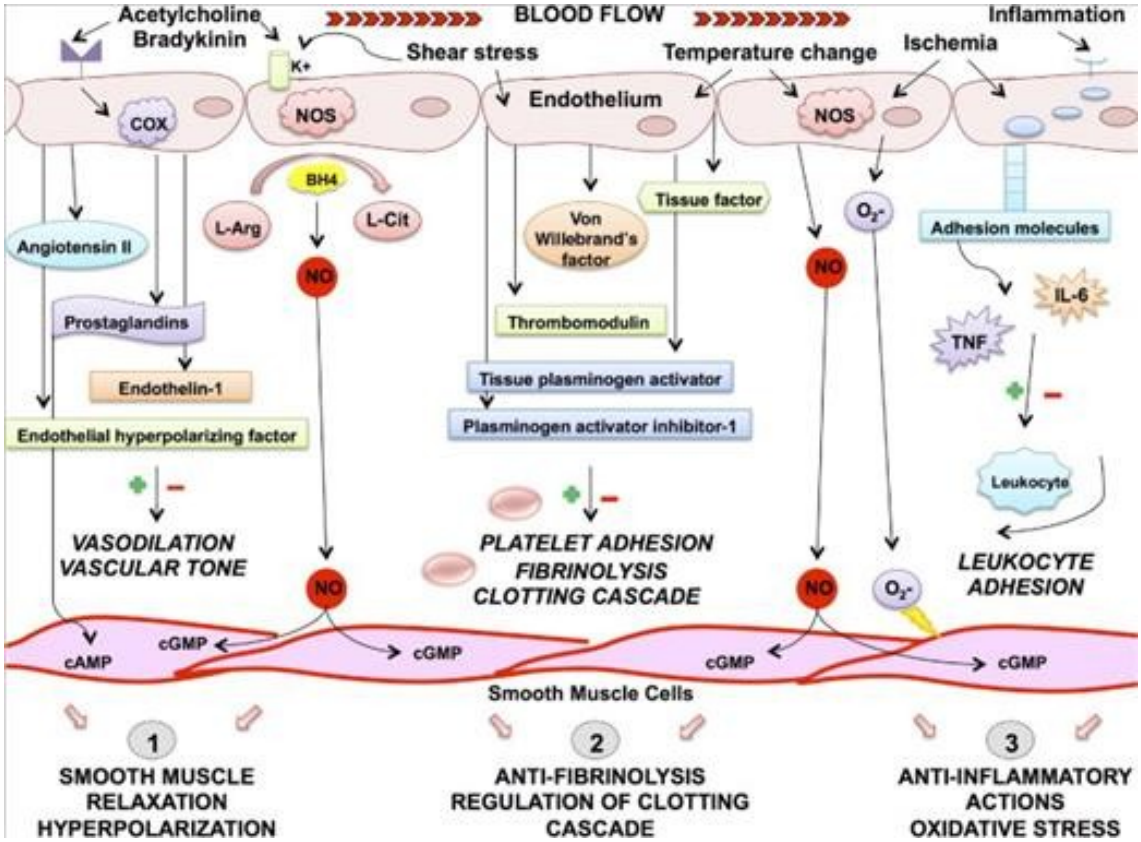

Figure 8 Endothelial cell function (reproduced from: http://content.onlinejacc.org/article.aspx?articleid=1361775)

1.2.3.2 Circulation

The heart is a dual pump that drives blood through two circulations – the pulmonary and systemic circulation – which are operated in series. Physiologically the blood flow is only one-directional (as determined by the cardiac valves). The deoxygenated blood from the body enters the right atrium through the superior and inferior vena cava. The blood then flows into the right ventricle through the right AV valve also called the tricuspid valve. From the right ventricle it is pumped across the pulmonary valve into the pulmonary veins that transport the blood to the lungs where it is oxygenated. The blood loaded with oxygen returns to the heart via the pulmonary veins and enters the left atrium. The left atrium connects to the left ventricle through the mitral valve. The blood is finally pumped across the aortic valve into the aorta and therefore the systemic arteries and organ tissues. From the tissues the blood is drained into systemic veins that return the blood to the venae cavae (Costanzo, 2011).

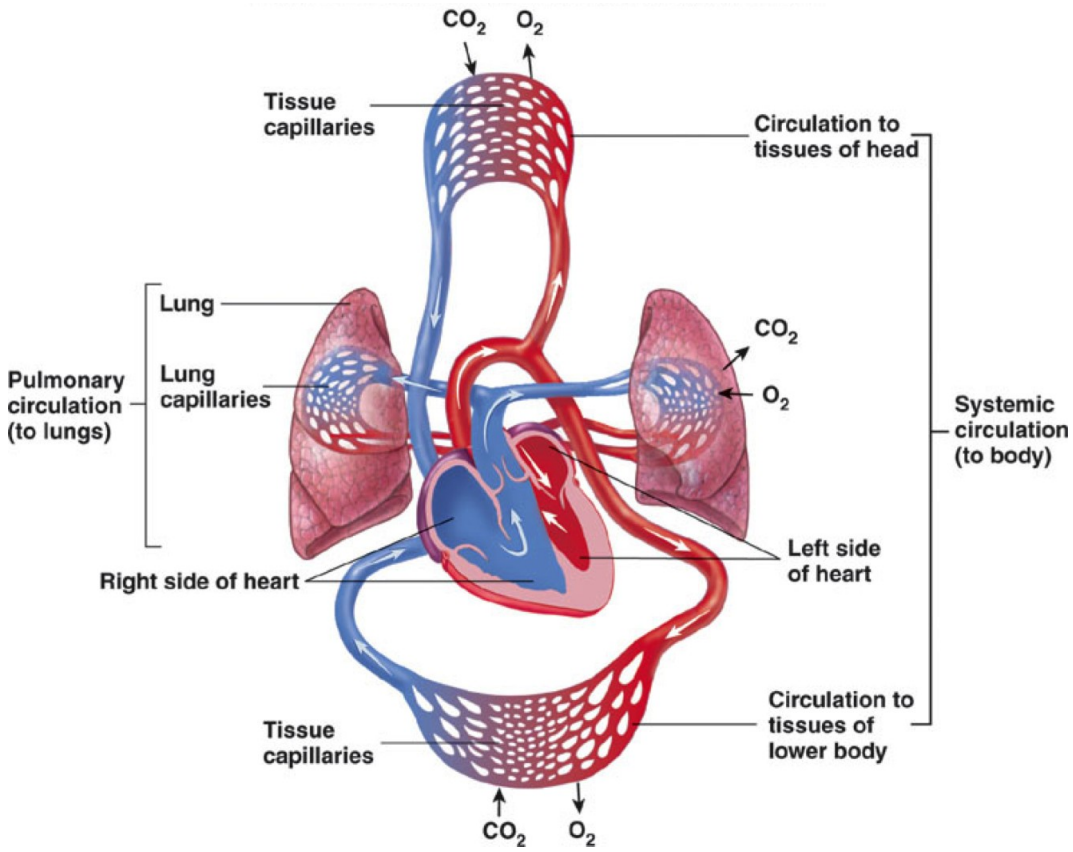

Figure 9 Systemic and pulmonary circulation (reproduced from: http://classconnection.s3.amazonaws.com/544/flashcards/666544/png/two_pumps1320107212642.png)

1.2.3.3 Blood Flow

The blood flow or cardiac output can be calculated by dividing the blood pressure gradient by the total peripheral resistance (which means the sum of resistances of the peripheral arterioles and capillaries). The viscosity of blood, the length of the vessel and the fourth power to the radius of the vessel determine the resistance of each vessel (Hagen-Poiseuille's equation) (Guyton and Hall, 2006; Boron and Boulpaep, 2005).

The cardiac output can also be expressed as the stroke volume multiplied with by the heart rate. It is usually defined in liters/min, approximately 5 l/min in adults. Due to the principle of continuity, the cardiac output from the left and right heart have to be the same because they are operated in series (Boron and Boulpaep, 2005).

The cardiac output is affected by age and size but also by the present metabolic state and activation level. That means that organs don't always receive the same amount of blood flow and are ranked differently in their importance. (Guyton and Hall, 2006).

The physiological blood flow in "straight" vessels follows a laminar pattern in most vessels. Because of adherence, the blood closer to the wall will move slower then the blood passing in the center of the vessel. This is called the parabolic profile of velocity (Guyton and Hall, 2006). At arterial curvatures, branching points and bifurcations, "eddy currents" may occur. This means that the blood components are "whirled up" passing these obstacles. This results in a higher resistance and lower blood flow and ultimately can disturb hemodynamics. The effect of these changed conditions can be seen in an altered biology of endothelial cells at these locations (predisposition for atherosclerosis) (Aird, 2007).

1.2.3.4 Arterial Blood Pressure

Blood pressure is defined as a "*force exerted by the blood against any unit area of the vessel wall* (Guyton and Hall, 2006)". It is usually measured in millimeters of mercury but can alternatively also be shown on the scale of centimeters of water (1mmHg equals 1.36cm H_2O) (Guyton and Hall, 2006).

During the cardiac cycle (systole and diastole), the blood pressure changes; it is pulsatile. The maximal (systolic) pressure should be around 120mmHg in the systemic and 25mmHg in the pulmonary circulation. It is measured when the blood is in the ejection phase. The diastole however is the phase with the lowest pressure – 80mmHg in the systemic and 8mmHg in the pulmonary circulation. It reflects the phase when the heart is relaxed and the blood is returned to the heart (Costanzo, 2011).

1.2.4 Pathophysiology

1.2.4.1 Endothelial Activation and Dysfunction

Endothelial activation and dysfunction have become widely used terms in connection with atherosclerosis and the pathogenesis of cardiovascular diseases.

Endothelial activation refers to the changes within the endothelial cell as a response to various stimulants. The activation of the endothelial cells causes a shift in the cell function. New/different adhesion molecules are expressed and other sets of cyto- and lymphokines, growth factors and vasoactive substances secreted. The endothelial surface becomes pro-thrombotic and pro-adhesive and the permeability increases (compare to physiological endothelial cell function as seen in Figure 8). While endothelial activation can be adaptive or non-adaptive endothelial dysfunction is always the latter (Ross and Pawlina, 2011).

Endothelial dysfunction (ED) therefore describes a state when the endothelium function becomes abnormal. The bioavailability of NO and the effects on vasodilation seem to have an important role as well. Endothelial dysfunction is seen as a precursor of cardiovascular disease although the assessment (as described in the chapter "Assessment of Vascular Function") hasn't yet found its way into daily clinical routine (Aird, 2007).

1.2.4.2 Arteriosclerosis vs. Atherosclerosis

The term arteriosclerosis includes three pathologies that are defined as a "hardening of the arteries". 1) Arteriolosclerosis affects smaller arteries and arterioles and is connected to arterial hypertension. 2) The Moenckeberg sclerosis describes a medial calcific sclerosis that doesn't include the intima. 3) Atherosclerosis is the most common form of arteriosclerosis that will mainly be discussed here. It is characterized by a variable combination of intima changes in mid-sized and large arteries. Atherosclerosis is considered the starting point of most arterial diseases and is currently the most common morbidity in the world (Böcker and Denk, 2008; Copstead and Banasik, 2013).

Risk Factors for Atherosclerosis

There are many "traditional" risk factors for atherosclerosis divided into two groups – modifiable and non-modifiable – that are believed to have an impact on the multifactorial etiology of atherosclerosis. These predispose to the occurrence and progression of the disease and are shown in Table 4 (Böcker and Denk, 2008).

Pathogenesis of Atherosclerosis

There have been many theories about the initiation of the atherosclerotic process in the past, the "response-to-injury hypothesis" being the most ascertained. As mentioned above, "disturbed" hemodynamics can already change the biology and gene expression of endothelial cells (Aird, 2007). This might explain why atherosclerosis often forms at sites with turbulent flow such as bifurcations and vascular curvatures. When the endothelium is damaged, the endothelium is activated and an inflammatory response is launched. Leukocytes are drawn to the site. Since the permeability of the inner vascular layer is increased LDL-lipoproteins can invade the intima from the blood (see figure below). Radicals from endothelial cells and leukocytes oxidize the LDL to mo-LDL. During the inflammatory phase, the mo-LDL accumulates, which enhances the production of chemokines, followed by monocyte attraction. Further adhesion molecules like ICAM-1, VCAM-1, PCAM-1, P-Selectin and E-Selectin are expressed where monocytes can dock and then immigrate into the intima. There they convert to macrophages. The now highly oxidized LDL binds to the scavenger receptors of the macrophages, which turns them into foam cells. Accumulations of foam cells in the intima can morphologically be identified as "fatty streaks". These changes are still considered reversible. At the same time, platelets adhere to the site of injury and release platelet-derived growth factor (PDGF). This in turn allows the migration of smooth muscle cells from the tunica media to the intima where they start to proliferate – a fibrous plaque begins to form (compare to figure below). Morphologically an atherosclerotic plaque can be described as a necrotic core of cellular debris and lipid accumulation covered by a fibrous cap consisting mostly of smooth muscle cells and connective tissue surrounded by macrophages and leukocytes. As the plaque continues to grow, the lumen of the artery is obstructed. The blood flow will diminish when the remaining vascular wall cannot compensate for the size of the atheroma anymore (Aird, 2007; Böcker and Denk, 2008).

