Diplomarbeit

Effects of Tuberculosis and HIV Co-morbidities on the Cardiovascular System and Cardiovascular Disease: A Review of Current Literature

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Eda Calisman

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Institut für Physiologie

unter der Anleitung von

Ass. -Prof. Priv.-Doz. Dr.med. PhD Nandu Goswami
Ao.Univ.-Prof. Mag.Dr.rer.nat.Andreas Rössler

Graz, am 09.06.2017

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Graz, am 09.06.2017 *Eda Calisman eh*

Acknowledgement

Firstly, I would like to express my deep gratitude to my supervisor, Ass. -Prof. Priv.-Doz. Dr.med. PhD Nandu Goswami. His patience and guidance helped me with my work on this diploma thesis during the past year. I appreciated his advice, by which he always provided me with food for thought.

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German abstract

Hintergrund: HIV und TB stellen eine große Belastung für unsere Weltgesundheit dar. Gerade die HIV/TB Koinfektion, die vorwiegend einkommensschwache Länder betrifft, bedeutet eine Herausforderung für die jeweiligen Gesundheitssysteme. Gleichzeitig steigt die Inzidenz von Atherosklerose und kardiovaskulären Erkrankungen in Entwicklungsländern. Allen voran ist Afrika südlich der Sahara mit einer zunehmenden dreifachen Last durch HIV, TB und kardiovaskuläre Erkrankungen konfrontiert. TB und HIV korrelieren jeweils mit einem Risiko für kardiovaskuläre Erkrankungen. Ungeklärt ist allerdings noch, wie die Koinfektion mit HIV und TB das Erkrankungsrisiko des kardiovaskulären Systems beeinflusst.

Ziele: Das Ziel dieser Diplomarbeit ist es, die derzeitige verfügbare Literatur im Hinblick auf die Fragestellung, wie die HIV und TB Koinfektion die Entstehung von kardiovaskulären Erkrankungen beeinflusst, zu untersuchen. Zusätzlich hinterfragt diese wissenschaftliche Arbeit, wie die HIV und TB Therapie das Risiko für kardiovaskuläre Erkrankungen verändert.

Methoden: Eine systematische Analyse der primären und sekundären Literatur wurde durchgeführt, um Informationen zu den zentralen Fragestellungen zu gewinnen. Medizinische Datenbanken lieferten dazu die aktuellen Publikationen. Die Bibliotheken der Medizinischen Universität Graz und der Karl-Franzens-Universität Graz gestatteten Zugang zu Lehrbüchern über Infektions- und Tropenkrankheiten. Richtlinien und aktuelle Daten von der Weltgesundheitsorganisation und den Zentren für Krankheitskontrolle und Prävention wurden mit in die Diplomarbeit integriert.

Diskussion: M. tuberculosis und das Humane Immundefizienz-Virus tragen durch direkte und indirekte Mechanismen zur Entstehung von Atherosklerose und kardiovaskulären Erkrankungen bei. Immunaktivierung, chronische Entzündung und autoimmune Vorgänge induzieren die Progression von atherosklerotischen Plaques und darausfolgenden kardiovaskulären Komplikationen. Es ist jedoch unklar, wie die Koinfektion mit HIV und TB das kardiovaskuläre Risiko beeinflusst. Diese Literaturrecherche hebt die Notwendigkeit von Forschung in diesem Bereich hervor. EndoAfrica, ein Forschungsprojekt, welches sich mit der kardiovaskulären Gesundheit in Südafrika befasst, untersucht die Zusammenhänge zwischen HIV Infektionen, antiretroviraler Therapie und endothelialer Dysfunktion, welche zu kardiovaskulären Ereignissen in der

südafrikanischen Population führt. Diese Diplomarbeit weist im Besonderen auf Studien wie das EndoAfrica Projekt hin, welche die oben erläuterten Zusammenhänge untersuchen.

Abstract

Background: HIV and TB impose an immense burden on our global health. Especially HIV/TB co-infection that affects particularly low and middle-income countries, challenges health systems on site. At the same time, the incidence of atherosclerosis and cardiovascular diseases is growing in developing countries. Above all, sub-Saharan Africa is increasingly confronted with a triple burden of disease (HIV, TB and cardiovascular diseases). TB and HIV are respectively correlated with cardiovascular diseases. However, it is still questionable by now, how HIV and TB co-infection alters the risk of cardiovascular diseases.

Aims and objectives: The aim of this diploma thesis is to search current literature about the pathophysiological effects of HIV and tuberculosis co-infection on the development of cardiovascular diseases. Additionally, this thesis examines how treatment of HIV and TB modifies the risk of cardiovascular disease development.

Methodology: A systematic review of primary and secondary literature was carried out to gather information about the main questions of this thesis. Medical databases provided current publications. The libraries of the Medical University of Graz and the Karl-Franzens-Universität Graz allowed access to textbooks concerning infectious and tropical diseases as well as to cardiovascular diseases. Guidelines and recent data from the World Health Organisation and the Centers for Disease Control and Prevention were added to this thesis.

Results: 207 references were surveyed for this diploma thesis. Of those, 41 full text publications were identified as relevant for the discussion part.

Discussion: It appears that the infectious agents m. tuberculosis and human immunodeficiency virus contribute to atherosclerosis formation and cardiovascular disease evolvement through direct and indirect pathways. Immune activation, chronic inflammation and autoimmune mechanisms fuel the development of atherosclerotic plaques and consequently cardiovascular complications. However, it remains unclear how the co-infection of HIV and TB affects the cardiovascular risk. This review highlights the need for further research in this context. EndoAfrica, a research project that studies cardiovascular health in South Africa, explores interactions between HIV infection, antiretroviral therapy regimens and endothelial dysfunction leading to cardiovascular events in the South African population. Attention needs to be drawn to systematic studies like the EndoAfrica project in which this relationship can be investigated.

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Abbreviations

ACS	Acute coronary syndrome
AIDS	Acquired Immunodeficiency Syndrome
AMI	Acute myocardial infarction
ART	Antiretroviral therapy
BCG	Bacillus Calmette–Guérin
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
C. pneumoniae	Chlaymdophila pneumoniae
CDC	Centers for Disease Control and Prevention
CMV	Cytomegalovirus
CVD	Cardiovascular disease
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DOTS	Directly observed treatment, short course
DST	Drug suspectibility testing
HAART	Highly active antiretroviral therapy
HDL-C	High-density lipoprotein cholesterol
HHV8	Humane Herpesvirus 8
HIV	Human immunodeficiency virus
HSP	Heat shock protein
HT	Hypertension
IDU	Intravenous drug users
IGRA	Interferon-gamma release assay
IL	Interleukin

IRIS	Immune Reconstitution Inflammatory Syndrome
LDL-C	Low-density lipoprotein cholesterol
M. tuberculosis	Mycobacterium tuberculosis
MSM	Men who have sex with men
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NO	Nitric oxide
NCD	Non-communicable disease
NRTI	Nucleoside reverse transcriptase inhibitors
PI	Protease inhibitors
PLHIV	People living with HIV
PPD	Purified protein derivate
RNA	Ribonucleic acid
SIV	Simian immunodeficiency virus
TB	Tuberculosis
TLR	Toll like receptor
TNF	Tumour necrosis factor
TST	Tuberculin skin test
WHO	World Health Organization

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1 Introduction

1.1 HIV and AIDS

The human immunodeficiency virus (HIV) causes infections that can remain undetected in the host´s organism for several years. During that time transmission to other individuals is likely to happen, which makes this infection particularly insidious. Immunosuppression and progress to the Acquired immunodeficiency syndrome (AIDS) are the crux of HIV. Since AIDS is number six among the top ten leading causes of death, it still is a burden for humankind. Emphasis should be put on enlightenment and prevention of HIV and AIDS particularly in developing regions on our earth with less medical coverage of the population and high transmission and death rates (1).

1.1.1 The Human immunodeficiency virus

1.1.1.1 Structure and Genome

HIV 1 and HIV 2 belong to the family of Retroviridae or more specifically to the Lentivirus subfamily. Generally, genetic information is transmitted from deoxyribonucleic acid (DNA) to ribonucleic acid (RNA) whereas retroviruses take the opposite way. Making use of the enzyme reverse transcriptase allows HIV to transfer its own genome, which is two identical RNA single strands, into DNA. As can be seen in the figure below, HIV contains two more enzymes - Integrase und Protease - in its core, that is covered by the protective capsid protein p24. All together they form the nucleocapsid. The nucleocapsid itself is in turn surrounded by the envelope, meaning two layers that emerge when the virus is released out of the host cell membrane. Surface and transmembrane glycoprotein complexes - gp120 and gp41 – adhere to the outmost part (1). Gp120 and gp41 are connected, whereby gp120 enables HIV to bind to target cells that express CD4 receptors on their surface, like T-helper lymphocytes and dendritic cells (2).

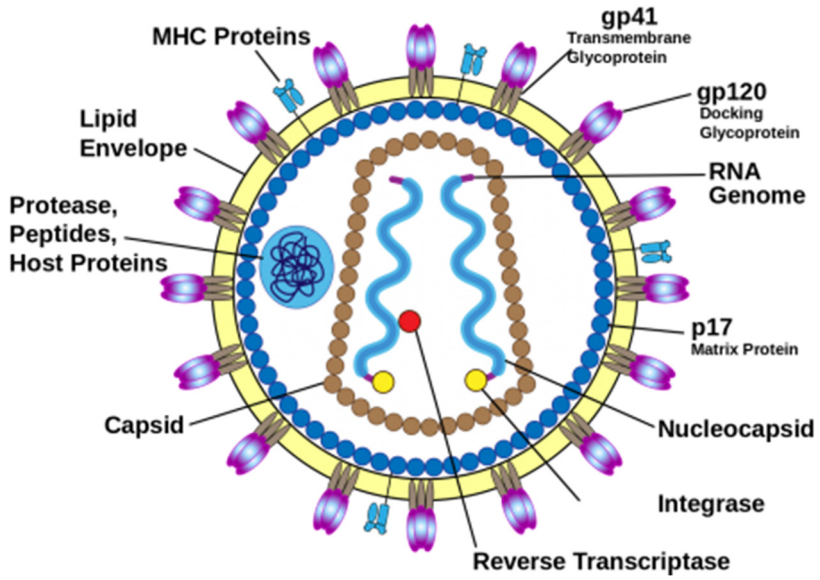


Figure 1: Structure of HIV
(reproduced from: http://guardianlv.com/2013/11/virulent-a302-hiv-strain-leads-to-aids-within-five-years/, Access 15.7.2016)

Genes that comprise the code for structural proteins in all Retroviridae are *gag, pol* and *env*. Apart from that HIV has six more regulatory and accessory regulatory genes as figure 2 points out.

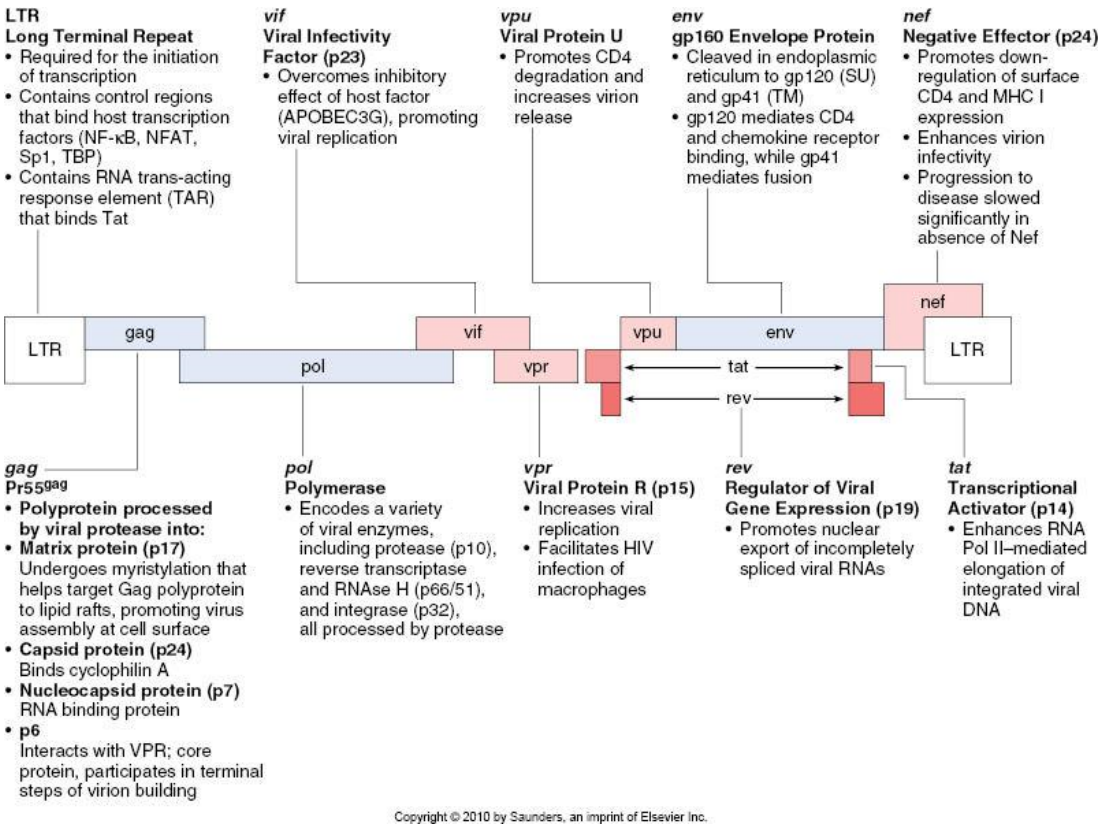


Figure 2: Genome of HIV
(reproduced from: https://classconnection.s3.amazonaws.com/174/flashcards/1804174/jpg/0060441349591360981.jpg, Access 19.7.2016)

1.1.1.2 Variants of HIV

HIV 1 and HIV 2 are the two different types, in other words two antigenic variants of the human immunodeficiency virus (1). HIV 1, transmitted from apes, contains the groups M, N, O and P. These groups result from separate cross species transmissions, from chimpanzees and gorillas (3). The HIV pandemic itself started through spreading of group M that again can be divided up into nine subtypes (A-K), of which B is most common in the western population. Subtype C by contrast can mostly be found in Africa and India - the regions that are hit the hardest by the pandemic. HIV 2, transmitted from sooty mangabeys, is predominant in West Africa and is not as severe as HIV 1 as it is showing lower transmission rates (4). Nevertheless HIV 2 prevalence is decreasing in West Africa whereas HIV 1 infections are getting more common (5).

1.1.2 History

As mentioned before, HIV was transmitted to humans from African primates, that were infected with the simian immunodeficiency virus (SIV). It is still uncertain how exactly the transmission to humans took place, but there is evidence that strongly suggests a communication of the disease through hunting of bush meat. First occurrence of HIV was detected in 1959 in Kinshasa, the Democratic Republic of Congo (6). The AIDS pandemic, however, started in June 1981, when four otherwise young and healthy homosexual men were reported with rare infections like Pneumocystis carinii pneumonia, severe mucosal candidiasis and viral infections such as cytomegalovirus (CMV) (7). In July of the same year Kaposi´s sarcoma, a rare tumour triggered by human herpesvirus 8 (HHV8) infection occurred in eight gay men in New York. A few months later intravenous drug users developed the same immunodeficiency symptoms. This development proved that the new disease was not restricted to male having sex with male (MSM) as scientists had supposed, but also included other groups of the population. Soon haemophiliacs, who had received blood products, female sexual partners of infected men and infants of infected mothers followed (8). In 1983 HIV was reported to be the cause of the Acquired Immunodeficiency syndrome that patients had suffered from (9). Not long after the successful isolation of the virus serological testing was unfolded in 1985 and first prevention strategies were originated. With the development of antiretroviral therapy (ART) in the late 1990s mortality was decreasing, at least in the western world. The

number of infected patients getting access to ART in the developing world started to increase much later (10).

1.1.3 Epidemiology

According to the UNAIDS there were 36.7 million people living with AIDS in 2015. The number of people newly infected with HIV, meaning the incidence, amounts 2.1 million. Moreover 1.1 million patients died of AIDS in 2015. As the figure below points out Africa, or more precisely, sub-Saharan Africa has the highest prevalence of HIV infections on earth. By now, about 19 million people are infected in Eastern and Southern Africa, which makes up more than half of the global amount (11). Furthermore, sub-Saharan Africa carries the highest burden as it comprises the highest amount of infants with HIV and deaths through AIDS (12). The global prevalence of HIV infections has increased from 28.9 million in 2000 to current numbers mentioned before (11). This is not least because more people are getting access to antiretroviral therapy and thus living longer (4). The incidence has still remarkably decreased from 3.2 million in 2000 to 2.1 million people in 2015 (11), since less heterosexual transmissions are taking place (4). Concerning homosexual transmission, particularly MSM, incidence still remains high despite the use of highly active antiretroviral therapy (HAART) (13).