Table 4 Risk factors for atherosclerosis (modified from: (Levinson, 2010))

Modifiable	**Non-Modifiable**
Smoking	Age
High blood pressure	Gender (Male)
Diabetes mellitus type 2	Genetic disposition
Hypercholesterolemia	Ethnicity
Hyperlipidemia	
Obesity Low physical activity Poor stress management	

The progression of atherosclerosis goes along with various serious complications. With increasing size and the inhibition of extracellular matrix production by INF-γ and proteinases, the plaque becomes more instable. This can result in the ulceration of the

plaque and thrombosis of the developed lesion. If lipid debris is carried off in the blood stream an emboli can form. The neovascularization at the proximity of the plaque can lead to hemorrhage if a capillary ruptures. Aneurysms, a bulging of the arterial wall, can form when the remaining vascular wall overcompensates the size of the atheroma (Böcker and Denk, 2008).

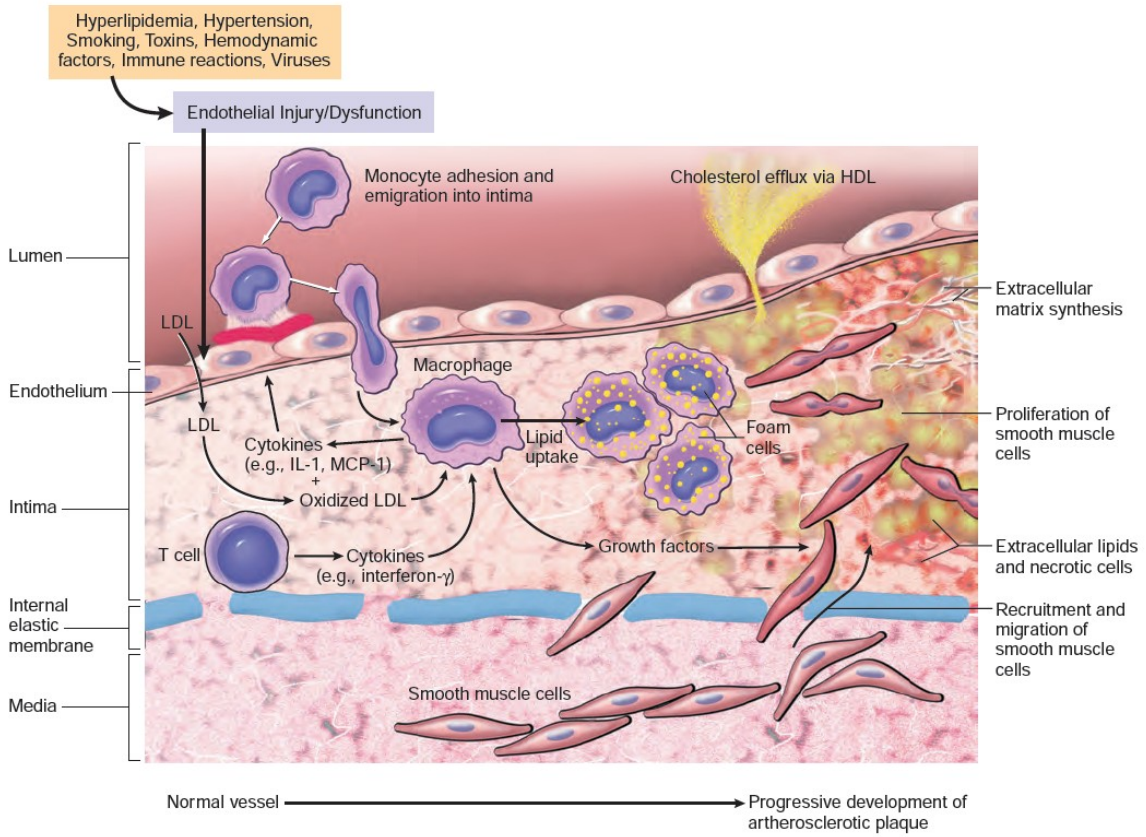

Figure 10 Pathogenesis of atherosclerosis (reproduced from: (Kumar et al., 2015))

1.2.5 Cardiovascular Diseases in HIV Patients

HIV patients can show a wide variety of cardiovascular problems, depending on their current HIV status (viral load, CD4+ cell count and class of antiretroviral therapy). The following chapters aim to provide information on cardiovascular diseases that were mainly seen in the pre-HAART era in comparison to today. The latter are also used as end points in studies connecting HIV to an increased cardiovascular risk.

1.2.5.1 Cardiovascular Manifestations of HIV Before the HAART Era

Prior to the introduction of HAART, cardiovascular manifestations of HIV were often connected to opportunistic infections. These can still be seen in patients with a low CD4+ cell count level of < 200 cells/mm^3 (Mandell et al., 2010).

Agents such as Toxoplasmosis gondii can commonly cause infectious myocarditis, although seldom as an isolated cardiac manifestation. Mycobacteria and fungi can also cause myocardial disease (Mandell et al., 2010).

A rather underdiagnosed problem is the HIV-related cardiomyopathy with left ventricular dysfunction. The patients present very unspecific symptoms such as fatigue and dyspnea on exertion, which could also account for wasting in advanced HIV stages. Other co-infecting viruses such as Epstein-Barr virus or Cytomegalovirus may also play a role in the evolution of dilated cardiomyopathy in HIV patients (Mandell et al., 2010).

Pericardial effusions are often found in patients in late stages and advanced illness especially in the hospital setting. Opportunistic infections and malignancies at other sites are the most common cause. The effusions are mostly asymptomatic and don't evolve to cardiac dysfunction (Mandell et al., 2010).

The acceleration of atherosclerosis by some antiretroviral drugs has been mentioned lately, but higher incidences of common cardiovascular pathologies aren't described in textbooks as of yet (Mandell et al., 2010).

1.2.5.2 Arterial Hypertension

By WHO definition, arterial hypertension is an elevated blood pressure above 140mmHg systolic to 90mmHg diastolic respectively. It can principally be divided into two groups based on etiology – primary or essential hypertension with a multifactorial genesis and no secondary causes and secondary hypertension as a consequence of an underlying disease (for example renal or endocrine). A single elevated blood pressure doesn't result in the diagnosis arterial hypertension; the WHO recommends three independent measurements at various times of day on at least 3 days (Herold, 2015).

Arterial hypertension isn't only a serious risk factor for atherosclerosis but has direct impact on the cardiovascular system. The increase in systemic vascular resistance, for example, coincides with an increased afterload, therefore the heart has to work harder to eject blood into the systemic circulation. The myocardium of the left ventricle hypertrophies which results in a decrease in the relaxation abilities of the heart muscle. The

relaxation phase of the heart – the diastole - is essential for the perfusion of the coronary arteries (Copstead and Banasik, 2013).

1.2.5.3 Coronary Heart Disease

Coronary heart disease (CHD) is also known as coronary artery disease (CAD). It is currently the number one cause of death in industrial countries (with higher incidences with increasing age). It describes the manifestation of atherosclerosis in the coronary arteries. The obstruction of the lumen of the arteries causes a lower blood flow and an imbalance between the oxygen demand and supply in the myocardium arises (coronary insufficiency). This results in myocardial ischemia (Herold, 2015).

Coronary stenoses can be distinguished into three grades, 1 being the least significant (25-49%), 2 being significant (50-74%) and 3 being critical (75-99%). In critical stenoses, an exercise-dependent angina pectoris is to be expected if there are insufficient collateral circuits. The main symptom is retrosternal chest pain, also called sternocardia, which can radiate in various directions (especially in women) (Herold, 2015).

In angina pectoris (AP), a stable and unstable form are usually differentiated. While stable AP is triggered by certain mechanisms (mostly exercise/exertion) and reacts well to nitrate therapy, unstable AP is more frequent, longer in duration and appears at rest. It is considered to be an acute pre-state to myocardial infarction without elevation of Troponin T or I (Herold, 2015).

1.2.5.4 Acute Coronary Syndrome and Myocardial Infarction

The Acute Coronary Syndrome (ACS) includes three entities: the unstable AP that was described above, the non-ST-elevation myocardial infarction (NSTEMI) and the ST-elevation myocardial infarction (STEMI). ACS is considered an immediate emergency and should be seen in hospital as soon as possible. NSTEMIs show an elevation in Troponin T or I but no distinctive changes in the EKG as seen in the STEMI (Herold, 2015).

Myocardial infarction occurs on the basis of CHD with a critical stenosis or complete occlusion of the artery. Usually a vulnerable and complex atherosclerotic plaque ruptures and causes a thrombotic closure of the artery lumen. This occlusion leads to ischemia in the tissue served by the affected coronary artery. Depending on the location of the infarction, left heart insufficiency and arrhythmias can complicate the outcome. The patients usually display instable angina pectoris, vegetative symptoms, arrhythmias, blood

pressure variations and/or dyspnea as a sign of left ventricular insufficiency. The therapy of choice would be fast revascularization of the occluded artery (Herold, 2015).

1.2.6 Assessment of Vascular Function

As discussed before, endothelial dysfunction can be seen as a precursor of cardiovascular disease. Therefore the assessment of endothelial and vascular function has become of major interest today. An easy, reliable and quantifiable method of measurement might help to set up preventive measures against further progression at a very early stage.
Vascular function can be assessed in one of the following ways: quantification of plasma markers, measurement of endothelium-dependent vasodilation and imaging for anatomic changes.

1.2.6.1 Plasma Markers

Plasma markers have to fulfill the criteria of being directly measurable and of not being produced by other cell types, which would make them non-specific. Therefore von Willebrand Factor, soluble Thrombomodulin, E-Selectin and Endothelin should be preferred over ICAM-1 and VCAM-1. Nitric oxide isn't directly measurable in laboratories (Aird, 2007).
Von Willebrand Factor (vWF) is continuously expressed by the endothelium. It is important in the upholding of hemostasis. It is part of the blood coagulation cascade and stabilizes/binds factor VIII. It also organizes platelet adhesion at vascular injury sites. Levels can be elevated in some physiological situations such as pregnancy but pathological findings are usually connected to atherosclerosis and inflammatory connective tissue diseases. High numbers of vWF are shown to have a poor prognosis on cardiovascular disease progression. VWF is currently considered the most reliable of the various plasma markers (Aird, 2007).
Thrombomodulin is a transmembranous receptor for Thrombin on the endothelial cell surface. It prevents the formation of thrombi in the vessel. When the Thrombomodulin is lost from the cell surface in pro-thrombotic conditions, it can be measured as elevated soluble Thrombomodulin levels in plasma or serum. The relevance as a predicting factor for cardiovascular events remains uncertain (Aird, 2007).
Endothelin-1 is a very potent vasoconstrictor and is constitutively expressed by the endothelium for the balance of the vascular tone. An increased level is associated with

almost every cardiovascular risk factor but isn't related to prognosis of further cardiovascular events. Endothelin binds to Endothelin A and B receptors found in vascular smooth muscle cells but also in macrophages, endothelium and other cells. A blockage in ETA receptors has shown an improved endothelial function (Aird, 2007).
While the abovementioned markers are always found in the plasma at a certain level, soluble E-Selectin is a distinct marker for endothelial activation through various stimulants (but not limited to cardiovascular diseases) (Aird, 2007).

1.2.6.2 Non-invasive Vascular Reactivity Measurements

There are various methods to measure the grade of vasodilation of endothelium, which is used as a surrogate marker for endothelial dysfunction (impaired function goes along with impaired vasodilation).
The first technique to be used for assessing vascular reactivity was the venous plethysmography. A mercury in-silastic strain gauge coupled to a plethysmograph is placed on the upper arm and an air-cuff around the wrist to occlude the blood flow to the hand. This serves to achieve a laminar arterial flow to the forearm. The baseline forearm blood flow is assessed before intra-arterial infusions are started. The measurements are usually ascertained from both arms (one arm serving as control). For endothelium-dependent vasodilation measurements acetylcholine is commonly infused. If nitroprusside, a NO donor, is injected instead, the endothelial-independent vasodilation can be assessed. The vascular reactivity (the change in the forearm blood flow) is measured in milliliters per minute per 100 milliliters of tissue.
Celermajer and colleagues on the other hand started using standard ultrasound techniques for flow-mediated vasodilation (FMD) measurements in 1992. The aim is to measure the baseline and highest post-inflation diameter of the brachial artery in a longitudinal ultrasound plane. A pneumatic cuff is placed around the upper arm or forearm and inflated to a pressure of 50mmHg above systolic blood pressure. This occludes the artery, which is maintained for 5 minutes and induces local ischemia (hypoxia, tissue pH changes and collection of deoxygenated blood). When the cuff is opened, the brachial artery dilates as a direct reaction to the ischemia. This creates additional shear stress and turbulent flow in the artery followed by an activation of the endothelium (vasodilation). The FMD represents the percentage the artery diameter increases post occlusion. The limiting factor of this technique is the high interobserver difference; it requires careful personnel training (Aird, 2007).

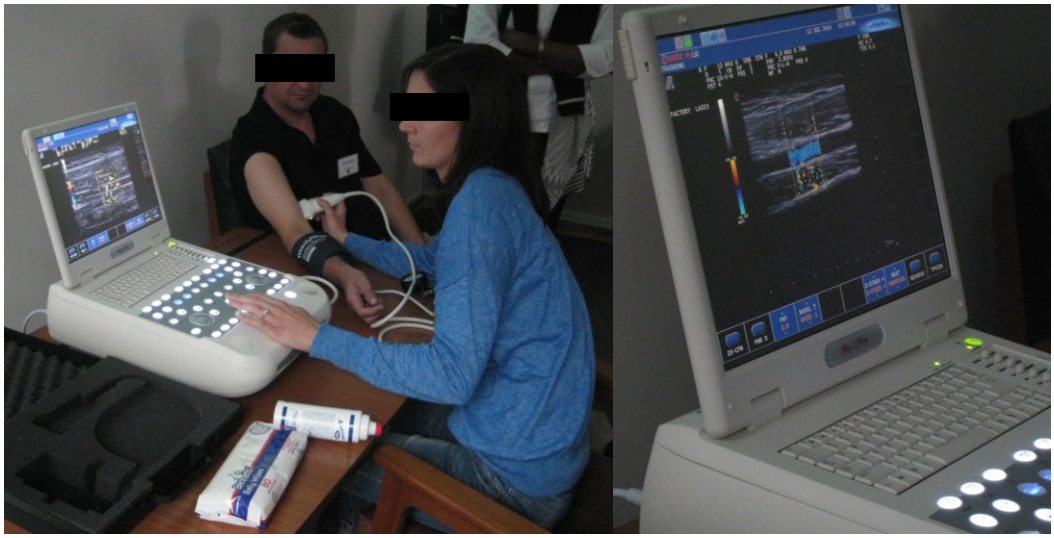

Figure 11 Demonstration of FMD measurements at the Stellenbosch University, South Africa[2] (for the study protocol a probe holder will be used to ensure easier handling and more exact measurements)
Figure 12 Doppler ultrasound of the brachial artery post-occlusion

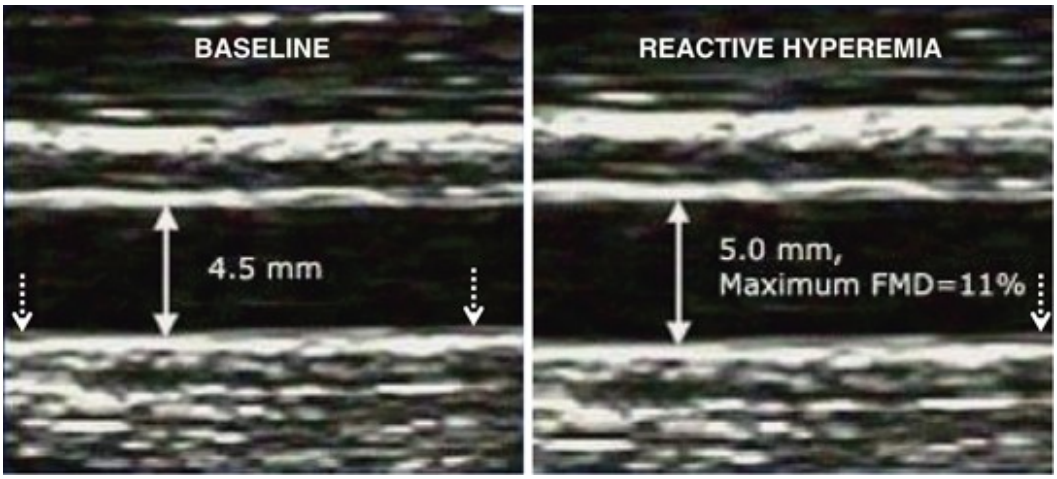

Figure 13 Standard ultrasound images for the measurements of the brachial artery diameter at baseline and after occlusion release (reproduced from: http://www.nature.com/ijir/journal/v22/n2/fig_tab/ijir200959f3.html)

1.2.6.3 Anatomic Changes

Anatomic imaging can also help to assess changes in the vascular wall in atherosclerotic patients. One of the first changes of atherosclerosis that can be readily measured is the increased intima-media thickness of the carotid artery. In high resolution B-mode ultrasound imaging, a typical "double line pattern" can be displayed from which measurements of the tunica intima and tunica media are taken. Studies have shown that an

[2] Permission was obtained from persons shown in the picture.

increase of the intima-media thickness (IMT) is associated with the occurrence of cardiovascular events (Aird, 2007).

A technique that is less widely applied is the measurement of the pulse wave velocity (PWV), which is considered a surrogate marker for arterial stiffness. Using Doppler probes the pulse pressure wave is documented at two different arterial sites and the time delay subsequently calculated. The carotid-femoral pulse wave velocity is used most commonly, but other sites such as carotid-dorsalis are also possible (Aird, 2007).

1.2.6.4 Invasive Vascular Reactivity Measurements

The endothelial function in the coronary arteries can still only be assessed invasively through coronary angiography. A catheter is usually placed in the left coronary artery and a vasoactive substance – acetylcholine – is injected. Physiologically this would cause vasodilation; in endothelial dysfunction the paradox reaction of vasoconstriction can be seen. In addition the blood flow can be measured using intravascular Doppler. Today measurements of the peripheral arteries are often used as comparable surrogate markers for assessing the risk of cardiovascular events (Aird, 2007).

2 Purpose and Objectives of this Diploma Thesis

Since the 1990s with the introduction of highly active antiretroviral therapy, the course of HIV infection and AIDS was greatly altered. Today the average time of clinical latency until disease progression has increased to over 30 years from a mean of 7 years in days without treatment. Other non-AIDS-related diseases most importantly cardiovascular diseases, have now pushed their way into the foreground concerning the management of HIV infected individuals (Francisci et al., 2009).

The aim of my diploma thesis is to compare the knowledge about the effects of HIV/AIDS and antiretroviral therapy on the cardiovascular system from literature such as textbooks (presented in the Introduction chapters) to the "state-of-the-art" publications found in medical databases and science journals.

The pathophysiologic mechanisms of cardiovascular disease in uninfected individuals have been widely studied. In HIV/AIDS, many pathways and the specific impact of HIV/AIDS and antiretroviral therapy still remain unclear. Articles concerning cardiovascular risk in HIV infected individuals (treatment-naive or under HAART treatment) will be discussed in this review. A special focus will be placed on endothelial dysfunction in HIV patients and whether it can be considered a precursor for cardiovascular disease in this population as well. A lot of research on HIV/AIDS and ART is conducted in Europe, North America and Australia, while studies from the most affected parts of the world such as Africa seem quite rare. I will try to provide additional information on geographical differences if applicable.

While HIV/AIDS might not seem a prevalent problem in Austria it has changed the socio-demographics of many parts of the world. My interest in HIV was especially evoked through the involvement in an EU-Africa project namely the EndoAfrica Project that was initiated by my supervisor. My motivation was further heightened by a trip to South Africa where I could see and experience the work of the research team firsthand, which focuses on the cardiovascular effects of HIV/AIDS in an endemic country.

3 Methodology

This diploma thesis is a literature review on the topic of "Effects of HIV/AIDS and antiretroviral therapy on the cardiovascular system". The information that is presented was gathered in a systematic literature research.

The Introduction chapter aims to give background information on both HIV/AIDS and the Cardiovascular System as the two most important topics covered in my diploma thesis. Secondary literature as well as two papers on the history of the AIDS epidemic and the current guidelines by the European AIDS Clinical Society (mainly information about ART extracted) were used for this section. I received access to these books through the Institute of Physiology at the Medical University of Graz and through the libraries at both the Medical University of Graz and at King's College in London during a placement.

The systematic literature research is mainly based on the two online databases PubMed and Web of Science accessed in the months from May 2014 to April 2015.
The first step was a PubMed search using free text using the following phrases in combination with "HIV/AIDS":

- Cardiovascular effects
- Cardiovascular disease
- Cardiovascular system
- Cardiovascular risk factors
- Endothelial dysfunction
- Endothelial function
- Atherosclerosis
- Antiretroviral Therapy
- HAART

This was repeated using human immune deficiency virus and acquired immune deficiency syndrome instead of the abbreviations. Synonyms and associated terms such as HAART or combined antiretroviral therapy for antiretroviral therapy were used where applicable.
In a second step, I identified the relevant MeSH terms (controlled vocabulary) which I connected with boolean operators such as AND and OR in the PubMed Search Builder to limit my search results. Subheadings such as pathologies, abnormalities and etiology were

used for HIV, AIDS, cardiovascular disease and vascular endothelium. Some terms such as endothelial dysfunction have yet to be included into the MeSH term database.
To support my search, I also used Web of Science to gain further information on the authors as well as how often and where the articles were cited. This allowed me to review and add appropriate references to my search results.

I evaluated literature based on the following criteria that also helped me to refine my search further (using filters):

- Objectives of the studies relevant to my review
- Articles published in the past 20 years
- Articles published in English (regardless from which country)
- Publications concerning adults
- Animal and in vitro studies were excluded
- Studies with co-infections (HBV, HCV, TB etc.) and opportunistic infections were excluded
- Articles without abstracts available even after an extensive web search involving search engines such as Google were also excluded

The free text and MeSH term based search resulted in over 600 titles (including duplicates) that were evaluated for their relevance. About 200 titles were then downloaded to my citation manager (EndNote, software provided by the Medical University of Graz). Fifty-two remaining duplicates were identified and erased before starting the review of abstracts. Twenty-five articles were excluded because of missing abstracts and a further 47 were deemed irrelevant for this review based on the abovementioned criteria.
In the end, 85 articles remained that fit the abovementioned criteria to which I added articles that I had previously received from my second supervisor and a researcher from the EndoAfrica group. After reading the full text publications, I categorized them based on their study objectives (with subcategories for studies performed in North America/Europe vs. Africa)

- Vascular function - Endothelial function /dysfunction
- Cardiovascular risk factors
- Cardiovascular disease (including specific end points such as myocardial infarction)
- Antiretroviral Therapy (including drug classes)

4 Review of Current Literature

In 1996 Zietz et al. already presented data about a possible increase in risk of cardiovascular disease in HIV patients. His postmortem study revealed that HIV-infected men at a young age already showed atherosclerotic changes in their vascular system (Monsuez et al., 2009; Zietz et al., 1996).

Since then many studies are focused on the possibly increased risk of cardiovascular disease in HIV-infected individuals and on the following three questions: 1) Are HIV-infected individuals a subpopulation that simply has a higher prevalence of traditional cardiovascular risk factors (independent of infection and antiretroviral therapy)? 2) Do HIV and ART increase the risk of developing traditional risk factors such as dyslipidemia, hypertension and insulin resistance? 3) Do HIV and ART (dependent on drug class) affect the pathogenic process leading to atherosclerosis through direct, indirect and even synergistic effects (on multiple levels) and, if so, how (Boccara et al., 2013; Currier et al., 2008)?

Lately the effects of HIV and ART on endothelial function, a precursor of cardiovascular disease, have become of major interest as well, although there is still little data available (mostly studies with small sample sizes) (Currier et al., 2008; Boccara et al., 2013).

The following chapters will shed some light on the study results concerning the effects of HIV and ART on vascular function, the prevalence of cardiovascular risk factors and the actual risk of cardiovascular disease in the HIV population. There might be a certain degree of overlapping concerning the results of studies and their categorization in chapters.

4.1 Vascular Function

The effects of HIV on the endothelium are very complex and include many direct and indirect effects. HIV probably directly infects endothelial cells via chemokine receptors, galactosyl-ceramide receptors and the CD4 antigen (Gresele et al., 2012). Certain types of endothelial progenitor types are greatly affected by HIV infection and their depletion leads to decreased secretion of vascular endothelial growth factor (Teofili et al., 2010). Furthermore the endothelium can be directly activated by viral proteins such as *gp120* and *tat* that are believed to alter the nitric oxide synthase system, or can be disturbed indirectly

by cytokines secreted by other infected cell lines (for example mononuclear and adventitial cells). The chronic inflammatory state, the continuous immune activation (often referred to as "emerging" risk factors) and possible metabolic changes (dyslipidemia, dysglycemia) caused by HIV and ART can also be connected to vascular injury (Gresele et al., 2012; Mondy, 2008; Andrade and Cotter, 2006). The influence of ART on endothelial function remains debated as will be discussed with the results of various studies (Monsuez et al., 2009). The figure below displays the factors that probably influence the development of endothelial dysfunction.