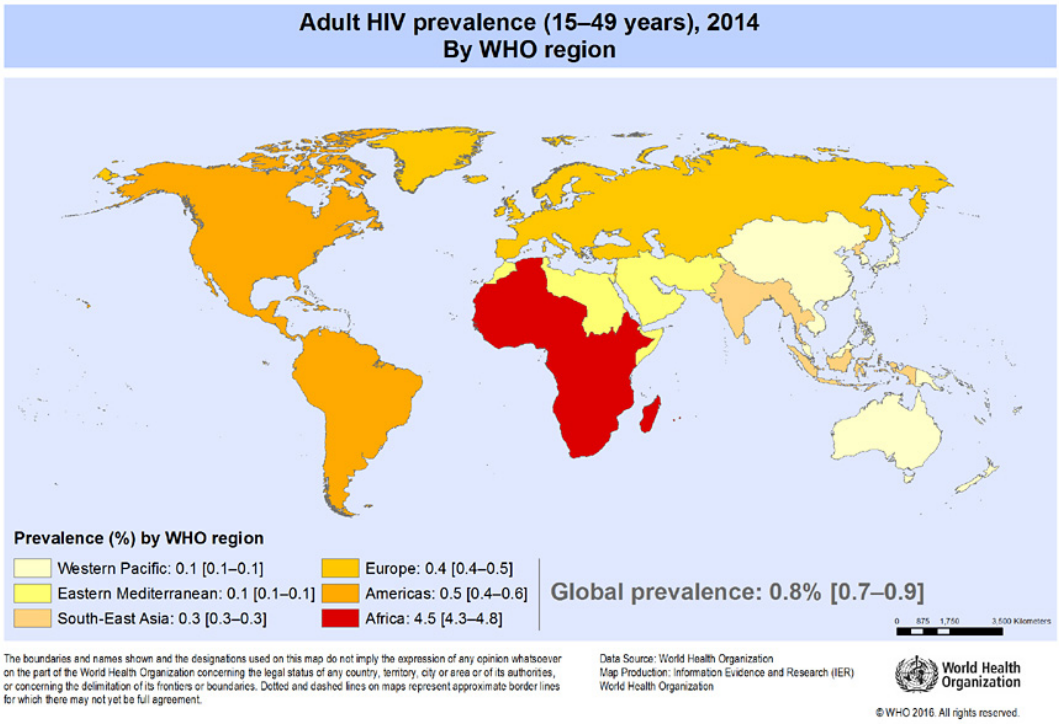


Figure 3: Prevalence of HIV in percent in adults (15-49 years), 2014
(reproduced from: http://www.who.int/gho/hiv/hiv_013.jpg?ua=1, Access 16.7.2016)

1.1.4 Transmission

In 1982 AIDS was referred to as the „gay plague“ since the first cases reported were members of the MSM community (8). This theory was soon abandoned as Haitians, patients suffering from haemophilia, women, infants and injection-drug users presented with similar immunodeficiency syndromes (14). After that, HIV was found in particularly blood, semen and cervical secretions. Today we know that the transmission of HIV and therefore the outbreak of AIDS takes place in three transmission modes. They consist of sexual intercourse (anal, vaginal, oral), vertical transmission from mother to infant (in utero, at birth, while breastfeeding) and parenteral transmission through contact with infectious blood (affecting intravenous drug users (IDU´s) and patients that receive blood products or donated organs) (15). However, the most prevalent cause of transmission today is heterosexual, especially in sub-Saharan Africa (12)(14). Nevertheless, the incidence of infants getting infected decreased from 490.000 in 2000 to 150.000 in 2015 as current data show (11). This is not least because of better coverage of infected mothers with HAART (15).

1.1.5 Target cells and Replication cycle

The human immunodeficiency virus requires a CD4 receptor on target cell surfaces, to get access to the host cell. These host cells include activated and resting T Helper lymphocytes, macrophages, Langerhans dendritic cells and microglial brain cells. Moreover, chemokine coreceptors of CD4 like CCR5 and CXCR4 are necessary for HIV to penetrate the host cell membrane (16). The replication cycle of HIV starts with the attachment of gp 120 on the surface of the HIV envelope to the CD4 receptor. This interaction triggers a conformational shift of the viral envelope, allowing the gp120-CD4 complex to get in contact with CCR5 or CXCR4. Fusion of viral and host membrane results from this step and viral contents open up into the target cell (1). After the viral RNA inside the capsid enters the cell, a sequence of events follows. Firstly, reverse transcriptase catalyses the production of a single DNA strand, using viral mRNA as a templet. Subsequently the emerged single strand is formed into ds DNA as HIV incorporates its own genome in the one of the target cell. Therefore, double stranded DNA is needed. Viral RNA is degraded and the ds DNA is actively transported into the nucleus. Integration into the host genome is accomplished by the enzyme Integrase (17).

The so called „provirus“ stays dormant in inactive cells, whereas activated cells produce viral RNA out of DNA and proteins with the help of RNA Polymerase (16). These immature viral particles are then released out of the cell in a process called -„Budding “. This last step results in formation of infectious virions that have now the ability to attack other target cells (17). The figure below illustrates the presented mechanisms. Knowledge of how HIV replicates is required to understand how antiretroviral therapy works.

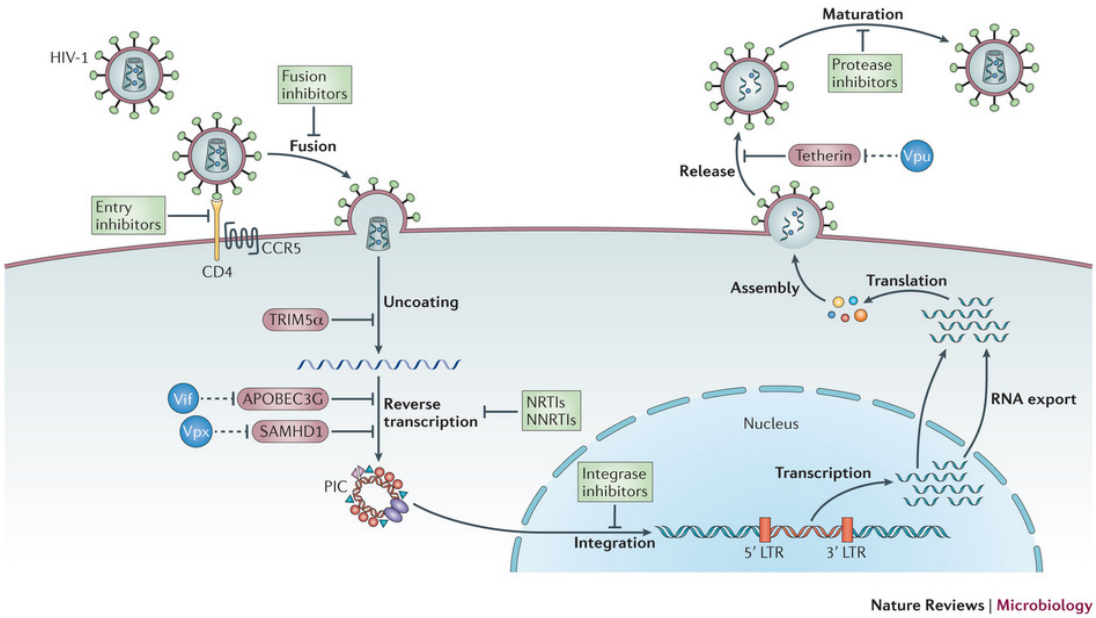


Figure 4: The replication cycle of HIV
(reproduced from: http://www.nature.com/nrmicro/journal/v11/n12/fig_tab/nrmicro3132_F2.html, Access 16.7.2016)

1.1.6 Clinical stages

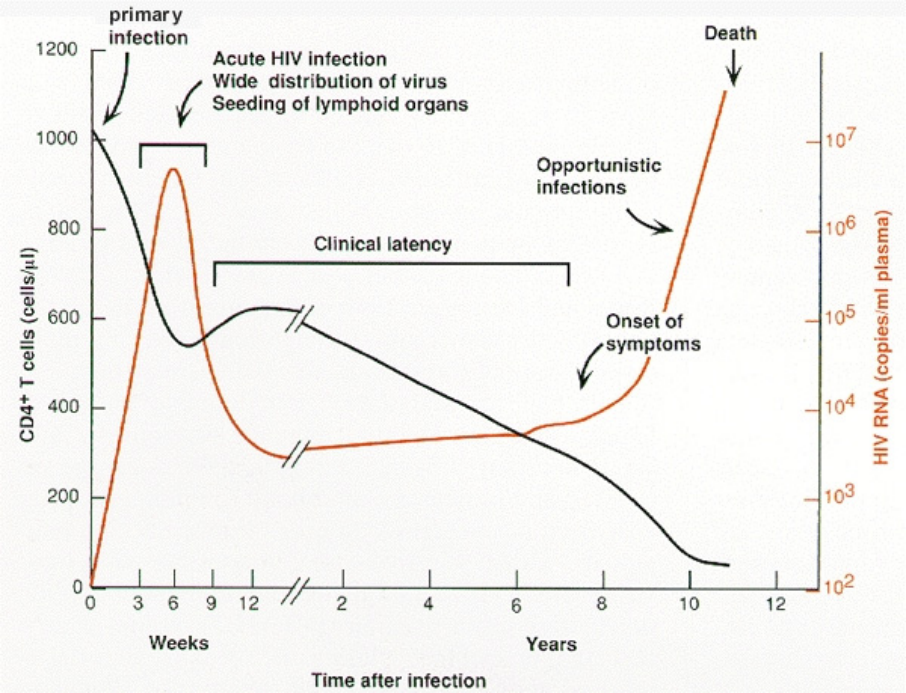


Figure 5: Typical course of HIV infection
(reproduced from: http://www.ncbi.nlm.nih.gov/books/NBK19374/figure/A6274/?report=objectonly, Access 12.7.2016)

As can be seen in the figure above, the HIV infection can be classified into the stages of acute infection, clinical latency, and the symptomatic stage, eventually leading to late stage disease with opportunistic infections. Primary/acute infection covers the period from the first contact between host and virus (particularly through sexual transmission) to the production of antibodies against it. Between 0-6 weeks after the primary infection HIV replicates explosively and reaches its maximum in body fluids after about 3 weeks. In this period the individual is still seronegative, but highly contagious (17). At the same time patients complain about flu-like symptoms including fever, lymphadenopathy, pharyngitis and myalgia because of a dramatical decrease of the CD4 positive cells (15). For symptoms of an acute HIV 1 infection being very nonspecific, making the right diagnosis can easily be missed, if there is no concrete suspicion (18). A recent study shows, that most of the patients identified to suffer from an acute HIV infection did either have just vague complaints or did not even notice any symptoms (19). Three to four weeks after primary infection antibodies occur in most individuals. The time between primary infection and seroconversion is called the „window period“ (20). The phase of clinical latency,

which follows, can last up to 12 years, and is characterized by decreasing viral load, low replication of HIV and high CD4 count. Meanwhile the individual has the ability to infect others (2). The higher the viral load becomes through progression in HIV replication, the more CD4 positive cells decline. Therefore the immune system of the affected individual gets weaker and more prone to other infections and malignancies (15). The breakdown of the immune system exposes the vulnerable host to opportunistic pathogens. Whereas individuals with a functioning immune system can cope with the natural bacterial flora and commensals, HIV infected patients develop opportunistic infections. These are caused by bacteria like *mycobacterium tuberculosis* (*m. tuberculosis*), viruses including cytomegalovirus and fungi, particularly referring to mucosal candidiasis and protozoa. Mostly, these infections occur when the CD4 count declines to lower than 200 cells per µ litre (1). The Centers for Disease Control and prevention (CDC) established a clinical staging system for HIV infections that is nowadays used most widely besides the World Health Organisation (WHO) staging system and was recently altered in 1993. The severity of the HIV infection is classified by connecting the CD4 cell count with the occurrence of HIV related diseases. Category B covers symptoms that do not fall in category C, but are causally attributable to HIV infection or indicate a dysfunction of immune defence, such as bacillary angiomatosis and oropharyngeal candidiasis. In contrast, Category C captures AIDS defining diseases, such as pneumocystis jiroveci pneumonia, Kaposi sarcoma and cerebral toxoplasmosis, to quote only few of them. A CD4 cell count lower than 200 cells per µl or the presence of AIDS defining diseases and malignancies allows reliable diagnosis of Acquired immunodeficiency syndrome (21). Still it should be critically reviewed that the viral load, which is particularly important for the prognosis of HIV infection is not considered in this classification. Moreover, a patient that was once categorized in a certain stage, remains there notwithstanding his current condition (21). The figure below provides a coherent view of the clinical staging system.

CD4 cell count, cells/mm^3 (CD4 cell percentage)	CDC classification		
	A[a]	B[b]	C[c]
⩾500 (⩾29%)	A1	B1	C1
200–500 (14%–28%)	A2	B2	C2
<200 (<14%)	A3	B3	C3

NOTE. From [32].

[a] Asymptomatic, persistent generalized lymphadenopathy, or acute HIV infection.

[b] Symptomatic (not A or C).

[c] AIDS indicator condition.

Figure 6: CDC Classification
(reproduced from: https://www.researchgate.net/figure/8356820_fig1_Table-9-Centers-for-Disease-Control-and-Prevention-CDC-staging-system-for, Access 12.7.2016)

1.1.7 Treatment

The initiation of highly active antiretroviral therapy was one of the most effective approaches in the history of AIDS in the 1990s. Since a complete eradication of the virus has not possible down to the present day, it remains to be a lifelong infection. Treatment with HAART can transform HIV infection into a manageable chronic condition (4). Moreover constant intake of antiretroviral drugs that enhance the CD4 count over 500 µl and suppress viral load ensure a normal life expectancy in comparison to the healthy population (22). Looking at the life cycle of HIV as imaged below, basic mechanisms of antiretroviral drugs can be explained. Standard treatment with HAART consists of two nucleoside reverse transcriptase inhibitors and either a non-nucleoside reverse transcriptase inhibitor, a protease inhibitor (PI) or an integrase inhibitor (4). Single drug therapy showed to cause resistances very early and had less benefit than HAART, the highly active antiretroviral therapy with three agents. Reverse transcriptase inhibitors such as nucleoside reverse transcriptase inhibitors act as analogues to physiological nucleosides of the target cell. Chain termination is caused, when viral reverse transcriptase inserts a nucleoside reverse transcriptase inhibitors (NRTI) into the viral DNA strand. This group of drugs prevents the elongation of the DNA chain. Particularly, the combination of Tenofovir and Emtricitabinide is established today since it proved to have advantages concerning dosing and side effects (23). Non-nucleoside reverse transcriptase inhibitors (NNRTI´s) dock directly to reverse transcriptase. This newly formed complex with a different confirmation

blocks the binding site of the enzyme and thus its activity (17). Nevirapine and efavirenz are just two examples of the first generation that showed the highest efficacy with undetectable viral load in combination with two NRTI´s. PI´s inhibit protease – the enzyme that oversees forming mature viral parts by cleaving structural proteins. Consequently, non-infectious viral particles are produced (16). Dyslipidaemia and lipodystrophy are side effects of long-term medication with protease inhibitors. Raltegravir is the prototype of integrase inhibitors that prohibits incorporation of proviral DNA into the host genome. Apart from that entry inhibitors and chemokine receptor antagonists find use in HIV infection therapy today (17).

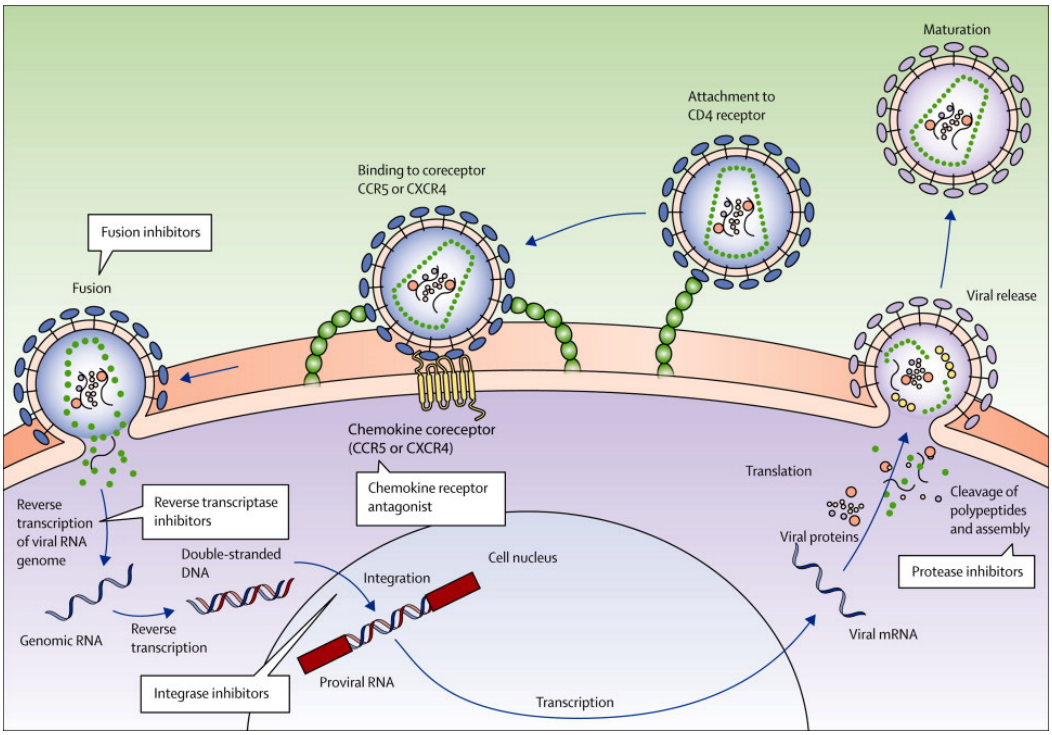


Figure 7: Targets for antiretroviral drugs in the HIV life cycle
(reproduced from: http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(14)60164-1/fulltext?_eventId=login, Access 19.7.2016)

The WHO recommends to start antiretroviral drugs in all HIV infected individuals irrespective of clinical stage, CD4 count and viral load (24). A recent study from 2015 proved, that immediate initiation of HAART in patients with a CD4 count of more than 500 µl revealed benefit compared to waiting for the cell count to decrease under 350 µl. AIDS related diseases occurred less frequent in patients who received early treatment (25). Moreover transmission rates in discordant couples sank due to reduced viral load in plasma and semen under HAART (10). However, one of the major challenges in treatment remains the emergence of resistance against antiretroviral drugs, since error frequency during the

replication of HIV leads to a high mutation rate (26). Fortunately, AIDS related deaths decreased globally around 26% between 2010 and 2015, due to wide-ranging distribution of antiretroviral drugs, as the figure below points out (11).