The last paragraph described pathogenic pathways that have been found thus far in in vitro studies and that were mentioned in various reviews. The following in vivo studies concentrate on the assessment of vascular functions and the alterations caused by HIV and HAART. The methods that can be used for the measurements of vascular function were described in the chapter "Introduction – Assessment of Vascular Function". Some additional/new approaches in the assessment of vascular function such as retinal imaging will be reviewed as well.

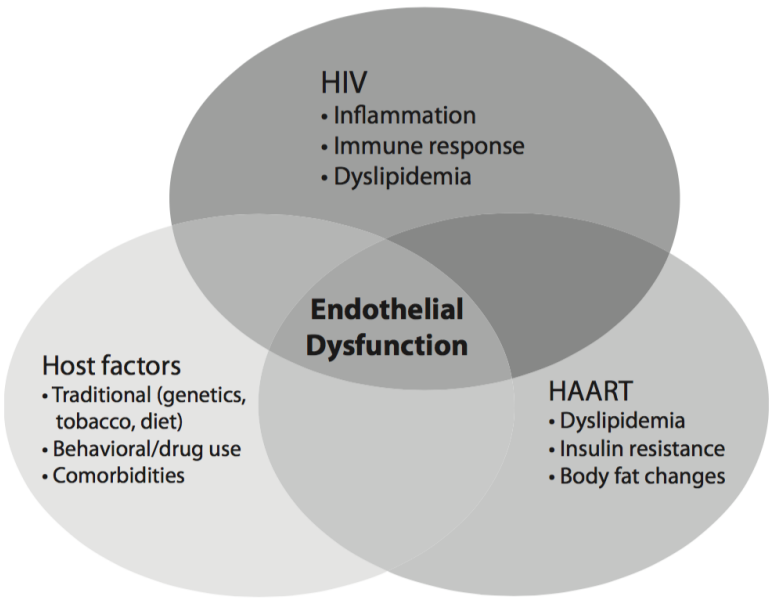

Figure 14 Relationship of factors probably contributing to endothelial dysfunction in HIV (reproduced from: (Mondy, 2008))

4.1.1 Flow-Mediated Dilation and Endothelial Plasma Markers

It is of note that standard FMD protocols differ slightly between the studies (arterial occlusion time, occlusion cuff pressure and time of artery diameter measurement post occlusion). Additionally NMD (nitric oxide mediated dilation) is often added to the

protocol for endothelium-independent vasodilation for comparison to the endothelium-dependent vasodilation measured in FMD. Sublingual nitroglycerin (usually 0.04mg) is therefore applied before the occlusion protocol and the vascular diameter measurements are repeated. This serves as a sort of internal control and seldom shows any differences between cases and controls. (Blanco et al., 2006; Kristoffersen et al., 2010; Solages et al., 2006; Torriani et al., 2008).

Blanco et al., Solages et al. and Kristoffersen et al. all came to the conclusion that antiretroviral treatment (various regimens) is connected to significantly lower FMD results than healthy controls. Blanco et al. studied a group of 28 HIV-infected patients with a low cardiovascular risk (young age, cardiovascular risk factors and diseases except for smoking were excluded). They found that even this low risk HIV population presented endothelial dysfunction. Treatment-naive HIV-infected patients were also included who only showed an intermediate impairment that didn't reach significant value, possibly due to the small sample size (Blanco et al., 2006). Solages et al. additionally associated the viral load with endothelial dysfunction. They named BMI, sex, HIV status and smoking as independent risk factors as well. Intravenous drug users displayed the worst FMD measurements within the HIV-seropositive group (Solages et al., 2006). In comparison to a minimum time of 6 months on HAART in Blanco et al.'s study, unfortunately no mean treatment time was mentioned in Solages et al.'s publication, which minimized the interpretation of effects of HAART duration on the measurements. There was also no differentiation of drug classes listed (Solages et al., 2006; Blanco et al., 2006).

Kristofferson et al. compared cardiac perfusion positron emission tomography (PET; using intravenous ^{13}N-ammonia) and FMD measurements in 12 HIV-infected individuals prior and post ART initiation (mean time of HIV infection 37 months; tests performed a mean of 35 days after start of treatment). FMD of the brachial artery decreased significantly from a mean of 8.68% to 4.58%. The PET images were taken at rest, during a cold pressor test (sympathetic activation) and during infusion of dipyridamole (myocardial hyperemia). While no changes in myocardial perfusion were found during the cold pressor test, the maximal myocardial perfusion decreased by 31% and the myocardial perfusion reserve by 20%. This proves a decline in peripheral but also in coronary vascular function in HIV-infected individuals (Kristoffersen et al., 2010).

This data contradicts several other studies that have found an increase in endothelial function after the initiation of HAART. Torriani et al. from the AIDS Clinical Trials Group (ACTG) compared FMD measurements and blood levels (HIV infection markers, lipid

panel, and inflammatory markers) of 82 treatment-naive HIV-infected individuals at baseline (pre-HAART) and 4 and 24 weeks after initiation of therapy. The blind test persons randomly received one of three treatment regimens to further gain information on effects of the various drug classes. At baseline, FMD was similar across the three treatment groups with a mean of 3.68% (no correlation with viral load or CD4+ cell count). Surprisingly the FMD significantly increased by 0.74% at the set point of 4 weeks and 1.48% at 24 weeks. The changes at 24 weeks were inversely proportionate to the viral load. Additionally, the whole lipid panel showed an increase at both set points with only one difference between the 3 treatment regimens - solely the total cholesterol level was significantly higher in the NRTI-sparing arm, which is composed of a NNRTI and a PI. Protease inhibitors are connected with dyslipidemia in several other studies discussed later (Torriani et al., 2008).

They conclude that endothelial function improves after the initiation of antiretroviral therapy. Of course long-term changes in the lipid and glucose metabolism cannot be taken into account in the short follow-up time of 24 weeks. The cause of improvement remains unclear: it could either be connected to an ART-induced mechanism, the viral suppression or an altered immunologic and chronic inflammatory status (Torriani et al., 2008).

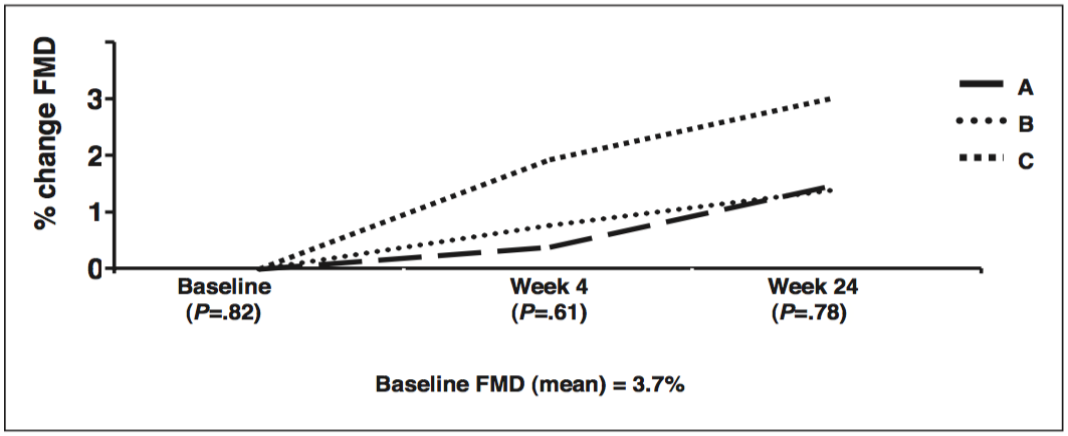

Figure 15 ACTG study results of changes of FMD over 24 weeks of treatment of previously treatment-naive HIV-infected individuals by Torriani et al.; FMD improved independent of treatment arm ((A) NRTI + NNRTI, (B) NRTI + PI, (C) NNRTI + PI) (reproduced from: (Mondy, 2008))

While the short follow-up time displays a limitation in this and many other studies, Francisci et al. measured endothelial and platelet activation markers in plasma samples of 56 HIV-infected individuals starting HAART treatment compared with 10 treatment-naive infected persons and 28 healthy controls taken at regular periods up to 24 months (Francisci et al., 2009). The findings coincide with Kristoffersen et al. (study on

biomarkers from 2009) and Arildsen et al. (Kristoffersen et al., 2009; Arildsen et al., 2013). VWF, sVCAM-1, sP-Selectin, and MCP-1 were all elevated at baseline (prior to start of treatment limited to two different regimens) as shown in the figure below. A significant decrease in levels was found beginning at 6 months with a return to the level of the healthy controls by the time of study end at 24 months (independent of lipid profile). There was no difference between the HAART regimens. IL-6 and IL-8 were not detectable, and t-PA and sCD40L did not show any difference to the healthy controls. In the group naive to treatment (because of CD4+ counts > 200cells/mm^3) the plasma markers remained elevated during the whole period of 24 months. A significant association of HIV RNA with sVCAM-1, MCP-1 and vWF was calculated. Additionally, Francisci et al. showed the typical lipid changes with increased TC and TG, only found in the protease inhibitor based regimen (Francisci et al., 2009).

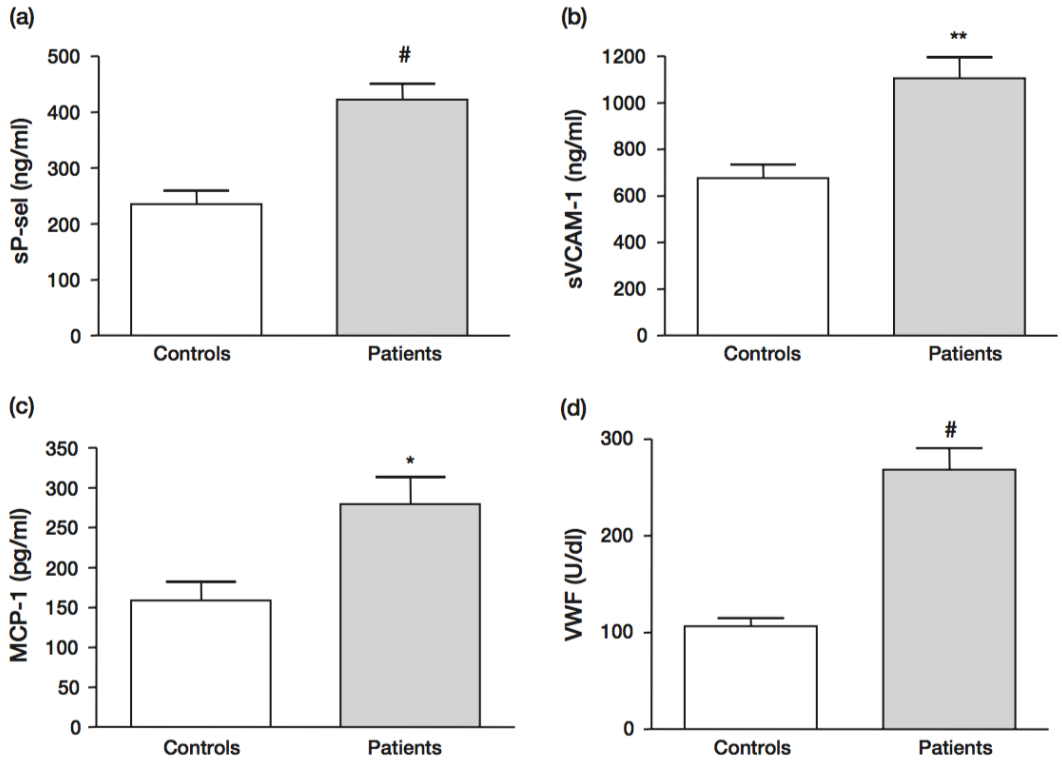

Figure 16 Plasma markers sP-Selectin, sVCAM-1, MCP-1 and vWF at baseline comparing controls to HIV-infected individuals before initiation of treatment (* p <0.05, ** p <0.005, # p <0.0001 vs. controls) (reproduced from: (Francisci et al., 2009))

Stein et al. compared FMD, IMT and biomarkers in 331 treatment-naive patients as part of another study of the AIDS Clinical Trials Group. As opposed to other studies, they postulated that there is a stronger correlation of impaired FMD and increased IMT with traditional cardiovascular risk factors in a low risk HIV population than with CD4+ cell count, viral load and inflammatory biomarkers (Stein et al., 2013).

The abovementioned studies were all conducted in Europe and North America. According to my literature search only the HART group (Hypertension in Africa Research Team) in South Africa has studied vascular function in HIV-infected persons in Sub-Saharan Africa, the region most affected by HIV. Fourie et al. have published several papers on the results of substudies of the PURE (Prospective Urban Rural Epidemiology) study. Since HIV subtype C is more prevalent in Sub-Saharan Africa one of their aims was to investigate whether differences in the cardiovascular effects can be seen to subtype B and the results from Europe/North America (Fourie et al., 2011b).

In a substudy from 2011, Fourie et al. studied the differences in endothelial function and biomarkers as well as coagulation and inflammatory markers in 300 newly diagnosed HIV-infected persons versus 300 uninfected individuals. Although all activation markers (ICAM-1, VCAM-1 and IL-6) were elevated only VCAM-1 showed a significant increase. Fibrinogen, PAI-1 and hs-CRP on the other hand showed no differences in comparison to the healthy control group. Since the cases were all newly diagnosed infections, the duration of the infection is unknown but can be assumed to be rather short. This and all other PURE substudies show no major differences between HIV subtype B and C (Fourie et al., 2011a).

4.1.2 Intima-Media Thickness and Pulse Wave Velocity

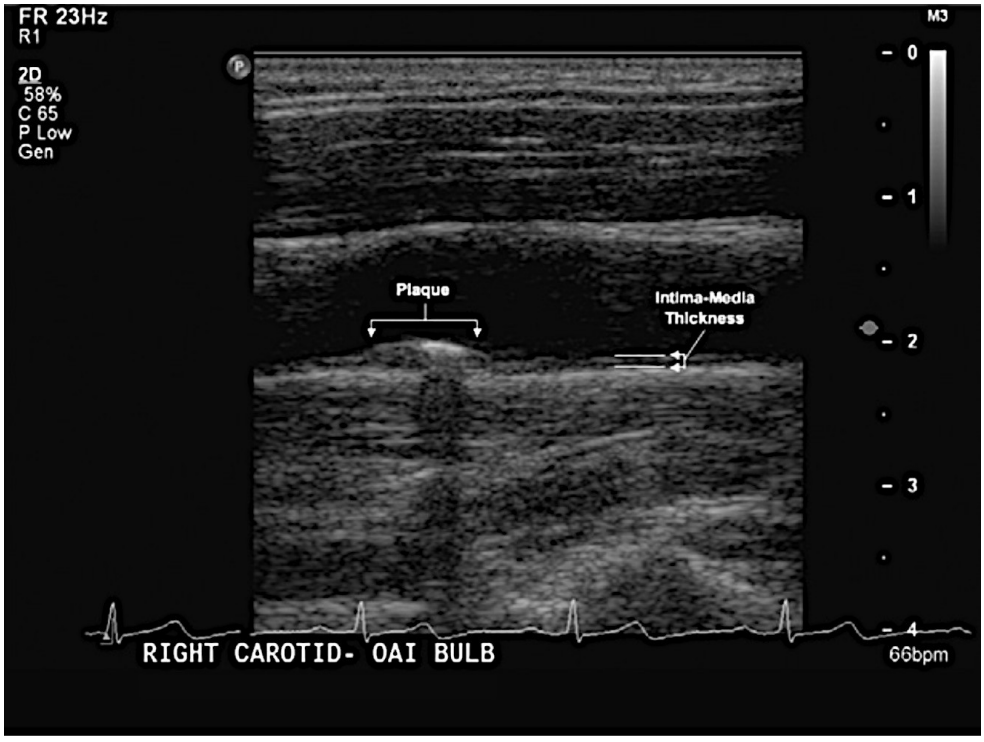

Figure 17 Carotid artery ultrasound image for measurement of IMT ("double line pattern"); atherosclerotic plaque present (reproduced from: (Longenecker and Hoit, 2012))

Staying in Africa, another substudy of the PURE study compared 144 infected persons (about 50% each treatment-naive and on HAART for a mean of 2.86 years) with 165 healthy controls. While dyslipidemia was only found in the ART group, increased ICAM-1 and VCAM-1 levels were detected in the whole HIV-infected group. No differences in hs-CRP levels were presented. Surprisingly neither pulse-wave velocity nor IMT showed any differences to the control group. Therefore no changes in vascular structure or function could be detected, perhaps because the mean time of infection was too short or because there are geographical and ethnical differences in the pathogenesis of atherosclerosis (Fourie et al., 2015). Similar results from another PURE substudy were published by Botha et al. (Botha et al., 2014).

In a European study from 2009, IMT, arterial stiffness (distensibility and compliance of the carotid, brachial and femoral arteries) and endothelial markers were compared over a period of 24 months in a longitudinal prospective study. Thirty-seven initially treatment-naive HIV-infected men were randomized to two drug regimens that showed no difference in the otherwise significant increase of IMT over the study period. The overall IMT progression of 0.061 mm/year and 0.044 mm/year (van Vonderen et al., 2009a) is higher than the 0.01 mm/year suggested in a study of carotid intima-media thickness of the general population (Howard et al., 1993). The arterial stiffness (after ART initiation) was only significant in the femoral artery. Different artery segments are known to be susceptible to different risk factors. Changes in the distensibility of the femoral artery are most often seen in metabolic changes, while aging and hypertension are correlated with changes in the carotid artery. The endothelial markers behaved exactly the opposite to the IMT measurements and arterial stiffness with a decrease in levels after initiation of ART. This suggests HIV and ART might have different effects on the micro- and macrovasculature or that arterial stiffness is connected to non-endothelial related mechanisms (van Vonderen et al., 2009a).

In a case-control study by van Vonderen et al., also from 2009, HIV-infected patients had a 10.8% higher IMT of the common carotid artery than controls. No differences between treatment-naive and treated individuals in IMT were measured. Arterial stiffness on the other hand was worse in infected persons but even more so in the treated HIV-infected group (only the femoral artery). This again implies that HIV and ART effects might vary along the arterial system. Pulse-wave velocity didn't show any differences at all (van Vonderen et al., 2009a) and no association with HIV infection markers were found (Delaney et al., 2010; van Vonderen et al., 2009e). Delaney et al. speculated that the

missing correlation might be due to the long duration of HIV infection and ART in their study group (time since infection mean of 13 years, minimum time of ART 2.6 years). Perhaps traditional risk factors overpower the effect of HIV in long-term infection (Delaney et al., 2010).

Grunfeld et al. adds that the difference in increase in IMT measures between HIV-infected and -uninfected groups remains significant even after adjusting for demographics and traditional cardiovascular risk factors. The association between the increased IMT and HIV was stronger in the internal carotid artery than in the common carotid artery (Grunfeld et al., 2009).

Hsue et al. postulate that HIV status alone should be considered a significant independent cardiovascular risk factor and not the quantifiable infection marker levels. They compared HIV-seropositive individuals with "elite controllers". This term defines HIV-infected individuals that are treatment-naive and have still maintained an undetectable viral load (due to unknown mechanisms). Surprisingly, elite controllers also displayed significantly increased carotid intima-media thickness. They believe that early onset of atherosclerosis in HIV patients is independent of cardiovascular risk factors, detectable viremia levels and antiretroviral drug exposure (Hsue et al., 2009).

Participants in the study by Lekakis showed an increased carotid IMT that was comparable to a group of patients with coronary artery disease (HIV-infected group: mean of 0.64mm, CAD group 0.66mm and controls: 0.55mm). The higher IMT measures within the HIV group were mainly based on individuals treated with protease inhibitors. FMD on the other hand presented a less drastic picture, with measurement results in the range between those for CAD patients and controls. Unfortunately no information was given on the mean time of infection and HAART although used in their univariate and multivariate analyses (Lekakis and Ikonomidis, 2010).

While Mercie et al., Kaplan et al. and Baker et al. also concur on the increased IMT progression and absolute IMT measures among HIV-infected individuals, they found associations with the CD4+ cell count and the suppression of the viral load respectively (Mercie et al., 2005; Baker et al., 2011; Kaplan et al., 2008).

Other studies e.g. by Maggie et al. and Currier et al. are mentioned in the chapter concerning "Antiretroviral Therapy" because their findings are mostly concerned with differences between drug classes.

4.1.3 Retinal Imaging

Retinal imaging (or fundus photography) has been widely used among ophthalmologists to monitor diseases of the retina, optic disc and macula. However, it also allows an easy way of visualizing an individual's microcirculation. It has lately been established as a validated marker for microangiopathy and systemic cardiovascular disease. Usually 30° pictures are taken from the retina (both or only one eye) with a fundus camera. This can be performed with or without pharmacological pupil dilation. The image should be centered on the optical disk. Afterwards the 6 largest vessel calibers each of venules and arterioles in a specified diameter from the optic disc are processed and summary variables – the central retinal arteriolar/venular equivalents (CRAE/CRVE) – calculated with the help of semi-automatic computer software that is offered by various companies (Pathai et al., 2012). There are currently very few studies concerning retinal vascular parameter changes in HIV patients available.

The image below shows retinal images of a HIV-infected individual in comparison with a healthy test person.

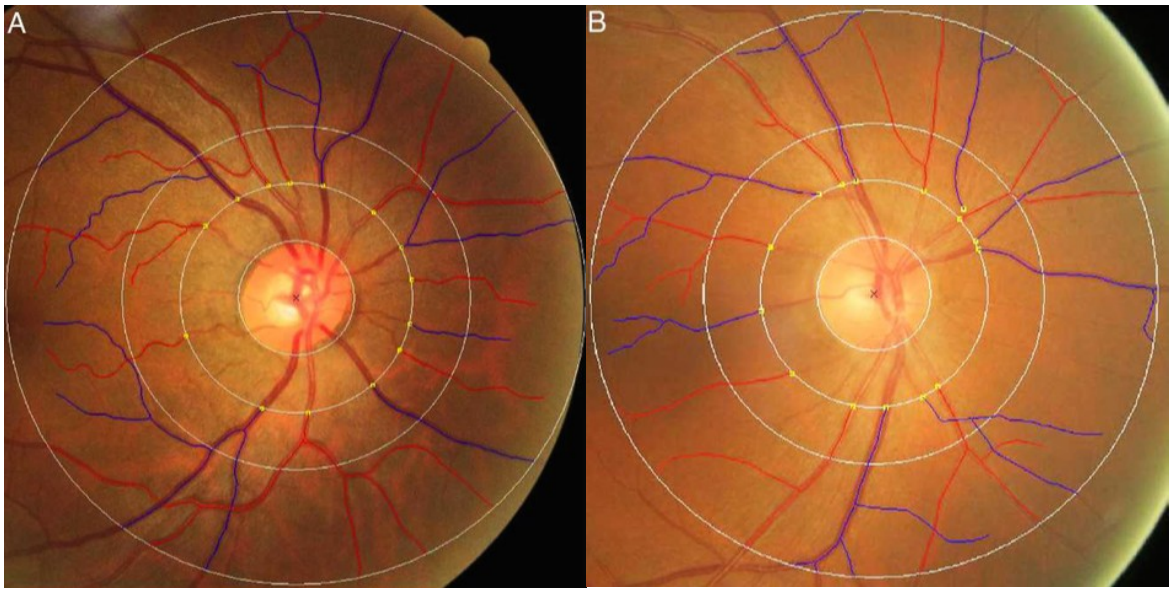

Figure 18 Retinal photographs assessed with ophthalmic software - arterioles are marked in red, venules in blue. A) Image of an HIV-infected patient (higher tortuosity). B) Image of a healthy control subject (reproduced from: (Tan et al., 2013))

Tan et al. compared the retinal imaging of 85 infected individuals to a matched control group of 251 in Asia. Three technicians separately graded the images and assessed several retinal vascular parameters including branching, tortuosity, projected vascular calibers and the arteriolar-venular ratio (AVR). Apart from HIV patients showing more tortuous

vessels, none of the other parameters revealed any significant differences between the two groups. While HIV viral load (RNA) and decreased arteriolar caliber and ARV are significantly associated, CD4+ cell count and HAART exposed no correlation. The latter might be connected to the very short mean of 7 months of exposure to antiretroviral therapy. The authors speculate that HIV induces hypoxia but also mention lipid metabolism changes, endothelial activation through viral proteins and systemic inflammatory dysregulation as possible backgrounds for the retinal vascular changes (Tan et al., 2013).

As part of the NIH funded Longitudinal Study of the Ocular Complications of AIDS (LSOCA), a cross-sectional prospective epidemiologic study, Gangaputra et al. published results of retinal vascular parameter changes of 1712 AIDS patients with a mean follow-up time of 6.1 years. In comparison to Tan et al. only the CRAE, CRVE and AVR were evaluated (Gangaputra et al., 2012).