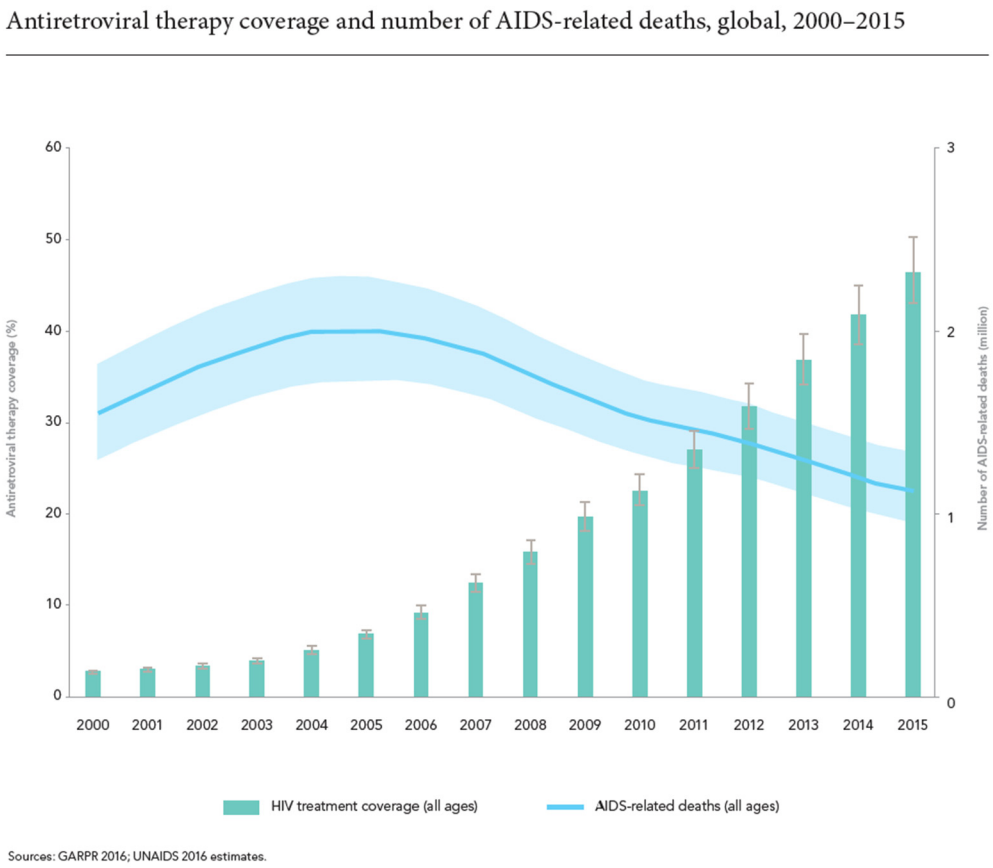


Figure 8: Antiretroviral therapy coverage and number of AIDS-related deaths
(reproduced from: https://www.chskenya.org/wp-content/uploads/2016/06/Screen-Shot-2016-06-08-at-10.43.20-AM.png, Access 19.7.2016)

1.2 Tuberculosis

Tuberculosis (TB) is an infectious disease that has claimed many deaths in the last centuries and still contributes to worldwide suffering. The group that is affected the most are individuals who live in poverty in low income countries with delayed medical care (27). With still no efficient vaccine on the market and more resistances to therapy regimens occurring, TB remains a challenge not just for certain risk groups but for our society (28). To prevent negligence against this global burden, the WHO developed "The END TB strategy" in May 2014 with the vision of a world that is free of tuberculosis with no more deaths, diseases, and ailment. The three main pillars of this aim include more efficient

prevention and care, a daring policy and engagement of the communities and intense research to combat TB (29).

1.2.1 History

„Phthisis “, „the white plague “, „consumption “and „the captain of the men of death “are names that humankind has given tuberculosis in the last centuries (30),(16). Until now, tuberculosis has caused hundreds of millions of deaths (16). Still it takes about 2 million lives ever year and thus remains to be one of the most important diseases of our times (31). Historically, first evidence of the genus mycobacterium occurred 150 million years ago. About 5000 years from now the wasting disease was detected in ancient Egypt, where spinal tuberculosis with its skeletal deformities was documented in mummies (32).
In the 19th century in the midst of the industrial revolution, crowded living conditions fuelled the outbreak of tuberculosis in Europe and North America (33). Landmarks in the history of TB were the identification of its pathogen *mycobacterium tuberculosis* by Robert Koch in 1882 (30) and Selman Waksman’s successful isolation of streptomycin as the first antituberculotic substance in 1943. The therapy of TB got even more efficient with the development of isoniazid, rifampicin and other medication. However, occurrence of drug resistances resulted in an increasing number of deaths again. Whereas infection rates still declined in developed settings, poverty and malnutrition have become the main drivers of TB in the low income countries till today (31).

1.2.2 The tuberculosis pathogen and transmission

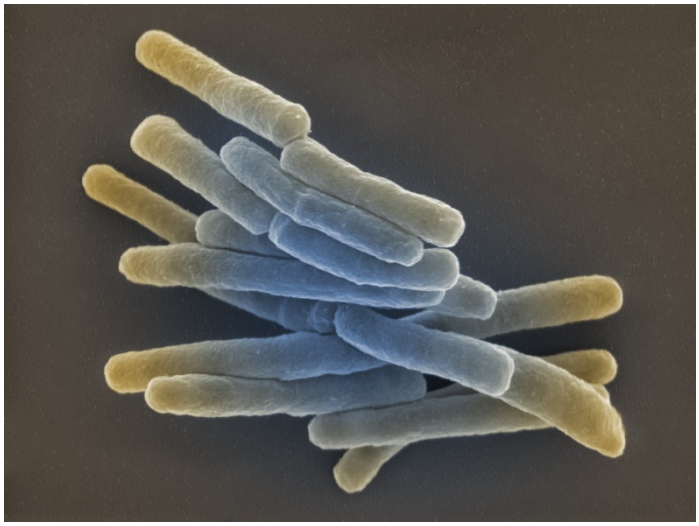


Figure 9: Mycobacterium tuberculosis
(reproduced from: https://www.rki.de/SharedDocs/Bilder/InfAZ/Tuberkulose/R2013-0011-05_TBC_ADC_19_color_JPG.jpg?__blob=publicationFile&v=3, Access 22.7.2016)

Tuberculosis is an airborne infectious disease, caused by the *mycobacterium tuberculosis* complex (30), including *m. tuberculosis, m. bovis* and *m. africanum. M.tuberculosis* has the highest prevalence and thus causes the majority of the reported tuberculosis cases in humans, whereas *m. bovis* also has the ability to infect cattle (33). These germs are slowly growing, non-spore forming, immotile, with a lipid rich cell wall and responsible for similar clinical presentation in patients (34). One of the properties that makes *m. tuberculosis* so harmful to humankind is its ability to remain undetected in its host for a long time. The period in which the host´s immune cells keep the bacilli under control is called the latent stage of infection (35). Close contact with *m. tuberculosis* infected patients can cause transmission by inhalation of infectious particles in droplets (30). Most transmission events occur in public transport and households, where contact between healthy and sick individuals is close. Shared spaces and workplaces are reported to be less relevant (36). In high income and low prevalence settings transmission takes place in particular locations like poor and neglected districts of big cities and urban areas with high prevalence of other infectious diseases as well (16).

1.2.3 Epidemiology

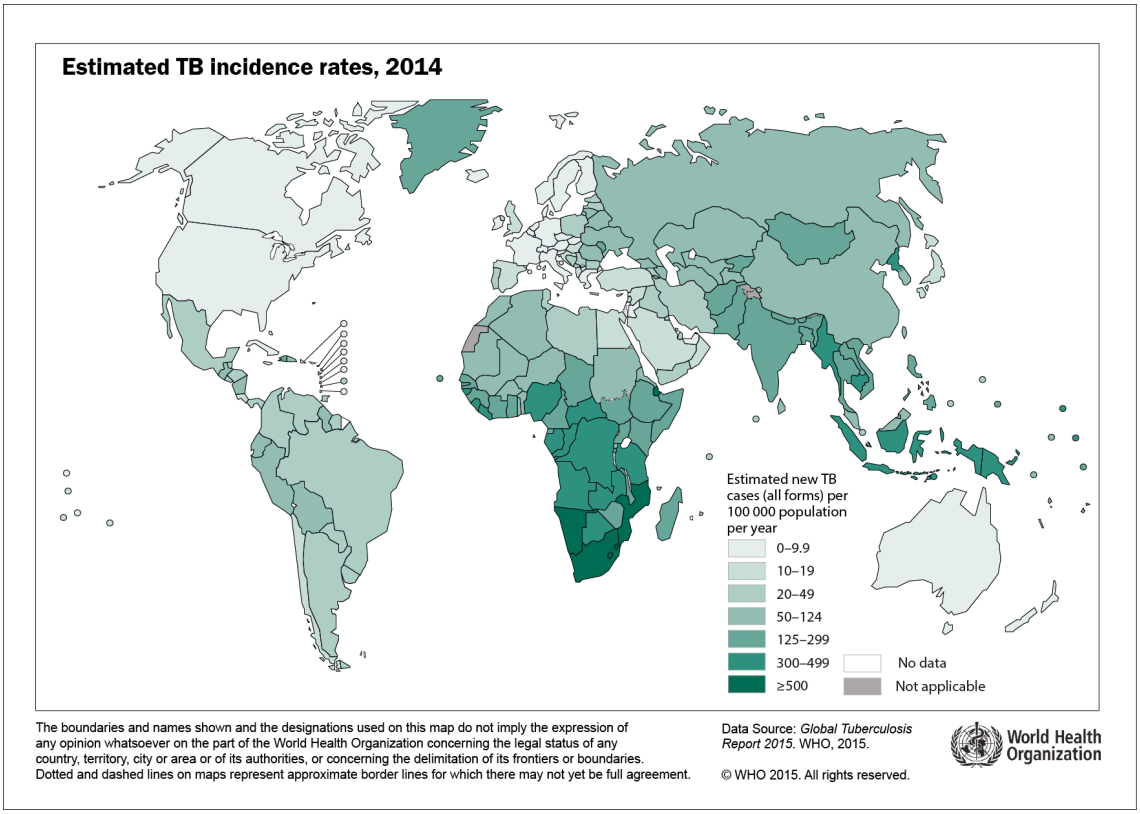


Figure 10: TB incidence rates 2014
(reproduced from: http://gamapserver.who.int/mapLibrary/Files/Maps/Global_TBincidence_2014.png, Access 22.7.2016)

Tuberculosis and HIV together counted to the leading causes of death worldwide in 2014. The same year, around 9.6 million people suffered from TB globally, 12 % of these patients had co-infection with HIV and 1.5 million died in total. The incidence of tuberculosis worldwide was calculated 6 million. The figure above points out, that new infections have the highest prevalence in South Africa. HIV and TB still remain a major burden of tropical Africa with the uppermost documented death rate (37). By now, 22 developing countries make up 80% of all TB cases worldwide, which is significant (28). The group of people that is most likely to get tuberculosis includes young male adults, health care workers with contact to infected patients and particularly HIV positive individuals, who show low immune competence, which is the strongest risk factor for TB after all (30). Apart from that, the epidemiology is linked with the socioeconomic status. Poverty, overcrowded living conditions, malnutrition and delayed medical care are drivers of the spreading of tuberculosis (27).

1.2.4 Immunology and pathology

After the airborne infection of the host by exhaled droplets, *mycobacterium tuberculosis* reaches the alveolar level, where it is phagocytosed by alveolar macrophages – the first non-specific, immunologic barrier within the lungs (38). The bacilli have certain properties that enable them to survive inside the air-space macrophages and thus escape the host´s immune response mechanisms. Keeping *m. tuberculosis* under control is only manageable with additional cellular immune response (33). Infected macrophages transport the bacilli to regional lymph nodes and by the bloodstream to all other organs (34). The first steps can be summarized as the primary complex. Pro inflammatory cytokines are released by macrophages and mycobacterial antigens are presented that consequently induce proliferation and activation of CD4 positive cells. The resulting inflammatory cascade leads to the formation of granulomas, which are specific and characteristic for TB and other mycobacterial infections (38). Granulomas are a merger of monocytes, macrophages, and neutrophils. Macrophages develop to foamy macrophages, epithelioid macrophages and giant cells that enter and surround the complex. Moreover, neovascularization and recruitment of fibrocytes provides solidity to the granuloma.
This formation provides a barrier in which m. tuberculosis is kept under control. Progression of TB infection leads to necrosis and caseation inside the inflammatory complex and reduces the integrity until eruption causes the distribution of bacilli into the alveoli and bronchi, ready to infect other individuals (39). The figure below points out the described steps of immune response and granuloma formation. Nevertheless, the immune system of the affected host develops defence against the pathogen only after 2-12 weeks (30). Until T cell response occurs in the host, the stage is defined as latent infection, meaning a suppressed and inactive form of the disease (30). Lower respiratory parts of the lung comprise the highest number of bacilli because of good ventilation conditions. However, apical parts of the lung that are perfused more efficiently allow *m. tuberculosis* to reproduce massively in an aerobe setting (38).