Patients on HAART showed narrower arteriolar calibers, but only a weak association with a low CD4+ count and the duration of AIDS could be presented. This could point in the direction of the atherogenic effect of HAART. Limiting in this hypothesis is the lacking quantification of the lipid panel in the study group. Furthermore, a correlation of wider venules and a larger AVR was found in patients with a low hematocrit. This can probably be explained by the higher prevalence of anemia in AIDS patients (Gangaputra et al., 2012). Various studies of non-HIV-infected patients have shown a correlation between age, gender, ethnicity/race and caliber changes. This was confirmed in this study with older patients and Blacks having significantly narrower arterioles. AIDS cannot be assumed to be the only influencing factor, but might be related to a faster vascular aging process (Gangaputra et al., 2012). This was also mentioned and quantified at 5 to 8 years in the study by van Vonderen discussed earlier (van Vonderen et al., 2009e)

304 patients died during this study, but unfortunately only about a third of the death certificates (documenting the cause of death) could be obtained. Gangaputra et al. postulate a 12% increase in mortality risk per quartile, by which the AVR decreases (with a significance of $p = 0.02$). The finding that retinal vascular changes predict an increased risk of death coincides with non-HIV studies (Gangaputra et al., 2012).

Pathai et al. agrees that HIV infection might cause acceleration in immunological and vascular aging because of chronic inflammation and immune dysfunction. They compared 242 infected individuals with 249 age and gender matched healthy controls from rural and urban areas in Sub-Saharan Africa. 12% of the case group was treatment-naive, a too small

sample size to appropriately display differences in retinal vascular parameters. The duration of HAART (especially with high viral loads while on HAART) were proven to have a significant narrowing effect on the CRAE with 167.83 mm in patients < 3 years on HAART vs. 158.89 mm > 6 years of HAART exposure (after adjustments for age). It seems reasonable to suggest that HAART is associated with a higher cardiovascular risk. A high viral load was additionally correlated with a wider venular diameter (before adjustments). Greater CRVE have been shown in connection with endothelial dysfunction independent of present cardiovascular risk factors (Pathai et al., 2012).

Although Gangaputra et al. postulated that the risk of death is elevated in HIV patients with retinal vascular changes, it remains unclear whether retinal imaging can be used to predict the cardiovascular risk in HIV-infected individuals.

4.1.4 Endothelial Progenitor Cells

While retinal imaging is a new way of assessing the microvascular situation in HIV patients, evaluating and culturing circulating endothelial progenitor cells might also give some indication of the pathophysiologic mechanisms involved.

Teofili et al. performed a study on 14 treatment-naive HIV-infected patients in comparison with healthy controls. Circulating colony-forming unit-endothelial cells (CFU-ECs) and endothelial colony-forming cells (ECFCs) are both engaged in neoangiogenesis and endothelial damage repair. The former mainly acts through secretion of vascular endothelial growth factors (Teofili et al., 2010).

HIV-infected patients presented a significantly lower number of CFU-ECs, which has been connected to a higher cardiovascular risk in previous studies of non-HIV-populations. No connection to viral load or CD4+ count was recognized. ECFCs however were quantified at elevated levels but not to a significant degree. The higher levels are most likely caused by stimulation through infected T cells and monocytes and not by HIV itself. The distinct changes in progenitor cell levels found in HIV patients are probably related to the different cell lineage. It seems that CFU-ECs are highly permissive to HIV infection (as proven by proviral DNA detection), while ECFCs remain relatively “untouched” by HIV (Teofili et al., 2010).

Another study on treatment-naive HIV-seropositive individuals from Brazil in 2011 examined the levels of endothelial progenitor cells (EPCs) expressing $CD34^+$, $CD133^+$ and KDR^+ and endothelial and platelet derived microparticles presenting $CD51^+$ and

$CD31^+/42^+$ antigens respectively. Microparticles are released from the cells when they are activated, injured or decay. In healthy persons only a small number of particles derives from endothelial cells (EMPs). In the study group the levels of EPCs were low in comparison to the controls, while microparticles were found to be elevated (see the dot plots in the figure below). This represents an imbalance between endothelial apoptosis and regeneration, which also explains the impaired endothelial function shown by low FMD measurements. The exact causative mechanism in depletion of endothelial progenitor cells remains unclear (da Silva et al., 2011).

The effect of antiretroviral drugs on endothelial progenitor cells is waiting to be studied.

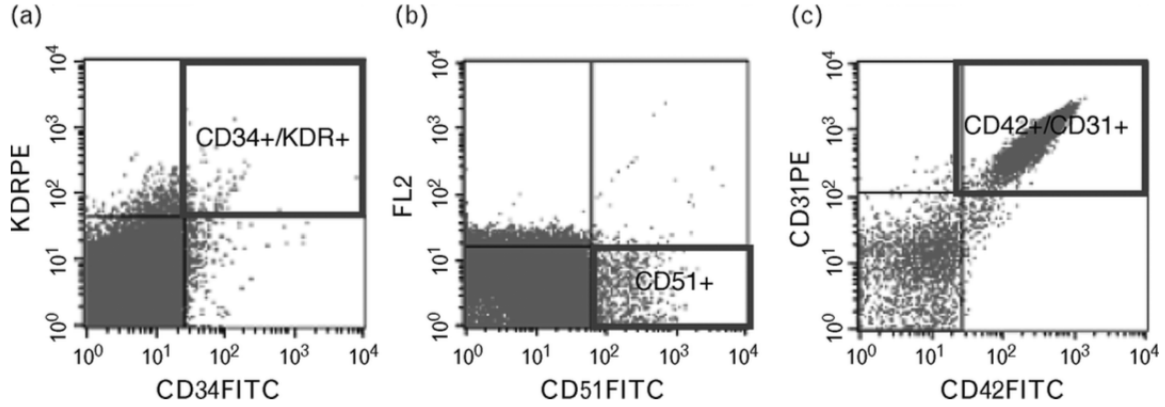

Figure 19 Representative dot plots of flow cytometry measurements of a) circulating endothelial progenitor cells that express CD34+/KDR+, b) CD51+ endothelial microparticles and c) CD42+/CD31+ platelet microparticles (reproduced from: (da Silva et al., 2011))

4.2 Cardiovascular Risk Factors

The traditional risk factors such as hypertension, dyslipidemia and insulin resistance have a higher prevalence in the HIV population (Boccara et al., 2013; Barbaro and Barbarini, 2011; Friis-Moller et al., 2010; Glass et al., 2006). One of the biggest HIV studies to date is the DAD study (Data Collection of Adverse Effects of Anti-HIV Drugs) that followed 33,389 HIV-infected individuals over the course of 6 years in centers all over the world (Europe, America and Australia). Table 5 shows their data (of 17,852 participants) on traditional risk factors at baseline. 25% of the cohort is at risk because of higher age, > 50% smoke, 20-25% display elevated lipid levels etc. (Friis-Moller et al., 2003). During follow-up, these risk factors actually worsened (apart from smoking), with patients becoming older the prevalence of history of CVD, diabetes mellitus type 2, hypertension and cardiovascular events such as myocardial infarction and stroke increased. The

significantly higher usage of lipid lowering medication might explain why the myocardial infarction rate over time decreased all the same. It still remains unclear however, how effective these drugs are in HIV-seropositive persons (DAD Study Group et al., 2008).

Table 5 Cardiovascular risk factors in the DAD study cohort at baseline according to current antiretroviral drug classes (reproduced from: (Friis-Moller et al., 2003))

	Total	Naive	No current ART	NRTI only	Combination with NNRTI	Combination with PI	Combination with PI plus NNRTI	P^a	Missing data (%)
Number of patients	17852	2315	1082	1898	3493	7749	1315		
Age > 45 years male, > 55 years female (%)	24.7	13.1	18.8	23.7	27.6	26.2	34.8	< 0.001	0.1
Body mass index (kg/m^2) [median (IQR)]	23 (21–25)	23 (21–25)	23 (20–25)	23 (21–25)	23 (21–25)	23 (21–25)	22 (21–24)	< 0.0001	13.9
Body mass index > 30 kg/m^2 [% (95% CI)]	3.5 (3.2–3.8)	4.8 (3.9–5.8)	3.7 (2.5–4.9)	3.0 (2.1–3.8)	3.9 (3.2–4.6)	3.1 (2.8–3.7)	2.3 (1.5–3.4)	< 0.001	
Current smoker [% (95% CI)]	51.5 (50.3–52.7)	55.1 (51.9–58.4)	59.0 (54.1–63.9)	52.7 (49.0–56.5)	47.5 (44.9–50.1)	51.6 (49.8–53.3)	45.9 (41.7–50.1)	< 0.001	18.5
Family history of CHD [% (95% CI)]	11.4 (10.8–12.0)	11.4 (9.8–13.1)	9.0 (6.9–11.1)	12.5 (10.3–14.6)	12.6 (11.1–14.1)	10.8 (9.9–11.8)	13.1 (10.5–15.8)	< 0.09	37.2
Previous CVD [% (95% CI)]	1.4 (1.2–1.6)	0.6 (0.3–0.9)	1.3 (0.6–2.0)	1.8 (1.2–2.5)	1.9 (1.3–2.2)	1.3 (1.1–1.6)	1.9 (1.1–2.6)	< 0.002	2.1
Hypertension [% (95% CI)]	8.5 (8.1–8.9)	6.1 (5.0–7.1)	8.7 (6.8–10.5)	7.0 (5.8–8.3)	9.6 (8.5–10.6)	8.9 (8.2–9.5)	10.1 (8.3–12.0)	< 0.001	8.0
Diabetes mellitus [% (95% CI)]	2.5 (2.2–2.7)	1.2 (0.7–1.7)	1.1 (0.4–1.7)	2.4 (1.7–3.1)	3.5 (2.9–4.2)	2.3 (2.0–2.6)	4.2 (3.1–5.3)	< 0.001	3.3
Total cholesterol (mmol/l) [median (IQR)]	5.1 (4.2–6.0)	4.4 (3.7–5.2)	4.4 (3.7–5.2)	4.6 (3.9–5.4)	5.3 (4.5–6.1)	5.3 (4.5–6.3)	5.9 (4.9–7.1)	< 0.0001	17.6
Total cholesterol ⩾ 6.2 mmol/l [% (95% CI)]	22.2 (21.4–22.9)	7.7 (6.4–8.9)	9.5 (7.3–11.4)	9.8 (8.2–11.3)	22.8 (21.0–24.6)	27.0 (25.7–28.2)	44.1 (40.1–47.9)	< 0.001	
HDL-cholesterol (mmol/l) [median (IQR)]	1.1 (0.9–1.4)	1.1 (0.9–1.4)	1.1 (0.8–1.3)	1.1 (0.9–1.5)	1.2 (1.0–1.5)	1.1 (0.9–1.4)	1.1 (0.9–1.4)	< 0.0001	54.2
HDL-cholesterol ⩽ 0.9 mmol/l [% (95% CI)]	25.7 (24.6–26.8)	25.5 (22.6–28.5)	35.0 (30.0–39.9)	24.8 (21.5–28.6)	19.1 (16.7–21.5)	27.1 (25.5–28.8)	23.8 (20.0–27.7)	< 0.001	
Triglycerides (mmol/l) [median (IQR)]	1.7 (1.1–2.8)	1.3 (0.9–1.9)	1.5 (1.1–2.3)	1.4 (0.9–2.2)	1.6 (1.0–2.7)	1.9 (1.2–3.1)	2.5 (1.6–4.2)	< 0.0001	17.6
⩾ 2.3 mmol/l [% (95% CI)]	33.8 (32.7–34.6)	15.2 (13.8–17.3)	25.9 (22.5–29.3)	22.7 (20.3–25.0)	31.8 (29.7–33.9)	40.0 (38.5–41.5)	54.3 (49.9–58.7)	< 0.001	
Lipodystrophy [% (95% CI)]	25.4 (24.6–26.1)	2.2 (1.6–2.8)	20.6 (17.9–23.3)	20.8 (18.7–22.8)	31.2 (29.3–33.1)	29.7 (28.5–31.0)	35.1 (31.9–38.3)	< 0.001	1.1

[a]Chi-square for comparison of frequencies, Kruskal–Wallis for comparison of distributions. IQR, Interquartile range; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; CI, confidence interval; CHD, coronary heart disease; CVD, cardiovascular disease; NA: Not applicable.

Glass et al. identified smoking (proportion twice as high as general Swiss population), low HDL, high triglycerides and high blood pressure as the most common cardiovascular risk factors among his cohort of 8033 HIV-infected individuals. Apart from smoking and reduced percentage of individuals with high cholesterol (probably due to higher prescription rate of lipid-lowering medication), the risk factors remained relatively stable over the study period of 6 years (Glass et al., 2006).

Besides the traditional cardiovascular risk factors, HIV and ART trigger the development of emerging risk factors. Chronic infection, low grade chronic inflammation, an altered immunological state and co-infections with other viruses such as CMV have shown an impact on the progression of atherosclerosis in HIV patients (Boccara, 2008). While Kristoffersen et al. and Ross et al. find an association of hs-CRP, an inflammation marker, with endothelial dysfunction and increased carotid intima-media thickness, Fourie et al. cannot confirm this correlation (Fourie et al., 2015; Kristoffersen et al., 2009; Ross et al., 2009). Whether HIV and HAART themselves should be considered as "emerging" risk factors is unclear, but Lorenz et al. postulates that they are actually independent risk factors for early carotid atherosclerotic changes in long-term infection and treatment regardless of the vascular risk profile (Lorenz et al., 2008).

Coming back to traditional risk factors, dyslipidemia is probably most discussed in the HIV population. Not only does HIV itself cause dyslipidemia, rather many especially older antiretroviral drugs have dyslipidemic properties as well (dependent on drug class). A study with healthy uninfected participants that took ritonavir for 2 weeks showed that LDL and triglycerides already increased significantly in that short period of time. While newer drug agents show favorable adverse effect profiles, the older medications are still widely used in rollout programs in developing countries. The usual lipid panel of a HIV-infected individual includes high total/LDL-cholesterol and triglycerides and low HDL- cholesterol levels (Feeney and Mallon, 2011).

Elevated triglycerides levels are also the most prevalent marker besides high blood pressure and low HDL in a study by van Wijk et al. concerned with metabolic syndrome in HIV patients. High waist circumference and impaired fasting glucose level on the other hand were rather scarce. The defining levels for metabolic syndrome can be found in Table 6. In the HIV group 15 patients with metabolic syndrome (MetS) and 22 without were compared to 14 healthy men and 13 diabetes mellitus type 2 patients, who also have MetS. FMD measurements showed lower dilation percentages in HIV-infected in comparison with the healthy controls. HIV positive and MetS positive individuals had the worst results,

whereas HIV positive and MetS negative participants had measurements comparable to the diabetes 2 patients. This implies that HIV patients are at increased cardiovascular risk and have endothelial dysfunction even without major metabolic changes present. It is questionable whether the presentation of MetS in the HIV population differs from the general population (van Wijk et al., 2006).

Table 6 ATP III (Adult Treatment Panel) definition of the metabolic syndrome (reproduced from: (National Cholesterol Education Program, 2001))

Risk factor	**Cut-off levels (men/women)**	
Waist circumference (abdominal obesity)	> 102 cm	> 88 cm
Triglycerides	≥ 150 mg/dL	
HDL cholesterol	< 40 mg/dL	< 50 mg/dL
Blood pressure	≥ 130 / ≥ 85 mmHG	
Fasting glucose	≥ 110 mg/dL	

Bonfanti et al. evaluated the prevalence of metabolic syndrome in HIV-infected individuals at 20.8% in comparison to 15.8% in controls, which translates into a twice as high age- and gender adjusted risk. No difference between treatment-naive and treated patients was found (Bonfanti et al., 2007) This data is contradicted by findings by Fourie et al. in 2010 who didn't find any differences in MetS prevalence in her PURE substudy in South Africa between newly infected individuals and healthy controls, although typical lipid abnormalities were found. This could be based on ethnic differences between Africans and Caucasians especially in body composition and a usually more favorable lipid metabolism (higher HDL values) (Fourie et al., 2010). Miller et al. also found that Black men presented a lower prevalence of coronary artery calcification and less obstructive coronary artery disease although coronary risk factors were higher (Miller et al., 2014).

Van Rooyen et al. performed a study to evaluate the prevalence of cardiovascular risk factors in Sub-Saharan Africa comparable to the Swiss study by Glass et al. The cohort of 140 HIV-infected Black Africans in South Africa showed significantly lower systolic blood pressures, total cholesterol and HDL-cholesterol, but higher TC/HDL and TG/HDL ratios as the most important risk factor (van Rooyen et al., 2014). A meta-analysis by Dillon et al. is also concerned with cardiometabolic factors and their influence on the progression of cardiovascular disease in HIV patients in Sub-Saharan Africa. 52 studies provided data on 29,755 participants that showed that HIV is connected to a lower mean Body Mass Index, higher TG and lower HDL levels (marked heterogeneity among standardized mean differences). The results from the statistical evaluation of studies on the effects of ART were inconsistent due to lacking information and differentiation between therapy regimens (Dillon et al., 2013)

4.2.1 Cardiovascular Predictive Risk Scores

There are several cardiovascular predictive risk scores such as the Framingham risk score or the PROCAM score that have been developed for the general population of developed countries unexposed to HIV or ART (Friis-Moller et al., 2010). For the Framingham risk score (10 year risk in %), points are given for levels of total cholesterol and HDL cholesterol, lipid-lowering medication, systolic blood pressure, smoking, age and sex (Framingham Heart Study, 2015). The PROCAM score adds even further parameters such as diabetes, fasting glucose levels, triglyceride level and familial disposition to the calculation (CHD Taskforce, 2010). Their specificity is generally ill established for the HIV population. The DAD Study Group found that especially the Framingham score overestimates the cardiovascular risk for HIV patients, because they are generally younger and are often exposed to antiretroviral therapy. They have developed their own CHD equation for a 5-year risk estimate of the risk of CVD end points based on a HIV-infected population including traditional cardiovascular risk factors along with certain antiretroviral drug classes and time of ART exposure (Friis-Moller et al., 2010).

While Friis-Moller found an overestimation of the Framingham score, Law et al. is more differentiated in his comparison of the predicted risk from the Framingham score with the actually observed cases of myocardial infarctions in the DAD study cohort. While the score overpredicted events in patients not on ART, it underpredicted the risk in HIV-infected individuals on therapy. Both, predicted and observed, rates however were associated with the duration of exposure (Law et al., 2006)

The three mentioned scores were compared in a cross sectional study on 294 HIV-infected patients in Brazil by Nery et al. They call their study informative but cannot give an assessment of the accuracy of the predicted outcome of cardiovascular end points. As shown in the figure below, the agreement of the scores is relatively good and the majority of participants were placed in the low risk category (Nery et al., 2013).

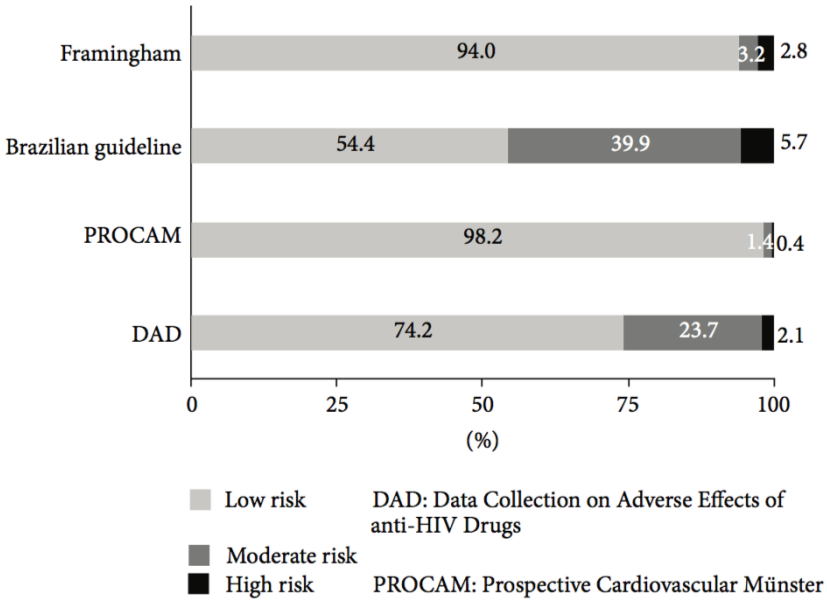

Figure 20 Comparison of cardiovascular predictive risk according to the Framingham score, the Framingham score with aggravating factors (= Brazilian guideline), the PROCAM score and the DAD CHD risk equation (reproduced from: (Nery et al., 2013))

4.3 Antiretroviral Therapy

Results concerning the influence of antiretroviral therapy on the cardiovascular system have already been discussed in some other chapters. They will only be mentioned shortly here. Unfortunately, apart from studies that include measurements from the treatment-naive state, the specific impact of HIV itself and HAART are hard to differentiate. They are probably both independent risk factors for cardiovascular disease in HIV-infected individuals (Lorenz et al., 2008).

Complicating the matter, not all antiretroviral drug classes (and not even agents within one class) seem to cause the same changes in the lipid and glucose metabolism and therefore their effect on the cardiovascular system might be diverse (Friis-Moller et al., 2007). The results are inconsistent especially those concerning protease inhibitors. Currier et al. aimed to compare a PI based regimen to other drug classes and healthy controls based on IMT measurements. In their study however no differences between the regimens or even the HIV-infected and HIV-uninfected group became evident. Torriani et al., Francisci et al. and van Vonderen et al. didn't find any differences either between treatment regimens in their FMD, endothelial plasma marker and IMT measurements respectively (Torriani et al., 2008; van Vonderen et al., 2009a; Francisci et al., 2009). The former two and Asztalos et

al. however did associate higher changes in lipid levels with regimens including protease inhibitors (Francisci et al., 2009; Torriani et al., 2008; Asztalos et al., 2006). Protease inhibitors can cause lipodystrophy as an adverse side effect, a condition of fat loss and redistribution. It is characterized by a fat collection at the upper back ("buffalo hump"), an increase of abdominal obesity and potentially an increased breast size as well as a loss of subcutaneous fat of the face, buttocks and limbs. It is connected to an abnormal lipid panel (Barbaro and Barbarini, 2011).

After the DAD Study Group presented a correlation between antiretroviral drugs and the incidence of myocardial infarction, they conducted a substudy to differentiate the impact of the various drug classes. They observed 345 documented myocardial infarctions of variable impact in 94,469 person-years. After adjustments the relative rate of myocardial infarction per year was 1.16 for HIV-infected individuals taking protease inhibitors and 1.05 for individuals on NNRTIs. The combination of both these drug classes roughly resulted in a higher MI incidence. No further differences were found between women and men or older and younger age. However a correlation of the cardiovascular risk with the duration of HAART treatment was calculated (Friis-Moller et al., 2007).

Maggi et al. confirms these findings in their study on carotid lesions. They described an earlier onset and more rapid evolution of the lesions in the PI regimen group in comparison to both the NNRTI-based group and the control group. Some participants had to be switched from the protease inhibitors to a treatment with NNRTIs and NRTIs, which was associated with a lower rate in lesion progression. They add, however, that although their groups were well matched for age, sex and cardiovascular risk factors, the PI group presented more advanced infection stages. This can be explained by the potency of protease inhibitors that was often used in advanced stages before new agents became available (Maggi et al., 2007).

A meta-analysis found that not all antiretroviral drugs are associated with an increased cardiovascular risk. Principally PIs were again considered more harmful than reverse transcriptase inhibitors, lopinavir/ritonavir and abacavir specifically presenting a greater risk. The time that infected individuals are on ART is an important factor in the calculation of individual cardiovascular risk (Islam et al., 2012).

An often cited study concerned with the adverse effects of antiretroviral drugs is the SMART study (Strategies for Management of Antiretroviral Therapy) from 2006. They compared a continuous therapy scheme aimed for viral suppression to an episodic CD4+ cell count controlled treatment plan (initiation of treatment at 250 cells/mm^3 and

continuation until at least above 350 cells/mm^3). Primary end points were opportunistic infections or death by any cause; secondary end points were defined as major cardiovascular, hepatic or renal disease. Contrary to expectations the incidence of all primary and secondary end points increased in the group on interrupted treatment to a point that the study had to be terminated early. They postulated that episodic treatment based on CD4+ cell count is "*deleterious*" and that adverse and other effects of antiretroviral drugs are probably connected to the grade of immune deficiency (El-Sadr et al., 2006).

All the studies presented here are relatively old and concern whole drug classes. Since individual drug agents have different risk profiles and more agents are becoming available, further studies on antiretroviral drugs will be necessary to establish their adverse effects on the metabolic and cardiovascular system and clarify the mechanisms they trigger (Friis-Moller et al., 2007). Nonetheless, antiretroviral drugs have decreased the morbidity and mortality of HIV-infected individuals tremendously. Therefore the benefits definitely exceed the risk (Nery et al., 2013).

4.4 Cardiovascular Diseases

While early studies on the association of HIV and cardiovascular events were based on cardiomyopathy and infectious cardiac manifestations, interest has shifted towards accelerated atherosclerosis in the HIV population today (Triant, 2013). Cardiomyopathy, pericardial effusions and marantic endocarditis however are still highly prevalent in developing countries (Barbaro and Barbarini, 2011) where still only a relatively small number of infected individuals has access to antiretroviral therapy (e.g. 40% in South Africa) (Botha et al., 2014).

The etiology and pathogenesis of cardiovascular diseases in the HIV population isn't quite clear, but is probably multifactorial (as the image below shows). As thoroughly discussed, HIV itself as well as ART and traditional cardiovascular risk factors seem to have an impact on the immunological and inflammatory state of the infected individual, which seems to facilitate the development of cardiovascular diseases.