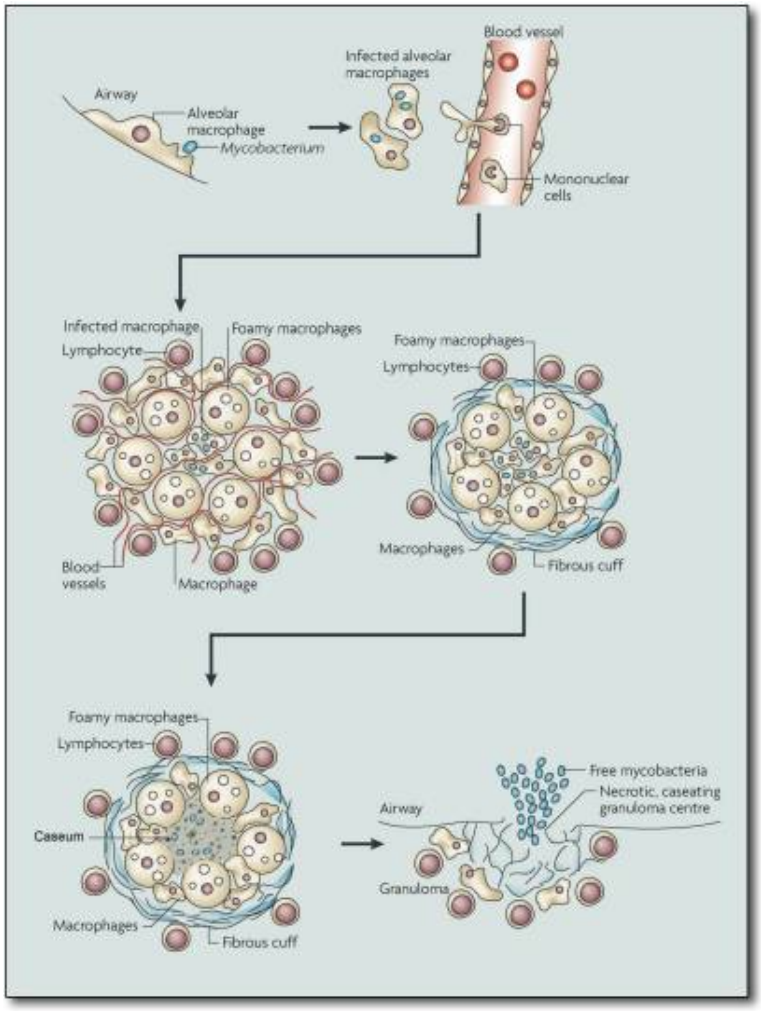


Figure 11: The life cycle of m. tuberculosis
(reproduced from:
http://www.ncbi.nlm.nih.gov/core/lw/2.0/html/tileshop_pmc/tileshop_pmc_inline.html?title=Click%20on%20image%20to%20zoom&p=PMC3&id=2872107_nihms-200284-f0001.jpg, Access 22.7.2016)

1.2.5 Symptoms

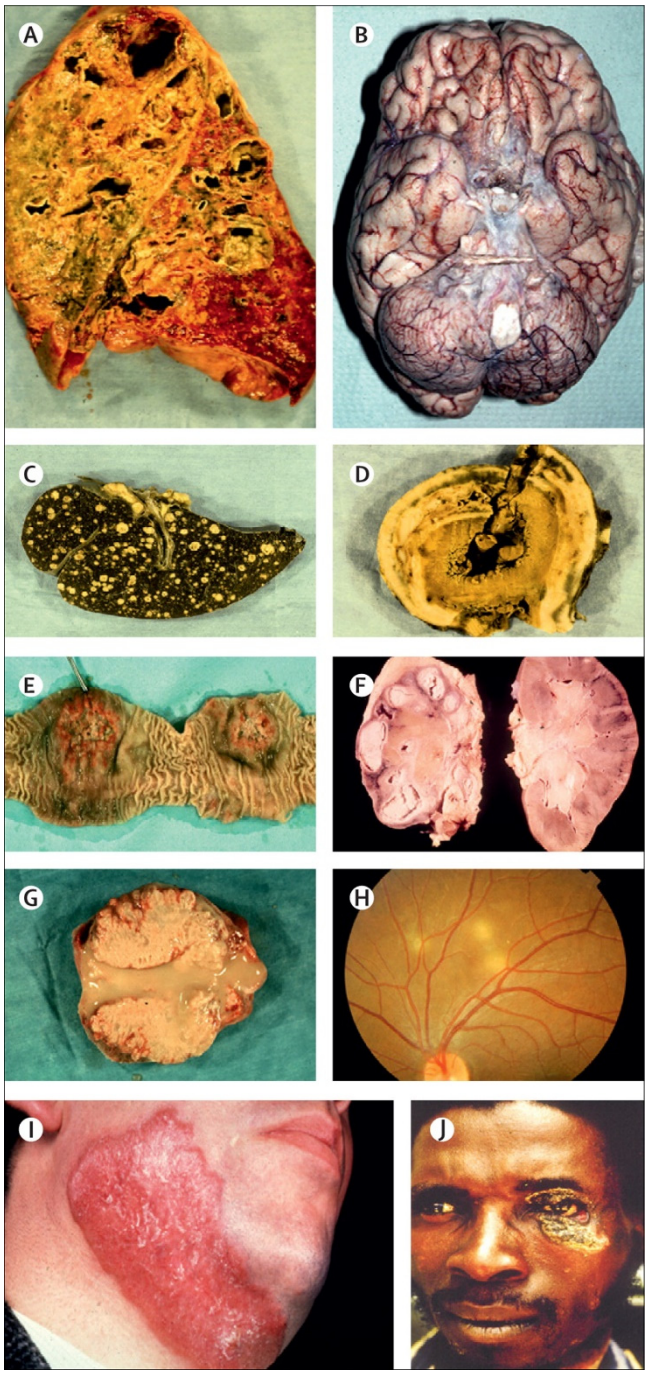
It is anticipated that one third of all individuals worldwide are infected with *m. tuberculosis,* but do not present with any signs and symptoms (40). An estimated number of 90% of these remain in the stage of latent infection (41). Still, there is a high risk of developing active tuberculosis in the years, following the initial infection (42). The clinical features of active tuberculosis are very miscellaneous. Active TB mostly affects the lungs; nevertheless, extra pulmonary symptoms are reported to emerge in 10-42% of the patients (41). However about 50% of patients with AIDS co-infection are hit harder by *m. tuberculosis*, showing several other organ involvements (43).

1.2.5.1 Pulmonary tuberculosis

Initial TB infection is asymptomatic and may be detected randomly on a chest x-ray (33). Later in progression appetite and weight loss, fever and night sweats and also slowly developing chronic cough strongly suggest pulmonary involvement (41). Additional productive cough with mucopurulent sputum occurs in further progress. Haemoptysis, only present in 10% of the infected individuals, is an indication of blood vessel damage. However, penetration of larger vessels causes massive blood streamed sputum (34). Furthermore, severe chest pain due to inflammation of the pleura and pleural effusion can emerge. Ulcers of the pharynx, tongue and mouth, hoarseness and dysphagia show involvement of also other parts of the respiratory tract (33). Dyspnoea results from a destruction of lung parenchyma, bronchial obstruction, and pleural effusion. Persistent complaints for weeks until presentation are the rule rather than the exception in low and middle income countries with delayed medical care and a high rate of infectious diseases, particularly co infections with HIV (16).

1.2.5.2 Extra pulmonary tuberculosis

Tuberculosis can imitate a broad spectrum of many clinical presentations in several organs, apart from the predominantly affected lung. Consequently, clinicians are required to be suspicious and remember TB in a list of differential diagnosis (28). Both cases, isolated extra pulmonary TB and concurrent infection with pathology of the lungs are possible (44). Tuberculous lymphadenitis, particularly affecting the cervical and supraclavicular lymph nodes, is frequently encountered and emerges through the drainage of bacilli out of the lungs (45). These lymph nodes are firm, enlarged, not tender and can develop to fistula, if they are not treated at the right time (43). Tuberculous pleurisy, the second most common manifestation with pleural effusion, as its consequence is counted to extra pulmonary TB, but causes the same clinical features described above, like fever, chest pain and non-productive cough (46). The *m. tuberculosis* infection, however, does not only take the direct and lymphatic way, but also the hematogenic route. This can result in involvement of the kidneys, other organs of the genitourinary tract, the bones, the central nervous system, and the gastrointestinal system. Tuberculosis in various other organs is illustrated in the figure below, pointing out the major extra pulmonary sequalae's (28).



While figure (A) makes clear what is meant by caseating necrotic regions in the lung, (B) shows an exemplar of basal tuberculosis meningitis, the most severe form of extra pulmonary involvement with fever, headache and neck stiffness (44). Inclusion of spleen (C) and tuberculous pericarditis (D) can occur during infection as well. Ulcers in the ileum with complains like diarrhoea, abdominal pain, weight loss and melena can emerge through particularly consuming food that was contaminated or swallowing sputum of infected individuals (E). Involvement of the kidneys (F) often remains undetected because of it being asymptomatic and is mostly randomly diagnosed by routine urine analyses with sterile pyuria presentation (16). (G) illustrates the classical tuberculous lesion in a lymph node while (H) shows an infected eye fundus.

Figure 12: Tuberculosis in various organs
(reproduced from:
http://thelancet.com/cms/attachment/2001012353/2003804574/gr6_lrg.jpg, Access 29.7.2016)

(I) is a specimen of granulomatous lesions of the skin. 35% of extra pulmonary cases present as bone and joint tuberculosis, most often affecting the spine. Destruction of vertebrae und discus as well as abscesses can occur through the infection. Pain or cord compression symptoms provide an indication of the so called “Pott´s disease” (43).

1.2.5.3 Miliary tuberculosis

Massive dissemination of *m. tuberculosis* and lack of immune competence of the host describe the phenomenon "miliary tuberculosis", where small infiltrates occur all over the lung with the appearance of millet seeds (16). This condition with its typical chest x-ray findings can be potentially fatal (47). With the spreading of HIV/ AIDS and the number of patients receiving immunosuppressive medication particularly after organ transplant or for treatment of certain medical conditions, the incidence of miliary tuberculosis rose in the last 30 years. Miliary TB can affect nearly all organs and thus present with various symptoms. Nevertheless, non-specific complaints like fever, chills and rigor, weight loss, night sweat and weakness occur very often (48).

1.2.6 Diagnosis

Early diagnosis of TB infection is a major issue since it results in an early onset of treatment and lower morbidity and transmission rates (49). Accurate tests with high sensitivity and specificity can prevent 22% of all tuberculosis deaths (50). Gold standard in detecting TB infection is the culture method, where growth of bacteria confirms the diagnosis. Since this takes 4-8 weeks and furthermore requires labs that have the equipment and qualified collaborators, it is not feasible at all in low income settings (49). The technique that is used most widely to prove m. tuberculosis infection is sputum smear microscopy with examination of the Ziehl-Neelson stained specimen. This method is universally available but is not sensitive enough, especially in early and latent infection (34). The most accurate tests that are recommended for detection of latent TB infection by the CDC include the tuberculin skin test (TST) and an interferon-gamma release assay (IGRA). They are equal in sensitivity, however, the former is even more cost-efficient (41). Intracutaneous injection of tuberculin purified protein derivate (PPD) causes a delayed-type hypersensitivity reaction when exposure to *m. tuberculosis* took place in the individual, either through vaccination or real exposure to active TB (30). TST cannot demarcate latent infection from disease (16). However, IGRA, which measures the response of T cells that interact with antigens, has the ability to distinguish Bacille Calmette-Guérin (BCG) vaccine from TB. (30) All in all, these two methods are only acceptable, but not accurate enough (51). Since TB infection shows characteristic features on the chest x-ray, it can be used as primary method in radiographic evaluation. This technique is not specific for the diagnosis of TB and thus must be followed by a sputum

smear microscopy. Cavitary opacities in the upper parts of the lungs are classical features, that occur in TB infection. Chest x rays can also be used in case follow up and control of several complications (52).

1.2.7 Treatment

Evidence based guidelines for the accurate treatment of tuberculosis are provided and updated regularly by the WHO. Control of TB started with the DOTS Strategy (directly observed treatment, short course) with standardized treatment methods in sputum smear positive patients and was enhanced by the "Stop TB strategy" in 2006 (53)(54) . The major pillars of DOTS include government commitment, diagnosis through microscopic sputum smear, standardized therapy, continuous drug supply and steady monitoring. This strategy entailed remarkable achievements in treatment (55). There are five main agents that form the basement of tuberculosis treatment. Isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin. A combination of these available drugs and an intensive dosage in the first two months, followed by lower dosage in the following four months turned out to be most efficient. The selection of drugs changes due to the stage of the disease. Latent infection should be treated different from active infection, where it should be distinguished between drug resistant and non-drug resistant cases. To prevent further drug resistances that occur either way because of the high mutation rate of *m. tuberculosis,* this mixture of agents is required. Depending on resistance factors and the stage of infection therapy of at least six months may have to be continued up to 20 months (30). Drug susceptibility testing (DST) of *mycobacterium tuberculosis* plays an essential role in the individual treatment of patients, whereby this mostly lacks, especially in regions where the equipment and infrastructure is sketchy (28). Drug sensitive active tuberculosis requires isoniazid, rifampicin, pyrazinamide and ethambutol as first line drugs for at least six months with only isoniazid and rifampicin in the last four months. If there are any risk factors, therapy should be continued for three more months. Patients with latent TB must be treated with single drug use, particularly isoniazid, to prevent progress into active tuberculosis. An even better alternative is to administer isoniazid plus rifampicin for three months (41). Therapy of multidrug resistance TB remains a challenge because of the occurrence of intolerance and toxic effects after the intake of a combination of several agents, other than in the treatment of drug sensitive TB. In these cases, the intensive administration is supposed to be not two, but eight months. At least 20 months of treatment are necessary to eradicate *m. tuberculosis* here (41).

The table below gives an overview of current therapy regimens.

Table 1: Treatment of tuberculosis
(reproduced from: http://www.nejm.org/doi/full/10.1056/NEJMra1200894, Access 31.7.2016)

Table 1. Current Recommendations for Tuberculosis Treatment.

Type of Infection	Recommended Regimen	Comments
Active disease		
Newly diagnosed cases that are not multidrug-resistant	Isoniazid, rifampin, ethambutol, and pyrazinamide for 2 mo (intensive phase), followed by isoniazid and rifampin for 4 mo (continuation phase)	Pyridoxine supplementation recommended to prevent isoniazid-induced neuropathy
Multidrug-resistant disease	Four second-line antituberculosis drugs (as well as pyrazinamide), including a fluoroquinolone, a parenteral agent, ethionamide or prothionamide, and either cycloserine or para-aminosalicylic acid if cycloserine cannot be used	Initial treatment based on local disease patterns and pending drug-susceptibility results; later-generation fluoroquinolones (e.g., moxifloxacin or levofloxacin) preferred
Latent infection	Isoniazid at a dose of 300 mg daily for at least 6 mo and preferably for 9 mo	Recommended for 9 mo or more in HIV-infected persons; daily administration for 6 mo also an option but with lower efficacy; extension to 36 mo further reduces risk among HIV-positive patients in regions in which tuberculosis is endemic
	Isoniazid at a dose of 900 mg plus rifapentine at a dose of 900 mg weekly for 3 mo (directly observed therapy)	Studied with directly observed therapy in predominantly HIV-uninfected persons; higher completion rates and equal efficacy, as compared with isoniazid for 9 mo
	Rifampin at a dose of 600 mg daily for 4 mo	Shown to be effective in persons with silicosis
	Isoniazid at a dose of 300 mg plus rifampin at a dose of 600 mg daily for 3 mo	Effective alternative for HIV-infected persons
	Isoniazid at a dose of 900 mg plus rifampin at a dose of 600 mg twice weekly for 3 mo	Another effective alternative for HIV-infected persons

1.3 Co-infection of Tuberculosis and HIV

1.3.1 Epidemiology

Of all the opportunistic diseases that occur in early and late stage HIV infection, TB is the most common and severe one, particularly in resource limited countries. Thus, HIV remains to be the strongest risk factor for exacerbation of a recently acquired TB infection into an active disease. Tuberculosis is the cause for 25% of all AIDS related deaths worldwide with a mortality rate of 350.000 individuals in the year 2010 (56). Because of the high impact that this dual infection has on our global health, it was even named the "accursed duet" (57). HIV positive patients that present with fever, cough and weight loss should make clinicians suspicious of a possible tuberculosis infection. Updated WHO guidelines even demand a preventive clinical screening for a potential *m. tuberculosis* infection in people living with HIV (PLHIV) that attend health centres. This could provide an early diagnosis and treatment could be started immediately to reduce mortality (58). As can be deduced from the figure below, HIV is a strong driver for TB infections in both low and high income settings.

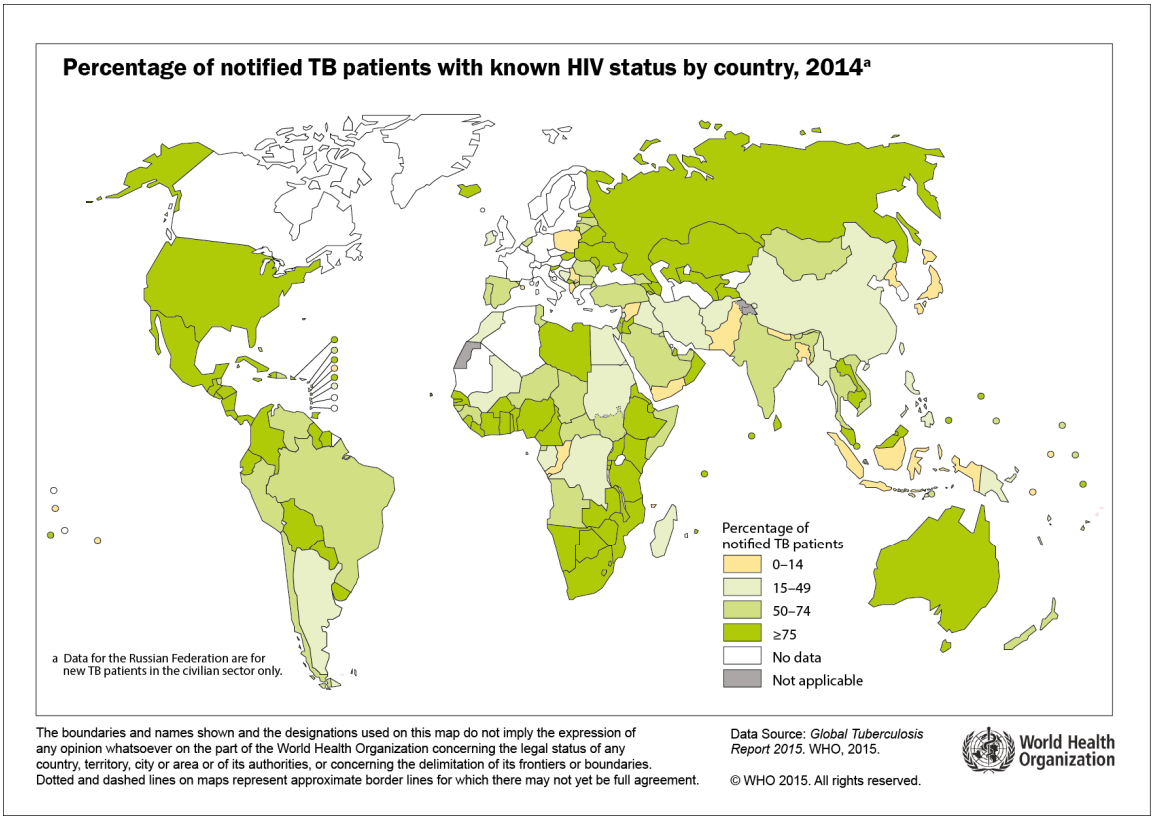


Figure 13: TB patients with HIV status in %
(reproduced from: http://gamapserver.who.int/mapLibrary/Files/Maps/Global_TB_Patients_HIV_2014.png, Access 31.7.2016)

1.3.2 Pathogenesis

It is decisive for the comprehension of co-infection with HIV and tuberculosis to note that the former causes a decline in CD4 positive cells, which are essential to curb the invasion and proliferation of *m. tuberculosis*. Consequently, HIV interferes with the host´s ability to combat *m. tuberculosis* (59). HIV is not only increasing the risk of initial infection with TB, it is also providing the reactivation from the latent to the active form, as well as it fuels re-infections after exposure and the development from one stage to another more severe stage (60). It is documented that HIV positive patients have a higher susceptibility to tuberculosis, whereas some steps in the pathogenesis remain unclear and need further investigation. There are several ideas, taken from clinical and animal studies that explain how these two pathogens interact. Some of them will be presented in this context. As pointed out before, a drop in CD4 count is a risk for TB disease. However, study results report that developing severe and active TB in HIV positive patients has its highest risk in the first twelve months, after acquiring HIV, when the CD4 cells are still high. In further studies, it was made clear, that HIV can lower the amount and function of *m. tuberulosis* specific T cells in the blood and in the lungs in co-infected patients. This reveals, that

human immunodeficiency virus attacks particularly host defence mechanisms against TB bacilli (61). HIV multiplies in CD4 cells and macrophages. These immune cells form major parts of the granulomas, which play an essential role in TB disease as they provide the containment of *m. tuberculosis* inside the granulomas. When CD4 positive T cells drop, the cohesion of the granuloma is not warranted anymore. Disruption and reduced integrity of this complex increase the likelihood of activation of a latent TB infection (59). These results are substantiated as higher viral loads were detected in bronchoalveolar lavage fluid (BAL) and pleural fluid in TB infected patients rather than in otherwise healthy individuals. This underlines the fact that HIV preferably replicates at sites of tuberculosis (62). The programmed cell death, apoptosis, is an essential part of the host´s immune system, enabling him to respond m. tuberculosis infection by killing bacilli inside alveolar macrophages in the very first step during the pathogenesis of TB. Recent studies found out that HIV changes the function of these macrophages and prohibits their ability of intracellular killing of bacilli. In the following process macrophages release less tumour necrosis factor (TNF) and lose their ability to acidify vesicles, making the host more susceptible for TB (59). Moreover, HIV needs CCR5, a co-receptor on cell surfaces, to invade the target cells in the early infection. Later, the receptor CXCR4 becomes even more essential. Throughout m. tuberculosis infection the expression of CXCR4 receptors in alveolar macrophages rises, making establishment of HIV in the target cells easier (62). Furthermore, recent studies suggest a genetic correlation of HIV and a higher risk of acquiring TB, where inflammasome gene polymorphisms play an essential role. However, these hypotheses still need further investigation (61).

1.3.3 Clinical features

The clinical presentation of a dual infection is depending on the immune status of the individual. Diffuse infection of lower lobes of the lungs, mediastinal lymphadenopathies and miliary tuberculosis are likely to develop. Nevertheless, fever, cough and weight loss are the most common clinical features observed in several studies (62). In further course, extrapulmonary tuberculosis occurs much more often, earlier and with a higher mortality in co-infected patients than in immunocompetent individuals. Impact on the lymphatic system and the pleura occurs frequently .Moreover, bone-, joint-, soft tissue and central nervous systems can be affected at the same time (63).

Table 2: TB characteristics associated with CD4 count
(reproduced from: http://www.ijidonline.com/article/S1201-9712(14)01712-3/abstract, Access 3.8.2016)

CD4 count, cells/ml	TB risk and disease characteristics
≥500	Increased TB risk irrespective of CD4 count, but appreciably lower risk compared to patients with a CD4 count <500 cells/ml
<500	WHO recommendation for ART initiation[36]
350–499	Mostly still typical upper zone infiltrates with/without cavitation[36]
	Increased atypical TB features[37]
	Increased bacterial pneumonia (also super-infection with TB)[36]
	Tuberculin skin test more likely to be false-negative[37]
200–349	As above (more pronounced)
100–199	Cavitation and positive sputum smear microscopy less common
	Middle and lower lung zone infiltrates, intra-thoracic lymph node enlargement and miliary pattern more common on chest radiograph[36]
	Extra-pulmonary disease more common[37]
<100	As above (even higher rates of disseminated and/or extrapulmonary TB)[50]
	TB-IRIS (unmasking and paradoxical)[38]
	Recommendation to use combination of Xpert MTB/RIF (on sputum) and Determine TB-LAM assay (on urine) as TB screening test[47]

The table above shows how the risk for developing TB and clinical features change in correlation to the CD4 count.

1.3.4 Immune Reconstitution Inflammatory Syndrome (IRIS) and Treatment

In the context of dual infection, the Immune Reconstitution Inflammatory Syndrome will be explained further. IRIS is a phenomenon that is still not completely understood and may possibly occur in HIV positive patients that receive HAART while they suffer from undiagnosed TB or just recently diagnosed TB. During HAART, CD4 counts rise and immune competence is restored, leading to a hyperactive immune response of the individual to bacilli, particularly *m. tuberculosis.* An exacerbation of the pulmonary and extra pulmonary symptoms may develop despite or in other words because of increasing CD4 cells and declining viral load (64). IRIS can arise as an unmasking and a paradoxical scenario. In unmasking IRIS, active TB resulting from an occult infection develops during the treatment with HAART. Paradoxical IRIS, the second pattern, can occur as worsening of TB symptoms in patients who are receiving both HIV and TB treatment (58). In some cases, occult multidrug resistant tuberculosis can present like IRIS and thus should be clarified. It is essential that subclinical TB is diagnosed before HAART initiation,

however, there are diverse opinions concerning treatment and its timing in HIV / TB co-infection (64). On the one hand, delayed initiation of HAART can prevent the incidence of IRIS, as well as drug interactions with anti-tuberculosis drugs and hepatotoxic events (58). On the other hand, early treatment with HAART in HIV positive patients is beneficial as early initiation of HAART improves immune function, reduces relapse risk, prevents the occurrence of opportunistic infections and sexual transmission to others and lastly reduces mortality (58). The eradication of *m. tuberculosis* is the ultimate objective to prevent spread of the infection to other individuals. Currently, it is recommended to immediately start HAART, when the CD4 count is lower than 50 cells/mm^3 (58). According to several recently published studies, an initiation of HAART within two weeks of anti - tuberculosis treatment was reported to be more advantageous. However, there are varying views on treatment regimens, whereby clinicians should weigh up the pros and cons. Still it is strongly suggested that an early initiation of HAART contributes to benefits in the rate of TB (58)(64). Understanding this profound impact that HIV and m. tuberculosis have on each other is essential to improve treatment and reduce mortality. A simultaneous therapy management will help to prevent this accelerating disease progression and improve quality of life (64). Above all, prevention of TB in HIV positive patients should be the ultimate objective. Therefore, the "Three I´s for HIV/TB" were established as an approach to efficient prevention, care, and treatment. Intensified case finding with the benefit of earlier diagnosis and treatment, Isoniazid preventive treatment with reduction of the risk of active TB and infection control for TB are the main pillars (66).

1.4 The Cardiovascular system

Non-communicable diseases (NCD) contribute to many deaths worldwide. Among these, cardiovascular diseases (CVD) represent the major cause of death not only in developed, but also in developing countries. In 2015 ischaemic and hypertensive heart diseases, stroke and diabetes mellitus (DM) were responsible for 29.6% of all demise globally. Ischaemic heart diseases alone made up about 13.2% of them (67). These numbers show that the cardiovascular system has a high impact on our global health.

1.4.1 Basic information

The cardiovascular system functions as a transport system, whereby blood is the means of transportation and the vessels represent the pipes, through which the blood flows and reaches its target cells and organs. The heart, as a muscular hollow organ acting as a pump, provides blood to the system with its left ventricle and to the pulmonary system with its right ventricle. Together with the blood heat, gases, nutrients like amino acids and electrolytes, immune cells and hormones are distributed, whereas wasting products are collected concurrently (68). In the systolic phase, blood leaves the left ventricle with high pressure to flow into the arterial system. Via arteries and arterioles blood reaches the capillary system, where the organs are supplied with nutrients and oxygen, before wasting products are removed back to the right ventricle with venules and veins. Basically, the structure of all types of blood vessels consists of three layers. The outmost part is called the adventitia with connective tissue as its major component. The Tunica media is a layer of smooth muscles that can constrict or dilate according to the physiological requirements (69). The innermost part is referred to as endothelium and plays an essential role in the pathogenesis of cardiovascular diseases.

1.4.2 The Endothelium

The endothelium makes up the innermost surface of the vessels, consists of only one layer of simple squamous epithelium and forms the interface between intra and extravascular space, thus between tissue and blood (68). Furthermore, it represents a selectively permeable membrane and helps to regulate the blood flow by producing endogenous agents like prostacyclin, nitric oxide (NO), and endothelium-derived hyperpolarizing factor that can dilate vessels. Moreover, the monolayers´ influence on inflammatory processes should not be disregarded. In a healthy organism, contact between leucocytes in the blood and the endothelium does not automatically lead to an inflammation, but with an activation of the endothelium by bacterial endotoxins and pro inflammatory cytokines, this is more likely to happen as leukocytes bind to activated endothelial cells. Atherosclerosis is a condition, where this endothelial dysfunction fuels the docking of immune cells and their accumulation. The endothelium also has an impact on the hemostasis and thrombosis with heparan sulfate glcosaminoglycans on its surface having antithrombotic function and inhibiting smooth-muscle proliferation. Of all the layers of blood vessels, the endothelium

with its barrier function has to most important influence on the development of vascular diseases like atherosclerosis, hypertension (HT) and renal disorders (70).

1.4.3 Endothelial dysfunction and Atherosclerosis

Atherosclerosis is defined as a disease of chronic condition, characterized by chronic inflammation, endothelial dysfunction, and lipid aggregation on the inner surface of vessels (71). The most important and influenceable risk factors for atherosclerosis are hypertension, diabetes, hypercholesterolemia, and smoking. All these risk factors are associated with endothelial dysfunction (72). To current knowledge endothelial dysfunction is recorded as an early precursor of the development of atherosclerotic plaques (73). As a first step, endothelial activation results either from a physiological reaction to a disturbance in the immune response, or is triggered by the risk factors outlined above (74). Thereafter, the health enhancing endothelial functions change in character to pro-inflammatory and pro-thrombotic nature, including a higher expression of leukocyte adhesion receptors and cytokines and accompanied by reduced NO release (72). Although the pathogenesis of atherosclerosis is not completely clarified, the response-to-injury hypothesis is the fundamental principle today. This theory builds upon the assumption that endothelial damage triggers a cascade of events that finally lead to plaque formation and narrowing of the blood vessels lumen. Branching points in the circulatory system are under high mechanical stresses, and can cause injury of the endothelium, particularly in combination with high blood pressure. Lipid integration into the endothelial wall and adhesion of monocytes and thrombocytes follow, whereby monocytes develop to macrophages that release reactive oxygen radicals, damaging the cell and rendering NO ineffective. This dysfunction of the endothelium can furthermore cause an additional spasm of the smooth muscles of the vessel. This leads to an alteration of the low-density lipoprotein (LDL) molecules by the modified oxygen radicals. Macrophages phagocyte these LDL molecules and convert them to foam cells. Proliferation of smooth muscles and their recruitment to also foam cells fuels this process of plaque formation even more. If this cascade of reactions continues over months and years, a progressive stenosis of arteries with myocardial infarction, stroke and peripheral arterial disease develops (75)(76)(77).

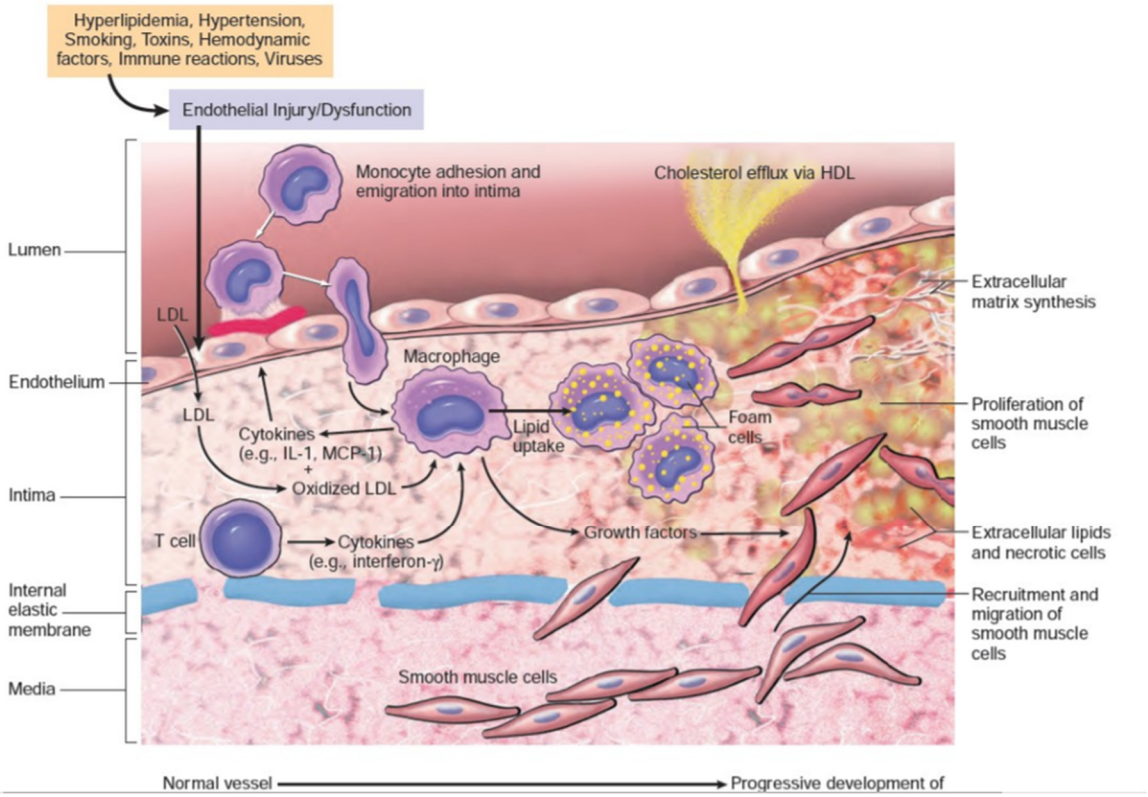


Figure 14: Pathogenesis of atherosclerosis
(reproduced from: http://www.slideshare.net/drmustansar/atherosclerosis-40196416, Access 5.8.2016)

Figure 14 aims to show the steps that are required in atherosclerosis formation, as they were illustrated above.

2 Aims and Objectives

HIV and tuberculosis are both among the top causes of death worldwide. These two infectious diseases are individually responsible for suffering and death especially in developing regions, whereas incidence is also increasing in the high-income countries. As a combination HIV and TB impose an immense burden on our global health (58). Additionally, clinical observations of the last years revealed, that infectious diseases correlate with the occurrence of atherosclerosis and cardiovascular complications, reporting the highest mortality rates worldwide (78). Indeed, both pathogens (*m. tuberculosis* and HIV) are associated with an increased cardiovascular risk (79)(80).

The aim of this diploma thesis is to examine, how co-existence of HIV and TB modulates the cardiovascular risk. It is hypothesized that the co-infection aggravates cardiovascular diseases, as both HIV and TB infection have a negative impact on the cardiovascular health with increased atherosclerosis risk (79)(80).

Moreover, it will be investigated, how the treatment of tuberculosis and HIV alters the risk and development of cardiovascular diseases. It is postulated that concurrent treatment of the co-infection decreases the cardiovascular risk, since a prudential selection of antiretroviral drugs can minimize the cardiovascular risk (81) and untreated TB infection has severe consequences such as unstable angina and acute myocardial infarction (AMI)(82).

To verify these hypotheses, literature was reviewed from secondary literature and recently published primary literature from medical databases.

This review of the literature is important as co-infection of HIV and tuberculosis, mainly a challenge of the low-income countries and developing regions on our earth, is now widely associated with cardiovascular diseases. While cardiovascular diseases were mostly a phenomenon of industrialized populations, epidemiological changes are taking place in this context, and are now affecting developing countries as well (74). This literature review is also important in terms of improved therapy and better access to treatment options in low-income regions.

This work is inspired from my personal experiences in infectious diseases, particularly HIV and tuberculosis, which I obtained during my internship in a regional hospital in Accra/Ghana in August 2014. With this diploma thesis, I want to impart clinicians a deeper knowledge about the connection between infectious diseases and non-communicable diseases, the health challenges of our times per se.

3 Methodology

This diploma thesis is a review of current literature on the subject of "Effects of Tuberculosis and HIV Co-morbidities on the Cardiovascular System and Cardiovascular Disease".
A systematic literature research was done to collect all the information that is presented in this thesis.
The introduction contains basic information about HIV/AIDS, tuberculosis, co-infection of HIV and TB, as well as the cardiovascular system to give the reader an overview of these three major pillars.
For the creation of the introduction, both primary and secondary literature was made use of. Current publications as well as textbooks were systematically searched to gather the essential information. Recently updated data and guidelines from the World Health Organisation and the Centers for Disease Control and Prevention were added to this thesis. Access to textbooks was provided by the libraries of the Medical University of Graz and the Karl-Franzens-Universität Graz, whereas two of them were downloaded as e-books from the internet.

The methodical research of current literature was started in July 2016 and finished in May 2017, mainly resting upon the medical database PubMed. However, the platforms Google Scholar and UpToDate were additionally reviewed.
Access to some of the articles from the New England Journal of Medicine was provided by the Centre for infectious diseases in the Zentralkrankenhaus Bozen, whith the help of a fellow student. Afterwards the NEJM database was searched thoroughly whereby some articles contributed to this thesis.

First searches included the combination of the phrases "HIV/AIDS" and

- -history
- -epidemiology
- -replication cycle
- -diagnosis
- -transmission
- -clinical stages
- -treatment.

This was repeated with the term “Tuberculosis” in combination with the phrases listed above and additionally:

- *m. tuberculosis*
- pulmonary tuberculosis
- extrapulmonary tuberculosis
- miliary tuberculosis
- co-infection HIV

In a third approach, the phrase “cardiovascular” was combined with the terms

- tuberculosis
- HIV/AIDS
- Infection.

Boolean operators like “AND”, “OR” and “NOT” were used in this research process.

To confine the results to my search keywords and relevant aspects of my thesis, the article chosen were selected based on the following criteria:

- Full text articles, where possible
- 10 years, concerning the publication dates, except for few publications with information about historical details.
- Only articles that were written in English.

Particular attention was paid to how often the articles were cited by other authors, as it reflected the importance of the publication. Moreover, references of all reviewed papers were searched systematically in order to find more relevant literature and to ensure that any literature that was missed during my literature search was covered.
In the end 207 references were added into Mendeley, my citation manager. Finally, I identified 41 publications as relevant for my discussion. Additionally, I considered articles about the EndoAfrica study that my supervisor provided me.

4 Review of current literature

Based on my extensive literature research and gathered information I divided my thesis into four sections.

In **section 1** is discussed **how infectious diseases influence cardiovascular disease evolvement in general**. Current seroepidemiological and experimental studies suggest that bacterial and viral pathogens contribute to atherosclerosis formation. Infectious agents were identified in atherosclerotic plaques and increased cytokines. Furthermore, acute phase proteins during infection were shown to hasten atherosclerotic progression.

Section 2 deals with the **connection between HIV and cardiovascular diseases**. Immune dysfunction and chronic inflammation, changes in blood lipid composition and pro-coagulant activity in HIV infection trigger pro-atherogenic mechanisms and accelerate cardiovascular diseases.

Section 3 examines how **tuberculosis modifies the cardiovascular risk**. Studies outline that TB causes immune activation, pro-inflammatory cytokine release and autoimmune mechanisms that can lead to cardiovascular dysfunction.

In **section 4** the **causal relationship of HIV, TB and CVD** is outlined. It is found that currently there is a lack of both epidemiological and pathophysiological information making further investigation necessary.

There is a chance of overlapping regarding the theories and results of the articles and their classification in the following chapters.

4.1 Section 1 - The role of infection in atherosclerosis

To obtain information on the role of infection in atherosclerosis progression five articles were selected. They include clinical trials and reviews (see Table 3).

Table 3: Articles dealing with Infection and CVD relation

Author	Year	Major finding	Article type
Ramirez	1996	*C. pneumoniae* was isolated from atherosclerotic plaques of patients with CAD	Clinical trial
Campbell and Rosenfeld	2015	Infection is interrelated with plaque progression	Review article

Elkind	2010	Aggregate burden of infections may lead to atherosclerosis	Review article
Epstein et al.	2009	Pathogen burden, immune and autoimmune responses may lead to atherosclerosis	Review article
Rosenfeld and Campbell	2011	C. pneumoniae and some periodontal pathogens may contribute to atherosclerosis and evidence for other pathogens is weaker	Review article

The following section discusses the mentioned articles in detail. As outlined in the introduction, traditional risk factors for the development of atherosclerosis are widely believed to be hypertension, hyperglycaemia, smoking, hypercholesterolemia, and genetic disposition. However, epidemiological evidence suggests that infections can equally contribute to endothelial inflammation and the emergence of atherosclerotic plaques. As reported by **Campbell and Rosenfeld (2015)**, there could be a direct and an indirect pathway by which infectious agents induce atherosclerotic lesions. Firstly, infectious agents were detected in plaques on the inner surface of the endothelium, which strongly emphasises the role of a direct pathway. Secondly, high antibody release against several pathogens was recorded to be correlated with cardiovascular dysfunction. That in turn suggests an indirect mechanism (71).

4.1.1 Direct mechanisms

The question arises, which mechanisms lead to an increased cardiovascular risk through direct infection. The association of Chlamydophila pneumoniae (*C. pneumoniae)* and atherosclerotic plaque occurrence has been investigated most intensively up to date. Supporting the idea of direct invasion of the pathogen into the cells, **Ramirez (1996)** extracted and reproduced *C. pneumoniae* from patients with coronary artery disease (83). Moreover, **Elkind (2010)** describes the detection of Cytomegalovirus, Epstein Barr Virus, HIV, Helicobacter pylori and several other infectious agents that could, however, not be cultured in atheromatous tissue (84). After attacking the endothelial cells directly, pathogens can induce either latent infection, low replication, or abortive infection as **Epstein et al. (2009)** claim. Additionally, CMV and *C. pneumoniae* can invade the cell barriers by infecting circulating monocytes. These immune cells can storage the pathogens and release them steadily into inflamed tissue, after invading the endothelial cells and developing to macrophages. When immune cells discharge contained viral products, they

fuel inflammation processes within the vessel wall (85). It is supposed, that *C. pneumoniae* is stored in a similar manner in endothelial cells as CMV is inside alveolar macrophages of the lungs and expressed at times. After pathogens release into the endothelium, smooth muscle cell proliferation, elevated cytokine and chemokine release and increased cellular adhesion molecules evolve. Additionally, CMV and HSV invasion can change properties of the endothelium towards procoagulant activity. This can lead to an elevated tissue factor production and increased thrombin output. In sum, the mentioned mechanisms cause atherosclerotic plaque progression and increased cardiovascular events. Some infections, however, are of persistent manner, with continuous expression of viral gene products without viral replication and host cell apoptosis. Nevertheless, these so called "abortive" infections still represent a pro-atherosclerotic condition (85).

4.1.2 Indirect mechanisms

Campbell and Rosenfeld (2015) describe studies, which support the role of indirect mechanisms via systemic effects that could contribute to atherosclerosis development during infection, especially based on the evidence from *C. Pneumoniae*. Hyperlipidaemic animal models show elevation of cytokines and acute phase proteins in the plasma of *C. pneumoniae* infected mice, leading to progression of atherosclerosis (71). These released factors reach the systemic circulation and nurture the already ongoing chronic inflammation inside the atheromatous plaque (86). Concerning CMV infection, experimental studies show that even latent infection without active viral replication can lead to increased T- cells and plaque formation due to systemic cytokine response. Additionally, once CMV infected, T Lymphocytes discharge interleukin 6 (IL-6) when they get in contact with the pathogen again. Acquiring further mediators and recruiting more monocytes and T- cells into the vessels fuels local inflammation and progression of atherosclerotic lesions. **Epstein et al. (2009)** maintain that constant circulation of cytokines like TNF, Interleukins and Interferons itself is a risk for atherosclerosis, regardless of the active presence in the endothelium (85).
According to **Campbell and Rosenfeld (2015)** studies in other animal models showed a significant decrease of atheroprotective high-density lipoprotein (HDL) in hyperlipidaemic mice models. Plaque destabilization markers occurred much more often in experimental mice as well (71). Further indirect effects of pathogens on atherosclerosis progression are provided by molecular mimicry mechanisms between host and pathogen antigens (86).

This autoimmune phenomenon, especially regarding to tuberculosis infection will be explained in chapter 4.3.1.

The additional role of Toll-like receptors (TLR) in the development of atherosclerotic plaques was outlined by **Epstein et al**. **(2009)** (85). TLR´s are part of the early non-specific immune response and detect foreign molecules on infectious agent surfaces. In a cascade of events, TLR´s acquire the nuclear factor -κB and induce mitogen-activated protein kinase reactions. Thus, TLR´s augment an inflammatory response when they get in contact with the infectious pathogen to prevent the progression of infection. Due to the fact that TLR´s are expressed on atherosclerotic plaques, the pathogen induced immune activation can cause an exacerbation of atherosclerosis. Animal models indicate, that a decrease of TLR´s seems to be advantageous concerning atherosclerosis prevention. At the same time, elevated TLR´s in infected mice create a continuous proinflammatory condition, provoking plaque generation (85).

Latest observations by **Rosenfeld and Campbell (2011)** refer to the "pathogen burden" as a composition of several pathogens rather than just one infectious agent causing atherosclerosis. Evidence based studies support the idea of an "infectious burden". Three fourths of individuals, suffering from coronary artery disease with exposure to none less than three microorganisms showed an elevated CAD risk. The higher the number of pathogen exposure, the higher was the CAD prevalence (86).

Figure 15 summarizes the outlined correlation between infectious pathogens and atherosclerosis development. Both the induction of inflammation and autoimmune mechanisms result in an increased cardiovascular risk.

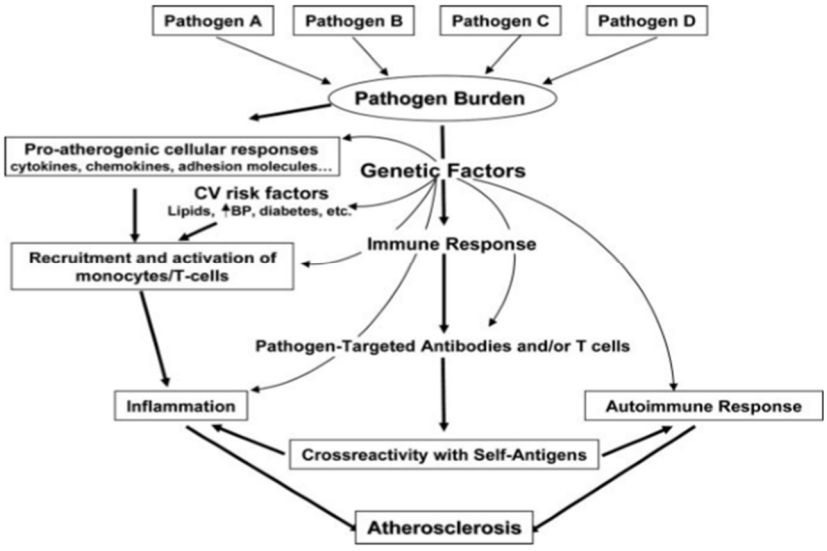


Figure 15: How infection leads to atherosclerosis
(reproduced from: http://circ.ahajournals.org/content/119/24/3133, Access 19.3.2017)

However, despite all the above-discussed evidence related to infection and atherosclerosis, **Epstein et al (2009)** observed that **infections do not trigger atherosclerosis** and cardiovascular dysfunction. This was examined in patients suffering from coronary artery disease and who were receiving treatment with macrolide antibiotics. These macrolides were specifically effective against *C. pneumoniae*, as this pathogen was assumed to have the biggest impact on atherogenesis. It was expected that antibiotic treatment could prevent myocardial infarction or death in the cohort. The conducted studies showed no treatment effect regarding the cardiovascular event rate and all-cause mortality. Lastly, a contradictory increase of mortality in the treated group was recorded. Even though these trials have limitations as well, they should invite us to critically review the association between pathogens and atherosclerosis (85).

4.2 Section 2 - HIV and the cardiovascular system

Twelve publications (see Table 4) were considered as relevant in this context.

Table 4: Articles covering CVD and HIV connection

Author	Year	Major finding	Article Type
Barbaro and Barbarini	2011	HAART may cause lipodystrophy syndrome and induce atherosclerosis acceleration	Review article
Thienemann et al.	2013	HIV is associated with cardiac diseases and HIV induced immune activation triggers CVD	Review article
d´Ettorre et al.	2016	Immune activation in HIV causes accelerated atherosclerosis	Review article
Baker and Lundgren	2011	Untreated HIV infection enhances pro-atherogenic mechanisms via immune activation, inflammation, coagulation, and lipoprotein changes; ART decreases and increases CVD risk	Review article
Kuller et al.	2008	HIV induced inflammation and coagulation and ART interruption increases risk of death by elevated IL-6 and D-Dimer	Clinical trial

Grinspoon and Carr	2005	Metabolic and body-fat abnormalities occur often in PLHIV under ART; evidence suggests a resulting increased risk for cardiovascular diseases	Review article
Sliwa et al.	2012	De novo HIV cases are strongly related to cardiomyopathy and pericardial diseases	Clinical trial
Barbaro	2002	Cardiac and pulmonary complications are sequelae of HIV; they may result after long lasting immunosuppression and interaction of mediators within opportunistic infections, autoimmune responses, ART-related cardiotoxicity	Review article
Ntsekhe and Hakim	2005	Pericardial disease might be the first manifestation of HIV Infection	Review article
Barbaro and Silva	2009	HAART reduces opportunistic infections and myocarditis, leading to decreased cardiomyopathy prevalence. HAART is associated with metabolic syndrome, leading to cardiovascular events in PLHIV	Review article
Chastain et al.	2016	Cardiovascular dysfunction due to HIV infection is particularly seen in significant immunodeficiency with opportunistic infections and malignancies	Review article
Barbaro	2010	Significant reduction of HIV related cardiomyopathy since HAART in developed countries; HAART related lipodystrophy and cardiovascular risk is increasing.	Review article

According to **d´Ettorre et al**. clinical observations suggested that particularly HIV is associated with a significantly high risk for developing cardiovascular diseases. There are several factors within HIV infection that when combined together increase the risk of cardiovascular complications (87).

4.2.1 Pathophysiologic aspects

Baker and Lundgren (2011) suggest that immune activation and inflammation, alterations in lipids, pro-coagulant activity and vascular injury form a complex with negative influence on the cardiovascular health (88).

HIV is a state of immune dysfunction and inflammation with severe consequences for the individual. Drivers of the chronic inflammation in HIV infected persons are excessive

replication of HIV in host cells and dysregulation of leucocytes. Activation of lymphatic cells and inflammation are characterized by high C-reactive protein (CRP) and cytokines like IL-6, produced from monocytes and lymphocytes (88). These biomarkers, which are elevated in untreated HIV within a chronic immune activation condition, correlate with high CVD risk, as **Kuller et al. (2008)** claim (89). Even in effective treatment with HAART, a much higher risk remains for developing CVD in HIV positive patients than in the healthy population (88).

Moreover, **Baker and Lundgren (2011)** point out that HIV changes the composition of the blood lipids and lipoproteins. Human immunodeficiency virus infection with persistent high levels of cytokines that inhibit the efficacy of the enzyme lipase lead to a decline in triglyceride elimination. Furthermore, a higher low-density lipoprotein cholesterol (LDL-C) and a decline in high density lipoprotein cholesterol (HDL), which is a predictor of cardiovascular event risk, is commonly detected in HIV positive individuals. The loss of atheroprotective functions of HDL like reverse cholesterol transport, antioxidant and anti-thrombotic functions results in a higher exposure of the vessels to harming agents (88).

Barbaro and Barbarini (2011) postulate that HIV infected individuals who are treated with antiretroviral drugs may develop HIV-associated lipodystrophy (80). **Grinspoon and Carr (2005)** characterize this condition by lipoatrophy of the face, limbs and buttocks and accumulation of fat in the dorso-cervical and abdominal region, as well as in the liver and the muscles. The duration of the treatment and the combination of antiretroviral drugs, particularly nucleoside analogues and protease inhibitors has a strong impact on the emergence of lipodystrophy, whereby only few cases of HIV-associated lipodystrophy were reported in untreated patients. Among individuals with fat distribution, higher triglycerides and lower HDL were reported (90). These metabolic alterations and dyslipidaemia result in an increased risk for cardiovascular events (80).

Thienemann et al. (2013) identified Diabetes mellitus as another metabolic disease that is associated with high cardiovascular risk. Increased insulin resistance was observed in HIV positive patients without treatment, however, studies that prove a cause and effect relationship between HIV and diabetes mellitus are still lacking and need to be established. Indeed, antiretroviral drugs, particularly protease inhibitors induce insulin resistance and the risk for diabetes mellitus development and consequently for cardiovascular events (81).

Moreover, **Kuller et al. (2008)** and **Baker and Lundgren (2011)** postulate that HIV infection is a state of high coagulability. D-dimer, fibrinogen and von Willebrand factor are all markers of coagulation and associated with a high CVD risk and mortality both in

PLHIV and in the general population (89). Nevertheless, studies recently detected an elevated D-dimer in PLHIV. Pro-coagulant activity is provided by HIV replication and immune activation, but detailed information in this context is still missing (88).
As already mentioned HIV replication is followed by a high production of pro-inflammatory cytokines and an activation of the endothelium. This activation leads to an impaired function of the endothelium. This connection was particularly detected in untreated HIV infection, where HIV RNA levels were high.
HAART was reported to improve vascular function in several studies, however, an intake of antiretroviral medication, particularly protease inhibitors for more than five years, was also associated with a reduced brachial flow-mediated dilation, which is used to evaluate endothelial function. There are several other studies that highlight the ambivalence of HAART use. Consequently, atherosclerosis puts a burden on HIV patients in both groups treated and untreated individuals (88). The following figure illustrates the hallmarks in the development of CVD in PLHIV that were outlined above.

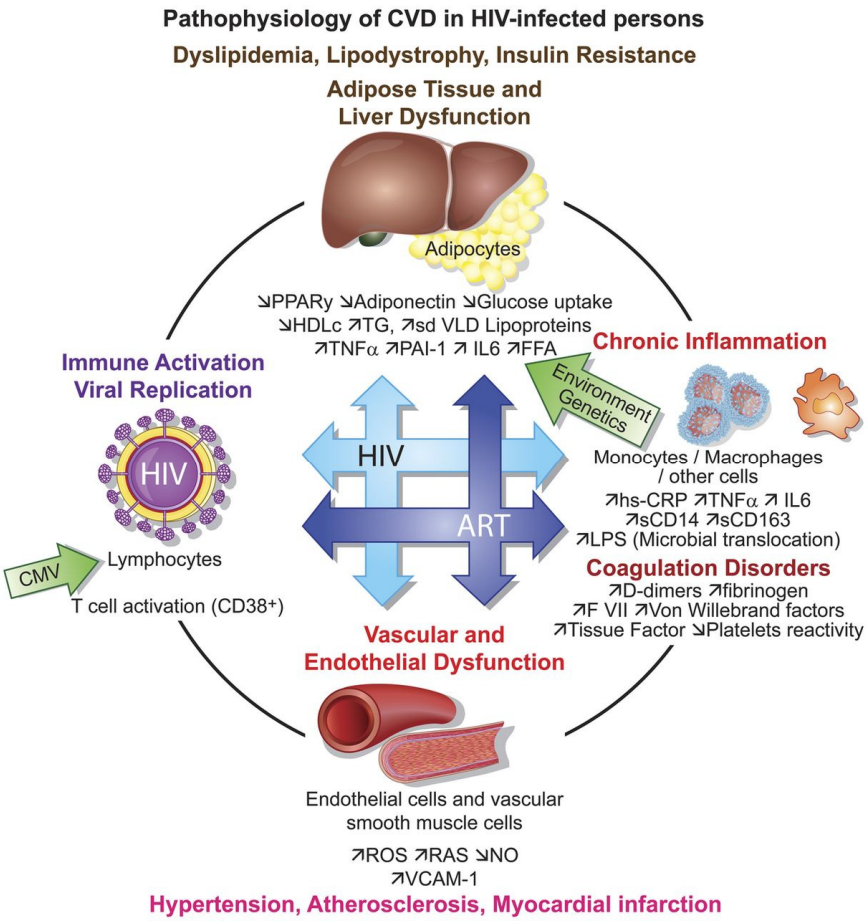


Figure 16: Pathophysiology of CVD in PLHIV
(reproduced from: http://eurheartj.oxfordjournals.org/content/early/2014/01/08/eurheartj.eht528, Access 7.8.2016)

4.2.2 Clinical features

Sliwa et al. (2012) investigated that the most widespread diagnosis in HIV infected individuals is HIV related dilated cardiomyopathy. It is, however, a manifestation, that rather occurs in late stages of disease and should, considering its severity be followed by HAART initiation (91). Dilated Cardiomyopathy mostly arises from a recent myocarditis either through direct infection with HIV or through infections with other opportunistic pathogens as **Barbaro and Barbarini (2011)** summarize (80). As already discussed in the chapters before, HIV infects CD4 positive cells and reproduces itself in them. Myocytes, however, are CD4 receptor negative but still belong to HIV´s target cells and get infected. The resulting cardiac myocyte dysfunction is caused by a nonspecific activation of TNF alpha, IL-1, IL-6, IL-19 through viral infection. TNF alpha decreases myocardial contractility and increases the release of nitric oxide, which in turn has a negative inotropic effect, particularly together with altered calcium levels in the myocytes through TNF alpha. Additionally, dilated cardiomyopathy can emerge out of an opportunistic infection with e.g. cytomegalovirus (CMV) as it often occurs within the context of HIV and immunosuppression. Either way certain reservoir cells keep HIV on their surfaces for a long time and constantly release cytokines that cause further tissue damage in myocardial cells (92).

Ntsekhe and Hakim (2005) mention that pericardial effusion is also commonly seen during HIV infection. Whereas the reason for this sequela in developed countries has not yet been clarified, individuals in developing countries acquire pericardial diseases in 86% through treatable microorganisms like *m*. *tuberculosis.* Other possible reasons for pericardial involvement include pyogenic infections, lymphoma and Kaposi sarcoma (93). **Barbaro and Silva (2009)** suggest that unexplained pericardial effusion and tamponade should make clinicians suspicious of a possible HIV Infection or at least be remembered in a list of differential diagnosis(94).

According to **Chastain et al. (2016)** coronary artery disease is likely to develop in PLHIV, however, there is yet no detailed clarification about this interaction. As discussed before lipid disorders and endothelial dysfunction contribute to the emergence of atherosclerosis in HIV positive individuals. In several studies, low CD4 counts were reported to be associated with coronary artery plaques and stiffness of the carotid artery, whereas high levels of CRP, IL-6 and D-dimer facilitated plaque rupture (95). Moreover HIV genome

sequences were found in coronary arteries of PLHIV, who died of acute myocardial infarction and coronary arteritis, as suggested by **Ntsekhe and Hakim (2005)** (93). HAART, particularly intake of PIs, is a strong risk factor for myocardial infarction. Clinicians however, should carefully consider the advantages and disadvantages of antiretroviral medication.

Additionally, HIV has a high impact on arterial hypertension. According to **Barbaro (2010)** several hypotheses, renal dysfunction as well as endothelial injury are the two major causes in the pathogenesis of HIV-related hypertension. In this context kidney function can either be influenced by HIV directly or by HAART, associated with PI caused lipid disorders (96). Figure 16 displays the various clinical patterns in cardiovascular diseases that may occur during HIV infection.

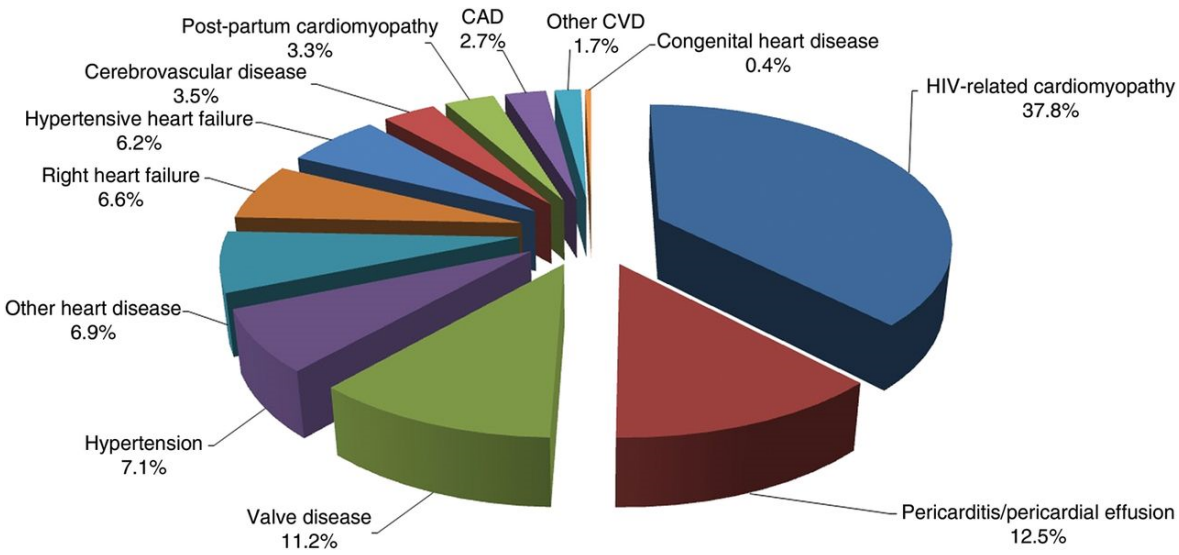


Figure 17: Primary cardiovascular diagnosis in HIV positive patients
(reproduced from: http://eurheartj.oxfordjournals.org/content/34/46/3538, Access 17.8.2016)

4.3 Section 3 - Tuberculosis and the cardiovascular system

17 publications were regarded as essential concerning this section (see Table 5).

Table 5: Articles discussing CVD and TB correlation

Author	Year	Major finding	Article Type
Rajendran et al.	2013	Endothelium is involved in coagulation disorders in infectious diseases	Review article

Huaman et al.	2015	Persistent immune activation in TB and molecular mimicry mechanisms with mycobacterial HSP 65 may cause CVD	Review article
Chung et al.	2014	TB patients have a higher risk of acute coronary syndrome than healthy individuals	Cohort study
Epstein et al.	2009	Atherogenic effects in infection are pathogen burden, inflammation, immune and autoimmune responses	Review article
Muhlestein and Anderson	2003	Direct vessel wall colonization through pathogen, vessel damage by initiating immunologic responses ma cause atherosclerosis	Review article
Liu et al.	2012	TB myocarditis can cause sudden cardiac death	Review article
Rodriguez et al.	2012	*M. tuberculosis* may the cause of coronary arteritis leading to sudden cardiac death	Case report
Rota	2005	Mycobacterial HSP 65 was found in atherosclerotic plaques; Mycobacterial phosphatidylinositol has procoagulant effects	Review article
Babu Chodisetti et al.	2012	Cross-reactive T cell epitopes between M. tuberculosis and humans may cause molecular mimicry/autoimmune response in TB infection	Bioinformatical research article
Kager et al.	2015	Primary and recurrent TB create a procoagulant state; decreased anticoagulant mechanisms within TB infection	Prospective study
Von Hinsbergh	2012	Immunologically acquired deficiencies and overreactions to pathogens alter endothelial functions	Review article
Jeon et al.	2010	TB prevalence in individuals with Diabetes mellitus is high	Review article
Ogbera et al.	2014	Patients with TB may have an increased risk of developing Diabetes mellitus	Observational study
Ogbera et al.	2015	Diabetes mellitus prevalence in TB infected individuals is three times higher than in the general population	Observational study

Li et al.	2012	DM prevalence is high in patients suffering from TB in China; infection can induce hyperglycaemia and falsely implicate DM	Prospective Observational study
Zhu et al.	2004	Antibodies against mycobacterial HSP65 correlate with coronary calcification; autoimmunity may induce atherosclerosis	Clinical trial
Huaman et al.	2017	TB is linked with an increased AMI risk	Retrospective cohort study

The pathogens of HIV and *m. tuberculosis* both enter the host cells and establish a chronic infection in the individual that can persist for years, before it gets activated. This latent and persistent inflammation can result in the development of atherosclerosis and subsequently cardiovascular diseases, as pointed out in the chapters before.

4.3.1 Pathophysiologic aspects

The correlation between TB and CVD was first described in case studies. Epidemiologic data outline, that any individual who suffered or recovered from TB still has a higher risk for cardiovascular events than a person, who has never been in contact with *m. tuberculosis*. Currently, **Huaman et al. (2015)** speculate that there are two main theories that link CVD and TB. Atherosclerosis formation through mycobacterial infections can either occur through direct invasion of the endothelium or through an autoimmune mediated process (79). A variety of relevant mechanisms in the development of CVD through TB is illustrated in the Table below, whereas several pathophysiologic aspects are highlighted in this chapter. **Liu et al. (2012)** mention that direct effects of *m. tuberculosis* to the myocardium in tuberculous myocarditis can lead to congestive heart failure and particularly ventricular tachyarrhythmia, possibly resulting in sudden cardiac death, as clinical case studies detected (97). Moreover, tuberculous arteritis caused by the mycobacteria may lead to myocardial infarction, as **Rodriguez et al. (2012)** explored (98). However, further discussion on direct effects of *m. tuberculosis* are out of scope of this DA.

Table 6: Mechanisms of CVD in TB
(reproduced fom: http://tdtmvjournal.biomedcentral.com/articles/10.1186/s40794-015-0014-5, Access 9.8.2016)

• Direct effect on the myocardium (tuberculous myocarditis)
• Direct effect on coronary arteries (tuberculous arteritis)
• Increased expression of pro-inflammatory citokines (i.e., IL-1, IL-2, IL-6, IFN-γ, TNF-α)
• Monocyte/macrophage immune activation
• $CD4^+$ TH1 and TH17 cell immune activation
• Auto-immunity mediated by antibodies against mycobacterial HSP65

To verify the claim that tuberculosis results in cardiovascular diseases via inflammatory pathways, it is important to demonstrate that mycobacterial infections initiate similar immunological host reactions as seen in the formation of atherosclerosis. According to **Rajendran et al. (2013)** increased immune activation and cytokine production are well established pillars in atherosclerosis development (also see chapter 1.4) (72). CD4+ cells, a subtype of TH1 cells that dock to adhesion molecules on the endothelium, are the main actors in this process. As they release interferons, tumour necrosis factor and interleukins, they accelerate plaque growth on the endothelial surface, cite **Huaman and collegues (2015).** In the process of atherosclerotic plaque formation, persistent inflammation can boost any step in the cascade of atherosclerosis development. Equally, *m. tuberculosis* infection initiates the host's adaptive immune response with the help of CD4+ cells. They trigger the cytokine, tumour necrosis factor and interferon cascade, in a similar manner to that in the development of cardiovascular diseases. T cells in tuberculosis patients, particularly those of the TH1 type release IL-17, IL-22 and interferons when they get in contact with antigens of *m. tuberculosis*. With TH1 initiated inflammation, the considerations between TB and atherosclerosis, more precisely CVD come full circle (79).

Rota (2005) and his work put particular emphasis on the correlation of heat shock proteins (HSP) and atherosclerotic plaque formation through autoimmune mechanisms. HSPs are in fact largely intracellular stored molecules that are presented on cell surfaces of the endothelium, macrophages and smooth muscle cells when inflammation and infection take place (99). With the sudden appearance of these antigens on extracellular environment, they are graded as foreign by the immune system which in turn leads to an autoimmune response. This phenomenon can be explained by means of the molecular mimicry mechanism, cite **Epstein et al. (2009)** (85). **Babu Chodisetti et al. (2012)** summarize that

as host proteins and pathogens having similar epitopes, the immune surveillance system cannot distinguish between self and non-self and triggers autoimmunity by the activation of autoreactive T-cells and B-cells. In fact, there is a significant number of potential cross-reactions between human proteins and *m. tuberculosis* epitopes, whereby HSP was identified to play an important role (100). HSP65 in mycobacteria has similar patterns with human HSP60. This provokes cross-reaction between these molecules, leading to autoimmune response through molecular mimicry. **Huaman et al. (2015)** claim that the resulting inflammation is responsible for the occurrence of atherosclerosis and plaque development (79). Figure 17 points out, how autoimmune antibodies against pathogen proteins attack endothelial cells with homologue surface components.

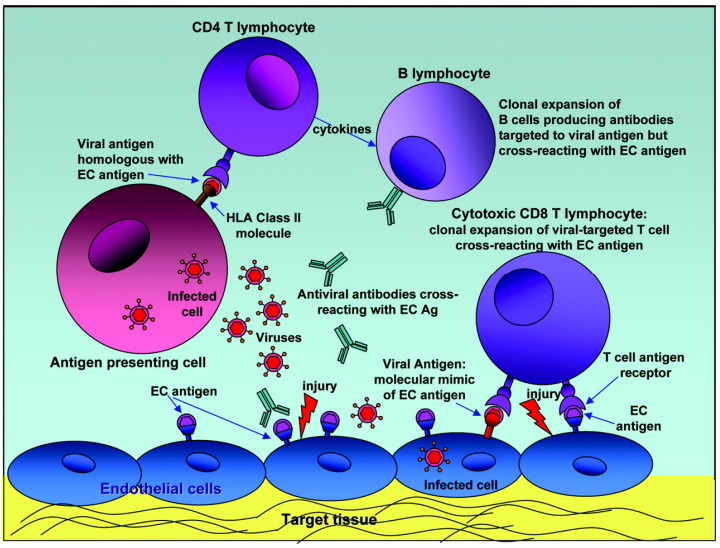


Figure 18: Molecular mimicry:an autoimmune mechanism contributing to atherogenesis
(reproduced from: http://circ.ahajournals.org/content/119/24/3133, Access 14.3.2017)

Apart from other components, the mycobacterial wall consists of several phospholipids. In prior studies **Rota (2005)** found out that some of these molecules, particularly phosphatidylinositol, play an essential role in prothrombin complex and thrombin induction. Moreover, surveys prove that cytomegalovirus and other herpesviruses provoke activation of coagulation due to phospholipids on their cell surface. For *m.tuberculosis* inhabiting phosphatidylinositol in its cell wall, it was claimed that procoagulant activity and therefore atherosclerotic progression can be induced during TB infection (99). Recently conducted research draws attention to procoagulant activity in tuberculosis cases, apart from the phospholipid hypothesis.

In an observational prospective study in Bangladesh, **Kager et al. (2015)** analysed blood and bronchoalveolar lavage fluid (BALF) from patients with primary and recurrent TB.

Increased thrombin-antithrombin complexes were recorded in the plasma of primary TB patients, creating a high coagulation status. Additionally, D-dimer values were considerably increased in individuals with recurrent tuberculosis infection. Likewise, plasma fibrinogen levels were recognized to be strongly elevated in the primary and recurrent TB cohort. In contrast, a down-regulation of anticoagulant mechanisms was recorded. In addition to the induction of coagulation, an enhanced systemic activation of the vascular endothelium by *m. tuberculosis* is presumed (101). One of the major functions of endothelial cells is to ensure blood flow and inhibit thrombus formation on the inner surface of vessels that can establish atherosclerotic plaques (102).

According to **van Hinsbergh´s (2012)** assumption, ADAMTS13 plays a central role in preserving endothelial integrity. ADAMTS13, a disintegrin and metalloproteinase is an enzyme that is secreted into the blood and able to denature von Willebrand factor, a multimer that can bind platelets in the endothelium. Therefore, it prevents clot formation and obstruction of the vessels. Dysfunctional ADAMTS13 leads to high von Willebrand factor levels. A reduction of ADAMTS13 efficacy has been recognized under few inflammatory circumstances, such as in tuberculosis. This enables to determine that m. tuberculosis infection results in activated endothelium (101)(102).

4.3.2 Clinical features

Diabetes mellitus is a high influence risk factor in the development of atherosclerosis and thus cardiovascular diseases. In this context, it is important to outline the link between metabolic disturbances and *m. tuberculosis* infection, which was illuminated by intense research. Patients with DM have a higher risk of developing active TB and the outcome in disease progression is significantly worse, as reported by **Jeon et al. (2010)** (103). Similar observations were made in studies from Lagos, Nigeria by **Ogbera et al. (2014 and 2015).** They observed that diabetes mellitus occurs much more often in *m. tuberculosis* infected individuals than in the healthy population. In Nigeria, the prevalence of diabetes mellitus is 12% among the group of TB infected patients, which is roughly three times higher than throughout Nigeria (105)(104). China, the country with the second highest reported tuberculosis case numbers, must also address the sharp increase in diabetes mellitus prevalence. However, **Li et al. (2012)** suggest that DM may not be associated with TB infection. Infection-related hyperglycaemia is a commonly observed phenomenon. Hence, an active tuberculosis infection can provoke hyperglycaemic circumstances. To underline

this fact, Li refers to four studies that recorded decreasing blood sugar levels during continuous tuberculosis treatment. Thus, false positive DM diagnoses may occur which is why screening for DM should not be done too early before therapy (106).

In addition to the interrelationship between DM and TB, further research has tried to study the relationship of tuberculosis with acute coronary syndrome (ACS). A nationwide population-based cohort study conducted by **Chung et al. (2014)** in Taiwan, which collected data between the years 1997 until 2010, proved that the probability of acquiring AC is 1.4 times higher in TB infected individuals than in the healthy population. Unstable angina and myocardial infarction with or without ST-elevation are significantly associated with *m. tuberculosis* infection (82). In fact, **Zhu et al. (2004)** reveal that antibodies of mycobacterial HSP 65 correlate with calcification of the coronary arteries and early atherosclerotic changes that subsequently induce coronary artery disease and CVD. This connection is significant, since traditional cardiovascular risk factors were already adjusted in this study (107). A recent study running between the years 2008 and 2010 in the U.S. verified data of 4052 patients, with half of them being part of the tuberculosis cohort and the other half being healthy. This research, carried out by **Huaman et al**. **(2015),** proved that AMI occurred much more frequently in the tuberculosis group with 3.3%, whereas the risk for myocardial infarction was 1.6 % in a non-tuberculosis cohort. Hence, this entails a twofold risk of acquiring AMI for patients suffering from TB in the U.S. population (108). Figure 18 summarizes the relationship of TB with AMI.

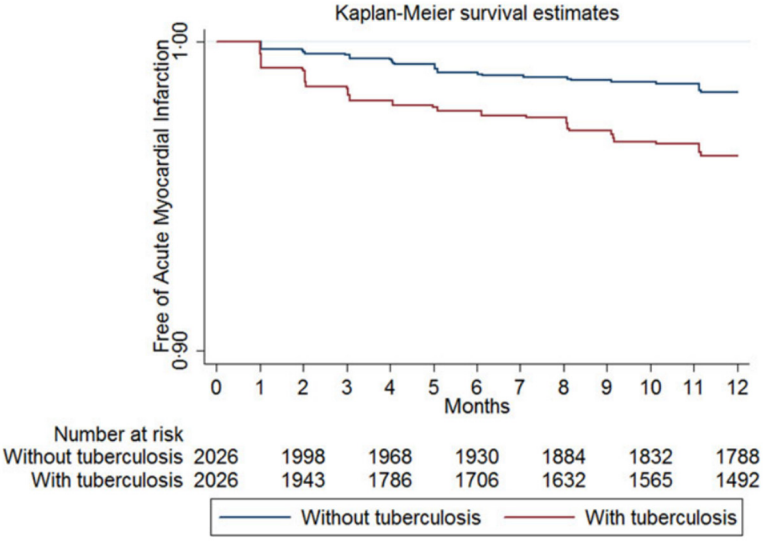


Figure 19: Acute myocardial infarction in the tuberculosis and non-tuberculosis cohort
(reproduced from: https://www.cambridge.org/core/services/aop-cambridge-core/content/view/611910AA988C2EAB8F162107112AB16E/S0950268817000279a.pdf/tuberculosis_and_risk_of_acute_myocardial_infarction_a_propensity_scorematched_analysis.pdf, Access 16.3.2017)

4.4 Section 4 - HIV and TB and the cardiovascular system

I identified four articles as being relevant for this section (see Table 7).

Table 7: Articles focussing on Co-infection and CVD risk

Author	Year	Major finding	Article Type
Oni et al.	2005	Dependency between infectious diseases and non-communicable diseases in peri-urban South Africa	Cross - sectional study
Young et al.	2009	Diabetes and cardiovascular risk correlate with TB and HIV infection	Review article
Angkurawaranon et al.	2016	Countries with high HIV prevalence may have a high burden of NCD as well	Review article
Strijdom et al.	2017	Is HIV infection and ART treatment in South Africa associated with endothelial dysfunction and cardiovascular risk?	Study protocol

This last and most challenging section discusses the **effects that the HIV and TB co-infection has on cardiovascular disease** development. Several studies have devoted themselves to the question how HIV and hepatitis C virus co-infection affects the cardiovascular system. Additionally, some studies analyse how a simultaneous CMV and HIV infection modifies the risk of acquiring non-communicable dysfunctions of the heart and the vessels. Unfortunately, not even a fraction of this relationship is known, especially with respect to HIV/TB co-infection and cardiovascular function. Only limited literature is currently available regarding this research question.

4.4.1 HIV and TB and traditional risk factors

From the foregoing sections it is evident that each HIV and TB are associated with cardiovascular diseases. To investigate the patterns of the coexistence between non-communicable diseases and communicable diseases more precisely, **Oni et al. (2005)** conducted a cross-sectional study in a primary health care clinic in South Africa covering data of 14364 individuals. Only patients with at least one chronic condition, either HIV, TB, hypertension, or diabetes mellitus were included. 22.6% of study participants suffered from multi-morbidity, mostly concerning coexistence of DM and hypertension and co-

infection of HIV and TB. However, an association between hypertension and HIV infection was recorded as well. In 19.7% of PLHIV who were receiving HAART, hypertension was identified. Without HAART, the hypertension rate was much less, suggesting a **possible negative influence of antiretroviral therapy** on the heart and the vessels. Additionally, 12.3% of PLHIV had comorbidity with DM. Moreover, 37% of TB patients were under hypertension treatment and 12% received antidiabetic therapy. However, only patients in whom any chronic disease was already diagnosed and who received treatment were included in this study. This in fact implicates a possible underestimation of the interdependency of TB, HIV and CVD (109).

Young et al. (2009) confirm the relation between TB and DM as well as they interrelate HIV and the metabolic syndrome in the setting of sub-Saharan Africa. Glucose intolerance, insulin resistance, a low HDL and elevated triglycerides as well as hypertension and a high body mass index contribute to the so-called "metabolic syndrome", which is one of the main drivers of cardiovascular diseases. It is claimed, that particularly HIV was associated with diabetes and cardiovascular disease emergence. However, antiretroviral treatment was also linked to the metabolic syndrome. For unknown reasons **NRTIs and PIs induce metabolic disturbances** including dyslipidaemia and insulin resistance (110). Figure 20 summarizes which overlaps and interactions between HIV, tuberculosis and cardiovascular risk factors are known so far.

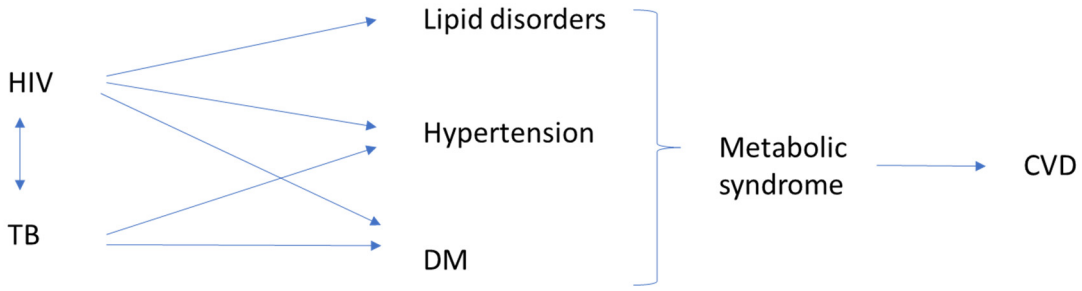


Figure 20: Co-infection and traditional risk factors

Angkurawaranon et al. (2016) examined the association between hypertension and HIV. In their publication, however, they stress the fact, that traditional risk factors of cardiovascular diseases are likely to explain the increased CVD risk in PLHIV. Due to treatment regimens for PLHIV life expectancy got higher and HIV turned more into a chronic state than a fatal disease. Thus, patients suffering from chronic infectious diseases like HIV and TB are likely to develop traditional risk factors for cardiovascular diseases. In terms of epidemiological transition these include lifestyle changes such as unhealthy

diet as well as a lack of physical activity and not at least aging. The epidemiological transition could be one of the possible reasons for the simultaneous occurrence of NCD and CVD (111).

4.4.2 The EndoAfrica project

Studies establishing the various aetiologies of cardiovascular diseases related to HIV and TB are in their early stages. Further investigation is needed to understand the multiple interactions in this context. Against this background, EndoAfrica, a research project by **Strijdom et al. (2017)** that studies cardiovascular health in South Africa is particularly important. As highlighted in the introduction, endothelial dysfunction is believed to lead to cardiovascular diseases. At the same time, the endothelium represents a target for both HIV and HAART. Thereby, HIV infection is reported to be associated with endothelial damage and activation due to increased inflammation. Several studies from developed countries describe an increased incidence of coronary heart disease and myocardial infarction in PLHIV, though adjusted cardiovascular risk factors. However, evidence from Africa - the continent that is affected the most by HIV is lacking. Therefore, researches of the EndoAfrica project started conducting a study to explore interactions between HIV infection, antiretroviral therapy regimens and endothelial dysfunction leading to cardiovascular events, particularly in the South African population (see Figure 21). HIV negative and HIV positive patients, of which some receive antiretroviral medication and others not, will be screened for traditional cardiovascular risk factors including obesity, smoking, hypertension, diabetes mellitus and dyslipidaemia, and biomarkers of cardiovascular diseases. Several methods such as flow-mediated dilatation measurements of the brachial artery, carotid intima-thickness measurements and quantitative retinal blood vessel measurements will be used to analyse the vascular and endothelial function. By now 231 individuals, with 60 of them being HIV negative, 133 being HIV positive and receiving ART and 38 being HIV positive without antiretroviral treatment participate. It is strived to complete the 18-months study until December 2018. It is hoped to shed some light on the following questions: How have the measured parameters changed between the baseline assessment and the follow-up assessment 18 months later? Is HIV and/or ART associated with an increased CV risk and endothelial dysfunction in South Africa? EndoAfrica is the first longitudinal prospective study, conducted in sub-Saharan Africa in this context. (112).

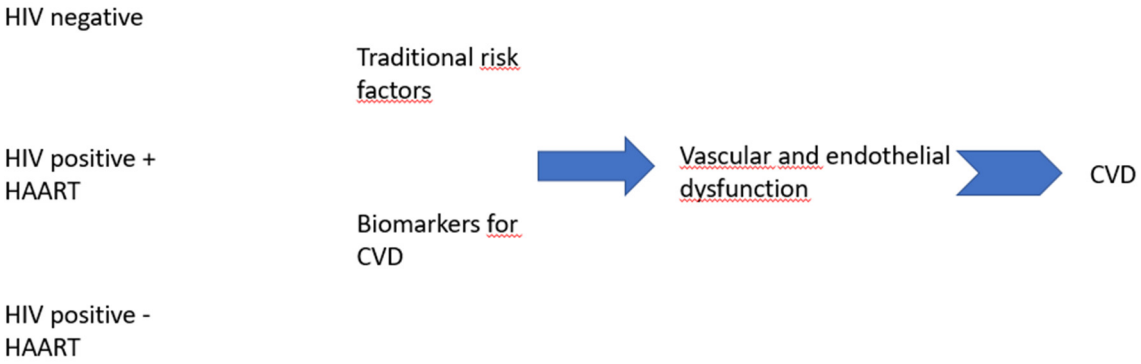


Figure 21: The EndoAfrica project aim

5 Conclusion

Future directions and perspectives

Future attempts should be made to establish a similar cohort to the EndoAfrica study, in which can be assessed **how** tuberculosis related inflammation induces endothelial function changes and CVD in PLHIV. That would entail including patients with TB and HIV who are receiving anti-retroviral treatment. By studying this, we can clarify to which **degree** TB infection affects the severity of cardiovascular events in HIV patients on therapy. Then we can really assess whether **antiretroviral therapy alters** the risk of endothelial dysfunction and CVD in HIV and TB co-infected patients. Furthermore, it would be interesting to assess how TB, in which immune function is rather augmented, affects HIV, in which immune function is compromised and, more importantly, how HIV and TB co-morbidities will modulate cardiovascular risk. Based on a review of the literature in this DA, I can hypothesize that the risk of cardiovascular diseases in patients with HIV and TB would be different from those who are only infected with HIV.

Studying cardiovascular disease risk in HIV patients on HAART and having TB would be particularly important as epidemiological data from the Global Burden of Disease Study 2013 outlines that, contrary to frequent assumptions, most of the cardiovascular disease related deaths occur in low and middle income countries (113). A significant increase of 81% of cardiovascular diseases was recognized between the years 1990 and 2013, mainly due to population growth and demographic changes (113). At the same time, communicable diseases such as tuberculosis and HIV gained ground as well, particularly in developing regions (114). As sub-Saharan Africa is increasingly confronted with a double (HIV and TB) – more specifically triple burden of disease (HIV, TB and cardiovascular diseases) attention needs to be drawn to a systematic study in which these diseases and co-morbidities can be investigated. Such studies will gain novel data to create **awareness of CVD occurrence/ risk in HIV/TB** infected patients and to **set up guidelines** for the early detection and clinical management of the affected patients. Establishing these guidelines, particularly in primary health care systems in sub-Saharan Africa will provide **benefit for the cardiovascular health** of co-infected patients.

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