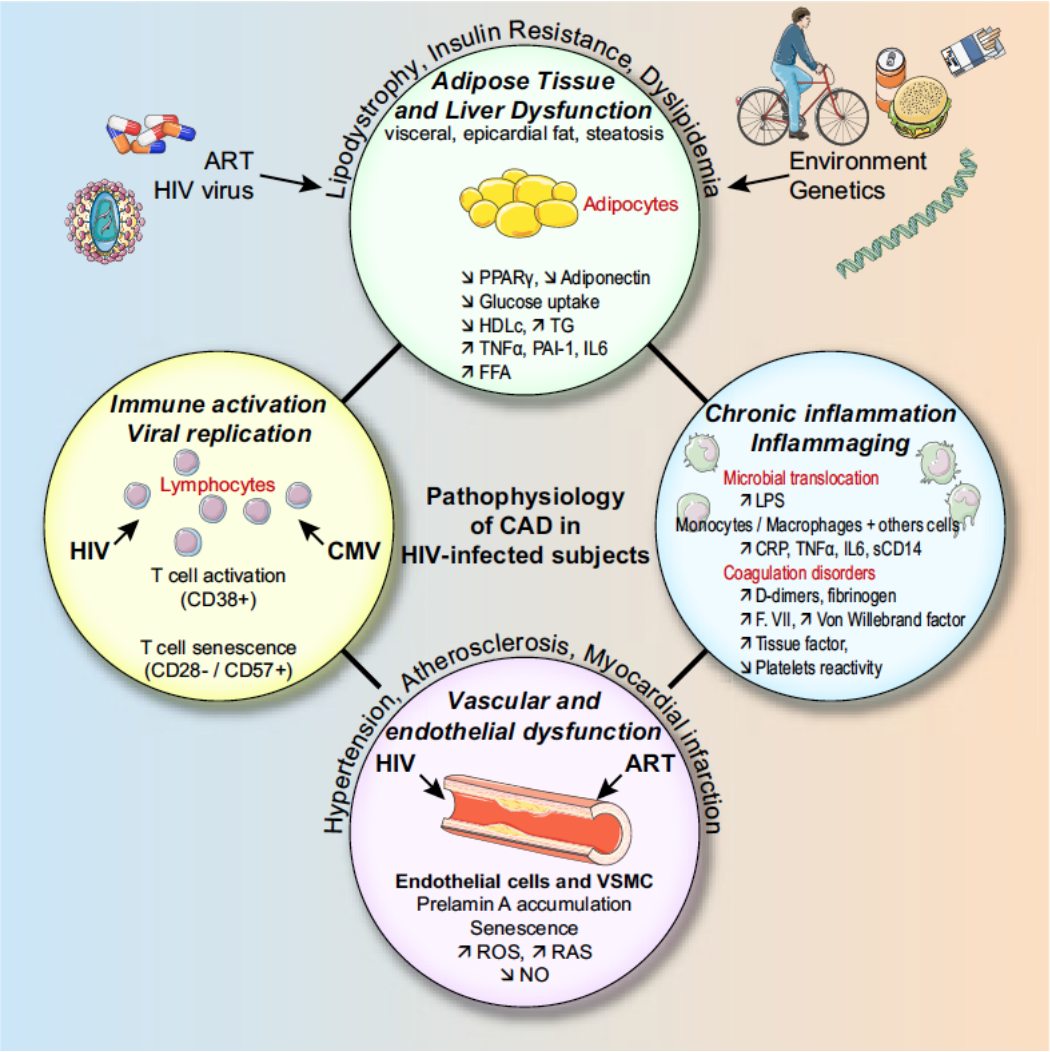

Figure 21 Hypothesis of pathophysiology of CAD in HIV patients treated with combination antiretroviral therapy (Abbreviations: PAI – plasminogen activator inhibitor; PPAR – peroxisome proliferator-activated receptor, ROS – reactive oxygen species, RAS – renin angiotensin system, TNF – tumor necrosis factor[3]) (reproduced from: (Boccara et al., 2013))

A rather early study of 36,766 HIV patients from Veterans Affairs facilities showed a decrease in admission due to cardiovascular and cerebrovascular disease and death from any cause. This can mainly be attributed to the use of antiretroviral therapy (Bozzette et al., 2003). Other studies such as a meta-analysis by Islam et al. calculated that the relative risk of cardiovascular disease of HIV-infected individuals on treatment is 52% higher than the risk of treatment-naive individuals. The type and duration of treatment plays a role as displayed in many studies. Overall the relative risk of HIV-infected individuals was postulated as 61% higher than that of healthy controls (Islam et al., 2012). According to Cheruvu and Holloway, the 1.5 to 2-fold higher risk of developing CAD is roughly

[3] Other/Common abbreviations are found in the list of abbreviations at the beginning of the diploma thesis.

comparable to the increased risk of CAD for uninfected persons that smoke, have diabetes or are 5 to 9 years older (Cheruvu and Holloway, 2014).

Most studies concerning cardiovascular events focus on acute coronary syndrome and myocardial infarction because the diagnosis is more precise and better documented than other cardiovascular diseases. Acute coronary syndrome often reveals the presence of CVD in HIV patients (Boccara et al., 2013). Hsue et al. speculated that the clinical findings and outcome of ACS might differ in HIV patients due to an altered and accelerated pathogenesis. The 68 HIV-infected patients that were admitted with ACS in a time period of 10 years were younger (mean of 50 versus 61 years of age in controls), more likely male and current smokers. Additionally a low HDL cholesterol level was correlated with ACS. Their coronary disease however was often less extensive, only affecting a single coronary vessel. The restenosis rate after percutaneous coronary intervention on the other hand was higher than expected (51% in HIV-infected individuals vs. 15% in controls) (Hsue et al., 2004). These findings concur with a retrospective chart review by Ambrose et al (Ambrose et al., 2003).

An early study on the DAD study cohort published a number of 126 persons with myocardial infarction in 36,199 person-years, which translates into 3.5 events per 1000 person-years. While the relative rate of myocardial infarction increases with every year of antiretroviral therapy, the absolute risk remains low. Traditional risk factors such as older age, smoking and male sex are associated with an increased risk for MI in this study while markers connected to HIV infection (apart from ART) are not (Friis-Moller et al., 2003). A newer case-control- study from France from 2012 contradicts this lacking association. Besides a higher prevalence of traditional cardiovascular risk factors, they found a significant correlation of increased MI risk with a high CD8+ T-cell level, a HIV RNA level above 50 copies/mL and a low nadir CD4+ cell count (Lang et al., 2012).

Given the increased risk of MI and CVD presented in many studies, the 3-fold mortality risk over a 5-year study follow-up period compared to healthy controls published by Cockerham et al. isn't surprising (after adjusting for demographic and cardiovascular risk factors). Risk of death was strongly associated with low CD4+ counts even at levels > 500 cells/mm^3 (up to 6-fold with < 200 cells/mm^3). Smoking and older age were also strong predictors (Cockerham et al., 2010).

4.4.1 Prevention of Cardiovascular Diseases in the HIV-Population

Since the connection of an increased cardiovascular risk and HIV/AIDS and ART was discovered, some clinical guidelines concerning the matter have been published. These are based on guidelines used for the general population and are mainly focused on identifying the cardiovascular risk (using the Framingham risk score for example) and monitoring modifiable cardiovascular risk factors. The EACS for example recommends annual risk assessments and EKGs for men older than 40 years and women older than 50 years without prior diagnosis of cardiovascular diseases. They concentrate on controlling blood pressure, coagulation, glucose levels and lipid levels by life-style changes at a first level and drug therapy if necessary (EACS, 2014).

5 Conclusion

Both HIV/AIDS and cardiovascular diseases are among the top 10 causes of death worldwide (ranking differs between high- and low-income regions) and many advances have been made in connecting HIV and ART to the increased risk of cardiovascular diseases in the HIV population.

Summarizing the results from the review of current literature and answering the questions mentioned at the beginning of the review: 1) It seems that cardiovascular risk factors are more prevalent in HIV-infected individuals, smoking and dyslipidemia being the most common. 2) HIV/AIDS and ART both have an effect on cardiovascular risk factors. In particular the glucose and lipid metabolism are disturbed. Besides the traditional risk factors, emerging risk factors such as chronic inflammation and immune activation seem to play an important role in the pathogenesis of atherosclerosis and cardiovascular disease in the HIV population. Additionally HIV and ART should be considered independent cardiovascular risk factors. 3) The etiology of cardiovascular diseases in the HIV population is most likely multifactorial and most of the underlying pathomechanisms remain unclear. The specific impact of HIV itself and ART are hard to differentiate.

It also remains unknown whether measurements of endothelial dysfunction (FMD, plasma markers) and early anatomic changes (IMT, retinal imaging) can be used as surrogate markers in the assessment of individual cardiovascular risk in HIV-infected individuals.

It is clear that further prospective studies on large cohorts over a longer period of time as well as in vitro studies will be necessary to provide further information. Studies conducted in Africa are especially important to shed some light on possible ethnical and geographical differences of HIV-infected subgroups and a possible connection between the high prevalence of HIV and the rising numbers of people affected by cardiovascular diseases in Africa. I believe that results from the EndoAfrica Project will help to provide background information for the creation of HIV-infected subgroup-specific guidelines for the management of HIV infection as well as the increased risk of cardiovascular disease. This might include the development of special treatment options that interact with HIV-specific pathomechanisms or the initiation of special screening programs (combining the communicable disease HIV with the non-communicable diseases of the cardiovascular system) that, for example, also include younger patients.

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Appendix

A) CDC HIV Classification and List of AIDS-Defining Conditions

Clinical AIDS-defining conditions (only found in stage 3 HIV infection) (reproduced from: (Centers of Disease Control and Prevention, 2008))
- Bacterial infections, multiple or recurrent*
- Candidiasis of bronchi, trachea, or lungs
- Candidiasis of esophagus
- Cervical cancer, invasive
- Coccidioidomycosis - disseminated or extrapulmonary
- Cryptococcosis - extrapulmonary
- Cryptosporidiosis, chronic intestinal (>1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes), onset at age >1 month
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy, HIV related
- Herpes simplex: chronic ulcers (>1 month's duration) or bronchitis, pneumonitis, or esophagitis (onset at age >1 month)
- Histoplasmosis - disseminated or extrapulmonary
- Isosporiasis - chronic intestinal (>1 month's duration)
- Kaposi sarcoma
- Lymphoid interstitial pneumonia or pulmonary lymphoid hyperplasia complex*
- Lymphoma, Burkitt (or equivalent term)
- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma, primary, of brain
- *Mycobacterium avium* complex or *kansasii* - disseminated or extrapulmonary
- *Mycobacterium tuberculosis* of any site – pulmonary, disseminated or extrapulmonary
- *Mycobacterium*, other species or unidentified species - disseminated or extrapulmonary
- *Pneumocystis jirovecii* pneumonia
- Pneumonia - recurrent
- Progressive multifocal leukoencephalopathy
- *Salmonella* septicemia - recurrent
- Toxoplasmosis of brain (onset at age >1 month)
- Wasting syndrome attributed to HIV

* These conditions are usually only found in children younger than 13 years old.

B) WHO Clinical Stages of HIV

Clinical Staging as defined by the WHO for adults and adolescents - Compare to staging based on CD4+ T cell count (see Table 1 on page 4) (reproduced from: (Mandell et al., 2010))	
Stage 1	- Asymptomatic - Persistent generalized lymphadenopathy
Stage 2	- Unexplained moderate weight loss (< 10% of body weight) - Recurrent respiratory tract infections - Herpes zoster - Angular cheilitis - Recurrent oral ulceration - Popular pruritic eruptions - Seborrheic dermatitis - Fungal nail infections
Stage 3	- Unexplained severe (>10% of body weight) - Unexplained chronic (duration > 1 month) - Unexplained persistent (> 37.6°C, intermittent or consistent, duration > 1 month) - Oral candidiasis – persistent - Oral hairy leukoplakia - Pulmonary tuberculosis (current) - Severe bacterial infections (pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteremia) - Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis - Unexplained anemia (< 8 mg/dL), neutropenia (< 0.5 x 10^9/L) or chronic thrombocytopenia (< 50 x 10^9/L)
Stage 4	- HIV wasting syndrome - *Pneumocystic jirovecii (carinii)* - Recurrent severe bacterial pneumonia - Chronic herpes simplex infection (orolabial, genital or anorectal - duration > 1 month; or visceral at any site) - Esophageal candidiasis/Candidiasis of trachea, bronchi or lungs - Extrapulmonary tuberculosis - Kaposi's sarcoma - Cytomegalovirus infection (retinitis or infection of other organs) - Central nervous system toxoplasmosis - HIV encephalopathy - Extrapulmonary cryptococcosis, including meningitis - Disseminated non-tuberculous mycobacterial infection - Progressive multifocal leukoencephalopathy

	- Chronic cryptosporidiosis (with diarrhea) - Chronic isosporiasis - Disseminated mycosis (coccidiomycosis or histoplasmosis) - Recurrent non-typhoidal *Salmonella* bacteremia - Lymphoma (cerebral or B-cell non Hodgkin's) or other solid HIV-associated tumors - Invasive cervical carcinoma - Atypical disseminated leishmaniasis - Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy