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Scienze Cliniche
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COORDINATORE Prof. Lorenzo Cosmi

Detection and prevention of cardiovascular diseases in HIV-infected patients

Settore Scientifico Disciplinare MED/11

Dottorando
Dott.ssa Giulia Pontecorboli

Tutore
Prof. Carlo Di Mario

Coordinatore
Prof. Lorenzo Cosmi

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Summary

Introduction:

Viral infections and cardiovascular disease

Over the last decades the link between viral infections and inflammatory heart disease has been widely studied. Carl Ludwig Alfred Fiedler was the first scientist who described in 1900 a case of sudden heart failure caused by an isolated acute inflammation of the myocardium with unfavourable prognosis due to invisible microorganisms[1], which later would have been identified as viruses. Many years later, the employment of endomyocardial biopsy, immunohistochemistry and molecular techniques allowed the in-vivo diagnosis and aetiology identification of myocarditis and viral agents were recognized as the most common causes of the disease[2]. A wide spectrum of cardiotropic viral genomes has been identified, including both RNA and DNA viruses such as enterovirus, parvovirus B19, adenovirus, influenza A virus, human herpes virus (HHV), Epstein–Barr, cytomegalovirus, hepatitis C virus, human immunodeficiency virus (HIV).

Recently, the emergence of severe acute respiratory syndrome coronavirus 2 **(SARS-CoV-2)** virus, which causes coronavirus disease, has rapidly grown into a pandemic, and a large proportion of affected patients have been reported to have underlying cardiovascular disease (CVD). Preliminary data suggest that the virus may cause cardiac injury in infected patients through multiple mechanisms such as 'cytokine storm', endotheliosis, thrombosis, lymphocytopenia etc[3]. Autopsies of COVID-19 patients reveal an infiltration of inflammatory mononuclear cells in the myocardium, confirming the role of the immune system in mediating cardiovascular damage in response to COVID-19 infection and also suggesting potential causal mechanisms for the development of new cardiac pathologies and/or exacerbation of underlying CVDs in infected patients[4].

In the 20th century the link between viral infections and cardiac disease was studied during the outbreaks of myocarditis. ***Coxsackievirus B*** was recognized as the most frequent cause of myocarditis from the 1950s through the 1990s. However, in the late 1990s, the spectrum of viruses detected in endomyocardial biopsy samples shifted to adenovirus and, most recently, parvovirus B19 and Human herpes virus 6 (HHV6) have become the most common causative agents, accounting of 50 % and 24% of the cases respectively[5,6].

The pathogenesis of virus-induced myocardial damage is common to all viruses and can be simplified into a three-phase process[7] starting in the acute phase with a direct cytotoxic injury causing cell lysis induced by the virus, subsequent myocyte injury leading to a cellular and humoral immune response that enhances tissue destruction and, finally, progression into dilated cardiomyopathy (DCM) due to tissue damage and repair mechanisms . In most patients, the pathogen is eliminated in the early phase and the immune reaction is down-regulated, whereas in others the virus or the inflammatory process may persist and contribute to the development of DCM. Cardiovascular magnetic resonance imaging has emerged as a highly sensitive and specific tool for the diagnosis of myocarditis due to its unique potential to perform tissue characterization, allowing visualization of edema, hyperemia and fibrosis of the myocardium[8].
Nevertheless, myocarditis is only one of the possible expression of cardiac diseases due to viruses. In fact, the specific mechanisms by which every virus produces damage of the heart vary with the specific entity and may involve not only the myocardium but also pericardium and the endothelium.
HHV-6 is a lymphotropic virus that infects a broad number of cell types including cardiomyocytes and the vascular endothelium. Intriguingly, HHV6 is able to integrate its genomes into telomeres of human chromosomes, which allows transmission of the virus via the germline. Chromosomally integrated HHV-6 can reactivate from its state and can be associated with an increased risk of heart failure[9].
Parvovirus B19, a single-stranded DNA virus, belongs to the genus of erythroviruses and replicates in erythroid precursor cells of the bone marrow. It is known that endothelial cells represent specific targets in parvovirus B19-associated myocarditis probably through blood group P antigen. Thus, parvovirus B19-related myocardial inflammation is associated to infection of vascular endothelial cells, leading to endothelial dysfunction and impaired myocardial microcirculation[10] .

There is a growing body of evidence suggesting that chronic inflammation, endothelial dysfunction and immune system play a critical role in the development and progression of atherosclerosis. Chronic infections represent one such stimulus that share the common pathophysiological milieu of chronic inflammation. Various viruses have been shown to have a direct effect on the vascular endothelium as well as an indirect effect by systemic cytokine release, both of which contribute to accelerated atherosclerosis. Infectious agents that have been linked to atherosclerotic disease include Chlamydia pneumoniae, Helicobacter pylori, influenza A virus, hepatitis C virus (HCV), cytomegalovirus (CMV), and human immunodeficiency virus (HIV). However, there are significant differences in the strength of the data supporting their association.

HCV is a single-stranded RNA virus belonging to the Flavivirida, causing chronic liver disease and many extrahepatic comorbidities, including CVD. HCV plays an important role in initiating and maintaining chronic inflammation and in generating oxidative stress, which is believed to trigger atherogenesis [20]. HCV also interferes with glucose and lipid metabolism, leading to insulin resistance and diabetes, known factors that promote atherosclerosis. Interestingly, the presence of cryoglobulinemia, cold-insoluble immune complexes containing rheumatoid factor, polyclonal IgG, and HCV RNA, is associated with vasculitis and cardiovascular events[11]. Recently, it has been shown that endotoxinemia, a component of the outer membrane of gram-negative bacteria that live in the intestine that is reported in cirrhotic disease, induces a pro-atherogenic inflammatory condition and an increase in oxidative stress levels that contribute to the development of atherosclerosis in chronic HCV patients[12].

HIV is a retrovirus, a single-stranded RNA virus responsible for the Acquired Immunodeficiency Syndrome (AIDS) epidemic, affecting roughly 38 million people worldwide [13]. Cardiac involvement of the infection, such as HIV-related perimyocarditis and cardiomyopathies, was appreciated early in the epidemic of AIDS even before the virus was isolated [14]. However, with the advent of antiretroviral therapy, survival of HIV-infected patients has dramatically increased and HIV has become a chronic disease with long life expectancy and a high burden of comorbidities. CVD now represents a leading cause of morbidity and mortality in HIV patients: in contemporary, observational studies the proportion of total deaths from CVD ranged from 6.5% to 15%, with HIV infection alone conferring a 1.5- to 2-fold increased risk of CVD compared with uninfected individuals [15,16].

The pathophysiology of CVD in HIV is complex and multifactorial, likely involving the interplay among factors such as inflammation [17], autoimmune mechanisms, direct HIV-induced myocardial damage [18], side effects of HIV medications [19], increased burden of traditional cardiovascular risk factors, and accelerated atherosclerosis [20,21] which is subject of intensive research.

Higher levels of inflammatory markers, such as C-reactive protein, interleukin-6, and tumor necrosis factor (TNF) have been found in HIV-positive patients compared with HIV-negative patients, suggesting potential benefits of therapeutic strategies which aim to directly or indirectly target systemic inflammation in order to reduce the risk of cardiovascular events [22,23].

Identifying the underlying pathophysiological mechanisms and the appropriate target pathways of cardiovascular disease would be of paramount importance to improve risk stratification, early

diagnosis and to develop specific therapies in order to decrease the burden of cardiovascular disease in patients with chronic viral disease.

CHAPTER 1:
HUMAN IMMUNODEFICIENCY VIRUS

The human immunodeficiency viruses (HIV) are two species of *Lentivirus*, a subgroup of retrovirus, that cause systemic T cell destruction and reduced cell-mediated immunity that leads to a wide range of opportunistic infections and cancers, gathered in a condition called *Acquired Immunodeficiency Syndrome*(AIDS).

AIDS was first recognized in the United States in the summer of 1981, when the U.S. Centers for Disease Control and Prevention (CDC) reported the unexplained occurrence of *Pneumocystis jirovecii* (formerly *P. carinii*) pneumonia and of unusual kind of tumors, like *Kaposi's sarcoma* (KS) and *non-Hodgkin lymphoma*, in previously healthy homosexual men and in injection drug users [24]. Indeed, the current CDC classification system for HIV infection and AIDS categorizes patients based on clinical conditions associated with HIV infection together with the level of the CD4+ T lymphocyte count. The syndrome has a spectrum ranging from primary infection, with or without the acute form, to the relatively asymptomatic stage, to advanced stages associated with opportunistic diseases[25].

1.1. Features of the virus

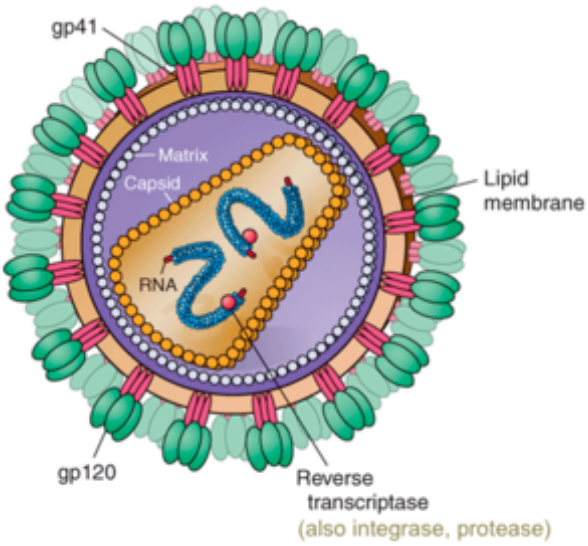
1.1.1. Structure

Similarly to other members of Lentivirus, HIV follows the same structural features. Virion is spherical and its diameter varies from 100 to 120 nm; the *core* has conic shape and is made by the capsidic protein p24[26]. The principal structural proteins are:

- *Matrix protein* forms the inner envelope, upon under the viral membrane
- Capsidic protein (*p24*) contains the viral RNA
- *Nucleocapsidic protein* keeps RNA stable inside the capsid

Polypeptide *Gag* (p55) works as precursor: it is processed by viral protease in order to yield structural proteins that will give origin to mature virion. Inside the capsid there are two identical RNA *strands*, strictly associated to RNA-dependent DNA polymerase and nucleocapsidic protein.

Envelope proteins derive from the cleavage of precursor glycoprotein gp160 into gp120, a protein located on the external envelope surface, and gp41, a transmembrane protein.



Structure of HIV-1, including the gp120 envelope, gp41 transmembrane components of the envelope, genomic RNA, enzyme reverse transcriptase, p18(17) inner membrane (matrix), and p24 core protein (capsid). *(Copyright by George V. Kelvin.) (Adapted from RC Gallo: Sci Am 256:46, 1987.)*

1.1.2. Cellular interaction

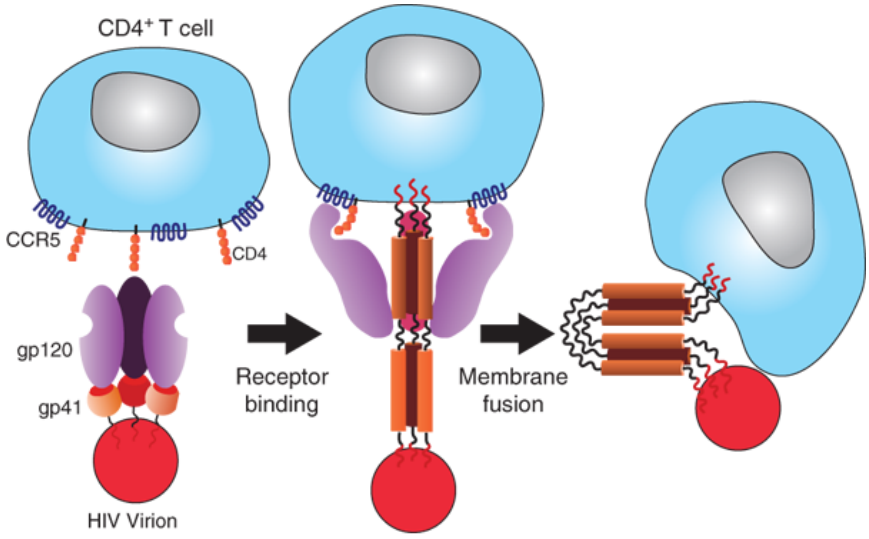
The two envelope proteins *gp120* and *gp41* form trimeric envelope spikes and cover a key role in fusion process with the target cell of HIV virus: CD4+ T cell.

Gp120 binds the receptor CD4, this induces a conformational change in the viral envelope facilitating the binding to one of two major co-receptors: CCR5 and CXCR4.

Both receptors belong to the family of seven-transmembrane-domain G protein–coupled cellular receptors, and the use of one or the other or both receptors by the virus for entering the cell is an important determinant for the cellular tropism of the virus.

Typically, in early phases the binding with CCR5 predominates, but in 40-50% of infected individuals the disease progression is preceded by a *switch* of the viral affinity from CCR5 to CXCR4. The importance of this passage comes from the much higher prevalence of the co-receptor CXCR4 on the CD4+ T cell (90%) respect to CCR5 (10%), thus this switch consents the infection of a very higher number of CD4+ T lymphocytes, accelerating the disease progression.

Following binding of the envelope protein to the CD4 molecule and co-receptor, fusion with the host cell membrane occurs via the newly exposed gp41 molecule penetrating the plasma membrane of the target cell and then coiling upon itself to bring the virion and target cell together.



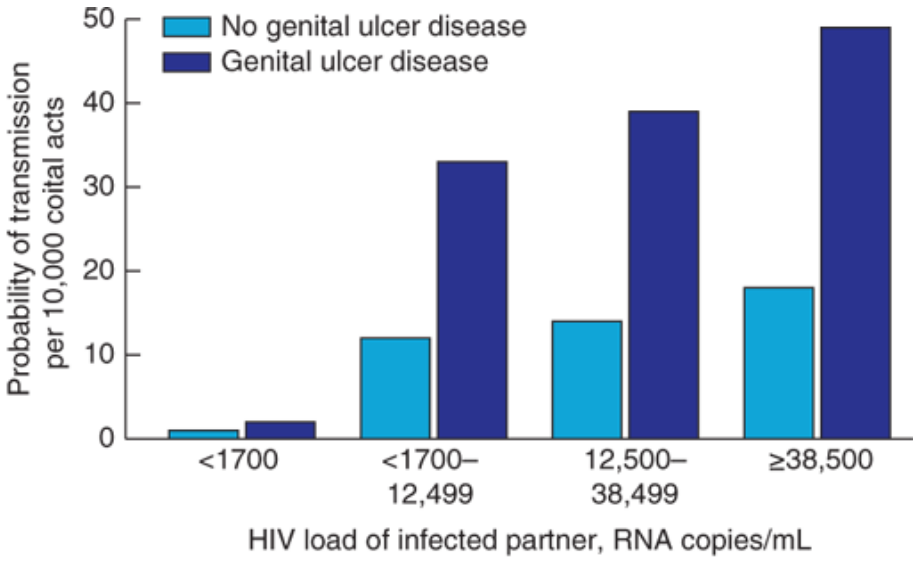
Source: J.L. Jameson, A.S. Fauci, D.L. Kasper, S.L. Hauser, D.L. Longo, J. Loscalzo: Harrison's Principles of Internal Medicine, 20th Edition

1.1.3. Transmission

Globally, the majority of new HIV infections are due to heterosexual transmission. Members of certain high-risk populations are disproportionately affected. Sex workers, people who inject drugs, transgender people, prisoners, homosexual men, other men who have sex with men, and their sexual partners accounted for 62% of all new HIV infections in 2019.

1.1.3.1. Sexual transmission

HIV infection is predominantly a sexually transmitted infection (STI) worldwide. By far the most common mode of infection, particularly in developing countries, is heterosexual transmission, although in many western countries male-to-male sexual transmission dominates. HIV has been demonstrated in seminal fluid both within infected mononuclear cells and in cell-free material. The virus appears to concentrate in the seminal fluid, particularly in situations where there are increased numbers of lymphocytes and monocytes in the fluid, as seen in genital inflammatory states such as urethritis and epididymitis, conditions closely associated with other STIs. The virus has also been demonstrated in cervical smears and vaginal fluid.



1.1.3.2. Injection drug use

HIV can be transmitted to injection drug users (IDUs) who are exposed to HIV while sharing injection material such as needles, syringes, the water in which drugs are mixed, or the cotton through which drugs are filtered. Parenteral transmission of HIV during injection drug use does not require IV puncture; subcutaneous ("skin popping") or intramuscular ("muscling") injections can transmit HIV as well, even though these behaviours are erroneously perceived as low-risk. Among IDUs, the risk of HIV infection increases with the duration of injection drug use; the frequency of needle sharing; the number of partners with whom material is shared; comorbid psychiatric conditions such as antisocial personality disorder; the use of cocaine in injectable form or smoked as "crack"; and the use of injection drugs in a geographic location with a high prevalence of HIV infection.

1.1.3.3. Transfused blood and blood products

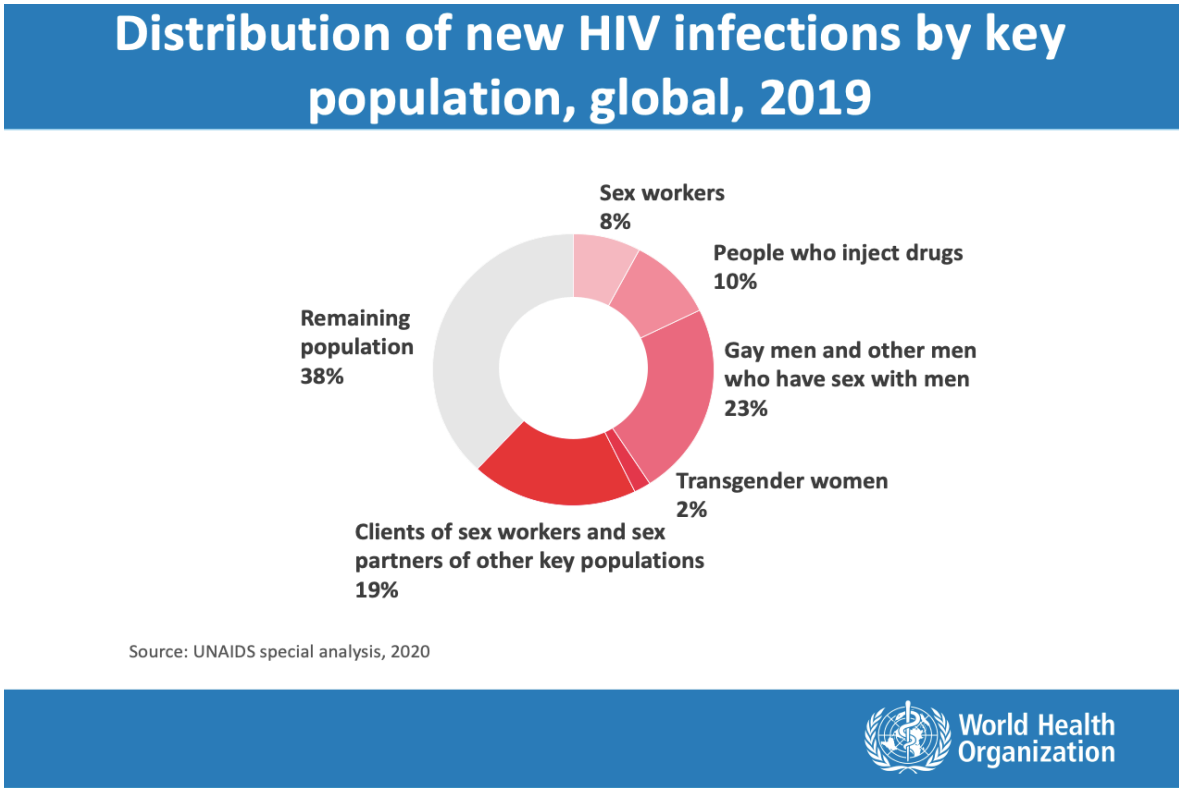
HIV can be transmitted to individuals who receive HIV-contaminated blood transfusions, blood products, or transplanted tissue. The vast majority of HIV infections acquired via contaminated blood transfusions, blood components, or transplanted tissue in resource-rich countries occurred prior to the spring of 1985, when mandatory testing of donated blood for HIV-1 was initiated. It is estimated that >90% of individuals exposed to HIV-contaminated blood products become infected. Transfusions of whole blood, packed red blood cells, platelets, leukocytes, and plasma are all capable of transmitting HIV infection. In contrast, hyperimmune gamma globulin, hepatitis B immune globulin, plasma-derived hepatitis B vaccine, and Rho immune globulin have not been associated with transmission of

HIV infection. The procedures involved in processing these products either inactivate or remove the virus.

1.1.3.4. Mother to child

HIV infection can be transmitted from an infected mother to her fetus during pregnancy, during delivery, or by breast-feeding. This remains an important form of transmission of HIV infection in some developing countries. HIV can be transmitted to the fetus during the first or second trimesters of pregnancy. However, maternal transmission occurs most frequently in the perinatal period. The 82% of pregnant women living with HIV had access to antiretroviral medicines to prevent transmission of HIV to their child in 2018.

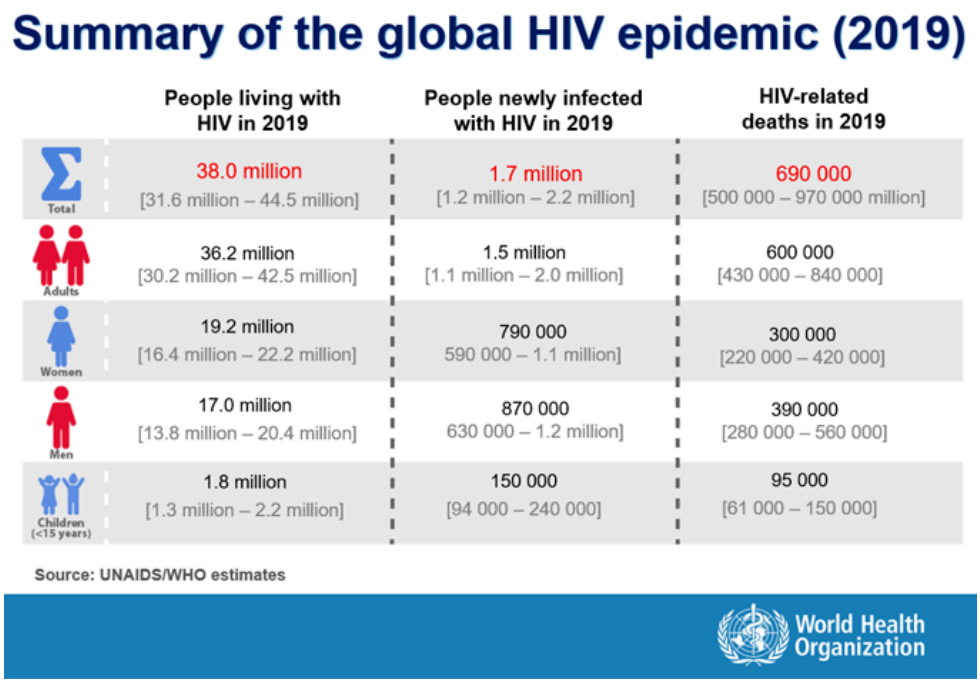
In the absence of antiretroviral therapy for the mother during pregnancy, labour, and delivery, and a prophylaxis for the child following birth, the probability of transmission ranges from 15% to 25% in industrialized countries and from 25% to 35% in developing countries.



1.2. Epidemiology

1.2.1. Worldwide

At the end of 2019, an estimated 38 million individuals were living with HIV infection, according to the Joint United Nations Programme on HIV/AIDS (UNAIDS). 36 .2 million were adults and 1.8 children (<15 years). Among the global number of infected individuals, just the 79% knew their HIV status and about 8.1 million people did not know that they were living with HIV. An estimated 95% of people living with HIV/AIDS reside in low- and middle-income countries and approximately 50% are female. The estimated number of people living with HIV has increased more than four-fold since 1990, reflecting the combined effects of continued high rates of new HIV infections and the life-prolonging impact of antiretroviral therapy[27-29].



1.2.1.1. Prevalence

In 2018, the global prevalence of HIV infection among people aged 15–49 years was 0.8%, with rates varying widely by country and region[29].

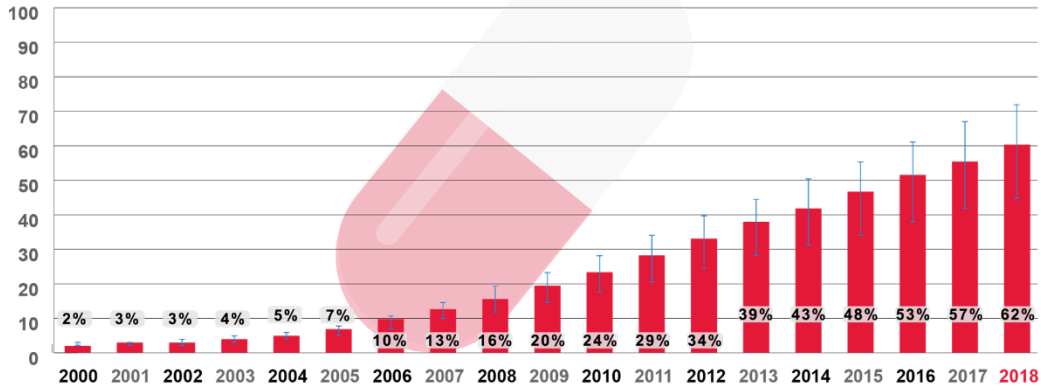
1.2.1.2. Incidence

In 2018, an estimated 1.7 million new cases of HIV infection occurred worldwide, compared to 2.9 million in 1997, and 160,000 of them were children <15 years. Since the peak in 1999, the new

infections have been decreased by 40%. Instead, from 2010, new infections have declined by an estimated 16%, from 2.1 million to 1.7 million in 2018. Also the new infections among children have receded by 41%[28,29].

1.2.1.3. Access to antiretroviral therapy

A great improvement has been recorded in people living with HIV (PLWH) accessing cART since 2010, bringing the coverage from 7.7 million to 23.3 million in 2018. Nevertheless, globally, it is reported that 62 % of PLWH is under treatment[28,29].



Source: UNAIDS/WHO

1.2.2. Italy

1.2.2.1. HIV

The Operative Center for AIDS (Coa) from Istituto Superiore di Sanità, since 1984 collects data related to Aids diagnostic cases and from the 2008 to the new HIV infections. This is a Surveillance system that notifies new clinical diagnosis of HIV infection. In 2018, 2847 were the new HIV infections diagnosis, 4,7 new cases per 100.000 inhabitants. In terms of incidence of new HIV infections, Italy ranks in the average among the European Countries (5.1 cases per 100.000 inhabitants). Furthermore, the incidence of new diagnosis is slightly diminished in the recent years. The highest incidence has been reported in Lazio, Liguria and Toscana[30]. Among people who discovered to be positive to HIV in 2018, 85.6% were males. The median age is 39 years for males and 38 for females. The highest incidence has been observed in the third decade of life. The majority of the new diagnosis was attributable to unprotected sexual contacts for up to 80.2% of all notifications (41,2% heterosexual; men who have sex with men 39%). 29,7% of the new diagnosis involved foreigners[30].

In 2018, more than half of people with a new HIV infection diagnosis has been found in an advanced stage of disease, in particular the 37,8% with a number of CD4 lymphocytes inferior to 350 cell/ μL and 57,1% with a number of CD4 lower than 200 cell/μL. 32,6 % of people with a new diagnosis undertook the test for the presence of symptoms suspected for HIV or AIDS, 14,4 % following an unsafe behaviour and the 11,3% after a routine control[30].

1.2.2.2. AIDS

Since the onset of the pandemic (1982), so far 70.567 cases of overt AIDS have been recorded, whose 45.347 died until the 2016. In 2018, 661 new cases have been diagnosed, with an incidence of 1,1 per 100.000 inhabitants, which is steadily and slightly declining over the past 4 years. The proportion of people already presenting a fungal infection at their initial diagnosis has diminished, while viral co-infections and tumors are now more commonly seen at the onset[30]. In 2018, 25% of people with a new AIDS diagnosis was under treatment before the diagnosis. Overt time, it increased from 20,5% (in 1996) to 74,6% (in 2018) the percentage of individuals with a new AIDS diagnosis ignoring their positivity for HIV, discovering to be infected just few months before the onset of the AIDS[30].

1.3. Pathogenesis and immune response to HIV

The multitude of the cells involved in immune response to HIV-1 infection is the real driver of this disease. Although the hallmark is the loss of CD4+ T cells, the immune activation of several cellular lines, no longer quiescent, dramatically influences the progression of the infection. Even highly active antiretroviral therapies (HAART) do not completely restore the physiological state of immune quiescence.

From the cardiovascular point of view, this persistent hyperactivity results important for other systems: the endothelium and the coagulation.

Several factors may contribute to immune activation, and the first aetiological factor is HIV-1 infection itself. On the one hand, HIV-1 production provides alloantigens that trigger the immune system directly. On the other hand, some HIV-1 components may directly stimulate immune cells. For instance, the Negative Factor (Nef) and Viral Protein R (Vpr) are two regulatory proteins encoded by viral DNA that stimulate monocytes and macrophages.

1.3.1. Microbial translocation

The local inflammation established by the infection increases the permeability of the gut epithelium. The gut barrier results disrupted and leave the way for gut-originating microbes to invade the organism, with the production of Lipopolysaccharides (LPS) and consequently boost the already activated immune system.

Moreover, HIV-1 infection demonstrated to alter the gut flora composition and this change in microbiota is associated with gut tissue inflammation, microbial translocation and increased T cell activation in periphery. The invasion from bacterial components can promote immune activation by inducing pro-inflammatory cytokines including IL-1 and IFN-c [7]. This phenomena remains, in part, despite HAART treatment.

1.3.2. CO-infections

Co-infection with other microbes during HIV-1 infection is another potential factor that provokes chronic immune activation. An example can be the hepatitis C virus (HCV) that if co-existent with HIV-1 in women present with a higher T cell activation than the single infection. Also, the HIV/HBV co-infection accelerates the inflammation and development of liver disease and contributes towards hepatic morbidity and mortality.

1.3.3. CD4+ T cell Lymphopenia

Low CD4 cell count is another factor favouring immune activation indirectly. Actually, it is a vicious cycle in which the loss of CD4 is cause and consequence of an immune activation, in particular of T-cells. A reason can be that lymphopenia induces the production of IL-7, a cytokine with pro-inflammatory activity on CD8+ T cell. This has been confirmed by an increase in CD8+ T cell activation after the administration of IL-7 to nonimmunological responders to HAART.

1.3.4. Immunosenescence

TNF-a and IL-6 are central in another deleterious phenomenon: the immune senescence. In vitro, it has been seen that senescent T cells overproduce these inflammatory cytokines and in vivo, aging has been associated with elevated levels of them. Therefore, also the premature senescence of immune cells is a factor potentially causing immune activation, and chronic immune activation, in turn, induces premature aging of the immune system, deteriorating the body's defence system.

1.3.5. T cell

The proportion of CD4+ T cells expressing activation markers on their surface, along with the percentage of dividing CD4+ T cells, is abnormally high in viraemic patients. As a consequence of chronic immune stimulation, the memory T cell subset expands. However, by competition the expanding subset induces a reduction of naive T cells, and thus of the pool of antigens that the organism is able to recognize. If T cell clones specific for a few antigens expand, on the other hand this results in a global reduction of T cell repertoire. Concurrently to the expression of activation markers, the CD4+ T cells show also augmented inhibitory receptors on their surface, such as PD-1, CTLA-4, TIM-3, LAG-3 and CD160. It appears as a consequence of persistent activation and limits the capacity of the cell to respond to new stimuli.

1.3.6. Apoptosis

One of the reasons of how activation induces a depletion of CD4+ cells is the post activation apoptosis, a programmed cell death. Chronic activation shortens the cells half-life and is now considered as the major cause of CD4+ T cell loss.
In addition, another factor involved in circulating CD4+ T cells reduction is that activated CD4+ T cells tend to be trapped in the secondary lymphoid organs. Seemingly, ART does not reverse all of these signs of CD4+ T cell activation.

1.3.7. B cell

Similarly to T cells, in peripheral blood B cells have been found an amplified number of activation, proliferation, differentiation and apoptotic markers. The increment involves not only the antibody secreted by circulating B cells and plasmocytes, but also the plasma level of immunoglobulins and auto-antibodies. Another potential consequence of this polyclonal B cell activation is the high frequency of B malignancies. Here again, not all of these abnormalities are corrected under HAART.

1.3.8. Monocytes

Macrophages are divisible into two groups.
The M1 macrophages, which produce inflammatory cytokines and ROS, and the M2 macrophages which produce anti-inflammatory cytokines (IL-10 and TGFb) and are involved in tissue repair. The infection may modify the balance between these two subsets of macrophages.

Also for circulating monocytes a state of activation has been noticed, displayed for example by an increase in cell surface of the adhesion molecules CD11b and CD18.
The production of inflammatory cytokines such as TNFa, IL-1 and IL-6, and of the chemokines CCL-3, CCL-4, CCL-5, MCP-1, have also been reported spontaneously elevated, likewise the expansion of a pro-inflammatory monocyte subpopulation overexpressing CD16. The impairment in oxidative metabolism, in microbicidal capacity and phagocytosis have been noted. Similarly to many other immunological defects induced by HIV, also the concentration of soluble markers of macrophage activation, such as neopterin, sCD14 and sCD16, may remain incremented after ART in blood plasma and cerebrospinal fluid.

Each individual may have his own combination of causes of immune activation that, in turn, establish his own profile. As previously said, many of these causal factors persist in spite of a full virological response to treatment. If on the one hand, viraemia remains under values detectable by the most advanced technique of molecular biology (<20 copies/μL) and the CD4+ T cell count recovers its physiological level (>500 cells/mL), on the other hand the immune activation often persists. This is what impair the immune recovery but also favour non AIDS-linked morbidities. Accordingly, it would be useful to monitor the level of immune activation in virological responders to HAART, especially because the diagnosis of the causes of immune activation in each patient may potentially lead to an aetiological treatment.

1.4. Antiretroviral Therapy

The treatment of HIV contains a wide variety of therapies: the antiretroviral therapy, the primary and secondary pharmacological prophylaxis, the therapies for Opportunist Infections (OIs) and HIV-related tumours and the supportive care. The following chapter will be focused on antiretroviral therapy, to understand how can be influent on cardiovascular disease in these subjects.

1.4.1. Rationale for Combination Antiretroviral Therapy

Combination antiretroviral therapy (cART), also referred to as highly active antiretroviral therapy (HAART), is the cornerstone of management of patients with HIV infection. In the United States in 1995–1996, with the advent of cART a marked decline in the incidence of most AIDS-defining conditions was noted. The rationale for the application of a multiple therapeutic regimen, made of at least 3 different drugs, comes from the aim of stopping and limiting the massive viral replication rate.

Indeed, in a patient not under treatment there are from 10.000 to 100.000 viral copies/mL in plasma, and the lost copies are replaced with a rate of 10 billion new copies per day. The matter is that the enzyme *reverse transcriptase,* in addition to its huge proliferation rate, commits several transcriptional errors, generating a high amount of mutations and, thus, favouring the onset of drug resistance phenomena.

There are now several classes of effective anti-HIV drugs, which target different phases of the HIV life cycle in human cells. They are used in combination

to prevent drug resistance and are effective in>70% of patients in bringing down blood viral loads to undetectable levels (ie<50 virus particles/ml blood) and enabling the blood CD4+ T cell count to rise to nearer normal levels (>500 cells/mm3 blood). Despite the blood viral load becoming "undetectable", there is no sterilization; there is always low-level, difficult-to-detect, latent infection in mononuclear cells, which will emerge when ART is stopped. Latently infected sites include lymphoid tissues, gut and brain[31]. Following the most updated guidelines on HIV management, it is recommended that the treatment must be started as soon as the infection is recognized[32]. This line has been decided thanks to the contribution of important trials such as START[33] and TEMPRANO[34], which showed that starting treatment when the CD4+ T cell was above 500/µL carries a lower incidence of AIDS and non-AIDS related events, improving the quality and expectancy of life.

1.4.2. Classes of Antiretroviral Drugs

There are 4 classes of drugs used to treat HIV infection in course of HAART.

1.4.2.1. Reverse Transcriptase Inhibitors

The HIV-encoded, RNA-dependent DNA polymerase, also called *reverse transcriptase*, converts viral RNA into pro-viral DNA that is then incorporated into a host cell chromosome. Available inhibitors of this enzyme are either nucleoside/nucleotide analogues or non-nucleoside inhibitors[25].
This class is considered the "backbone" of cART and is divisible into different sub-classes based on the analogue:

- **nucleoside reverse transcriptase inhibitors, NRTI :** they are administered as pro-drugs and need to be phosphorylated in order to yield active metabolites able to compete for being integrated in viral DNA. They are competitive antagonists of enzyme reverse transcriptase, disrupting the DNA synthesis.

- **nucleotide reverse transcriptase inhibitors, NRTI :** they inhibit viral enzyme without a previous phosphorylation.
- **non-nucleoside reverse transcriptase inhibitors, NNRTI:** they inhibit reverse transcriptase by directly binding to a hydrophobic pocket adjacent to the active site and inducing a conformational change that greatly reduces the enzyme activity. They act as non-competitive inhibitors.

The FDA-approved reverse transcriptase inhibitors include the *nucleoside analogues* Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir, and Emtricitabine; the *nucleotide analogues* Tenofovir Disoproxil and Tenofovir Alafenamide; and the *nonnucleoside reverse transcriptase inhibitors* Nevirapine, Delavirdine, Efavirenz, Etravirine, and Rilpivirine.

The non-nucleoside reverse transcriptase inhibitors are quite selective for the HIV-1 reverse transcriptase whereas the nucleoside and nucleotide analogues inhibit a variety of DNA polymerases in addition to those of the HIV-1 reverse transcriptase. For this reason, serious side effects are more varied with the nucleoside analogues and include mitochondrial damage that can lead to hepatic steatosis and lactic acidosis as well as peripheral neuropathy and pancreatitis. The use of either of the thymidine analogues zidovudine and stavudine has been associated with a syndrome of hyperlipidaemia, glucose intolerance/insulin resistance, and fat redistribution often referred to as *lipodystrophy syndrome.*

1.4.2.2. Protease Inhibitors

These drugs prevent proteolytic cleavage of HIV *gag* and *pol* precursor polypeptides that include essential structural and enzymatic (reverse transcriptase, protease and integrase) components of the virus. This prevents development of HIV virus particles into their mature infectious forms. One of them, Ritonavir, is typically used as a booster, due to its high affinity for several isoforms of cytochrome P450 (3A4, 2D6). It is a pharmacokinetic enhancer, increasing the plasma concentrations of co-administered drugs metabolized by these pathways[27]. The old generation protease inhibitors could increase the level of lipid plasma concentration, promoting cardiovascular disease and relevant phenomena of drug-drug interactions[26].

The HIV-1 protease inhibitors are Saquinavir, Indinavir, Ritonavir, Nelfinavir, Amprenavir, Fosamprenavir, Lopinavir/Ritonavir, Atazanavir, Tipranavir, and Darunavir.

1.4.2.3. Integrase Inhibitors

Raltegravir is the name of the first integrase inhibitor licensed in 2007. It blocks the catalytic activity of the HIV-encoded integrase, preventing integration of DNA virus into the host chromosome, interfering with the formation of covalent bonds[25]. The selectivity of the mechanism of action is given by their capacity to bind exclusively the complex made by integrase enzyme and viral DNA, interacting with magnesium ions, fundamental co-factor for the enzyme. Indeed, they are among the most potent and safest of the antiretroviral drugs and frequently part of initial combination regimens[25].

The four licensed integrase inhibitors are Raltegravir, Elvitegravir, Dolutegravir, and Bictegravir.

1.4.2.4. Entry Inhibitors

As well known, the virus exploits several proteins and receptors to fuse its pericapsid with the cell membrane and free the *core* within the cytosol. To avoid this, a few drugs have been developed. They are divided into *fusion inhibitors* and *CCR5 antagonists*.
Enfuvurtide inhibits fusion of the viral and cell membranes mediated by gp41 and CD4 interactions. It works as being a heterologous molecule, it mimics one of the two homologous domains of gp41, impeding their adequate interactions and function. Instead, Maraviroc is a chemokine receptor antagonist and binds to the host cell CCR5 receptor. The co-receptor is thus stabilized in an unfunctional conformation and it is no more identifiable by the virus itself.
The first available drug to be licensed in this class was Enfuvurtide, or T-20, then followed by Maraviroc and in 2018 the anti-CD4+ monoclonal antibody Ibalizumab.
It is fundamental taking into account the possible switch of viral tropism from CCR5 to CXCR4 as co-receptor. This could possibly carry to drug resistance and a faster disease progression.

1.5. Aging of HIV patients and comorbidities

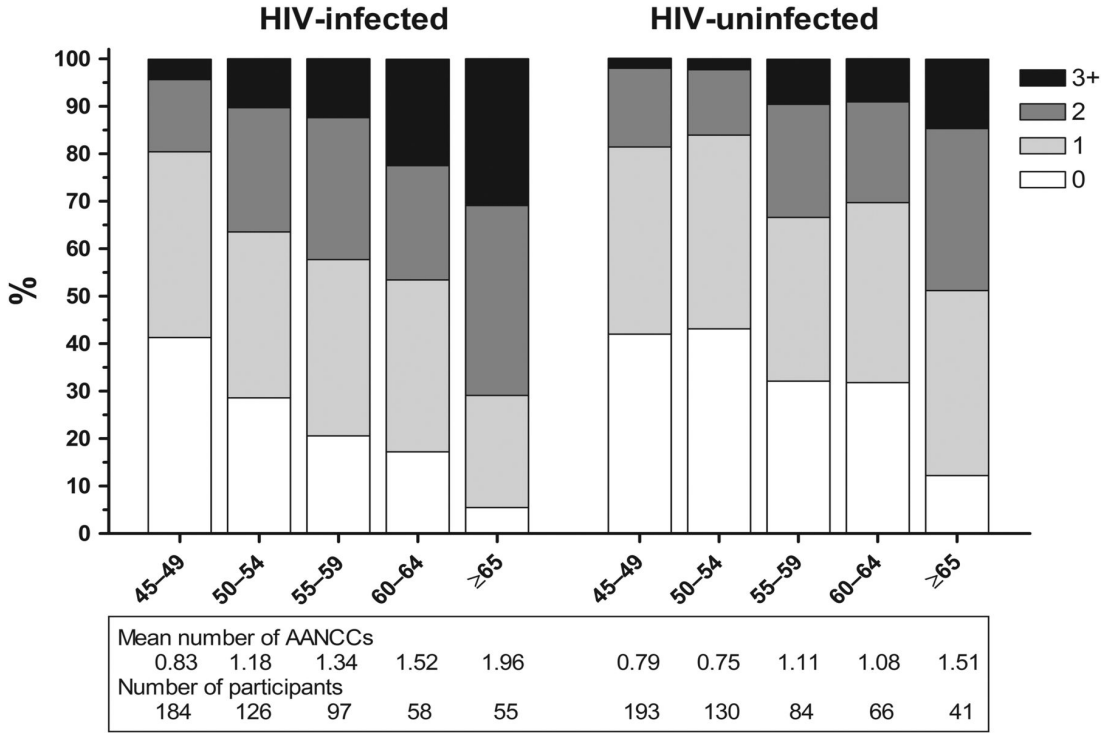
The majority of people living with HIV (PLWH) in the United States are now older than 50 years[35]. There is actually a global aging of HIV population and the reasons can be attributed mostly to the Antiretroviral Therapy. In fact, it permitted the suppression of the viral load, the restoration of acceptable CD4 cell counts and, thus, was able to prevent the onset of the so called AIDS-defining events. It has been noted a shift of the peak of disease onset from young adults towards older ages[28]. Indeed, HIV is no more a rapidly terminal disease, but a chronic and manageable condition with a

steady rising in life expectancy. However, despite substantial gains in health and survival, life expectancy in PLWH continues to lag by approximately 5 years, compared with the general population[36]. The reason can be attributed to the persistence of the same risk factors related to the infection present in young patients, and the progressive accumulation of age-related risk factors. The prolonged survival among PLWH is associated with an increased prevalence of comorbidities including cardiovascular disease and malignancies at all ages presumably due to the inflammation, immune activation and immune senescence associated with HIV infection[37].

HIV patients present an accentuated risk of age-associated comorbidities with polymorbidity. In a large, retrospective, cross-sectional study from Italy, polymorbidity (with at least 2 comorbidities) developed approximately 10 years earlier in PLWH compared with HIV-uninfected individuals[38]. Furthermore, 84% of PLWH in a large study from the Netherlands were projected to have at least one comorbidity by 2030, up from 28% in 2010, and 29% were projected with at least 3 comorbidities by 2030[39]. These observations illustrate the accentuated risk of comorbidities in PLWH compared to the general population, suggesting that HIV may accelerate aging[38]. There is growing experimental evidence of accelerated aging with HIV, and there are clinical evidence that non-AIDS-defining events have supplanted the typical AIDS-defining in this class of patients. As shown by D:A:D study, which investigated trends over time in all-cause mortality and for specific causes of death in people with HIV from 1999 to 2011, it is possible to affirm that also in the mortality rate we are observing a displacement of AIDS with non-AIDS-defining events as overall cause[40]. Evidence for such acceleration must take into account the increased prevalence of predisposing factors among PLWH. HIV infection, or its treatment, share many of these aging-associated hallmarks, including increased genetic instability, enhanced T-cell senescence, diminished naıve T-cell regeneration, and altered intracellular communication from deregulated nutrient sensing, heightened inflammation, and cytotoxic proteins[41].

Although the overall comorbidity burden increases with age, clinical emergence of comorbidities and attributed mortality occur also at younger ages in PLWH. Hanna and colleagues at the New York City (NYC) Department of Health examined the trends of CVD deaths among HIV-infected individuals compared to HIV uninfected. When they stratified mortality rates by age they found higher CVD mortality among PLWH in all age groups through 65 years old. Above 65 years old, however, CVD mortality was not different between HIV-infected and uninfected suggesting a threshold effect of cumulative CVD risk, and reinforcing the higher CVD mortality burden in younger HIV-infected adults[42]. In practice, it can be difficult to differentiate between what would be part of the normal aging process versus contributions from HIV itself, co-infections, adverse effects of medications, other comorbidities and psychosocial barriers. The HIV Medicine Association

developed primary care guidelines specifically for individuals with HIV in recognition that PLWH may need earlier and/or more frequent monitoring for potential comorbidities compared to the general population[43].



Distribution of the number of age-associated noncommunicable comorbidities stratified by age across both study groups. Abbreviations: AANCC, age-associated noncommunicable comorbidities. *Clin Infect Dis*, Volume 59, Issue 12, 15 December 2014, Pages 1787–1797

CHAPTER 2:

CARDIOVASCULAR DISEASE IN HIV PATIENTS

Cardiovascular disease (CVD) in the HIV population accounts for a large proportion of morbidity and mortality and, with the increased life expectancy, the burden of CVD is expected to rise[21] . In contemporary, observational studies of HIV patients, the proportion of total deaths from CVD has ranged from 6.5% to 15%, with HIV infection alone conferring a 61% increased risk compared with uninfected individuals[44,45.] As such, CVD has become an important health issue and the ability to identify HIV-infected patients at risk is now an essential component in their management in clinical practice.

The pathophysiology of CVD in HIV is complex and multifactorial, likely involving the interplay between several factors as inflammation, autoimmune mechanisms, direct HIV-Induced myocardial damage, side effects of HIV medications, nutritional factors, accelerated atherosclerosis and an increased burden of traditional cardiovascular risk factors.

HIV-infected patient can develop both ischemic heart disease, due to accelerated coronary artery disease (CAD), and non-ischemic heart disease, since the HIV infection may involve the pericardium, myocardium, cardiac valves and pulmonary vasculature.

2.1. Ischemic heart disease

2.1.1. Epidemiology

For several years the burden of cardiovascular disease among HIV-infected individuals has been noted to be elevated, and now different studies confirmed that consideration on a wider population of study. One of the biggest is a meta-analysis by Shah et al[46]. who estimated the temporal changes in the population-attributable fraction and disability-adjusted life-years (DALYs) from HIV-associated cardiovascular disease from 1990 to 2015 at a regional and global level. In 793.635 people living with HIV and a total follow-up of 3.5 million person-years, the crude rate of cardiovascular disease was 61.8 per 10 000 person-years. In comparison with individuals without HIV, the risk ratio for cardiovascular disease was 2.16. Over the past 26 years, the global population–attributable fraction from cardiovascular disease attributable to HIV increased from 0.36% to 0.92%, and DALYs increased from 0.74 to 2.57 million. There was marked regional variation with most DALYs lost in

sub-Saharan Africa and the Asia Pacific regions. Indeed, interestingly, in a study concluded in 2015 by Triant et al., besides the finding of a significantly higher incidence of myocardial infarction among HIV-positive individuals, with a relative risk (RR) of 1.75 adjusted for other traditional cardiovascular risk factors, a relevant aspect discovered was the statistical significance of the ethnic group, resulting in a much higher risk for individuals of african and hispanic origin than Caucasians[47].

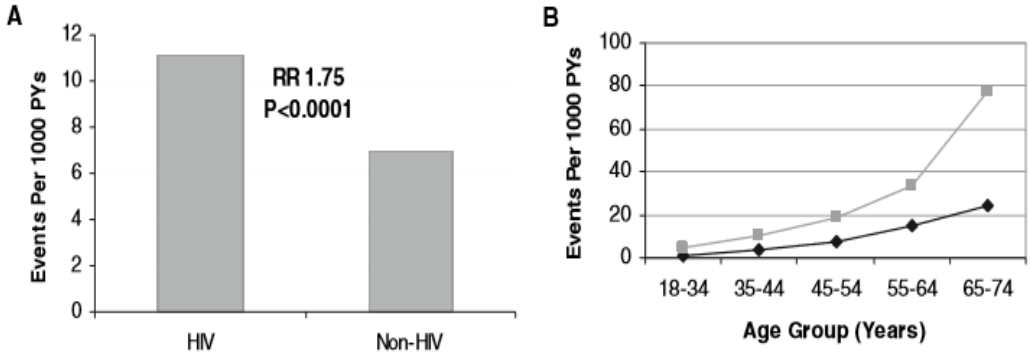


FIG. 1. A, Myocardial infarction rates and corresponding adjusted RR. *Bars* indicate crude rates of AMI events per 1000 PY as determined by ICD coding. RR and associated *P* value are shown *above the bars*. RR was determined from Poisson regression analysis adjusting for age, gender, race, hypertension, diabetes, and dyslipidemia. Associated 95% CIs for RR shown are 1.51–2.02. B, Myocardial infarction rates by age group. *Light line* indicates patients diagnosed with HIV disease. *Dark line* indicates patients not diagnosed with HIV disease. Data shown include both genders. Rates represent number of events per 1000 PY as determined by ICD coding. For associated 95% CIs, see Table 3.

2.1.2. Traditional risk factors

Traditional risk factors play a role in CVD in both HIV- negative patients and HIV-positive patients, but in the last population the pathogenesis may be accelerated by the combined effects of HAART and HIV infection. Moreover, patients with HIV infection often exhibit higher rates of conventional CV risk factors[48].

2.1.2.1.Smoking

Since the beginning of the HIV pandemic, the prevalence of smokers among HIV-infected patients has been observed being up to two and three times higher than that in the uninfected population. With the increased life expectancy, a lot of chronic conditions like cardiovascular disease and cancers have taken the place of the AIDS-defining disease. Thus, tobacco-smoking assumed a central role as risk factor for this group of patients. Indeed, the mortality for non- AIDS-related events is higher among HIV-positive-smokers than in HIV-negative-smokers[49]. In 2015, a study showed that in the United States up to 42,4% of HIV-infected individuals was smoking, the double if compared with smokers

among in general population (20,6% in 2009)[50]. In the same study, the HIV-infected individuals were less incline in quitting tobacco-smoking, with a withdrawal rate of 32,4% versus the 51,7% of the general population. There is reason to believe that social factors such as a low educational level, poverty, ethnicity, depression and abuse of illicit drugs can explain the major habit of tobacco-smoking among HIV-positive subjects[51].

Moreover, the D:A:D study group reported that in HIV-infected persons, there was almost a 3-fold increased risk of myocardial infarction in current smokers as compared with HIV-infected non-smokers[52]. Nevertheless, more studies are needed to better understand the enhanced nicotine metabolism in HIV patients and its pathological consequences. It has been proposed a drug-drug interaction between nicotine and some compounds of ART due to the common metabolic pathway passing through cytochrome P450 enzyme[53].

2.1.2.2.*Dyslipidaemia*

As for other traditional cardiovascular risk factors , changes in lipid profile has been highly observed in HIV patients compared with general population.

Dyslipidemia is a well-known predictor of atherogenesis and adverse cardiac events. The high incidence of this condition in PLWH can be attributed to, on one hand, the same origin implied in uninfected people, like unhealthy diet and smoking, but on the other hand HIV infection per se and ART may contribute to modulate the lipid values, finally inducing atherosclerosis.

How the disease exerts these consequences is not completely understood yet.

How heavy is the burden of dyslipidemia among HIV-positive individuals?

If the overall prevalence of dyslipidemia among HIV-positive patients does not deviate so much from that seen in HIV-negative individuals, elevated levels of TG and low HDL have been found higher in HIV-infected than uninfected individuals.

The disparities in the pattern of dyslipidemia seen in HIV individuals are not yet fully understood but are thought to derive from direct effects of the virus itself and inflammation.

A high HIV viremia and a low CD4 lymphocyte count are both independently linked to lower HDL-C levels, whereas HIV viremia alone is associated with reduced levels of LDL-C and increased levels of very low-density lipoprotein (VLDL) and TG.

The forms of dyslipidemia are also different between those patients on treatment and those who are not. Indeed, with the introduction of antiretroviral therapy, a successful long-term suppression of HIV, reduction in inflammation, and restoration to health have been achieved, but the problem of

dyslipidemia did not disappear. Instead, it emerged a different pattern of dyslipidemia, represented by significantly higher total cholesterol, triglycerides, LDL-C, and lower HDL-C.
To confirm that the advent of cART did not delete the problem of dyslipidemia, a recent meta-analysis including more than 37,000 individuals, found that the risks of hypercholesterolemia and hypertriglyceridemia are still significantly higher among cART-exposed individuals compared with cART-naive individuals[54].

Effects of HIV infection

The exact mechanism of how the virus induces dyslipidemia remains to be completely enlightened. The HIV virus has a protein called Nef ("Negative Factor"), whose function is to manipulate the host's cellular machinery and allow infection, survival and replication of the pathogen. It is a virulence factor that interact with the adenosine-triphosphate-binding cassette transporter A1 (ABCA1), a transmembrane transporter on macrophages surface. In normal situations, this transporter shuttles cholesterol from peripheral tissues to HDL. In an independent manner to HDL-C level, this cholesterol efflux is inversely associated with cardiovascular risk[55]. During HIV infection, Nef impairs cholesterol efflux by downregulating ABCA1. As a result, cholesterol accumulates in macrophages, triggering a key passage for atherogenesis: the transformation from macrophages to foam cells. These changes can be blocked by the initiation of cART[56].

Effects of Inflammation and Immune Activation

The pattern of dyslipidemia seen in HIV is common to other infections and inflammatory conditions and is believed to be initially protective, but prolonged inflammation resulting in persistence of this pattern could be atherogenic[57].
Treatment-naive HIV-infected people showed an increased de novo hepatic lipogenesis compared with normal controls, and this emerged to be linked to the levels of cytokines (TNF-a, TNF-b, interleukin (IL)-1). Three known pathways by which cytokines are able to affect lipid metabolism are:

1. Stimulation of the activity and expression of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG CoA) reductase, the rate-limiting enzyme in cholesterol synthesis[57].
2. Cytokines may also reduce the expression of ABCA1, impairing reverse cholesterol transport[57].
3. The modification of lipid particles and lipoproteins into atherogenic oxidized forms through the action of reactive oxygen species and phospholipase A2 enzymes. These modified lipid

species, such as oxidized LDL (oxLDL), can trigger a protracted inflammatory state seen in atherosclerosis by stimulating the innate immune system and also lead to lipid deposition in blood vessels[58].

Effects of Viral Suppression

To evaluate the benefits or the negative consequences from a strong viral suppression the SMART trial[59] (The Strategies for Management of Antiretroviral Therapy) aimed to give a clear insight of the problem. The study randomized 5472 HIV-infected individuals to receive either intermittent cART or continuous cART, demonstrating that cART, given continuously, not merely reduced opportunistic diseases AIDS-related, but also other serious events, and in particular cardiovascular diseases[23].
In a subgroup of individuals in this trial, those who were randomized to receive cART immediately were found to have improved lipid profile compared with those who deferred it. Interestingly, the magnitude of improvement was highly correlated with the level of inflammation at baseline (measured by high-sensitivity c-reactive protein [CRP] and IL-6), but not with CD4 count, HIV viral load, or traditional risk factors, suggesting that the degree of dyslipidemia was associated with immune activation[60]. Despite these beneficial effect, cART does not, however, completely reverse the effects of inflammation and immune activation. Residual immune dysregulation syndrome, as described by Lederman et al[23] is characterized by persistent T-cell activation and a proinflammatory and prothrombotic state that is still present also in well-controlled, virally-suppressed HIV-infected individuals[61]. Furthermore, Munger et al, using advanced lipoprotein phenotyping found that HIV-infected individuals on cART with seemingly normal lipid panels continue to have atherogenic profiles based on increased LDL particle number, decreased LDL size, reduced large HDL particles, and decreased HDL cholesterol efflux capacity. This effect may be minor but could in part explain why HIV-infected individuals continue to have a higher risk for cardiovascular events even when they are effectively suppressing HIV[62].

2.1.2.3.Insulin resistance and Diabetes Mellitus

Metabolic complications in HIV-positive patients are frequent, and insulin resistance and Type 2 Diabetes Mellitus (T2D) give an important contribution. In infected individuals the rates of disorders in glucose metabolism range between 2% and 14%[63]. The factors implied in the onset of glucose-intolerance and T2D like age, obesity, low HDL-C and high total cholesterol values, play a role in this group of population, but the pathophysiology regards also more complex mechanisms, like lipodystrophy and immunosuppression[64].

A few of the older antiretroviral medications, such as first generation PIs and thymidine-containing analogues reverse transcriptase inhibitors were associated with drug-induced T2D. The mechanism is possibly due to mitochondrial toxicity and glucose transporter GLUT-4 blocking[65]. The newer ARV drugs do not seem to lead to the development of glucose disorders[66].

The systemic immune response may be another factor associated with an increased risk of T2D, through an imbalance in hs-CRP and tumor necrosis factors 1 and 2 levels[67].

Metabolic dysfunction in PLWH can be further altered by additional medical and lifestyle factors, including diet, physical activity, medications, and aging. Few recent investigations have attempted to delineate the separate contributions of weight change, diet, and physical activity to altered metabolism in PLWH on ART: Reeds et al. demonstrated that women with HIV who lost similar amounts of weight to HIV-negative women did not have equivalent improvements in fatty acid metabolism and insulin sensitivity[68]. Engelson and colleagues conducted a 12-week diet and exercise intervention for weight loss among obese women with HIV. They reported a moderate decrease in body weight, subcutaneous and visceral adipose tissue, but no change in measures of insulin sensitivity and CVD risk biomarkers[69]. It is thus questionable the extent to which clinicians can extrapolate from knowledge of nutrition and physical activity impact on metabolism in HIV-negative cohorts when providing care for PLWH.

Monocytes and glucose metabolism

HIV-1 infection seems to modify not only the level of background activation of monocytes and macrophages, but also the type of activation. Lately, it has been shown that the expression of glucose transporter-1 (Glut-1) is increased in pro-inflammatory monocytes in treated and untreated HIV-1 infected patients. This finding suggests an increased glucose uptake and glycolytic metabolism[70].

2.1.2.4.Hypertension

Hypertension is a significant risk factor for total mortality and cardiovascular diseases. Individuals, including PLWH, who suffer from an increased arterial blood pressure are at increased risk for coronary heart disease (CHD), heart failure, atrial fibrillation, aortic valve disease, sudden cardiac death (SCD), sick sinus syndrome (SSS), left ventricular hypertrophy (LVH), and thoracic and abdominal aortic aneurysms (TAA/AAA) [71] Among PLWH, hypertension showed to worsen kidney disease and exacerbate renal dysfunction[72]. As emerged from a meta-analysis of 63 554 participants from studies published from 2011 to 2016, hypertension had an esteemed prevalence of 35% for PLWH on ART and 13% for ART-naïve PLWH[73].

This data can be explained perhaps from the state of vascular permeability due to the uncontrolled inflammatory microenvironment and endothelial dysfunction. Nevertheless, there are no compelling studies on whether the prevalence of hypertension is higher in HIV infected people under treatment or in uninfected people[74]. HIV-infected adults with hypertension had a 2-fold higher risk of incident acute myocardial infarction as compared with HIV-uninfected adults with hypertension[75]. About the mechanisms behind the hypertension in PLWH, many of them derive from a cluster of factors, common to PLWH, able to enhance the risk for cardiovascular disease. Systemic chronic inflammation, immune activation, lypodistrophy, endothelial dysfunction and dyslipidemia are non-traditional cardiovascular risk factors that persist in spite of a good virological and immunological response to ART. Nonetheless, a direct effect of ART cannot completely explain the higher risk of HTN in PLW. So far it has not been possible to quantify the contribute given from ART to increase the hypertensive risk, but several studies enlightened some independent risk factors for hypertension, like nadir CD4+ count <50 cells/μL, lypodistrophy, renal dysfunction and HIV tropism[76].

Protease inhibitors (PIs), beside the multiple dysmetabolic adverse-effects, are able to activate RAAS[77]. Conflicting data does not permit us to state if also nucleoside reverse transcriptase inhibitors (NRTIs) may also play some role.

2.1.3. Non-traditional Risk Factors

2.1.3.1.Inflammation and Immune Activation

Understanding the role of inflammation and immune activation in conferring CVD risk is critical to guide risk stratification, prevention, and management strategies.

Despite the great improvements in the effectiveness of ART and amplification in patient access to ART over the last two decades, virologic suppression with ART is often not sufficient to fully ameliorate the enhanced immune activation established during HIV infection, especially among those who fail to restore CD4+ T cell counts[78]. This residual inflammation may increase long-term CVD risk and severity[79]. Consistent with these data, a recent study suggested that the initiation of ART was insufficient to decrease arterial inflammation[80].

The potential association between HIV-specific inflammation and atherosclerosis was first demonstrated in the SMART study[81], in which increased CVD event rates were observed in the group undergoing to episodic treatment compared with the one in which there has been a good viral suppression with a continuous treatment (HR=1.57), concomitant with increasing viremia and inflammatory markers interleukin-6 (IL-6) and d-dimer[82-83]

Since the SMART study, multiple investigations have linked systemic inflammation and immune activation with CVD surrogate markers and outcomes. Recent data have shown increased risk of serious non-AIDS conditions and mortality with increasing levels of IL-6, soluble CD14 (sCD14), and d-dimer[84].

A greater prevalence of severe coronary stenosis and coronary artery calcification score has been observed with increased tumour necrosis factor α (TNFα) [85].

Inflammation also impacts outcomes following CVD events. In a study of HIV-infected patients undergoing percutaneous coronary intervention (PCI), CD8 T-cell level and persistent C-reactive protein (CRP) elevation at 6-months were significantly associated with angiographic restenosis[86].

Decreased CD4 counts have been linked to an increased CVD risk[87], supporting the role of immune activation in imparting risk. Recent studies have demonstrated a lower or inverted CD4/CD8 ratio, a surrogate marker of immune senescence, to independently predict increased CVD risk[88] and carotid Intima Media Thickness (cIMT) progression[89]

2.1.3.2.Antiretroviral Therapy

There are evidences that a prolonged use of ART contributes to pathogenesis of CVD. Individuals exposed to ART generally show higher levels of total cholesterol, LDL and triglycerides, whereas lower levels of HDL in comparison to general population. However, receiving a combined therapy it was difficult to know which one was the culprit medications for this phenomenon.

Older ARVs including nucleoside reverse transcriptase inhibitors (Zidovudine, Didanosine, Stavudine) and early protease inhibitors (PIs) historically contributed to an increased risk of myocardial infarction (MI) due to mitochondrial toxicity and associated metabolic effects such as insulin resistance, lipoatrophy, lipohypertrophy and dyslipidemia[90]. In 2001 a study showed how the protease inhibitor Ritonavir was able to impede the normal degradation of apolipoprotein B from hepatocytes, thus leaving more substrate available to synthetize a bigger amount of LDL. In 2003 with the D:A: study, an increased rate of myocardial infarction (MI) in subjects exposed for a longer time to ART was demonstrated. People who never started ART had a minor percentage of MIs. Also the exposition for less than a year to ART slightly increased cardiovascular risk[91]. 7 years later the same investigators demonstrated that a few protease inhibitors (Indinavir, Lopinavir and Ritonavir) were responsible for altering lipidic metabolism and, in turn, the risk of myocardial infarction[92]. However, in the era of newer, less toxic ARVs, the observed elevated risk of atherosclerotic cardiovascular events is less likely attributed to ART. Moreover, nowadays more attention is focused on drug interactions and correction of modifiable risk factors in HIV-positive patients.

2.1.3.3.Lipodystrophy

Lipodystrophy is a heterogeneous group of rare disorders with a common and variable degree of distribution and degeneration of body fat. This cluster of disease contains both genetic and acquired conditions. All together the lipodystrophies (count) / (amount is up to) 1.3 – 4.7 cases per million, not considering the antiretroviral therapy-induced lipodystrophy in PLWH (ranging from 10% to 80% among all PLWH worldwide).

HIV-associated lipodystrophy is an undesirable effect of anti-retroviral therapy (ART) that occurs due to the redistribution of adipose tissue, resulting in fat accumulation (lipohypertrophy), fat loss (lipoatrophy) or both.

Lipoatrophy (LA) is the loss of subcutaneous peripheral fat usually at the face, buttocks and limbs and has been associated with the use of thymidine analogues (zidovudine and stavudine). On the contrary, lipohypertrophy (LH) is the accumulation of visceral and central fat in the abdomen, anterior neck, dorsocervical region ("buffalo hump"), trunk and/or breasts[90].

The exact cause for lipodystrophy is unknown and the role played by ART in the development of this condition is not completely understood.

The culprit drug classes involved are Protease inhibitors (PIs) and reverse transcriptase inhibitors (NRTIs, specifically the thymidine analogues Zidovudine and Stavudine, associated with lipoatrophy), responsible for altering adipose and glucose metabolism.

The main effect of these drugs is realized by Adiponectin, a cytokine which plays a pivot role in the glucose regulation and fatty acid oxidation, decreasing its release from adipose tissue and resulting in an impaired adipocyte differentiation. Also, this provokes the suppression of lipogenesis, the increase of lipolysis, reducing the free fatty acids (FFA) uptake by adipocytes and boosting the release of stored triglycerides and glycerol in the systemic circulation[93].

Switching to newer NRTIs such as abacavir or tenofovir demonstrates to prevent worsening. Inversely, discontinuation of PI has not proved to revert fat accumulation.

Lipodystrophy also contributes to morbidity via the development of insulin resistance, hyperlipidaemia, and endothelial dysfunction, which can increase risk of cardiovascular disease and therefore identification and prompt management of HIV-associated lipodystrophy is of utmost importance[94]. Some recent research concludes that the use of HAART is not the only determinant of changes in nutritional profile (despite their direct association with adipose tissue and insulin resistance), but rather their sum with genetic, environmental and nutritional factors has to be considered[95].

2.1.3.4.Substance use

Tobacco-smoking, alcohol abuse and illicit drugs are all frequent modifiable risk in HIV infected individuals.

Alcohol abuse is associated to a major mortality risk among HIV-infected compared with general population, also speaking about low consume levels. A study used the VACS (*Veterans Aging Cohort Study*) index to measure organ damage and general mortality risk. It has been proved that, drinking the same amount of alcohol per month, both increased much faster among HIV-positive drinkers in comparison to HIV-negative[96]

With regard to illicit drugs, it is not easy to obtain correct data, nonetheless there is a little study[97] conducted in 2016 in the United States which showed that among 95 HIV-affected individuals over 50 years old, 48,4% was consumers. Marijuana was the most consumed substance (32,6%), followed by cocaine and crack both at 10,5%.

The Veterans Aging Cohort Study showed that among HIV-infected men, alcohol abuse was associated with a higher prevalence of CVD compared with infrequent and moderate drinking, even after adjusting for traditional CVD risk factors, antiretroviral therapy, and CD4 count[98]. In addition, recreational drug use including cocaine and methamphetamine contributes to cardiac toxicity[99].

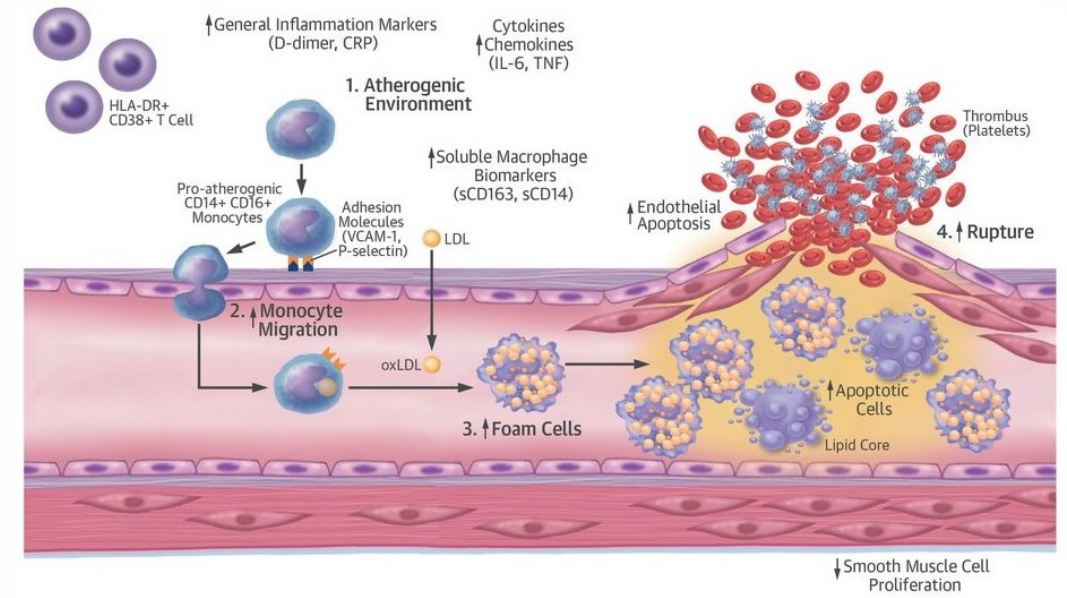
2.1.4. Pathogenesis of HIV-associated CAD

2.1.4.1.Atherosclerosis

After many years of research, the underlying mechanism behind atherosclerosis and CVD among HIV-infected individuals still remains not completely understood. CVD has multifactorial causes and is the result of the interplay among traditional risk factors and others more specific for the HIV subset of patients. The risk for various CVD manifestations are 1.5- to 2-fold greater for HIV patients compared with uninfected individuals[100]. As CVD has become one of the leading causes of death in PLWH, a considerable interest in the early detection of asymptomatic (subclinical) atherosclerosis has grown, in order to improve the risk assessment of CAD event, allowing an appropriate CVD prevention by early intervention[101].

A meta-analysis study carried out by D'Ascenzo et al. showed an association between noncalcified coronary artery plaques and reduced CD4 cell count in HIV patients, supporting the hypothesis of systemic inflammation and immune activation as drivers of an accelerated atherogenesis[102]. The

presence of noncalcified plaques detected by coronary computed tomography angiography (CCTA) in the general population is associated with higher rates of acute coronary syndrome when compared with mixed and calcified plaques[102-104] Indeed, there are studies showing a higher prevalence of subclinical coronary atherosclerosis[103] and a greater burden of coronary atherosclerotic plaque, particularly noncalcified inflammatory plaques, in HIV positive young men than in HIV negative individuals with similar cardiovascular risk factors. The plaques seen in these patients are more prone to rupture and thrombus formation, likely leading to the higher rate of HIV-associated acute coronary syndrome. This vulnerability is due to a necrotic core with an overlying thin fibrous cap and numerous macrophages. In conclusion, these studies support the substantial association between the presence of high-risk plaques and an increased immune activation and inflammation, seen in spite of ART and viremia[105].



Source: Hiv and atherosclerosis. ACC

2.1.4.2.The Emerging role of inflammation

The pathogenesis of atherosclerosis includes several traditional risk factors. Smoking, hypertension, dyslipidaemia and diabetes mellitus are some of the most important elements responsible for the development of an atherosclerotic lesion[27]. They can induce the accumulation of advanced glycation end products (AGEs), leading to increase reactive oxygen species (ROS) and retention of oxidized low-density lipoprotein (ox-LDL). Oxidative stress causes endothelial dysfunction and impairs the release of nitric oxide (NO) and endothelin-1 (ET-1). In atherosclerotic lesions, elevated tissue levels of ET-1 bind to ETB receptors on endothelial cells and cause expression of endothelial cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular-cell-adhesion molecule-

1 (VCAM-1). ET-1 promotes monocyte migration and activation by monocyte chemoattractant protein-1 (MCP-1), which is released from activated macrophages and endothelial cells. ET-1 also activates vascular smooth muscle cells (VSMCs) via ETA receptors to promote SMC proliferation. Oxidative stress also causes lower tissue levels of BH4 and induces the uncoupling of endothelial nitric oxide synthase (eNOS) and superoxide[106]. All these metabolic cascades are ruled by pro-inflammatory cytokines, chemokines and immunological cells. Indeed, atherosclerosis is a chronic inflammatory disease characterized by intense immunological activity but the underlying mechanisms are really complicated and remain largely unclear. However, for the first time, a study could target inflammation in order to obtain a decrease of CVD. This is CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study): a randomized, double-blind, placebo-controlled trial enrolling patients that had a history of myocardial infarction with an elevated hsCRP $\geq$ 2 mg/dL. At a median follow-up of 3.7 years, patients receiving canakinumab had a 15% decrease in the primary endpoint of nonfatal myocardial infarction or stroke, or cardiovascular disease death-driven largely by the reduction in nonfatal myocardial infarction[107]. Interleukin-1 is a major proinflammatory and prothrombotic cytokine, important in inflammatory cell recruitment and migration as well as stimulating further cytokine production promoting atherosclerosis. Further, interleukin-1β has been demonstrated to be upregulated in human coronary arteries with atherosclerosis[108]. Similarly to the general population, it is hypothesized that inflammation and immune activation could play important roles in developing of atherosclerosis also in the HIV subset of patients. It is well known that in HIV-infected individuals a systemic state of chronic inflammation persists for many years, even despite ART . Moreover, different studies demonstrated that activated monocytes and plasma macrophage-specific markers are correlated to HIV-related CVD[105]. Interestingly, Subramanian et al. showed how HIV positive patients had a higher level of aortic inflammation, measured with Fluorodeoxyglucose positron emission tomography (18F-FDG-PET) imaging, compared with non-HIV patients matched with the same Framingham risk score. Increased aortic FDG uptake correlate with plaque macrophage infiltration, and this inflammatory signal has been observed to be significantly associated to sCD163, a marker of monocyte and macrophage activation. Furthermore, it is known the correlation between the increased FDG uptake in the aorta and left main coronary artery and progression of atherosclerotic plaques. This suggest that patients with HIV have significant vascular inflammation, where monocytes and macrophage play a critical role, and an increased risk of CVD compared to general populations[109].

2.1.4.3.Endothelial dysfunction

The endothelium is now considered a spatially distributed organ, whose importance is given by the involvement in almost all disease states, often as a primary determinant. Endothelial cells (ECs) present a wide heterogeneity of functions and phenotypes, necessary for the adaptation to the multitude of organs vascularized by the blood vessels they coat.

The equilibrium between the mechanisms of vascular damage and repair is fundamental. Endothelium responds to injuries by activating endothelial progenitor cells (EPCs), plaque neovascularization and reverse cholesterol transport. The activation of all these mechanisms determines high levels of circulating ECs (cECs) and microvescicles. They are strongly associated to vascular damage and have been proposed as biomarkers of endothelial dysfunction.

It has been seen that in asymptomatic HIV+ young men with long-standing disease the rate of endothelial dysfunction and subclinical signs of atherosclerosis are higher compared with uninfected individuals[110].

HIV seems to impair also the mechanisms involved in vascular repair, decreasing the number of EPCs by a direct infection of these cells, characterized by the expression of the chemokine receptors CCR5 and CXCR4 on their cell surface.

Eventually, HIV set up a state with high levels of microvescicles and cECs, and a low level of EPC, resulting in a not complete capacity to restore the endothelial function.

The capacity of HIV virus to infect ECs has been studied in vitro. Actually, HIV virus has no tropism for ECs. Indeed, macrovascular ECs are generally resistant to HIV infection andthe direct infection of ECs by the virus has not been proved, suggesting that a HIV-driven endothelial dysfunction can be achieved just indirectly by:

1. Creating a pro-inflammatory environment by the release of cytokines and chemokines from infected cells
2. Capacity of HIV-encoded proteins to affect EC function

Role of cytokines and chemokines in EC dysfunction

The systemic chronic inflammatory disorder generated by the HIV infection is the result of continuous stimulation of the immune system. TNF-α, IFN-γ, IL-1β, IL-6, IL-10, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), macrophage colony-stimulating factor (M-CSF) and IL-18 are up-regulated by HIV-1 in T cells and monocytes cells[111].

The HIV-1-mediated inflammatory microenvironment causes a continuous recruitment of monocytes which migrate across activated ECs in blood vessels, differentiate into macrophages and produce proinflammatory cytokines, thus determining the progressive damage to vessel structures.

Furthermore, HIV-1 replicates in macrophages and induces activation and synthesis of several proinflammatory cytokines that in turn induce EC activation and leukocyte adhesion[112]. VCAM-1 and ICAM-1 levels are raised during HIV-1 infection[113] promoting trans-endothelial migration of immune cells and inhibiting their reverse migration[112], thus determining the localization of monocytes inside the vessel wall and promoting the formation of foam cells

Systemic chronic inflammatory disorders enhance the development of endothelial dysfunction atherosclerosis, arterial stenosis and damage and cardiovascular injury.

The role of Early HIV-encoded proteins in EC dysfunction

HIV early gene-encoded proteins, in particular Tat and Nef, contribute to the development of CVD in the HIV-infected population independently of traditional risk factors[113]

The Tat protein

The Trans-activator of Transcription (Tat) protein is a regulatory protein produced by HIV, essential for viral transcription. In the pro-inflammatory microenvironment, Tat activates ECs, favouring the monocytes transmigration to endothelium, facilitating the interaction of inflammatory cells with endothelium.

Fiala et al. (2004) analysed the pathogenesis of HIV-1-related cardiomyopathy, examining heart tissue from HIV+ patients and demonstrated the presence of HIV-1 DNA and RNA in inflammatory cells, but not in ECs or cardiomyocytes, although these cells suffered apoptosis. These authors also showed the *in vitro* ability of HIV-1 to invade - but not to replicate - in neonatal rat ventricular myocytes (NRVMs) and coronary artery endothelial cells (CAECs). However, they found that exogenous Tat protein was capable of activating Erk 1/2 phosphorylation, caspase-3 and apoptosis of both cells types, thus concluding that cardiomyopathy pathogenesis may involve HIV-1 replication in immune cells, release of pro-inflammatory molecules, apoptosis of ECs and cardiomyocytes through activation of Tat signalling. Tat is released by infected cells even during cART[115].

The Nef protein

Negative Factor (Nef) is an important pathogenic factor responsible for T cell activation and enhancement of virus production.

Wang et al. (2014) showed that Nef transfer itself from HIV-1-infected cells to ECs by nanotube-like conduits. Nef is able to activate and induce NADPH oxidase with its SH3 binding site and this activity is most important in Nef-induced EC apoptosis. Indeed, this viral protein significantly decreases nitric oxide production and, in contrast, increases superoxide an ion production, thus contributing to ROS production, cell oxide stress and cell death[116].

HIV-infected T cells showed to be more potent than free virus in activating coronary arterial endothelial cells. It has been noted that this effect is Nef-dependent, because Nef-deleted virus shows only residual activity, suggesting that Nef is the main contributor of HIV-induced endothelial activation.

2.1.4.4.Coagulopathy

The state of immune activation found in HIV individuals trigger, in turn, two other systems: the endothelium and the coagulation. Indeed, HIV-1 infection results in a hypercoagulable state, with an increased level of factor VIII[36] and von Willebrand factor in the plasma[117-119], and a decrease in the plasma level of the anticoagulant factors antithrombin, protein C and protein S . Consequently, high levels of the fibrinolysis biomarker D-dimer have been observed. This is, interestingly, not normalized under ART and may result from the direct effect of HIV-induced tissue injury and the indirect effect of immune and endothelial activations[119].The tissue factor, a trigger of the extrinsic pathway of the coagulation cascade, is abundant in the plasma as well as at the surface of monocytes of platelets of HIV-1 patients. Moreover, the percentage of monocytes expressing tissue factor is not normalized under viral suppression[118]. Another way for immune activation to promote coagulation in HIV-1 infected individuals is via the acute phase protein fibrinogen that is involved in platelet aggregation[120]. In addition to their roles in coagulation, platelets are major actors of the immune response. They sense pathogen-associated molecular patterns via pathogen recognition receptors that they express, and, once activated, produce inflammatory cytokines and chemokines, and other inflammation mediators. In addition, they may physically interact with leucocytes and activate them. Many studies report platelet activation in HIV patients. Activated platelets tend to bind to monocytes. Accordingly, platelet–monocyte complexes have been shown to be more frequent in HIV-1 infected than in uninfected persons , and persist in spite of efficient antiretroviral therapy[121].

2.1.5. Management

2.1.5.1.Risk assessment

Cardiovascular disease (CVD) risk prediction functions are tools widely used to predict CVD risk and prevent disease through identification of the profiles with the highest-risk, and individuate who deserves modification of risk factors. HIV-infected individuals face an increased CVD risk, with multiple studies demonstrating a 1.5- to 2-fold increased risk of myocardial infarction (MI) or stroke compared with non-HIV–infected individuals[122]. Evaluating the risk of CVD in HIV patients can be

challenging because the most commonly used CVD risk functions, including the FHS (Framingham Heart Study) functions for hard coronary heart disease (FHS CHD) and atherosclerotic CVD (FHS ASCVD) and the American College of Cardiology/American Heart Association (ACC/AHA ASCVD), use just risk factors common in the general population and do not take into account risk factors specific for the subset of HIV population[123].

2.1.5.1.1. Framingham Risk Score

The investigators of the Framingham Heart Study developed the Framingham Risk Functions (also called Framingham Risk Scores, FRS) to evaluate the likelihood of developing CVD in individuals. With a study in 2008 the investigators D'Agostino et al. resumed in a unique function the risk prediction for general CVD, replacing disease-specific algorithms with a single general CVD prediction tool. They presumed a parallelism between atherosclerosis in different vascular territories in terms of sharing a common set of risk factors, explaining why the general CVD risk function performs well for predicting the individual components[124].

These functions are multivariate functions (algorithms) that combine the information in CVD risk factors such as sex, age, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, smoking behaviour, and diabetes status to produce an estimate of developing a component of CVD (such as coronary heart disease, stroke, peripheral vascular disease, or heart failure) over a fixed time, usually 10 or 30 years. These estimates of CVD risk are often major inputs in recommending drug treatments such as cholesterol-lowering drugs[125]. The FRS has been built up on a white middle-class sample in United States. Its validation among non-Framingham population often required adjustments for the variability of some risk factors linked to the ethnic group of origin. An example could be the blood pressure in African Americans, that appeared as a stronger risk factors if compared with the with American population[126]. The Framingham risk score tends to overpredict CHD in the general HIV population but to underpredict CHD risk in the subgroups of females, former smokers and diabetic patients. This tool seems to be currently the best way to assess the individual cardiovascular risk in HIV-infected patients but does not yet include inflammatory or immunologic markers[127].

2.1.5.1.2. ASCVD score

The American College of Cardiology (ACC) and American Heart Association (AHA) 2013 Guideline on the Assessment of Cardiovascular Risk developed the Pooled Cohort Equations (PCEs) for 10-year ASCVD risk prediction to assist with decision-making for ASCVD prevention. These equations

derived from more diverse population-based cohorts than prior ASCVD risk prediction tools and estimate 10-year and lifetime risks for atherosclerotic cardiovascular disease (ASCVD), defined as coronary death or nonfatal myocardial infarction, or fatal or nonfatal stroke. The information required to estimate ASCVD risk includes age, sex, race, total cholesterol, HDL cholesterol, systolic blood pressure, blood pressure lowering medication use, diabetes status, and smoking status[128].

The release of ACC/AHA guidelines on CVD risk assessment and cholesterol treatment represents a turning point in the approach to CVD prevention in the general population, but the applicability of established functions in the setting of HIV, however, remains incompletely understood[123]. A recent study from the Centers for AIDS Research Network of Integrated Clinical Systems cohort found the ACC/AHA function exhibited acceptable discrimination and moderate calibration when used to predict MIs for HIV+ individuals from a multi-site U.S. HIV cohort with adjudicated MIs. Moreover, the incorporation of HIV-specific variables did not show to improve the MI risk [125-129] Triant et al[123] found ACC/AHA function to be inadequate when applied to individuals with HIV.

2.1.5.1.3. European SCORE

The Systematic COronary Risk Evaluation (the SCORE) model was developed in 2003. Development was based on a pool of datasets from 12 European populations or occupational cohort studies and considered a total of 205,178 individuals. The SCORE predicts the ten-year risk of cardiovascular mortality in individuals without pre-existing atherosclerotic diseases. The model is applicable to individuals, aged 45-64, with no previous history of cardiovascular disease (CVD) and takes into account such risk factors as gender, age, smoking status, lipids and systolic blood pressure[130]. These factors contribute multiplicatively to the overall risk and increase the likelihood of a future CVD event[131]. The SCORE model is based on either total cholesterol level or the ratio of total cholesterol to high-density lipoprotein (HDL) cholesterol. These were calculated for low- and high-risk European countries. Country-specific versions of the SCORE, adjusted to national circumstances and different tools such as charts, tables, computer programs and web-based tools, have been developed over the last few years. The SCORE may present several limitations not only on the estimation of the risk level but especially with regard to treatment.

Very high Risk:	Subjects with any of the following: ▪ CVD ▪ Type 2 diabetes, or type 1 diabetes & target organ damage ▪ Patients with moderate to severe CKD (GFR <60mL/min/1.73m²) ▪ SCORE ≥10%
High Risk:	Subjects with: ▪ Markedly elevated single risk factors such as: - Familial dyslipidaemias - Severe hypertension. ▪ SCORE ≥ 5% and <10%
Moderate Risk:	SCORE is ≥1 and <5% at 10 years, further modulated by: ▪ family history of premature CAD ▪ HDL-C ▪ abdominal obesity ▪ TG ▪ physical activity pattern ▪ hsCRP ▪ social class
Low Risk:	SCORE less than 1% and free of qualifiers

Source: European Guidelines on CVD Prevention in Clinical Practice (Version 2012) © European Society of Cardiology -

2.1.5.1.4. D:A:D

Existing risk score for general population may not accurately estimate risk in the HIV setting. Previous studies have suggested that CVD risk prediction functions in an HIV cohort systematically underestimate CVD risk in HIV. Discrimination and calibration are both suboptimal when applying the functions to a cohort of largely antiretroviral therapy–treated men engaged in HIV care, indicating that risk prediction algorithms developed for use in the general population may underperform and may be inadequate in their ability to appropriately order CVD risk and accurately calculate predicted risk. This mean that the actual functions may fail to identify patients at elevated CVD risk who would benefit from aggressive risk reduction. Adaptation of current CVD risk functions with the incorporation of new risk factors is likely to be needed for safe and accurate risk estimation in HIV[109]. That is what it has been attempted with the CVD risk model based on data from the D:A:D study with a risk score calculator (http://www.cphiv.dk/TOOLS/Framingham/tabid/302/Default.aspx) specific for HIV individuals. This model, using traditional risks for CVD in addition to HIV-specific risks, such as CD4þ count, abacavir use, and exposure to PIs and nucleoside reverse transcriptase inhibitors, give a slightly better prediction for CVD events then Framingham risk score. However, this and other HIV-specific risk calculators have not been widely validated or adopted. In the ART era, where the prevalence of PLWH became higher, developing accurate CVD risk score in HIV-infected individuals has been identified as a priority.

2.1.5.2.Modification of risk factors

Although there are no data from large-scale clinical trials to direct specific primary prevention for CHD in HIV-positive patients, recent research efforts have focused on the management of traditional CVD risk factors and use of newer antiretroviral agents in order to reduce drug interactions and adverse effects. Given the high prevalence of traditional risk factors for CVD in HIV-infected individuals, management of dyslipidaemia, hypertension and counselling for behaviour changes is the first priority.

Smoking

Because the prevalence of smoking in HIV-infected individuals is so high, the reduction of smoking would lead to the highest absolute reduction in CVD and should be strongly advocated. Life expectancy of HIV- smokers has been calculated to be, on average, 8 years less than that of HIV-non smokers[133]. However, this goal is the most difficult to achieve. Counselling for smoking cessation is of outmost importance and referring motivated patients to give up smoking clinics might have the highest impact on CVD risk reduction.

Dyslipidaemia

HIV-infected patients typically have low TC, and LDL-C and high-density lipoprotein cholesterol (HDL-C) levels, as well as elevated TG[134]. Antiretroviral treatment causes marked increases in TC, LDL-C, TGs, and a predominance of small dense LDL particles, while HDL-C remains low. HIV-infected patients have a higher risk for CVD when compared with HIV-uninfected individuals [relative risk (RR) 1.61, 95% CI 1.431.83], while ART (and especially older protease inhibitors) further increases this risk up to two-fold (RR 2.00, 95% CI 1.702.37) [54].

Treatment of dyslipidaemia in HIV-infected individuals receiving ART arise some issues in relation to possible drug interactions with antiretroviral drugs. There are no Randomized Controlled Trials where emerge the efficacy of antilipidemic drugs for relevant endpoints in HIV-infected individuals, but many RCTs comparing different antiretroviral have investigated the alteration of lipids in relation to different antiretroviral drug combinations. The newer PIs (atazanavir and darunavir), NNRTIs (Intelence), or integrase inhibitors (Raltegravir) have shown to induce less lipid changes then the older but, the switch of antiretroviral drug to less atherogenic ones may also lead to drug failure if the response of the patient to them has not previously been carefully considered[127].

Fibrates, niacin, and fish-oil have all been shown to effectively reduce ART-related increases in triglycerides in HIV-infected patients[135] However, the use of these compounds cannot be generally recommended. Meta-analyses of randomized trials with fibrates indicate a moderate reduction in

CHD events that is offset by an increase in non-CHD-related mortality[133]. Meta-analyses and clinical trials found no reduction in CHD mortality when niacin was compared with placebo or added to a statin. Large clinical trials of fish oil in patients at risk for CHD indicate no benefit[136]

Achievement of LDL-cholesterol treatment goals with dietary intervention and use of statins remains the first priority in the management of dyslipidaemia in HIV-infected individuals at moderate to high risk for CHD.

Few RCTs have investigated statins in HIV-infected individuals and found similar reductions of LDL-cholesterol compared with HIV negatives[137] , but drug interactions with ART need to be considered. Simvastatin, lovastatin, and atorvastatin are all metabolized in the liver via the cytochromeP450 3A4 isoenzyme system and are susceptible to drug interactions with PIs and the NNRTI efavirenz. Fluvastatin and, to a much smaller extent, rosuvastatin are primarily metabolized via CYP 2C9 and are vulnerable to interactions with PIs aswell[138]. Pravastatin is not significantly metabolized via the CYP isoenzyme system and is therefore a preferred statin in HIV-infected individuals. Interestingly, Pravastatin has been involved in REPRIEVE (Randomized Trial to Prevent Vascular Events in HIV) trial: a prospective, randomized, placebo-controlled trial of a statin strategy for the primary prevention of major adverse cardiovascular events (MACE) in PWH with low to moderate traditional risk. The use of simvastatin and lovastatin in combination with all PIs and efavirenz is not recommended. Atorvastatin, fluvastatin, and rosuvastatin when co-administered with PIs should be initiated at the lowest dose and patients must be carefully monitored because of the increased risk for potential drug interactions[138].

From the most updated ESC guidelines of 2019 on dyslipidaemia emerged that administering lipid-lowering therapy should be considered in HIV patients with dyslipidaemia to achieve the LDL-C goal in high-risk patients[139].

Recommendations for lipid-lowering drugs in human immunodeficiency virus patients

Recommendations	Class[a]	Level[b]
Lipid-lowering therapy (mostly statins) should be considered in HIV patients with dyslipidaemia to achieve the LDL-C goal as defined for high-risk patients. The choice of statin should be based on their respective potential drug–drug interactions.	IIa	C

HIV = human immunodeficiency virus; LDL-C = low-density lipoprotein cholesterol.
[a]Class of recommendation.
[b]Level of evidence.

ESC/EAS Guidelines 1

Hypertension

When hypertension is present, the magnitude of increased CVD risk associated with this risk factor probably is similar to the one observed in the general population. Patients with HIV who completed an intensive lifestyle intervention that included dietary and physical activity counselling, modelled after the Diabetes Prevention Program, experienced a significant reduction in blood pressure[140]. Guidelines for the effective diagnosis and management of hypertension have been widely adopted and should be applied to patients with HIV until further data are available[74]. Specific research needs related to hypertension and CVD risk in patients with HIV infection begin with the need to obtain a better understanding of the prevalence of hypertension among patients undergoing ART, as well as the extent to which it influences risk of cardiovascular disease. Pharmacokinetic and pharmacodynamic studies that evaluate interactions between commonly used antihypertensive therapies and ART are needed, given the overlap in metabolic pathways affected by ART and certain antihypertensive medications. Potential drug interactions with antiretroviral drugs have to be kept in mind: for instance, concomitant use of diltiazem and atazanavir is problematic and all PIs may increase levels of dihydropiridine calcium-channel-blockers[131]

Glucose metabolism and Diabetes mellitus

Impaired fasting glucose and impaired glucose tolerance are best treated non-pharmacologically with lifestyle interventions such as dietary changes and exercise, although medications can be considered[141]. In patients with HIV, regular exercise and an intensive lifestyle intervention that includes dietary and exercise counselling tends to shorten insulin resistance[142]. Metformin and thiazolidinediones tend to improve insulin resistance in patients with HIV; however, the long-term effectiveness of these agents for the prevention and treatment of diabetes mellitus in patients with HIV is not known[143]. Patients initiating ART should be screened for impaired fasting glucose and diabetes mellitus by measurement of fasting glucose levels at baseline, annually, and after changes are made to ART regimens. The optimal treatment strategies for diabetes mellitus in patients with HIV have not been determined. In addition, further research to characterize the CVD risk conveyed by disordered glucose metabolism in patients with HIV infection is needed, including research on the extent to which disordered glucose metabolism and its associated increase in CVD risk are reversed when ART is modified[144.]

2.2. Non ischemic heart disease

Before the advent of cART, the most frequent presentations of HIV-associated CVD were dilated cardiomyopathy, pericardial disease, pulmonary hypertension. Since the onset of ART, HIV patients are increasingly at risk for cardiovascular ischemic disease such as MI, stroke, and heart failure[145-146]. CVD is the second leading cause of non–AIDS-related mortality in the United States and third in Europe among HIV patients[147].

Recent data show that the proportion of infected patients expected to survive 5, 10 and 15 years after seroconversion in the ART era are respectively 99%, 93% and 89%[148]. With the increased life expectancy and decreased morbidity from opportunistic infections, the recognition of chronic complications associated with HIV-1 infection are becoming an important priority, and among complications cardiac diseases are frequent. The spectrum of heart diseases varies significantly between developed and developing countries and between pre-HAART and post-HAART eras.

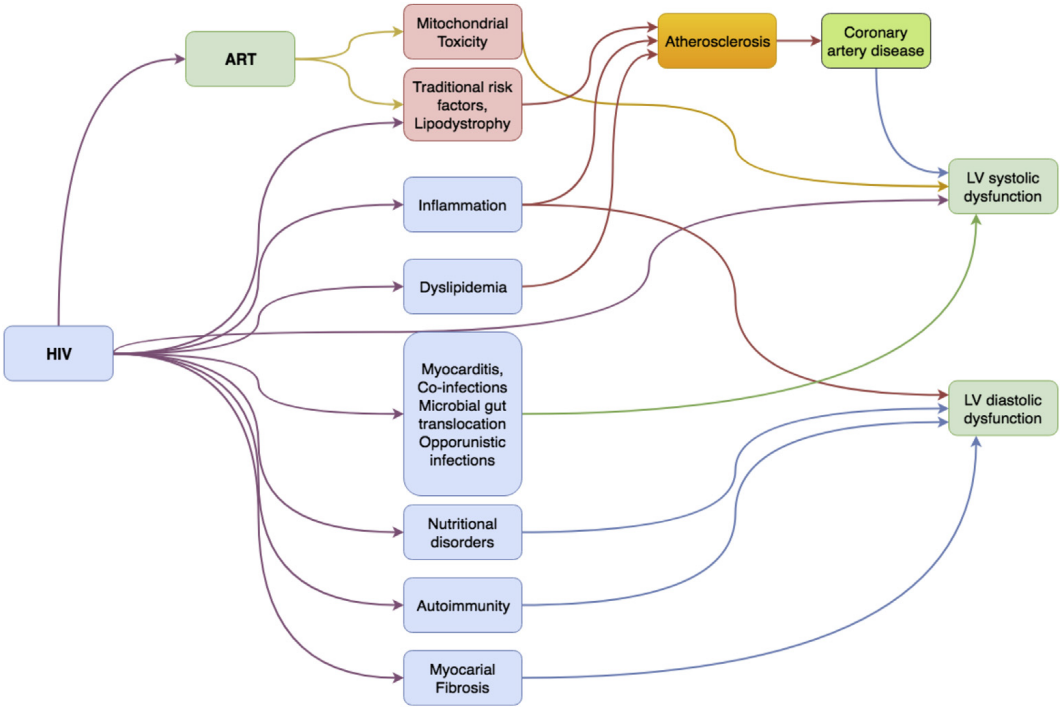
2.2.1. Myocardial disease

HIV-associated cardiomyopathy is currently recognized as a major long-term complication of HIV-1 infection in developing countries although still prevalent in developed countries[149].

Actually, there is no unifying terminology for identifying HIV-associated cardiomyopathy. However, it is useful to categorize the myocardial manifestations of HIV broadly into 3 categories: 1) focal myocarditis; 2) subclinical left ventricular (LV) or right ventricular dysfunction, which includes diastolic dysfunction and asymptomatic systolic dysfunction; and 3) symptomatic dilated cardiomyopathy with reduced ejection fraction[150].

The advent of ART has changed aetiology from a prevalent symptomatic systolic dysfunction to an asymptomatic or minimally symptomatic diastolic or mild systolic dysfunction[151]. With the spread of ART, the prevalence of systolic dysfunction has decreased, and nowadays the number of patients with severely impaired ejection fractions is quite low. On the contrary, the number of HIV-infected patients with abnormal diastolic parameters has increased significantly. The change in the profile of myocardial disease due to the advent of ART can be proved by two wide studies. A meta-analysis conducted in the cART era among 2242 well-controlled, asymptomatic HIV-1–infected patients reported a prevalence of systolic dysfunction of 8.3% and diastolic dysfunction of 43.4%. Risk factors for systolic dysfunction included high-sensitivity C-reactive protein >5 mg/L, tobacco use, and past myocardial infarction; for diastolic dysfunction, risk factors were hypertension and older age[152]. A study that collect data on HIV-infected population with a lower coverage of treatment is the The Heart of Soweto Study, that resemble the typical scenario before the advent of HAART. This investigation analysed 518 of the 5328 cases (9.7%) of newly diagnosed heart disease identified as HIV positive. Of those, almost one third presented with LV systolic dysfunction (n=148; 29%), and 196 (38%) had HIV-related cardiomyopathy[153].

HIV-related cardiomyopathy is characterized by a multifactorial etiology:



Direct HIV cardiac myocyte invasion has been supported by the findings of HIV-1 in myocardiocyte from humans with AIDS by in situ hybridization[154]. However, this mechanism is controversial, because cardiac myocytes lack CD4 receptors. It has been hypotized that HIV may enter the cardiac myocyte when the cell integrity has been disrupted by other cardiotropic pathogens, such as Epstein-Barr virus. An endomyocardial biopsy study of HIV-associated cardiomyopathy found histological evidence of myocarditis in 44% of 14 patients and a high prevalence of cardiotropic viruses, suggesting that these viruses may play a role in the pathogenesis of this condition[155]. Other studies suggest that the real reservoir for HIV is constituted by macrophages, dendritic cells and endothelial cells rather than myocardiocytes. Thus, a number of cytokines is produced, such as of tumour necrosis factor-alpha, interleukin-1 and -6, and other pro-inflammatory cytokines, resulting in myocardial injury and dysfunction induced by chronic inflammation[156].

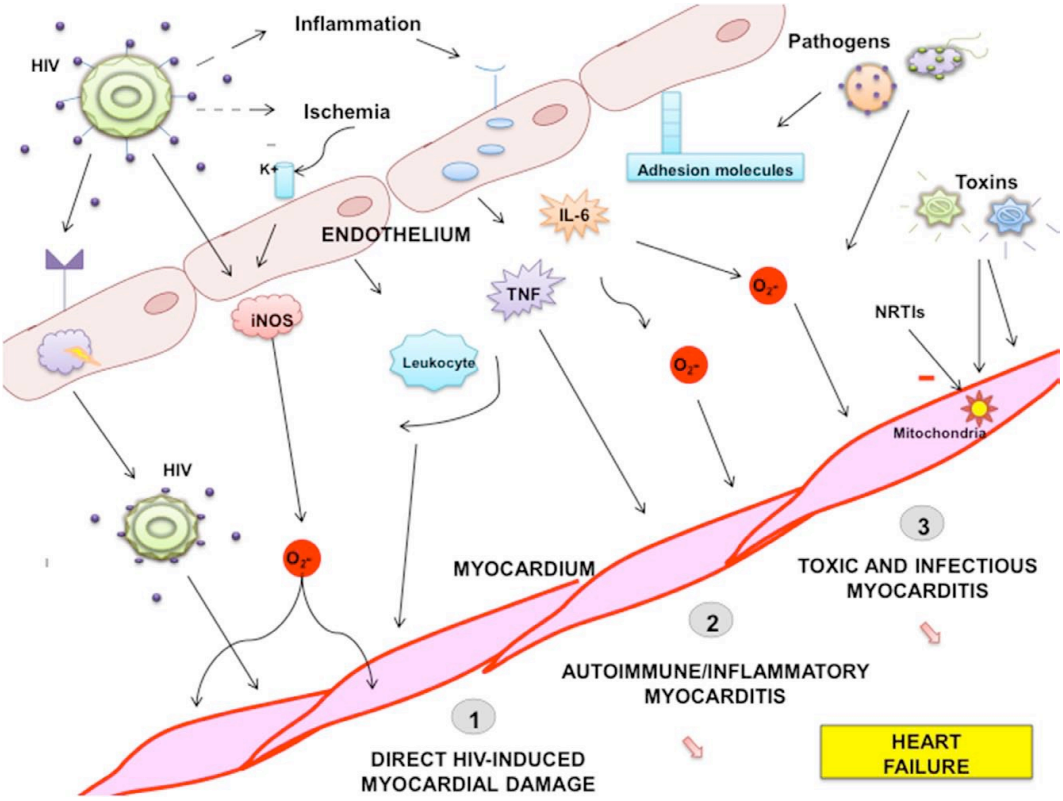
Cardiac magnetic resonance imaging data has reported abnormally increased myocardial tissue lipid levels and myocardial fibrosis compared with uninfected control subjects. Chronic inflammation is thought to induce myocardial fibrosis, whilst the cardiac steatosis seems to be secondary to the effects of antiretroviral therapies[150]. These findings correlate with impaired myocardial function in these patients.

Cardiotropic viruses alter surface antigens, leading to autoimmune reaction to endogenous epitopes. In addition, cardiac-specific autoantibodies are more common among HIV-infected individuals, especially those with evidence of myocardial disease. Increased myocardial expression of human leukocyte antigen class I antigens is commonly seen in patients with HIV and symptomatic systolic dysfunction. Interestingly, blocking some of these proteins might be cardioprotective, and monthly

intravenous immunoglobulins in HIV-1 infected children has been shown to improve LV dysfunction and reduce other markers of myocardial injury[151]

Proinflammatory cytokines, particularly interleukin-1ß and tumor necrosis factor (TNF) are presumably involved in HIV-associated cardiomyopathy, with impaired systolic function[135] Autopsies of HIV-associated cardiomyopathy patients suggest that TNF is a potent inducer of apoptosis[159].

Antiretroviral therapy itself may be directly toxic to the myocardium. For example, zidovudine causes myocyte mitochondrial damage, which is reversed after discontinuation of the drug[160]. This agent has been associated with an 8-fold increased risk in HIV-associated cardiomyopathy compared with the risk in patients not on zidovudine[161].



Of note, children and adolescents seem to be the most appropriate populations for the understanding of pathophysiological mechanisms of HIV-associated cardiomyopathy because they are less likely than adults to be exposed to other cardiovascular risk factors. In this way it is possible to observe how HIV infection and ART exposure lead to subclinical abnormalities of cardiac structure and function in children that may eventually result in symptomatic cardiomyopathy in adulthood. The prevalence of systolic dysfunction has decreased in developed countries; unfortunately, in the parts of the world where HIV is most prevalent and would be needed more, ART coverage is not adequate , and it is still common to find patients showing a severe left ventricular systolic impairment with high rates of morbidity and mortality[161].

2.2.2. Pericardial disease

Before the wide use of cART, the prevalence of pericardial effusions in HIV-positive patients ranged from 11% to 40%. In the Heart of Soweto study, pericardial effusions and pericarditis were the second most common (25%) HIV-associated cardiac manifestations[153]. On the contrary, in the post-ART era the prevalence of pericardial effusions is low. The majority of pericardial effusions are mild and largely asymptomatic[162]. As several reports have described significantly lowered CD4 cell counts in patients with effusions compared with HIV-infected patients without effusions, the immune status is believed to play a crucial role in the pathophysiology of this condition.

Pericardial effusion is frequently associated to the presence of co-infections with opportunistic pathogens like Cytomegalovirus, Nocardia and Cryptococcus. In South Africa and Tanzania more than 90% of HIV-infected patients with large pleural effusions have tuberculous pericarditis[163]. Also malignancies, such as lymphoma and Kaposi's sarcoma, have been noted to be associated with pericardial effusions. Hypoalbuminemia has been suggested as a potential cause of pericardial effusions in patients with advanced HIV disease[162]. In addition, it has been postulated that pericardial effusions could be the result of capillary leaks, which may be related to cytokine activation in advanced HIV infection. Finally, direct invasion of the pericardium and myocardium by HIV may be responsible for causing myopericarditis.

Diagnostic investigation should be guided by the epidemiology of the region and clinical presentation. Transthoracic echocardiography is a non-invasive, bedside, widely available diagnostic tool to identify the presence and the severity of pericardial effusion. Pericardiocentesis, with analysis of pericardial fluid biochemistry, cytology, and appropriate cultures for bacteria and tuberculosis, should be considered. Pericardioscopy and targeted pericardial biopsies, together with advances in contemporary pathology, virology, and molecular biology have improved diagnostic yield considerably[165]

In HIV-infected patients with AIDS and pericardial effusion, the mortality at 6 months from onset of the effusion was found to be almost 9-fold greater than the mortality for subjects without effusions[150].

Treatment of pericardial effusion in HIV-infected individuals is dependent on severity and etiology. Small, asymptomatic effusions do not need any treatment, other than follow-up. In patients with hemodynamic instability and cardiac tamponade, pericardiocentesis is indicated. Recently, a large randomized study clarified the role of immunotherapy and steroids in HIV-infected patients with tuberculous pericardial effusion. Both oral prednisolone and immunotherapy with Mycobacterium indicus pranii failed to reduce the primary endpoint of mortality. The use of oral prednisolone was associated with a reduced risk of constrictive pericarditis, but also with a 3-fold higher risk of HIV-associated cancer[166].

2.2.3. Pulmonary Hypertension

HIV-associated pulmonary artery hypertension (HIVPAH) is a distinct clinical condition, where the only recognized cause is the HIV infection. HIV-PAH is classified, along with idiopathic PAH, in the group 1 of the World Health Organization's classification[167].

HIV-PAH is a rare condition, occurring in 0.5% of HIV-positive patients in the pre-cART era. This prevalence comes from a large Swiss cohort of 1200 untreated patients, whose Pulmonary Arterial Pressure (PAP) has been evaluated by transthoracic echocardiography for unexplained respiratory symptoms[168]. A prevalence of 0.5% of HIV-PAH is remarkably high if compared with the general population, which has been estimated to be 5 to 25 cases per million[169]. Interestingly, the prevalence of HIV-PAH has not changed, despite the introduction of cART[170].

Patients with HIV-PAH have the same histological manifestations of idiopathic PAH. All forms of PH have common pathologic features indicative of pulmonary vascular remodelling, which include medial hypertrophy of muscular and elastic arteries, dilation and intimal atheromas of elastic pulmonary arteries. The hallmark of PAH is the plexiform lesion, a complex arterial lesion, characterized by focal proliferation of endothelial cells lined by myofibroblasts, smooth muscle cells, and connective tissue matrix[168]. Inflammatory mediators, growth factors, and vasoconstrictors contribute to the development of HIV-PAH leading to the development of the characteristic plexiform lesions. Cytokines, such as interleukin-6, alpha-TNF and platelet-derived growth factor induce a proinflammatory and procoagulant state. The absence of viral particles in these lesions suggests an indirect action of the virus, with inflammation acting as a trigger in predisposed individuals. HIV viral proteins, such as *glycoprotein-120*, transactivator of transcription protein, and *negative factor* interact with endothelial cells, inducing vascular oxidative stress, smooth muscle proliferation, and endothelial injury. Glycoprotein-120, a protein responsible for entry of HIV virus into macrophages and CD4 lymphocytes, has been shown to target human lung endothelial cells, stimulating the secretion of endothelin-1, which causes vasoconstriction[171].

Patients with HIV-PAH present in early adulthood with nonspecific symptoms, such as progressive dyspnoea, chest discomfort, dizziness, and syncope. The disease can progressively get worse. Echocardiography is the preferred initial investigation. Right Heart Catheterization is the gold standard for the hemodynamic evaluation of PAH. Hemodynamic assessment with RHC is mandatory before the initiation of PAH-specific therapy[172]. Although Pulmonary arterial systolic pressure has been found higher, on average, among HIV- infected individual than in HIV-uninfected patients, a screening for PAH in asymptomatic HIV-infected , especially in developed countries, is not recommended[150].

HIV-PAH carries a poor prognosis. Before cART and specific PAH therapy, the mortality rate was extremely high at 50% at 1 year. However, with current guideline-mandated therapy, overall survival has improved to 88% at 1 year[173].The effect of cART on patients with HIV-PAH is controversial. Retrospective studies have suggested a decrease in pulmonary artery systolic pressures after the introduction of cART. However, 2other retrospective studies demonstrated an increase in pulmonary artery pressures or no changes in right heart haemodynamic after the introduction of cART.

It is important to be aware of potential drug interactions in patients with HIV-PAH. In animal models, protease inhibitors (PI) have evinced an increase of pulmonary smooth muscle proliferation[174]. In HIV-PAH, the same treatment algorithm used for patients with idiopathic PAH should be adopted[175].

2.2.4. Valvular disease

Valvular heart disease is a common finding in HIV-positive patients. In an echocardiographic study of 803 HIV-infected individuals, a valve dysfunction was diagnosed in 77% of patients, of whom the majority had valvular regurgitation. However, with the exclusion of mild stages, the rate of significant valvular disorders (stage moderate to severe) decreased to 4.7%, and the rate of valve disease was not related to CD4 cell count or viral load[176]. The risk of infective endocarditis (IE) among HIV populations is comparable to that seen in other high-risk groups, such as intravenous drug users. CD4 cell count<50 cells/mm^3 and high viral load(>100,000 copies/mL) determine a 4-fold increased risk of developing IE[177]. There are differences between developed and developing countries, where the formers face a large prevalence of IE in HIV-positive patients who abuse intravenous drugs, whereas in the latters the predominant factor is rheumatic heart disease[178].

The clinical presentation of IE is similar in HIV-positive and HIV-negative patients. The overall clinical picture is defined by the type and location of valvular involvement. For instance, in intravenous drug abusers, the right-sided valves are usually involved whereas in developing regions it is more likely to find a left-sided valve disease. The most common pathogen in HIV-positive patients is Staphylococcus aureus[177-178].

There is no difference in outcome in patients with IE between HIV-positive or HIV-negative patients. Therefore, the HIV-infected individuals who are more severely immunocompromised and who have a mixed or left-sided valve involvement have a higher mortality[179]. Medical and surgical management should be guided by bacteriology and clinical status and should follow guideline-recommended therapy[150].

2.2.5. Arrythmias

From 2000 to 2009, Tseng et al conducted a retrospective study of 2860 outpatients with HIV, treated or not with ART, to examine all causes of death in this population. They reported that sudden cardiac death was the third leading cause of death (13%) after AIDS (57%). Remarkably, sudden cardiac death accounted for 86% of all cardiac deaths and was 4.5 times higher than in the general population. HIV-positive patients who died from sudden cardiac death had previously a higher burden of hypertension, heart failure, myocardial infarction and arrhythmias than persons with HIV who died of AIDS and natural causes[180].

Electrocardiographical abnormalities are more common among HIV-infected patients than non-HIV-infected individuals. Major ventricular conduction defects, including isolated ST-T abnormalities and prolongation of the QT interval, were found in 8% of HIV-positive patients and have been associated with an almost 2-fold increase in cardiovascular events[181.] HIV patients are often exposed to many potentially QT-prolonging agents, such as the azole antifungals, macrolide antibiotics, and tricyclic antidepressants. In addition, some antiretroviral agents, including PI, are reported to directly prolong the QT interval, whereas other antiretroviral agents may indirectly prolong the QT interval through interactions with cytochrome P450 (CYP450)-dependent QT-prolonging drugs[182]. Electrolyte disturbance is another factor that contribute to the high risk of QTc prolongation in HIV-infected patients and can promote malignant arrhythmias such as torsades de pointes and sudden cardiac death in patients with HIV[183].

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in clinical practice and is associated with an increased risk of stroke, heart failure, and overall mortality. The prevalence of AF is approximately 1% to 2% in the general population and increases to nearly 10% in the elderly[184]. Perhaps due to the younger age of those infected with HIV, AF seems to be relatively rare in HIV-positive patients. Only few studies, have examined the potential association between HIV and the development of AF. In one of those, Hsu et al reported a significant association between markers of HIV severity and AF. A low number of CD4þ T cells (<200 cells/mm3) and a high HIV viral load (>100,000 copies/mL) were both independently associated with the development of AF, even after adjusting for traditional AF risk factors[185]. Adaptive immune system changes and persistent inflammation have been recommended to be associated with increased risk of AF in HIV-infected individuals[1]

2.2.6. Vascular disease and Aortitis

Vasculopathy involving the aorta and its branches has been described in young adults with advanced HIV infection. HIV-associated vascular disease is a specific disease entity and differs from atherosclerotic disease in that patients are younger. It is characterized by occlusion of the vasa vasorum by an inflammatory cell infiltrate, leading to weakening of the vessel wall and aneurysm formation[186].

There have been case reports of HIV-positive patients with aortitis presenting with aortic regurgitation. This disease seems to affect the younger age group, as compared with degenerative aorto-sclerotic aneurysmal disease. In a study of children between 2 and 9 years of age, the mean aortic root measurements were found to be significantly higher in HIV-positive children than in HIV-negative[187].

CHAPTER 3:
IMAGING FOR CARDIOVASCULAR DIAGNOSIS IN HIV PATIENTS

The first evidence of premature atherosclerosis among PLWH was reported in 1998[188]. Successively, many studies demonstrated that PLWH have an increased risk of CAD events compared to the general population. This has generated considerable interest in the early, non-invasive detection of asymptomatic subclinical atherosclerosis in HIV-infected patients. Collectively, studies using imaging technologies supported the evidence of increased CAD risk in PLWH clarifying new pathways for investigating the pathophysiology ischemic and non-ischemic heart disease in patients with HIV infection.

3.1. Carotid Ultrasound (US)

The carotid arteries ultrasound with the mono-dimensional measurement of the thickness of the two inner layers (intima and media) is a reproducible, relatively inexpensive and with no radiation technique. The measurement of Carotid Intima-Media Thickness (CIMT) has been well validated in the general population as a surrogate marker of cardiovascular risk [189]. Literature demonstrated that the CIMT measurement correlates with CAD and can predict future CV events in the general population [190].

The study of morphology (such as evidence of ulceration) and size of atherosclerotic plaque are important predictors of risk and are better assessed with three-dimensional (3-D) ultrasound technique In addition, 3-D ultrasound is more sensitive than IMT for following plaque progression and response to treatment. Carotid plaque is associated with increased risk of cardiovascular events, independent of traditional risk factors[192-193]. Longitudinal carotid IMT progression also has been used as a surrogate marker of change in CAD risk, although this association is less clear.

Many studies have measured carotid IMT in HIV population. According to the results of a 2009 meta-analysis that included most of these studies (5456 HIV positive and 3600 HIV negative patients), PLWH tend to show a greater thickness in CIMT (0.04 mm thicker; 95% confidence interval: 0.02–0.06 mm, $p < 0.001$) when compared to HIV-negative controls. This difference in carotid IMT is of a magnitude that is consistent with the observed increased CAD risk in individuals with HIV

infection. The meta-analysis did not find higher carotid IMT values in HIV-patients exposed to protease inhibitors. However, this meta-analysis had significant heterogeneity because studies included had different population characteristics, study designs, sample sizes, length of follow-up, and various ultrasound techniques (IMT measurements of common carotid artery versus bifurcation and/or internal carotid artery) [194].

However, a subsequent report from the cohort with the second largest number of HIV-infected patients confirmed the result of the meta-analysis: the mean difference in common carotid artery IMT between HIV-infected and uninfected control patients was 0.033 mm (p =0.005). Of note, larger effects were observed in the internal carotid artery than in the common carotid artery.[195] These findings are not concordant with other studies not showing a difference or only weakly increased CIMT in PLWH[196-198]. Few studies evaluated the effects of HIV on the longitudinal progression of carotid IMT with discordant conclusion, but also with markedly different study design and participant characteristics (Figure 1).

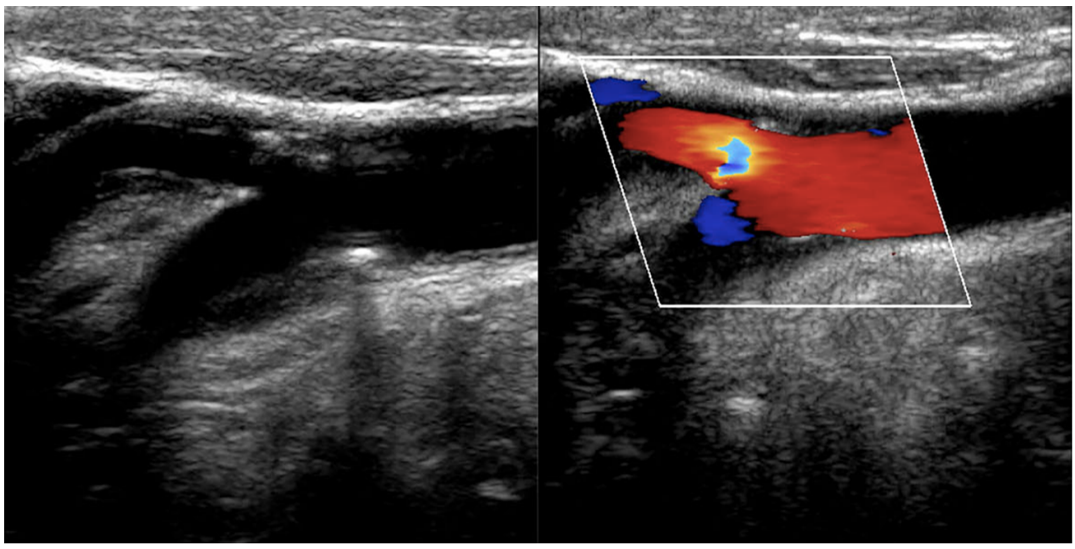


Figure 1: Significant atherosclerotic plaque with flow acceleration of internal carotid artery in a HIV patient.

3.2. Transthoracic Ecocardiography (TTE)

The average life expectancy in PLWH was prolonged with the use of ART and aggressive treatment against opportunistic infections. As a result, patients started to encounter long-term complications including, above all, cardiovascular disease. With its wide availability and low cost, transthoracic echocardiography (TTE) is usually the first-line imaging modality in diagnosing heart disease and is a mandatory step prior to advanced imaging. By far, echocardiography provides the highest temporal

resolution (~10 msec) among all other noninvasive imaging modalities. Furthermore, the use of color Doppler allows assessment of intra-cardiac blood flow and its direction, thereby facilitating estimation of transvalvular gradients. However, this modality is heavily dependent on operator experience and the exam can be limited by suboptimal acoustic windows in patients with a high body mass index or critical pulmonary disease. Serial echocardiography is useful to follow PLWH over time. The cardiac pathophysiology seen in these patients includes coronary artery disease, pericardial disease, valvular disease, vascular diseases and cardiomyopathy .

3.2.1 Diagnosis of CAD

While in the pre-ART era opportunistic infection causing pericarditis and myocarditis were the most important causes of heart disease, in the post-ART era, CAD, ACS and arrhythmias are the main causes of morbidity and mortality in PLWH.CAD and associated ACS are the third most common cause of death in HIV-infected patients in the United States [199].
ECG and echocardiography are the first medical evaluations for patients with ischemic symptoms: the presence of Q waves on the ECG and regional wall motion abnormalities on echocardiography usually indicate CAD. However, this diagnosis is not always corrected: a study demonstrated that 62% of the patients with coronary artery disease did not show Q waves on the ECG, whereas 13% of patients without coronary artery disease had Q waves. Similarly, almost 60% of the patients with CAD did not show regional wall motion abnormalities, whereas 17% of the patients without this disease did show regional wall motion abnormalities [200]. These findings highlight the need for additional imaging techniques to exclude or confirm CAD, such as invasive coronary angiography (ICA) and Coronary CT angiography (CCTA) providing anatomical information about CAD and functional imaging techniques (PET/SPECT, stress CMR, stress echocardiography) to evaluate the ischemic coronary burden.

3.2.2 Systolic and diastolic heart failure

HIV-associated cardiomyopathy comprises a large range of cardiac disease phenotypes. First, Kaposi's sarcoma with metastasis to the heart was reported, and later dilated cardiomyopathy was noted in HIV-positive patients[202] The pathophysiology of HIV-associated cardiomyopathy is likely multifactorial. In general, in the pre-ART era, HIV-associated cardiomyopathy was defined as symptomatic, systolic dysfunction with a dilated left ventricle.

The HIV cardiomyopathy phenotype has changed with the diffusion of ART. Heart failure with preserved ejection fraction (HFpEF) is the actual predominant manifestation of cardiomyopathy in the HIV population. Diastolic heart failure is difficult to diagnose because includes clinical heart failure (with or without symptoms) in the setting of normal or mildly abnormal left ventricular systolic function and evidence of abnormal left ventricular relaxation, filling, diastolic stiffness. This entity may be present in asymptomatic patients with hypertensive heart disease as well as in symptomatic patients with heart failure and a low ejection fraction. Echocardiographic studies in asymptomatic HIV-positive patients in the ART era reported a higher prevalence of diastolic dysfunction (DD) and elevated LV mass index, with low rates of LV systolic dysfunction [203]. DD is one of the earliest manifestations of cardiovascular involvement in HIV patients. If the mechanism responsible for the association between HIV and diastolic dysfunction is not well understood, it is clear that DD is independent of ART; various hypothesis for the development of DD and diastolic heart failure has been postulated, like a systemic pro-inflammatory state as the cause of reduced nitric oxide availability and decreased protein kinase G activity, leading to stiffer cardiomyocytes and increased interstitial fibrosis [204] Another possible explanation is a direct effect of HIV virus on cardiac function by infiltrating the myocardium.

3.2.3 Valvular Heart Disease

Valvular disease is a common finding in HIV-infected patients. Up to 78% of HIV- infected patients have pathologic function of one or more of the cardiac valves. Tricuspid regurgitation is the most commonly seen abnormality, but valvular insufficiency was present also in the mitral, pulmonary and aortic sites. However, most of these patients are asymptomatic or have mild presentations. Endocarditis is frequent in HIV patients, probably for the high rate of intravenous drug user among PLWH compared to the general population[205]. Even if most of valvular disorders are mild, a regular echocardiography evaluation in this patient is essential. Transesophageal echocardiography is more sensitive for detecting endocarditis than conventional transthoracic echocardiography.

3.2.4 Pulmonary Artery Hypertension (PAH)

PAH is a long-term complication in HIV infection [201]. Transthoracic Doppler echocardiography is an extremely useful tool for the diagnosis of HIV-PAH. The most frequent echocardiographic features are systolic flattening of the interventricular septum, enlargement of both the right atrium and ventricle, and a reduction in both left ventricular systolic and diastolic dimensions. Pericardial

effusion is also frequently detected. Doppler echocardiography may be used to estimate systolic pulmonary arterial pressure (PAP) on the basis of the peak velocity of the tricuspid valve regurgitant jet.

3.2.5 Pericardial Disease

In the pre-ART era pericardial effusion was one of the most common cardiac manifestations in HIV patients and it was an independent predictor of increased mortality. Effusions were more common in patients with advanced AIDS, most effusions were small and asymptomatic with unclear causes[206].
In the latest decades, the incidence of pericardial effusion has dramatically decreased, but not in areas of the world where ART is not readily available and where pericardial are prevalently due to tuberculosis[207]. Asymptomatic patients with pericardial effusions should have a follow-up echocardiogram every 1 – 2 years.
In general, patients with symptomatic HIV infection without cardiovascular abnormalities should have annual echocardiographic follow-up[208].

3.3. Speckle- Tracking Echocardiography (STE)

Speckle tracking is an echocardiographic technology that analyzes motion by tracking natural acoustic reflections (speckles, regions of interest) with relative angle independence, less noise interference and lower intra and inter-observer variability. Strain and strain rate are dimensionless measurements of deformation: strain is the myocardial deformation and strain rate (SR) is the rate of myocardial deformation (degree of shortening or stretching of fibers). The distances between speckles or their spatiotemporal displacement (regional strain velocity vectors) provide information about global and segmental myocardial deformation.
By estimating intra-tissue velocities, STE allows for discrimination between normal active myocardial segmental deformation versus passive displacement of a dysfunctional myocardial segment due to adjacent segment tethering and global cardiac motion.
As results of a large meta-analysis including 2,597 healthy volunteers, normal ranges of global longitudinal strain (GLS) varied from -15.9% to -22.1%. [209]A value above -20% with a standard deviation of +/-2% is likely to be normal.
The degree of shearing increases toward the sub-endocardium. The sub-endocardial region is assessed by longitudinal strain, while ischemic suffering of mid-myocardium and sub-epicardium leads to a reduction in circumferential and radial strain[210].

Since the introduction of ART, HIV-associated morbidity and mortality have decreased substantially, together with changing clinical presentation of cardiovascular disease in HIV patients. The decreasing prevalence of LV systolic dysfunction in PLWH has shifted the focus to more subtle changes in LV systolic and diastolic function which may be clinically silent particularly in the early stages of heart disease. LV function can be studied using various imaging techniques: among these, strain imaging is able to detect subclinical myocardial dysfunction at an earlier stage compared with other techniques[211].

Although conventional echocardiography is widely used to assess LV function [212], the GLS reflects this parameter more accurately detecting early dysfunction (LVSD) [213]. In addition, it has a prognostic value for patients with normal or near normal LVEF [214].

Some studies demonstrated that HIV-infected patients with normal LV systolic functions without cardiac symptoms had lower GLS than healthy individuals [215]. Literature offers conflicting results about a correlation between CD4 count and GLS in HIV-infected patients: a study demonstrated a directly proportional link between these values [216], unlike other studies [215,217]

3.4. Stress Echocardiography (SE)

SE is a robust and cost- effective method for both the diagnosis and risk stratification of patients with suspected or known CAD[218]. The appearance of reduced systolic wall thickening during SE, when myocardial oxygen demand exceeds myocardial blood supply, suggests the possibility of myocardial ischemia.

SE may be performed with exercise (treadmill or bicycle) or, in patients who are unable to exercise, with pharmacological agents such as dobutamine or dipyridamole. In any form of SE testing, echocardiographic images are acquired during rest intermediate and peak stress in parasternal long-axis, short-axis and apical 2-, 3- and 4-chamber views. These views of the heart visualize all three vascular territories. Particularly, dobutamine is used in patients who have an existing resting LV wall thickening abnormality. Low- dose dobutamine detects the presence of myocardial viability and high-dose dobutamine uncovers myocardial ischemia[218]. Dipyridamole is an alternative to dobutamine, but it is less likely to produce wall thickening abnormalities, even in the presence of significant coronary stenosis[219]. The presence of inducible wall motion abnormalities risk stratifies patients with suspected CAD: a normal SE carries an excellent prognosis and coronary angiography can safely be avoided.

Professional society recommended CAD screening in the HIV-infected population. However, specific tests and timing of cardiovascular disease screening in PLWH are not clear[220]. Some studies analyzed

these questions confirming that in the HIV population, dobutamine SE is highly specific for the noninvasive detection of obstructive symptomatic CAD. A cohort study revealed that SE risk-stratifies patients into a normal SE (low risk) and abnormal SE (high risk) groups. Patients with a normal SE have a benign prognosis, with an event rate comparable to the general population (<1% per year). Patients with an abnormal SE have poor outcomes and may benefit from aggressive interventions[221]. Moreover, evidence supported the value of Inotropic Contractile Reserve (ICR) in the risk stratification and prognosis of patients with ischemic and non-ischemic cardiomyopathy[222-223]. As a matter of fact, patients with HIV cardiomyopathy can be divided between high-risk (ICR absent) and low-risk (ICR present) groups. Patients without ICR had a yearly event rate 7 times higher than that those with ICR[224].

3.5. Cardiovascular Magnetic Resonance (CMR)

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique that combines a powerful magnetic field with radiofrequencies in order to produce three-dimensional detailed anatomical images without the use of ionizing radiation. Cardiovascular MRI (CMR) is gaining importance in clinical and interventional cardiology as the emerging diagnostic tool to evaluate the wide spectrum of cardiovascular disease. In a single exam, it can provide detailed information on cardiac and aortic anatomy, myocardial tissue characterization and valve morphology and function. CMR is considered the gold standard imaging modality to assess cardiac volumes and function and to quantify myocardial mass[225]. Recently, new sophisticated CMR-techniques of strain imaging have been introduced to study myocardial deformation and to detect subtle contractile changes. The most used technique is called Feature tracking-CMR and is is based on post-processing of standard cine images, similar to the strain analysis performed by echocardiographic speckle tracking, but with a lower observer variability [226] (Figure 2).

Valve pathology can be accurately evaluated through the use of different MRI sequences such as cine imaging and phase contrast velocity mapping. The greatest advantage of MRI over other imaging modalities is its unique ability to perform tissue characterization, providing an “in-vivo histology” based on the inherent different magnetic properties of human tissues. CMR is the gold standard noninvasive imaging modality to visualize and quantify myocardial oedema, by using T2-STIR and T2-mapping sequences and to assess myocardial fibrosis, by using late gadolinium enhancement (LGE) sequence for the evaluation of focal replacement fibrosis, and T1 mapping sequence, for assessing more diffuse patterns of interstitial fibrosis. Quantitative T1 imaging can be

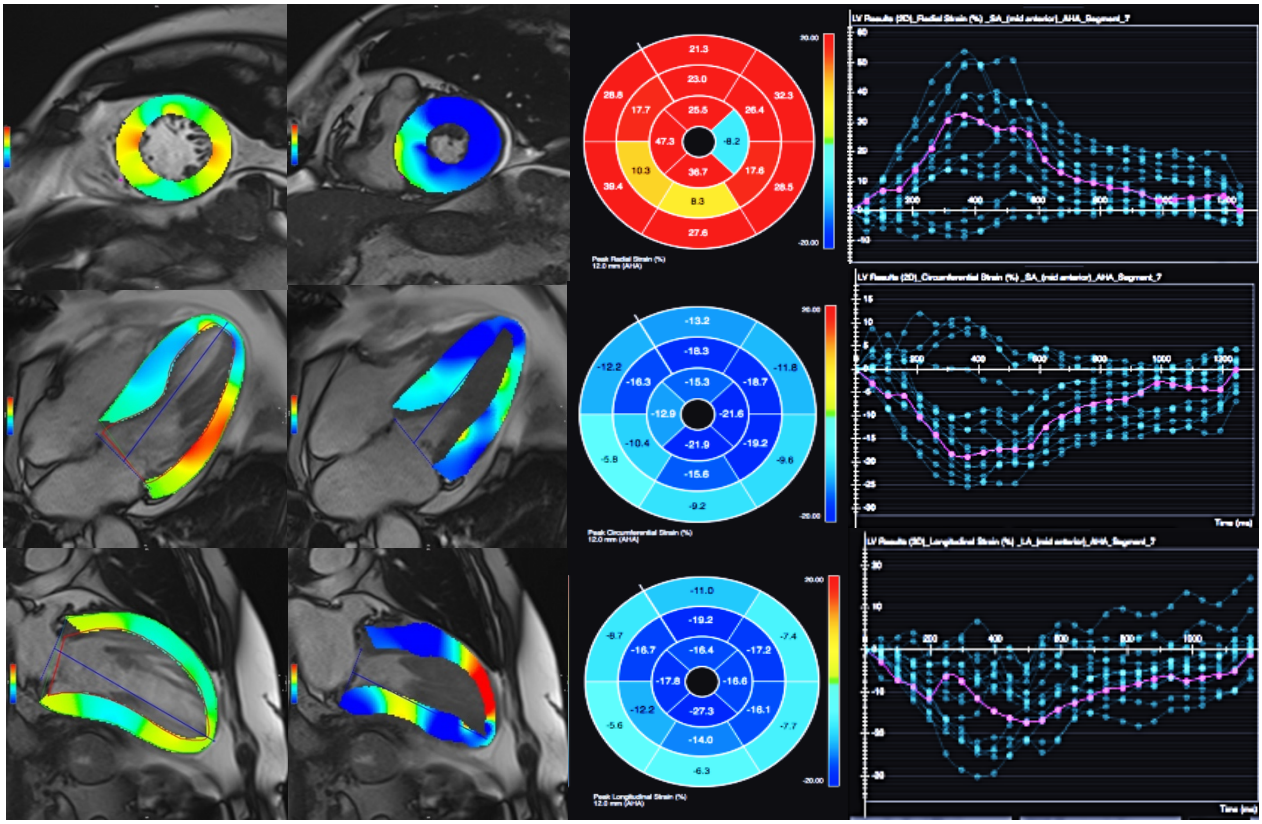


Figure 2: Myocardial deformation analysis by CMR- Feature Tracking. After the manual countouring of the left ventricle endocardial and epicardial borders in long- and short- axis cine images in end-diastole, radial, circumferential and longitudinal strain measurements are calculated by a dedicated software (Circle Medical imaging) in the 17 LV segments.

used to calculate the myocardial extracellular volume fraction (ECV), a measure of microscopic myocardial remodeling that has been associated with underlying diffuse fibrosis. CMR is an excellent tool not only in the diagnostic cardiovascular process, but also in prognosticating and in the long-term follow-up of patients. The evidence of myocardial late gadolinium enhancement is not only a marker of myocardial scarring/necrosis, as seen in ischemic heart disease but it represents a broader spectrum of myocardial damage in a variety of cardiomyopathies [227-228].

Aortic distensibility and pulse wave velocity are the two main parameters to assess aortic stiffness and can be accurately calculated by CMR. The 4D Flow MRI technique is one of the most valuable and visually-appealing example of a new tool with a great clinical potential as it allows for a sophisticated evaluation of the hemodynamics of the cardiovascular system both qualitatively and quantitatively [229]. The technique provides a three-dimensional representation of blood flow over time, thanks to a complete spatial and temporal coverage of a volume of interest by using the intrinsic magnetic properties of blood flow, without the use of a contrast agent. This technique provides a non-

invasive in vivo assessment of blood flow dynamics, enabling the analysis of parameters such as wall shear stress, turbulent kinetic energy, pressure difference and pulse wave velocity throughout the heart and major vessels of the cardiovascular system (Figure 3).

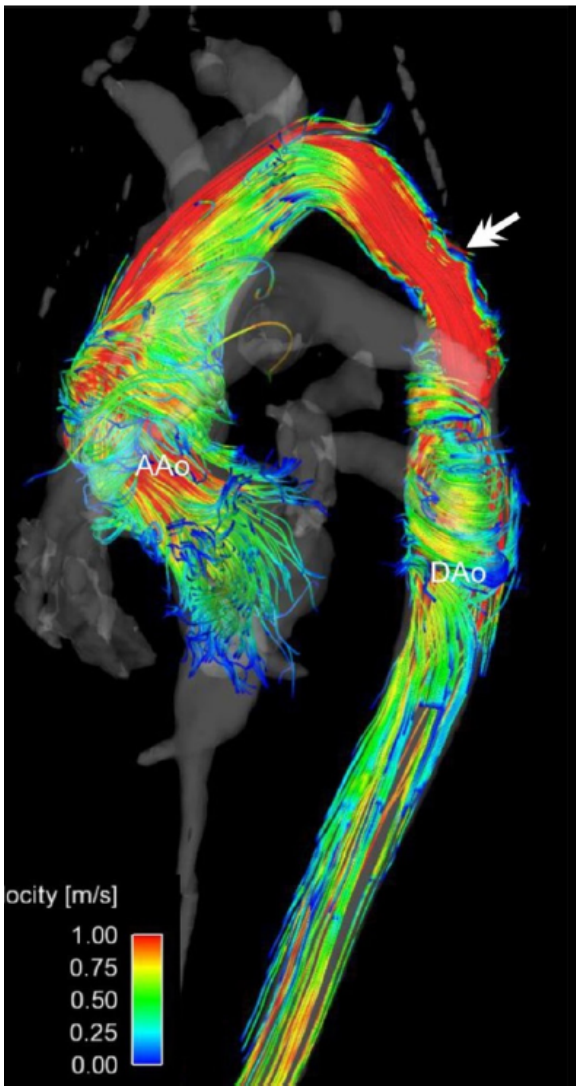


Figure 3: Aortic 4-D Flow MRI

To assess inducible myocardial ischemia, stress CMR uses myocardial perfusion sequences in the first pass of a gadolinium-based contrast agent bolus injection during pharmacologic vasodilation with adenosine, dypiridamole, dobutamine or regadenoson. In addition to stress and rest perfusion imaging for ischemia assessment, the basic stress perfusion CMR study also includes cine imaging for chamber volumes and function and late gadolinium enhanced imaging for scar and viability.

CMR myocardial perfusion imaging has a 2-3 mm of spatial resolution, highly superior to nuclear techniques, so that subendocardial ischemia is more easily identifiable[230].

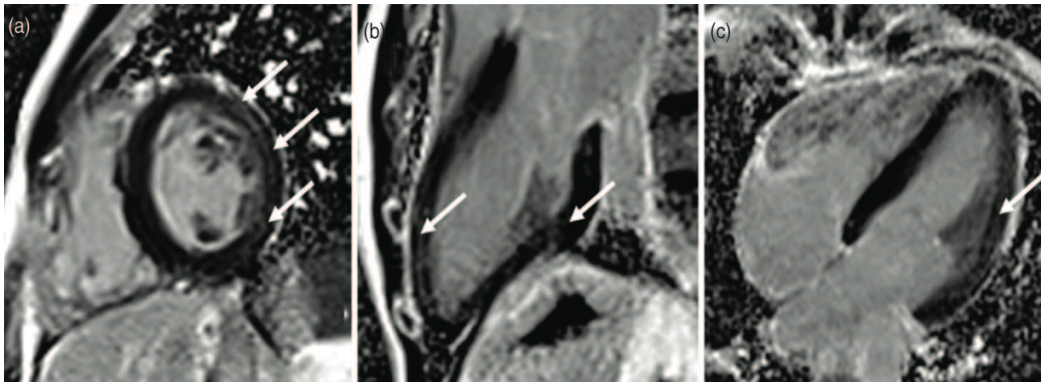


Figure 4. Cardiovascular magnetic resonance image of myocarditis in HIV. LGE image showing linear midwall fibrosis in the inferolateral and anterior walls in short-axis (a), vertical long-axis (b) and horizontal long-axis views (c).

Multiple studies demonstrated that myocardial inflammation and interstitial fibrosis, both focal and diffuse, investigated with T1 Mapping, have a higher prevalence in HIV-infected patients. Common findings in these CMR studies are impaired LV systolic function (as measured by strain parameters), lower LV ejection fraction, higher LV mass. [231] Holloway et al., in their CMR study, reported patchy myocardial fibrosis in HIV-infected patients predominantly in the basal inferolateral wall, a pattern consistent with previous myocarditis [231-232]. The higher sensitivity of CMR allowed demonstration of small pericardial effusions that even if not hemodynamically significant, support the presence a low-grade inflammatory state.

3.6. SPECT and PET

Myocardial perfusion imaging with Single-Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) is able to assess the functional significance of coronary lesions. With perfusion imaging during exercise and vasodilator stress, SPECT is used in a variety of clinical settings including stable angina and acute coronary syndrome [233]. Perfusion defects are observed not only in obstructive coronary disease but also in microvascular dysfunction and impaired coronary flow reserve. Few studies examined myocardial perfusion in HIV. A study of 105 HIV-infected adults and 105 matched controls found no difference in stress myocardial perfusion using

technetium-99 sestamibi SPECT[234]. Similarly, a Danish group found no difference in baseline myocardial perfusion and coronary flow reserve by PET in 25 HIV-infected subjects on ART compared with 14 controls[235].

3.7. Coronary Artery Calcium (CAC) Scoring and Coronary CT Angiography (CCTA)

Many CCTA studies tried to define atherosclerotic plaque in HIV-negative patients with CAD showing that fibrous and fibro-fatty plaque was present in 43.7% of patients, and calcific plaque was present in the remaining 56.3% of patients [236]. Coronary plaque characteristics in PLWH have been recently described. Autopsy studies in HIV-positive patients described eccentric atherosclerotic plaques determining up to 90% reduction of the vascular lumen, hyperplastic endothelial cells in a thickened intima and proliferation of smooth muscle cells and macrophages [237-238]. Cardiac CCTA studies conducted in asymptomatic HIV-positive patients found a higher prevalence of coronary atherosclerosis, coronary plaque volume and a greater number of involved coronary segments compared to HIV-negative patients with a similar Framingham 10-year risk for myocardial infarction [239] CAC scoring, using non-contrast enhanced Computed Tomography (CT), is a well-established tool for detection and quantification of coronary calcifications[240-241]. Applying different scoring systems, mainly the Agatston score, it is an excellent non-invasive atherosclerosis imaging modality characterized by high inter- and intra-observer reliability[242-243]. Coronary artery calcium was defined as CAC score >0 with the Agatston method [244]. Atherosclerotic plaque was defined as a lesion ≥1 mm2 within and/or adjacent to the vessel lumen [245]. Plaques were classified as calcified, non-calcified, or mixed. High-risk plaque is characterized by positive remodeling (remodeling index≥1.1) and/or low attenuation value expressed in Hounsfield Units (≤ 30 HU) [246]. In the general population, there is a strong correlation between CAC score and future CV endpoints. [242] In one large study, the predictive value of CAC for incident coronary heart disease and cerebrovascular disease exceed CIMT[247-248]. Literature suggested that CAC score change of ≥15% may suggest CAD progression[249]. CV event prediction may be improved when CAC is added to Framingham risk score [250]. CAC is present in almost all the atherosclerotic plaques causing obstruction of the coronary artery lumen, although it is also present in the majority of non-obstructive plaques [251]. Whether HIV infection accelerates atherosclerosis process is a matter of debate, but it is well-known that PLWH have a higher rate of cardiovascular events compared to controls of similar age and sex [252]. HIV-infected patients have significantly higher rates of myocardial infarction compared to controls, even in the setting of treated and suppressed viral disease. They have higher in-hospital mortality as compared to

uninfected individuals [253]. Current evidence about the link between HIV infection and CAC remains equivocal[254-255]. An increased CAC score was identified in Italian PLWH [256]. However, these findings were not confirmed in a Swiss cohort study [255]; several studies have found similar CAC scores in HIV-positive and HIV-negative persons. Moreover, in a study that compared CAC and carotid IMT in PLWH, more than 30% of HIV patients with undetectable CAC had markedly increased IMT, suggesting that in younger HIV-infected patients, carotid IMT may be a more sensitive indicator of subclinical vascular disease than CAC[256]. A major limitation of CAC is the inability to detect non-calcified plaque that represents an early stage of atherosclerosis and is more prone to rupture and thrombus formation compared than calcified lesions, then potentially leading to ACS .

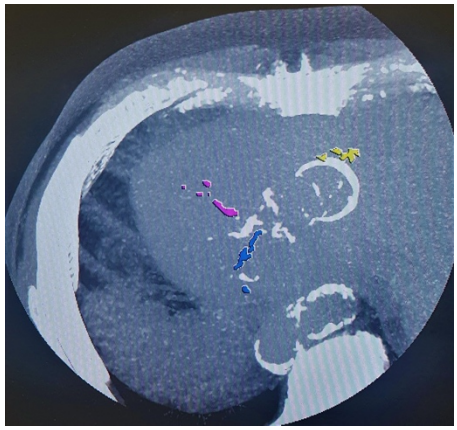


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Figure 5. Coronary artery calcium scoring in an asymptomatic HIV-positive patient

Coronary CT Angiography (CCTA) is a non-invasive imaging technique involving contrast-enhanced multi-slice CT that allows accurate morphological assessment of the coronary arteries [257]detecting calcified and non-calcified plaques (117). The use of electrocardiogram-triggered acquisition has contributed to lowering radiation dose exposure from 15–20 millisieverts (mSv) down to 2–3 mSv [258] CCTA is comparable to standard invasive coronary angiography in the detection of relevant coronary stenosis [259-261.] The vulnerable plaque is characterized by: a necrotic core of lipids and inflammatory cells surrounded by a thin fibrous cap; a positive remodeling (plaque segment diameter > reference segment diameter) [262]; adherent micro-calcifications[263]. It was demonstrated that non-calcified coronary artery plaque is predominant in patients <50 years of age (147). CCTA studies conducted in non-HIV-infected patients with ACS revealed that coronary plaques in ACS patients were more frequently vulnerable than plaques in non-ACS patients [264]. Sensitivity of CCTA in the detection of relevant coronary stenosis is similar to standard invasive coronary angiography .

From the histological point of view, in PLWH can be described predominantly non-calcified fibrous and fibro-fatty atherosclerotic disease. Necrotic core lesions were uncommon and dense calcified lesions were rare. Therefore, the presence of more vulnerable plaque could explain the higher prevalence and earlier onset of ACS in PLWH compared with HIV-negative patients. The mean age at presentation of ACS in HIV-positive patients is 50 years, a decade younger than the general population [265-266]. A meta-analysis (1229 HIV-positive and 1029 HIV negative participants) found a 3-fold higher prevalence of non-calcified coronary artery plaque on CCTA in asymptomatic HIV+ compared to controls. Disease severity may also contribute to atherosclerosis: it was reported the association between reduced CD4 cell count and the higher presence of non-calcified coronary artery plaque and coronary stenosis greater than 50% in PLWH compared to their uninfected counterpart (147). In the US-American multicenter AIDS cohort study (MACS) was recorded a higher prevalence of any coronary plaque and non-calcified plaque in 618 HIV+ compared to 383 HIV-negative men in the CCTA [267]. On the contrary, the Swiss HIV cohort study demonstrated a similar degree of non-calcified/mixed plaque and less coronary calcification with lower coronary atherosclerosis involvement in PLWH compared to their negative controls with similar Framingham risk score. These results are probably due to successful ART treatment, regular follow-up, and a lifestyle improvement.

A weakness of CCTA was the lack of the ability to predict hemodynamic relevance of coronary stenosis: the introduction of a novel technique called Computerized Tomography Fractional Flow Reserve (CT- FFR) allowed to exceed this limit. CT-FFR is a good technique for the assessment of hemodynamic effects of any stenosis. No data are available about the use of CT-FFR in PLWH.

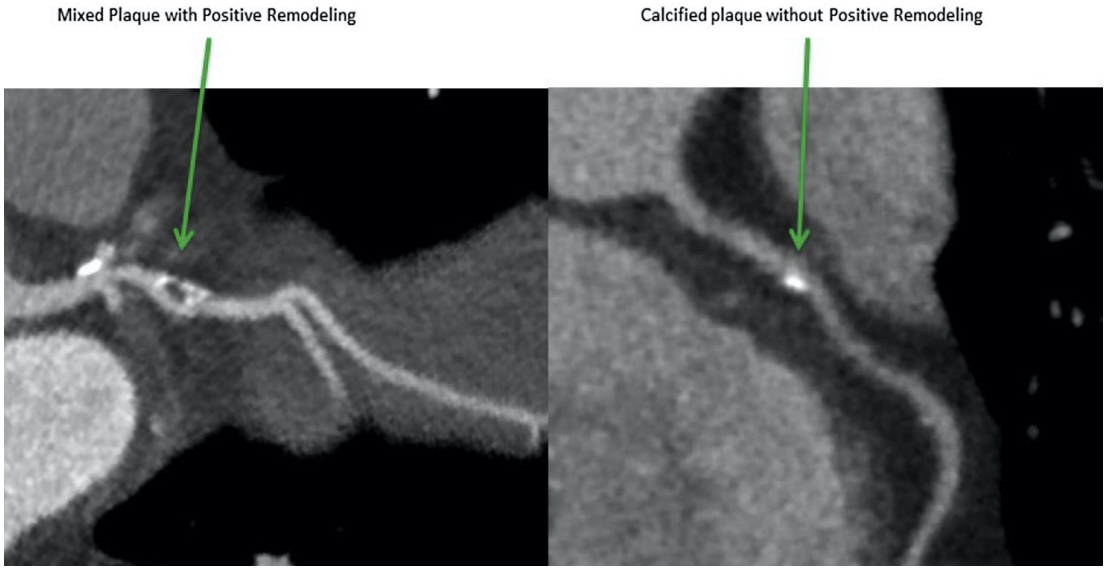


Figure 6. Example of calcified and mixed plaques in CCTA of HIV patient

3.8. Intravascular Ultrasound (IVUS) and Optical Coherence Tomography (OCT)

Intravascular methods of imaging atherosclerotic plaques such as IVUS and OCT are able to define more accurately the features of atherosclerosis in PLWH, including the prevalence of vulnerable plaque. These are invasive methods, so their use is limited to the detection of subclinical disease; however, they are useful in patients who are undergoing clinically indicated ICA. IVUS was developed over 20 years ago and is used widely to guide percutaneous coronary interventions. It can identify 4 kinds of plaque: soft, fibrous, calcified, and mixed[268]. OCT, with 10 times higher axial and lateral resolution compared to IVUS, can more accurately assess the thickness of an atheroma's fibrous cap, detect thrombus and coronary calcification; however, OCT cannot penetrate more than 1–3 mm into the vessel wall, limiting the ability to define the extent of vessel remodeling and total plaque burden[269]. To date, no studies have used intravascular imaging to characterize the features of atherosclerosis in PLWH.

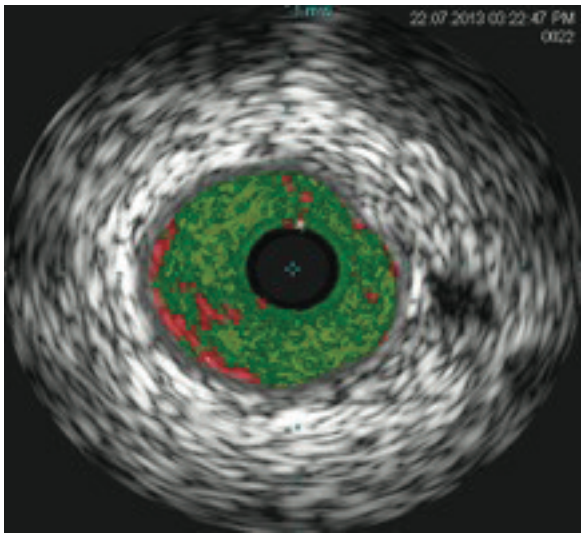


Figure 7. Example of intravascular ultrasound of non-calcified fibro-fatty plaque from HIV positive patient with acute coronary syndrome.

CHAPTER 4:
OUR STUDY

INTRODUCTION

The improved prognosis of HIV under the effective antiretroviral regimens increased the interest for other rampant comorbidities responsible for a rising share of the morbidity and mortality observed in these patients [15]. In contemporary, observational studies of HIV patients, the proportion of total deaths due to cardiovascular disease (CVD) ranged from 6.5% to 15%, with HIV infection alone conferring a 61% increased risk compared to uninfected individuals of comparable age and sex [15,16].

The pathophysiology of CVD in HIV is complex and multifactorial. The interplay of several items is involved, such as inflammation[17], autoimmune mechanisms, direct HIV-induced myocardial damage [18], side effects of HIV medications [270], nutritional factors, accelerated atherosclerosis [20] and increased burden of traditional cardiovascular risk factors.

HIV-infected patient can develop both coronary artery disease (CAD), due to accelerated atherosclerosis, and non-ischemic heart disease (NIHD), since the HIV infection may involve the pericardium, myocardium, cardiac valves and pulmonary circulation.

The clinical manifestations of CVD in HIV-infected patients differ from the general population, often presenting as unexpected and severe events suddenly interrupting a silent clinical history. A crucial issue is the lack of applicability of conventional risk scores, leaving healthcare providers without reliable decision-making instruments to select the appropriate diagnostic work-up and tailored treatment [123]. Thanks to the recent advances in technology, non-invasive multimodality cardiovascular imaging could be a powerful tool in providing new insights into the pathogenesis of CVD seen in the HIV-positive population and in identifying patients at-risk.

AIM OF THE STUDY

This study derives from a series of several admissions to our Unit of Structural Interventional Cardiology – AOU Careggi for ACS and end-stage heart failure among PLWH, with and without previous cardiovascular disease. The difficulty in recognizing and treating CVD in this subset of patients is due to the lack of tools for the identification of high-risk subjects. Clinical manifestations of CVD in PLWH are different from the general population, often presenting as unexpected and

severe events over a silent clinical history. Unfortunately, there are still many shadows and issues in the comprehension of the pathogenic mechanisms underlying cardiovascular disease in HIV-infected patients compared with general population.

The scope of the study is to identify:

1. The prevalence of cardiac disease and particularly of coronary artery disease in HIV-infected patients
2. The predictive factors of coronary artery disease in order to improve risk stratification in HIV population by analysing
   - clinical characteristics
   - molecular biomarkers
   - role of ART
3. An optimal strategy for early diagnosis of coronary artery disease in HIV patients, focusing on the use of multimodality diagnostic cardiovascular imaging modality.

METHODS

Patients selection

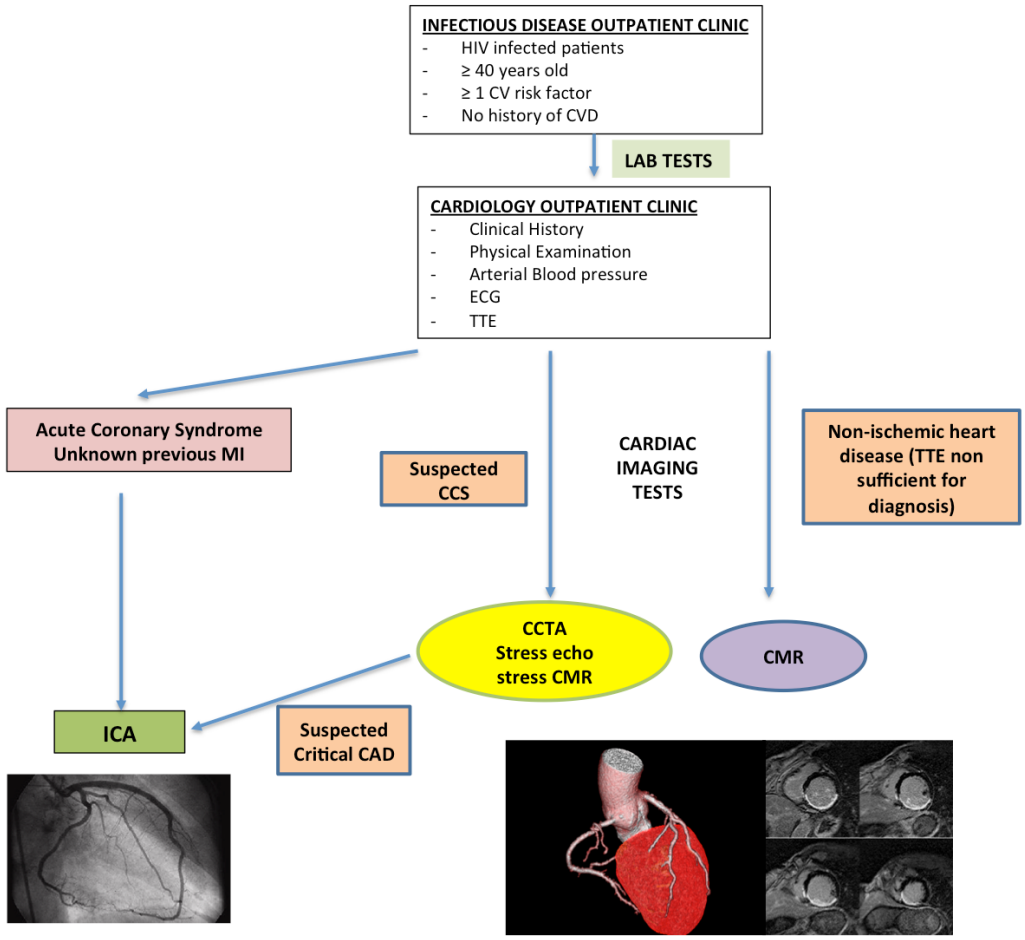
This study is a cross-sectional study conducted at the Infectious Disease outpatient clinic of our tertiary care university hospital. From November 2017 to March 2020, HIV infected patients meeting the inclusion criteria were consecutively enrolled and addressed to the cardiovascular diagnostic work-up program (Diagnostic Flowchart, Figure 1).

The patients have been recruited considering the following inclusion criteria:

- HIV-infection, as documented by positive HIV antibody test and confirmed by an antigen test
- Age>=40 years old
- At least one cardiovascular risk factor among current smoke, hypertension, dyslipidaemia, diabetes

Exclusion criteria were as follows:

- Inability to provide informed consent
- Known cardiac disease



TTE, transthoracic echocardiogram; CAD, coronary artery disease; CMR, cardiac magnetic resonance; CCS, chronic coronary syndrome; MI, myocardial infarction; ICA, invasive coronary angiogram, CCTA, computer coronary tomographic angiography.

***Figure 1**: Diagnostic flow chart of the cardiovascular work-up*

Lab tests

The viro-immunologic profile of each patient has been examined, focusing in particular on HIV duration, duration of antiretroviral therapy (ART) use, CD4+ nadir, zenith viremia, and the presence of detectable viremia. Likewise, we searched for other co-infections (HBsAg positivity, anti-HBc positivity, HCV co-infection) and recorded the antiretroviral therapy administered. The ongoing three drugs ART regimens at the time of the cardiologic visit were divided by the anchor drugs as follows: reverse transcriptase-inhibitor (NNRTI), protease inhibitor (PI), and integrase strand transfer inhibitor (INSTI). Where this definition was not applicable (three-drugs regimen with 1 or 3

nucleoside/nucleotide reverse transcriptase inhibitors [NtRTI/NsRTI], not boosted PI, PI monotherapy and more or less than three drugs) we classified as others. The panel of laboratory tests included general blood tests like full blood count, glycemia, HbA1c, renal and hepatic function, lipid profile (total cholesterol, LDH/HDL cholesterol, triglycerides, Lp[a]), NT-proBNP, hsPCR and IL-6 were collected within 3 months before the cardiology visit.

Cardiovascular diagnostic work-up

HIV-infected patients enrolled in the study were referred to the outpatient Cardiology clinic for a comprehensive cardiology evaluation with the following routine data collection:

- Clinical history and physical examination
- Arterial blood pressure measurement
- 12-lead ECG
- Transthoracic echocardiogram (TTE)

Clinical characteristics, laboratory test results and pharmacological therapy data were collected to assess the Framingham Risk Score and the DAD score calculated with the software elaborated by CHIP (*Centre for Health & Infectious Disease Research)* of the Copenhagen University, the DAD score. The ASCVD has been calculated by the "ASCVD Risk estimator" available on the official website of the American College of Cardiology / American Heart Association (ACC/AHA).

According to the diagnostic flow-chart (Figure 1), patients presenting clinical, ECG, and/or echocardiographic features of unstable angina or with evidence of previous silent myocardial infarction were admitted to hospital to perform an invasive coronary angiogram (ICA). Patients with clinical, ECG, and/or echocardiographic features suggestive for a chronic coronary syndrome (CCS) underwent a second-level non-invasive imaging test (coronary computed tomography angiography (CCTA) and/or stress echocardiography/stress cardiac MRI) as recommended by current guidelines[271].

If obstructive CAD was suspected (coronary stenosis > 50% at the CCTA examination and/or positive stress echocardiogram/MRI), hospital admission was scheduled to perform ICA. Liberal use of intracoronary physiologic measurements was encouraged when required.

Patients with evidence of cardiomyopathy, moderate-to-severe valvular disease not properly assessable by TTE, or with pericardial effusion underwent Cardiac Magnetic Resonance. Counselling for lifestyle modification and smoking cessation was offered to all patients with modifiable cardiovascular risk factors. The baseline therapy of all patients was carefully evaluated and modified,

if necessary, based on the current European guidelines on CVD prevention[272] and after careful verification of any drug interactions with the ongoing antiretroviral therapy, through consultation of the reference tabs drafted by the University of Liverpool (https://www.hiv-druginteractions.org/).

Non-invasive imaging tests for CAD

Non-invasive cardiac imaging tests were performed at the Radiology Unit and at the Cardiology outpatient clinic of Careggi Hospital.

CCTAs were performed using a 128-slice dual-source CTA system (SOMATOM Definition Flash, Siemens Healthineers, Forchheim, Germany) and followed scanning protocols satisfying Society of Cardiac Computed Tomography quality standards [273]. The detector collimation was 2×64×0.6 mm, and a flying focal spot technique and a gantry rotation time of 280 msec were used. Both tubes were operated at 100 or 120 kV, depending on the body mass index of the patient. Oral and/or intravenous beta-blockers or oral ivabradine were administered if necessary, in an attempt to achieve a target heart rate <60 beats/min. The contrast medium (Iomeron 400, Bracco Altana Pharma, Konstanz, Germany) and saline chaser were administered into an antecubital vein. Datasets for coronary arteries were reconstructed with a slice thickness of 0.6 mm, an increment of 0.4 mm, a field of view of 180 mm, a medium-soft convolution kernel (B26). All reconstructed images were transferred to a dedicated workstation (MMWP, Siemens Healthcare, Forchheim, Germany). Axial images, multiplanar reformations, and maximum intensity projections were used to evaluate coronary arteries (Figure 2).

Stress echocardiography was performed with a commercially available ultrasound scanner (iE33 platform; Philips Medical Systems, Andover, Massachusetts, USA) equipped with X3–1 (1– 3 MHz) matrix transducer and S5-1 (1–5 MHz) probe. Dipyridamole was administered at a dose of 0.56 mg/kg in 4 min followed by 4 min of no dose and then 0.28 mg/kg in 2 min, cumulative dose being therefore 0.84 mg/kg in 10 min; aminophilline was administered at the 12th minute after peak stress imaging completion. Echocardiographic images were semiquantitatively assessed using a 17 segments, 4-point scale model of the left ventricle (Figure 3). Wall motion score index (WMSI) was derived by dividing the sum of individual segment scores by the number of interpretable segments. Ischemia was defined as stress-induced new and/or worsening of pre-existing wall motion abnormality, or biphasic response (i.e. low-dose improvement followed by high-dose deterioration). A test was considered positive for ischemia when at least 2 adjacent segments of the same vascular

territory showed an increment of WMSI (worsening or regional function) of at least 1 point at peak stress.

Stress CMR were performed with a 1.5-T scanner (Siemens) according to the recommendations of the Society of Cardiovascular Magnetic Resonance (SCMR) [274]. Steady-state free precession cine acquisitions were then acquired at rest during held expiration in multiple short axis and three additional long-axis views (two-, three-, and four chambers) of the left ventricle. Vasodilatation was induced with dipyridamole injected at 0.84 mg/kg over 6 min. At the end of dipyridamole infusion, 0.1 mmol/kg of Gadolinium BOPTA (Multihence, Bracco, Milan Italy) was injected intravenously at 4 mL/s followed by saline solution with concomitant acquisition of three short-axis views (using the same geometry of rest imaging) of the left ventricle with first-pass perfusion technique using saturation-prepared T1-weighted fast gradient-echo sequence. Steady-state free precession cine acquisitions were then acquired at stress with the same geometry used at rest. Theophylline was intravenously injected (240 mg i.v.) to null the effect of dypiridamole at the end of the stress test. Ten minutes after contrast injection, breath-hold contrast-enhanced segmented T1-weighted inversion-recovery gradient-echo sequence was acquired with the same prescriptions for cine images to detect late gadolinium enhancement (LGE). The inversion time was individually adjusted to null normal myocardium. CMR datasets were transferred to a dedicated workstation and analyzed with a cardiac software using the 17-segment model for the myocardium (Figure 3). A perfusion defect was considered ischemic if ≥2 consecutive segments in at least three consecutive phases were affected.

Definitions

Cardiovascular disease was defined as any clinical condition involving heart, great vessels and coronary arteries requiring therapy and/or follow-up.
CAD was defined as the evidence of ≥1 coronary plaques ≥ 30% at the CCTA or ICA examination. Obstructive CAD was defined as coronary stenosis ≥75% in ≥1 vessels (≥50% in the left main coronary artery) at the ICA or if Fractional Flow Reserve (FFR) was <0.80.

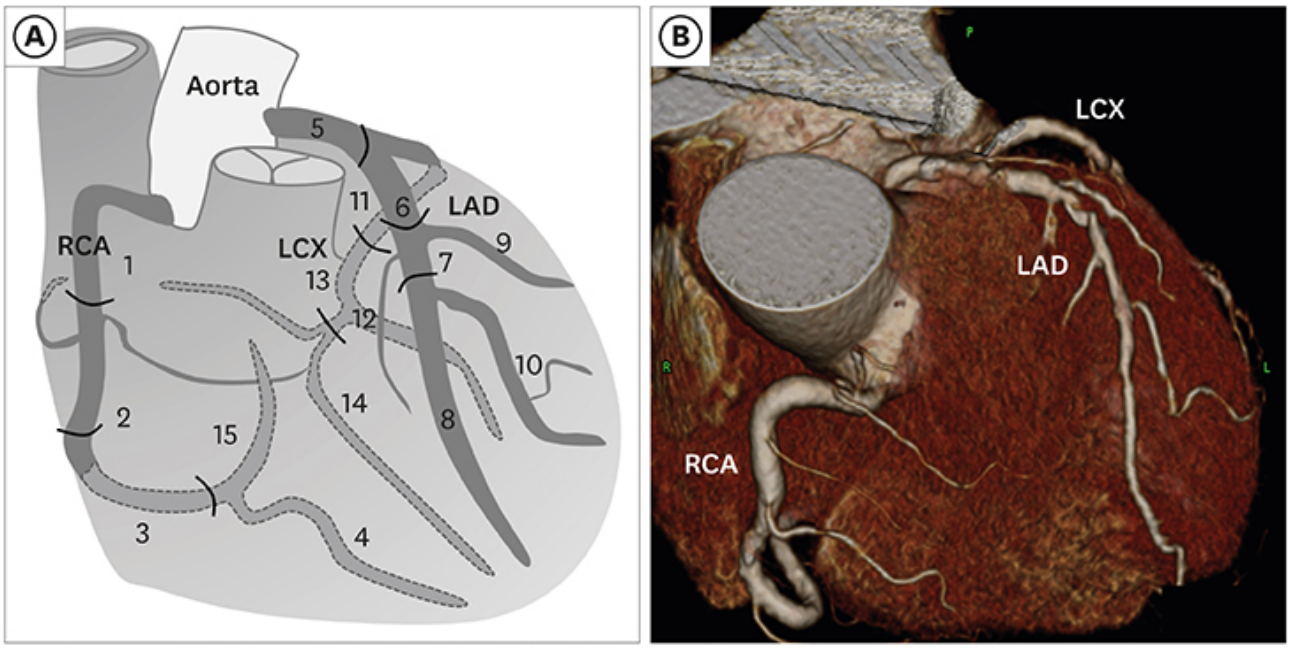


Figure 2. The anatomy of the coronary arteries (A) and the representative CCTA images of coronary artery disease (B).

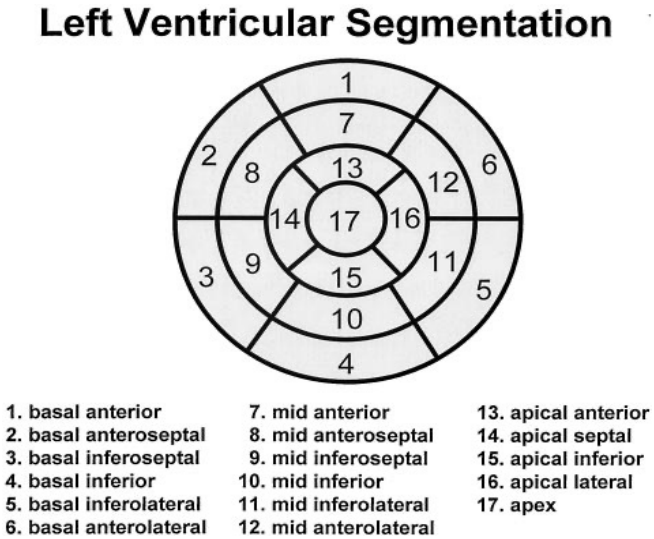


Figure 3: Display, on a circumferential polar plot, of the 17 myocardial segments and the recommended nomenclature for tomographic imaging of the heart.

STATISTICAL ANALYSIS

Continuous variables were expressed as mean (M) ± standard deviation (SD) when normally distributed or as median and interquartile range (IQR) when not normally distributed. Categorical variables were expressed as number (N) and percentage (%). The Student 2-sample t-test, Mann–Whitney–Wilcoxon rank-sum test were used to compare the differences between groups for continuous variables. X^2 and Fisher exact test were used for categorical variables where appropriate. All statistical analyses were performed using a standard software package (Stata, version 14.0; StataCorp). Two-sided probability values were considered significant at $p<0.05$.

RESULTS

Basic features of the investigated population

150 HIV-infected patients older than 40 years of age have been referred for cardiovascular diagnostic work-up. Of those, 117 patients met the inclusion criteria.

The baseline characteristics of the investigated population are summarized in **Table 1**.

Of the patients evaluated, 91 were males (77.7%) and 26 females (22.3%). The mean age was 56 years. The most frequent transmission route resulted in being sexual one: heterosexual in 50.8 % of cases, and 36.8 % in the male who have sex with males (MSM). 7.7% through intravenous drug use, 5.9 % of patients abused illicit drugs, and 5.9% of alcohol.

In **Table 2** the viro-immunologic characteristics at baseline of the population are listed. The median of years of positivity to HIV was 21 (range 0-34), while the median of ART treatment was 19 years [IQR 11-22]. 91.2 % had no detectable viremia (<20 copies/mL) in plasma. The co-infections recorded were 9.8 % HBV, 18.6 % HCV and 23.5 % syphilis. 14.7 % of patients had a previous AIDS event, and 5.9% had an acute retroviral infection. As for ART, the Integrase strand transfer inhibitors (INSTI) were the most used anchor drug (53%). As shown in **table 3,** 50% of patients had less than two comorbidities, 32.3 % had between 3 and 4, and 17.7% had more than four comorbidities. **Table 4** lists the cardiovascular (CV) risk factors, baseline therapy, arterial blood pressure, and ECG interpretation. 53% had dyslipidemia, 47% were current smokers, 37% suffered from hypertension, and 9% were diabetic. Of note, 29.1% of them presented a single CV risk factors, whereas 27.3% of them presented ≥ 3 CV risk factors.

Tab 1. Demographic characteristics

Demographic characteristics	***n=117 (%)***
Age (years)	56 ± 9
Sex	
Female	26 (22.2%)
Male	91 (77.7%)
Risk	
Heterosexual	59 (50.4%)
MSM	43 (36.8%)
IVDU	9 (7.7%)
other	6 (5.1%)
Ethnicity	
Caucasic	102 (87.2%)
African	6 (5.1%)
Hispanic	5 (4.3%)
Middle-Eastern	2 (1.7%)
African-American	1 (0.9%)
South-Est Asiatic	1 (0.9%)

MSM, men who have sex with men; IVDU, intravenous drug user

Tab 2. Viro-immunologic characteristics

Viro-immunologic characteristics	***n=117***
Duration HIV (y, median, IQR)	21, 13-26
Duration ART use (y, median, IQR)	19, 11-22
Nadir CD4+ (cells/µL), median, IQR	251, 159-332
Zenith viremia (copie/mL), median, IQR	60000, 7000-207000
Drug abuse	6 (5.1%)
Alcohol Abuse	6 (5.1%)
Detectable viremia	9 (7.7%)
HBsAg positivity	10 (8.5%)
anti-HBc positivity	32 (27.3%)
HCV co-infection	19 (16.2%)
Syphilis	24 (20.5%)

Previous AIDS event	15 (12.8%)
Acute retroviral infection	6 (5.1%)
Single Tablet Regimen	51 (43.5%)
Previous exposure to Abacavir	44 (37.6%)
Last therapy	
INSTI	53%
NNRTI	24%
PI	3%
NRTI	1%
Others	19%

ART, antiretroviral therapy; PI, protease inhibitors; NRTI, Nucleoside analogue reverse-transcriptase inhibitors; INSTI, Integrase strand transfer inhibitors; NNRTI, non-nucleoside analogue reverse-transcriptase inhibitors;

Tab 3. Charlson Comorbidity index

Charlson Comorbidity Index	
<2	50 %
3-4	32.7 %
>4	17.4 %

Tab 4. Results from cardiology visit

	n=117
Cardiovascular risk factors	
Smoke (current)	55 (47%)
Smoke (previous)	31 (26.5%)
Hypertension	43 (36.8%)
Diabetes	11 (9.4%)
Dyslipidemia	62 (53%)
Family history of CAD	27 (23.1%)
1 CV risk factor	34 (29.1%)
2 CV risk factors	51 (43,6%)

≥ 3 CV risk factors	32 (27.3%)
Framingham risk score	16.7 ± 12.9
Baseline Therapy	
Statins	19 (16.5%)
Ezetimibe	3 (2.6%)
Fibrates	3 (2.6%)
Diuretics	10 (8.7%)
Ca-antagonists	13 (11.3%)
ACE-I	19 (16.5%)
ARBs	11 (9.6%)
Alfa-blockers	3 (2.6%)
Beta Blockers	10 (8.5%)
Arterial Blood Pressure	
SBP (mmHg)	135, 121-143.5
DBP (mmHg)	80, 75-89
MAP(mmHg)	97, 90-106
ECG	
Sinus rythm	116 (99.1%)
Atrial Fibrillation	1 (0.09%)
Heart rate (bpm)	74±13

CAD, coronary artery disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; ACE-I, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers;

Results of the cardiovascular diagnostic work- up

Of the 117 patients evaluated in the study, one patient showed ECG and TTE findings of previous silent myocardial infarction, and one patient had features of unstable angina; both were hospitalized with high priority to perform an ICA. Non-invasive second level imaging test was performed in 27 patients with suspected CCS. CCTA was performed in 24 patients and stress-echocardiography in 8 patients and. Stress CMR was performed in 3 patients. Nine of them underwent ICA in the suspicion of obstructive CAD. 12 patients with evidence of NIHD underwent CMR.

Prevalence of cardiac disease

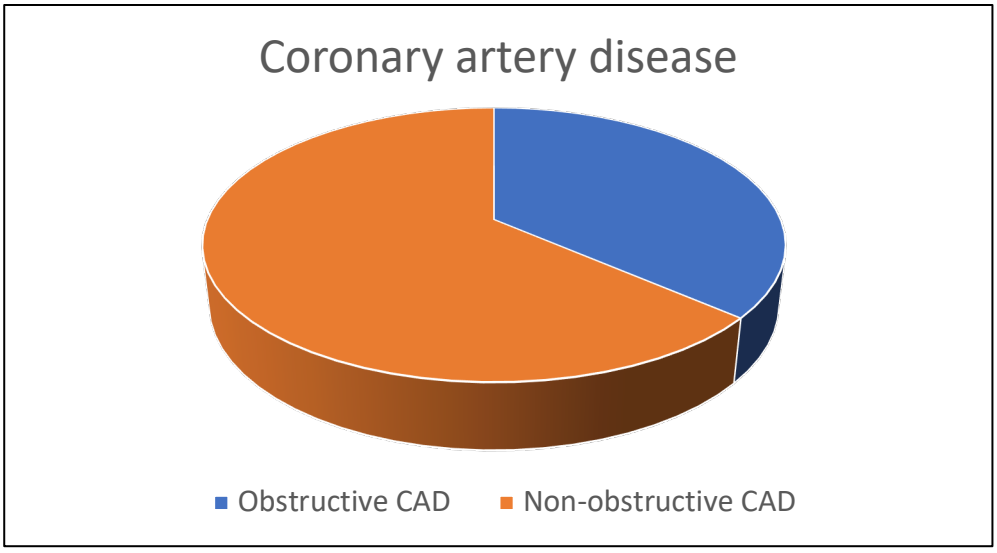
Of the 117 patients evaluated in the screening, 52 (45%) had evidence of cardiovascular disease (**Table 5**). Of them, 22 presented CAD, whereas 47 cases showed a NIHD. In 17 cases both conditions were present. ICA confirmed a obstructive CAD in 8 patients: 5 of them were treated with percutaneous coronary interventions, 2 with aorto-coronary by-pass (CABG), one presented a chronic coronary occlusion with non-viable myocardium and remained on medical treatment. Three patients undergoing ICA and 11 patients assessed non-invasively with CCTA showed a non-obstructive CAD requiring medical therapy and follow-up. Among the 8 patients with obstructive CAD, 4 were asymptomatic, 2 reported atypical chest pain, and 2 complained of exertional dyspnoea.

The NIHD group was broader and more heterogeneous. Hypertensive cardiomyopathy was the most frequent condition, with 21 cases diagnosed (18%). 12% of patients showed valvular disease of moderate or severe degree and 8% thoracic aortic dilatation (>40 mm). Pericardial effusion, pulmonary hypertension, and left ventricle (LV) systolic dysfunction were also found in some patients (Table 5, Figure 2). None of the five patients with pericardial effusion showed evidence of acute myocarditis at the CMR analysis. Two cases of hypertrophic cardiomyopathy and one patient with atrial fibrillation were also detected.

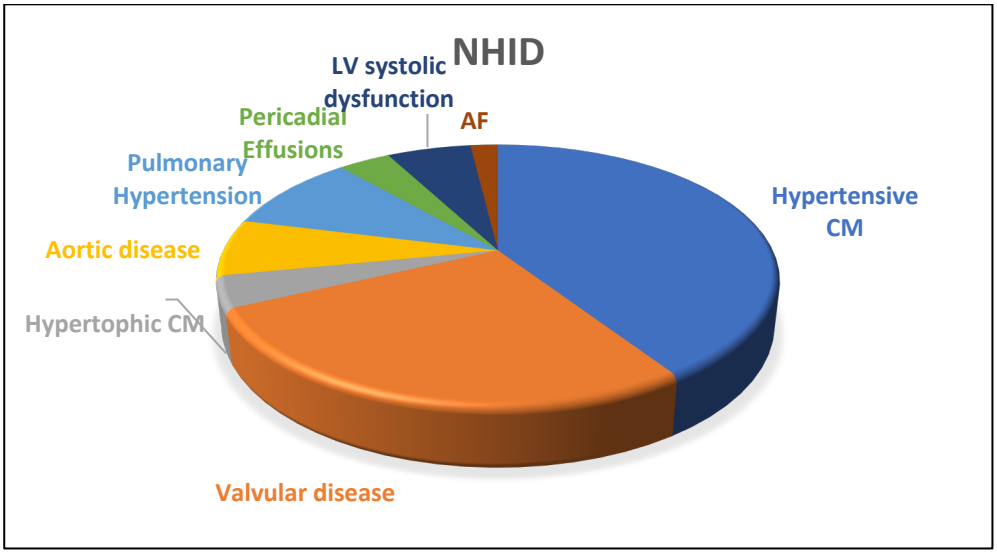
Tab 5. Results of the diagnostic work-up: prevalence of cardiovascular disease

Prevalence of CVD	**n=117**
CVD	52 (45%)
Coronary artery disease	22 (18.8%)
Obstructive CAD	8 (6.8%)
Non-obstructive CAD	14 (11.9%)
Non-ischemic heart disease	47 (40.1%)
Hypertensive Cardiomyopathy	21 (17.9%)
Aortic disease	9 (7.7%)
Pericardial effusions	5 (4.3%)
Valvular disease (moderate-to-severe degree)	14 (11.9%)
Atrial Fibrillation	1 (0.8%)
Hypertrophic cardiomyopathy	2 (1.7%)
Pulmonary Hypertension	5 (4.3%)
LV systolic dysfunction	3 (2.6%)

CVD, cardiovascular disease; CAD, coronary artery disease; LV, left ventricle



Graphic 1: Prevalence of CAD in our population



Graphic 2: Prevalence of NIHD in our population

Risk of CVD

As reported in **Tab 6**, patients with CVD were older than those without CVD (60.7±8.8 vs 53.4±7.3; <0.001). 15 patients were excluded from the analysis due to incomplete clinical or laboratory test. Hypertension and diabetes were significantly associated with the development of CVD (respectively $p<0.001$ and $p<0.05$), whereas smoking and dyslipidaemia did not show a significant association. Patients with higher levels of BP at the visit and glucose levels at the laboratory exams had an increased risk of CVD. The duration of HIV infection, the presence of detectable viremia and the impact of the last therapy on the CVD prevalence were not significantly associated with the development of CVD.

Tab 6. Univariate analysis: CVD vs. no-CVD

	CVD *n=52*	**no CVD** *n=50*	**P value**
Age (years)	60.7±8.8	53.4±7.3	**<0.001**
Males	43 (82.7%)	37 (74%)	0.28
Smoke (current)	25 (48%)	20 (40%)	0.36
Smoke (previous)	13 (25.5%)	15 (30%)	0.61
Hypertension	27 (53%)	10 (20%)	**0.001**
Diabetes	8 (15.7%)	2 (4%)	**0.05**
Dyslipidemia	30 (58.8%)	23 (46%)	0.20
Detectable Viremia (>50 copies/mL)	5 (9.6%)	4 (8%)	0.77
Duration HIV (years)	20.3±7.5	20.1±7.5	0.55
Duration ART use >10 years	41 (78.6%)	39 (78%)	0.92
Last Therapy			
INSTI	24	21	0.19
NNRTI	11	13	
PI	2	1	
NRTI	1	0	
SBP (mmHg)	140±17	128±14	**<0.001**
DBP (mmHg)	83±11	78±10	**0.02**
MAP (mmHg)	100±19	92±13	**0.02**
With Blood Cells (10^9/L)	6393±2003	6675±2013	0.48
HbA1c (mmol/mol)	39±12	38±7	0.56
Glucose(mg/dL)	109±37	94±14	**0.01**
Col Tot (mg/dL)	196±37	201±35	0.50
LDL (mg/dL)	117±40	119±36	0.84
HDL (mg/dL)	53±23	48±16	0.2
Tg (mg/dL)	168±117	173±167	0.87
Lp(a)	285±385	312±411	0.71
NT-proBNP	91±111	77±85	0.49
hs-CRP (mg/L)	2,4 + 1,7	3,4 + 3	0.20
IL-6 pg/ml	9,04±13,8	5,06±4,0	0.059
IL-6 (>10 pg/mL)	9 (19.6%)	5 (10.4%)	0.21

CVD, cardiovascular disease; ART, antiretroviral therapy; PI, protease inhibitors; NRTI, Nucleoside analogue reverse-transcriptase inhibitors; INSTI, Integrase strand transfer inhibitors; NNRTI, non-nucleoside analogue reverse-transcriptase inhibitors; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HbA1c, Glycated hemoglobin; Lp(a), lipoprotein(a); NT-proBNP, N-terminal prohormone of brain natriuretic peptide; LDL, low-density lipoprotein; Tg, triglycerides; hs-CRP, high sensitivity C-reactive protein; IL-6, interleuchin-6.

Risk of CAD

As for the CVD group, patients with CAD were older with a mean age of 60.2±7.0 years, compared to the 56.4±9.1 shown by the patients without CAD (p=0,049, Table 7). In the development of CAD, gender demonstrated to be statistically significant as all patients with CAD were male (p=0.007). Current smoking was positively associated to CAD (66.7% vs 38.0%; p=0.02), as well as hypertension (62% vs 30% p=0.007). Although the blood glucose and HbA1c level were higher in CAD group than in the group without CAD, the prevalence of known DM among patients with CAD was higher but not statistically significant when compared with the group without CAD (P=0,115). The lipid profile did not differ significantly in the two groups, with patients affected by CAD presenting even lower total cholesterol, LDL, triglycerides, and higher HDL levels compared to patients without CAD (Table 7). The inflammatory marker IL-6 was found to be to statistically higher in who had CAD respect to who did not (cut-off > 10 pg/ml; 28% vs 11,3%, P value 0.04).

Tab 7. Univariate analysis: CAD vs. no-CAD

	CAD n=22	**no-CAD n=80**	**p value**
Age (years)	60.2±7	56.4±9.1	**0.049**
Males	22 (100%)	59 (73.7%)	0.007
Detectable Viremia	3 (13.6%)	6 (7.5%)	0.77
Duration HIV (years)	21±7.5	19.9±7.6	0.444
Duration ART use (years)			
>10	18 (81.8%)	62 (77.5%)	0.363
Last Therapy			
INSTI	11	44	0.970
NNRTI	5	19	
PI	1	2	
NRTI	0	1	
Smoke (current)	14 (63.6%)	31 (38.7%)	**0.02**
Smoke (previous)	3 (13.6%)	25 (31.3%)	0.122
Hypertension	13 (59.1%)	24 (30%)	**0.007**

Diabetes	4 (18.2%)	6 (7.5%)	0.115
Dyslipidemia	10 (45.5%)	43 (53.8%)	0.617
SBP (mmHg)	144.7±18.6	131.5±15.7	**0.003**
DBP (mmHg)	87±11	79±10	**0.006**
MAP (mmHg)	106±13	93±19	**0.007**
White Blood Cells (10^9/L)	6955±2218	6421±1943	0.278
Glucose(mg/dL)	116±44	98±23	0.0196
HbA1c (mmol/mol)	44±18	37±6	**0.012**
Col Tot (mg/dL)	189±34	201±36	0.187
LDL (mg/dL)	115±35	119±39	0.731
HDL (mg/dL)	54±13	52±21	0.088
Tg (mg/dL)	174±117	170±149	0.894
Lpa	267±278	306±380	0.67
NT-proBNP	80.5±109	85.4±97	0.8437
hsCRP (mg/L)	1.9 + 1,7	3,3 + 3,2	0.23
IL-6 (pg/ml)	10.7 ± 11.6	6.1 ± 9.7	0.08
IL-6 (>10 pg/mL)	7 (32%)	8 (10%)	**0,04**

CVD, cardiovascular disease; ART, antiretroviral therapy; PI, protease inhibitors; NRTI, Nucleoside analogue reverse-transcriptase inhibitors; INSTI, Integrase strand transfer inhibitors; NNRTI, non-nucleoside analogue reverse-transcriptase inhibitors; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HbA1c, Glycated haemoglobin; Lp(a), lipoprotein(a); NT-proBNP, N-terminal prohormone of brain natriuretic peptide; LDL, low-density lipoprotein; Tg, triglycerides; hs-CRP, high sensitivity C-reactive protein;

From the results of the multivariate analysis (**Table 8)**, it is possible to evince that IL-6, current smoking and hypertension are strong risk factors for CAD in HIV infected patients. In particular, IL-6 > 10 pg/ml has been found to be positively associated to CAD with an Odd Ratio of 4,52 (IC 95%; p=0,042) also adjusted with the other variables.

Table 8. CAD in HIV patients, multivariate analysis

	Odds Ratio	**IC 95%**	**P value**
IL-6 (>10 pg/mL)	4,52	1,05 – 19,46	0,042
ART duration (years)	1,01	0,97- 1,05	0,42
Smoker (current)	6,13	1,51-24,85	0,011
Diabetes	3,16	0,59-16,82	0,17
Renal failure	1,53	0,10-23,64	0,75
Aids	0,95	0,21-4,14	0,95
Hypertension	8,04	1,99-32,35	0,003

Sub-analysis of CCTA data

A sub-analysis of our study considered all patients who underwent CCTA regardless of previous history of heart disease.
In our study a total of 40 patients underwent CCTA evaluation, of them 24 patients without previous history of cardiovascular disease. We excluded from the analysis 1 patients due to suboptimal image quality (severe artifacts impairing accurate evaluation) and 1 patients with history of coronary artery revascularization.

Al the 38 CCTA exams evaluated were performed during the period November 2017- October 2019 in our hospital as previously reported. Datasets for coronary arteries were reconstructed and transferred to a dedicated workstation (MMWP, Siemens Healthcare, Forchheim, Germany). Axial images, multiplanar reformations, and maximum intensity projections were used to evaluate arteries.

We performed 3 different analysis:

Per-patient analysis:
Based on the results of CCTA, we categorized patients into three groups by visual estimation of coronary stenosis:

1) No-CAD, patients with normal CCTA scans, or with evidence of minimal CAD, defined as a coronary stenosis <30% in principal branches of the left or right coronary artery; 2)
2) CAD, defined as a coronary stenosis ≥30% in principal branches of the left or right coronary artery; in this group we defined Non-obstructive-CAD, as ≥30-50% stenosis and Obstructive-CAD as >50% stenosis.

Per-segment analysis:
Coronary artery segments were classified according to a modified model of American Heart Association [10]. Segments were evaluated if the luminal diameter met or exceeded 1.5 mm.
Atherosclerotic plaque was defined as a lesion> 1mm in orthogonal reconstructions within and/or adjacent to the vessel lumen, not belonging to surrounding tissue.

For each patient we calculated:

1) The segment involvement score (SIS), a measure of overall coronary artery plaque distribution, is the total number of coronary artery segments exhibiting plaque, irrespective of the degree of luminal stenosis within each segment (minimum= 0;maximum=16).

2) The segment stenosis score, a measure of overall coronary artery plaque extent, is based on the extent of obstruction of coronary luminal diameter. Each individual coronary segment was graded as having no plaque to have severe plaque (scores from 0 to 3). Then the extent scores of all 16 individual segments were summed to yield a total score ranging from 0 to 48.

3) 3-vessel plaque score was calculated as 0 or 1 based on the coexisting presence of any plaque in the left anterior descending, left circumflex, and right coronary arteries, irrespective of severity.

Per-plaque analysis

Plaques were classified as calcified, non-calcified, or mixed (Figure 4).

1) Calcified atherosclerotic plaque was defined as any structure with attenuation >130 HU visualized separately from the intravascular lumen, identified in at least two independent planes.
2) Soft atherosclerotic plaque was defined as any structure that could be clearly assignable to the vessel wall, with a CT density less than the contrast-enhanced coronary lumen but greater than the surrounding connective tissue, identified in at least two independent planes.
3) Mixed plaque included lesions with less than 50% of plaque area occupied by calcium.

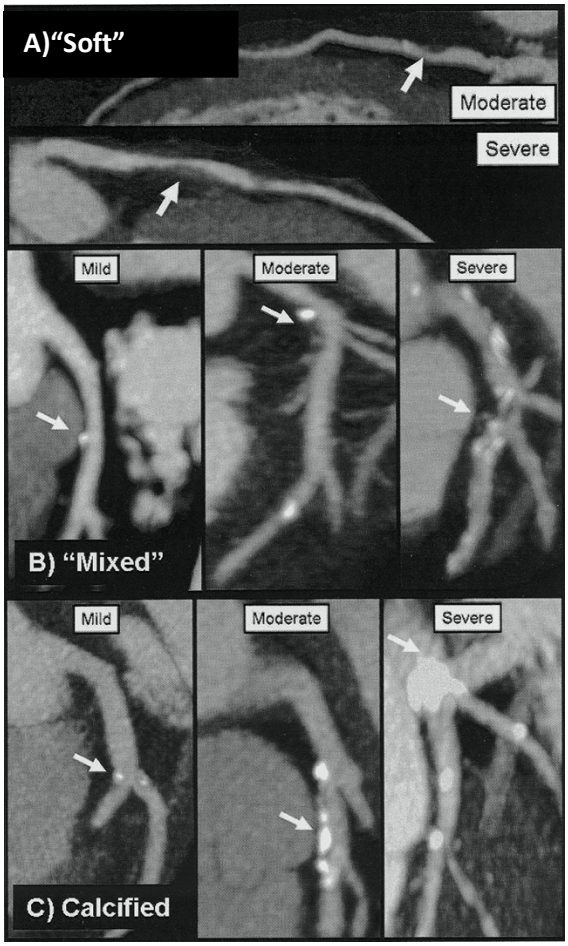


Figure 4. Grading of Plaque Severity: **(A)** Examples of moderate and severe extent of soft plaque of the left anterior descending artery. **(B)** Examples of mild, moderate, and severe extent of mixed plaque of the left anterior descending artery. **(C)** Examples of mild, moderate, and severe extent of calcified plaque of the left anterior descending artery.

Results

Of the 38 CCTA scans analyzed, patients averaged 59 ± 8.4 years of age, and 92% were males. Normal scans of evidence of minimal CAD was identified in 12 exams (31.5%), non-obstructive CAD in other 12 patients (31.5%) and Obstructive CAD in 14 patients (37%). As for plaque characteristics, (Table 9).

Table 9: Results from the CCTA analysis

Total CCTA	N= 38
No CAD	12 (31.5%)
CAD	26 (68.5%)
- Non-obstructive CAD	12 (31.5%)
- Obstructive CAD	14 (37%)
SIS (median, IQR)	3 (1-6)
SSS (median, IQR)	7 (2-13)
3 vessel plaque score	12 (31.5%)
N° segments with soft plaques (median, IQR)	0 (0-2)
N° segments with calcific plaques (median, IQR)	0 (0-1)
N° segments with mixed plaques (median, IQR)	0 (0-3.5)

CAD, coronary artery disease; SIS, segment involvement score; SSS, segment stenosis score, IQR, interquartile range.

We compared with t-student test and chi-square test the patients with and without evidence of CAD at CCTA. The two group did not differ significantly for age, sex, virological characteristics, traditional risk factors or Lipid profile. DAD score was the only score significantly higher in patients with CAD. Moreover, patients with CAD have increased values of IMT (2.2. ± 1.1 vs 0.9 ± 0.7, p = 0.03). Interestingly, none of the laboratory parameters examined differed in the two group, except from IL-6: patients with CAD showed higher levels of IL-6, with a mean of 9.5 ±2.9 pg/ml vs 2.3 ±2 pg/l of patients without evidence of CAD (p=0.01) (Figure 5) and in 75% of patients with CAD, IL-6 was > 5 pg/ml vs 10% of patients without CAD (p=0.001) (Table 10).

Table 10: Sub-analysis of CCTA: patients with no CAD vs patients with CAD

	No CAD	**CAD**	**p value**
Age (years)	56 ± 9	61 ± 8	0.170
Males %	96.4	80	0.098
Detectable Viremia (>50 copies) (%)	11	0	0.300
Duration HIV (years)	21 ± 5	21±7	0.966
Duration ART use	17 ±7	19 ± 6	0.463
Smoke (current) (%)	70.8	40	0.092
Smoke (previous) (%)	16.7	20	0.800
Hypertension (%)	50	40	0.605
Diabetes (%)	12	0	0.240
Dyslipidemia (%)	52	50	0.902
Charlson Comorbidity Score	2.9 ±1.6	3.7 ± 2.2	0.328
IMT mean (mm)	0.9 ± 0.7	2.2. ± 1.1	**0.030**
SBP (mmHg)	118 ± 44	137 ±35	0.178
DBP (mmHg)	71 ± 26	81 ±21	0.219
CV risk scores			
Framingham	15 ±16	21 ± 17	0.362
DAD	8 ± 4.6	18 ± 8.7	**0.013**
ASCVD	10 ± 8.3	17 ± 9.1	0.081
Lab tests			
With Blood Cells (10^9/L)	6746 ± 2484	7008 ± 1964	0.741
HbA1c (mmol/mol)	37 ± 8	40 ±10	0.436
Glucose(mg/dL)	100 ± 19	101 ± 19	0.829
Total Cholesterol (mg/dL)	192 ± 40	201 ± 35	0.481
LDL (mg/dL)	99 ± 31	117 ± 33	0.151
HDL (mg/dL)	46 ± 11	47 ± 14	0.783
Triglycerides (mg/dL)	186 ± 220	181 ± 102	0.926
Lp(a)	407 ± 540	281 ± 256	0.360
NT-proBNP	44 ± 31	90 ± 132	0.295
hs-CRP (mg/L)	2,1 ± 1,9	3,5 ± 3,1	0.213
IL-6 pg/ml	2.4 ± 2.2	9.6 ± 8.9	**0.018**
IL-6> 5 pg/ml (%)	10	75	**0.001**

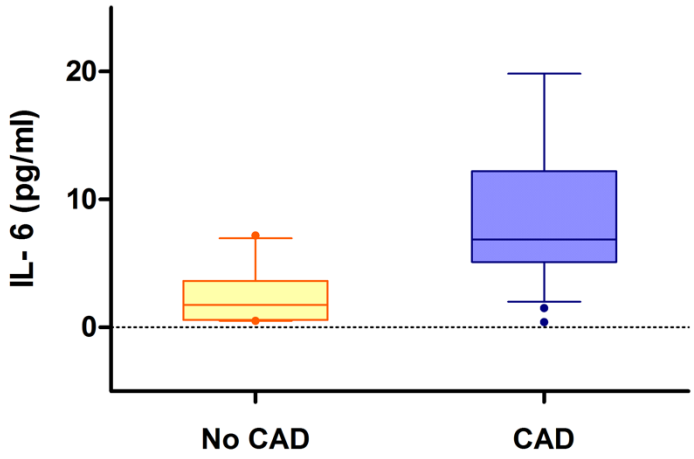


Figure 5: Blood levels of IL-6 according to the presence or absence of CAD ad CCTA

We repeated the analysis separating patients in other two groups, those who showed at the CCTA obstructive CAD (≥ 50% stenosis of the vessel lumen), and patient without obstructive CAD (0-50% stenosis). Increased levels of IL-6 appear to be strongly correlated to the presence of obstructive CAD: 85% of patients affected by obstructive CAD showed IL-6 levels >5 pg/ml, compared to 35% of those who had no obstructive CAD (p=0.003, Table 11)

Table 11: Sub-analysis of CCTA: No Obstructive CAD vs Obstructive CAD

	No Obstructive CAD	**Obstructive CAD**	**p value**
Age (years)	59 ± 9	60 ± 8	0.85
Males (%)	90	93.8	0.748
Detectable Viremia (>50 copies)	4.5	13.3	0.336
HCV positivity (%)	13.6%	13.3%	0.979
Duration HIV (years)	21 ± 7	21 ± 6	0.796
Duration ART use	17± 6	19 ± 7	0.456
Smoke (current) (%)	50%	83.3%	0.56

Smoke (previous) (%)	18%	16.7%	0.912
Hypertension (%)	40.9%	58.3%	0.331
Diabetes (%)	4.5%	16.7%	0.234
Dyslipidemia (%)	59.1%	36.4%	0.218
Charlson Comorbidity Score	3.2 ± 1.7	4 ± 1.5	0.260
IMT mean (mm)	1.6 ± 1.2	2.2 ± 1.2	0.317
SBP (mmHg)	131 ± 35	132 ± 45	0.936
DBP (mmHg)	78 ± 21	79 ± 26	0.972
CV risk scores			
Framingham	17 ± 13	22 ± 21	0.351
DAD	11.4 ± 6.0	21.4 ± 9	**0.01**
ASCVD	12.7 ± 7.2	20.1 ± 10.9	0.048
Lab tests			
With Blood Cells (10^9/L)	6640 ± 2046	7400 ± 2140	0.293
HbA1c (mmol/mol)	37.9 ± 6.4	43 ± 13.8	0.172
Glucose(mg/dL)	100 ± 16	101 ± 23	0.837
Col Tot (mg/dL)	196 ± 36	202 ± 37	0.641
LDL (mg/dL)	107 ± 30	119 ± 37	0.279
HDL (mg/dL)	51± 12	40 ± 12	0.018
Tg (mg/dL)	160 ± 154	218 ± 113	0.237
Lp(a)	404 + 427	196 + 181	0.095
NT-proBNP	39 ± 30	129 ± 161	**0.018**
hs-CRP (mg/L)	2.5 ± 2.8	3.8 ± 2.9	0.220
IL-6 pg/ml	5.5 ± 6.3	10.1 ± 10	0.105
IL-6> 5 pg/ml (%)	35%	85.7%	**0.003**

Finally we evaluated the CCTA characteristics of patients who expressed IL-6≥ 5 and IL< 5 pg/ml according to the severity of CAD, segments involvement and plaque characteristics **(Table 12).** 60% of patients without significant CAD expressed levels of IL-6 lower than 5 pg/ml whereas only a minority of them had IL-6 higher that 5 mg/ml (5.3%, p = 0.001). Conversely, the majority of patients who were found to have obstructive CAD (63%) had levels of IL-6 > 5 pg/ml and only 13,3% of them had lower IL-6 (p=0.003) (Figure 6). Increased levels of IL-6 correlated with the degree of coronary segment involvement (p=0.01 for SIS, p=0.03 for SSS) and with the burden of mixed plaques but no association was found between IL-6 and soft or calcific plaques.

Table 12: CTA angiographic characteristics of patients with blood level of IL-6 inferior and superior to 5 pg/ml.

	IL-6 < 5 pg/ml n=15	**IL-6 ≥ 5 pg/ml** n=19	**P value**
Analysis by patient			
- No CAD	9 (60%)	1 (5.3%)	0.001
- Non-obstructive CAD	4 (26.7%)	6 (31.5%)	0.5
- Obstructive CAD	2 (13.3%)	12 (63.2%)	0.003
Analysis by segment			
- SIS	1.9 ± 2.1	4.5 ± 2.9	0.01
- SSS	3.4 ± 4.3	10.2 ± 6.8	0.03
- 3 vessel plaque score	13.3%	44.4%	0.053
Analysis by plaque			
- N° segments with calcified plaques	0 (0-1)	0 (0-1)	0.526
- N° segments with mixed plaques	0 (0-0)	2 (0-5)	0.008
- N° segments with soft plaques	0 (0-0)	0 (0-2.2)	0.120

CAD, coronary artery disease; SIS, segment involvement score; SSS, segment stenosis score, IQR, interquartile range.

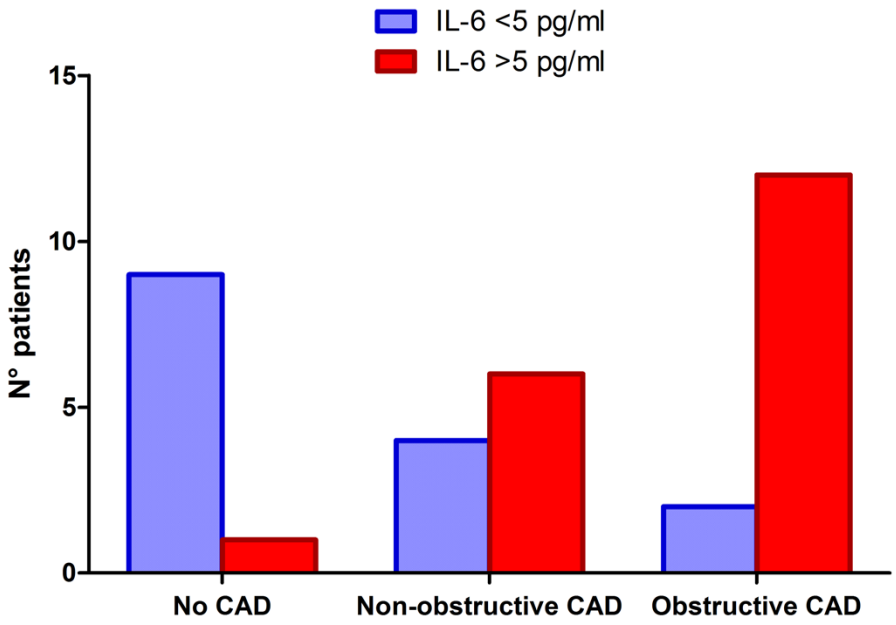


Figure 6. CCTA results in patients with levels of IL-6 inferior or superior to 5 pg/ml

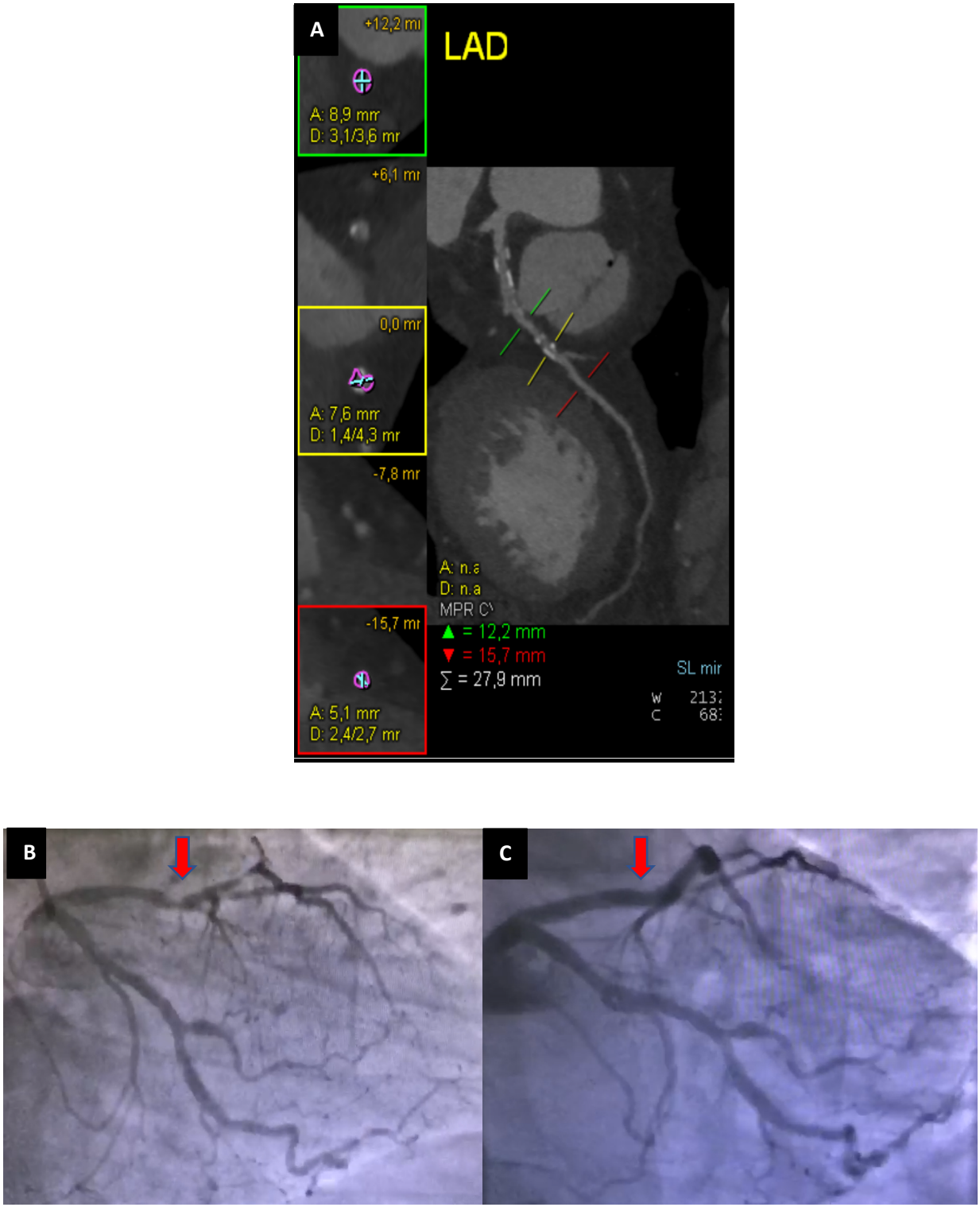


Figure 7. CCTA of a 53-years old HIV patients symptomatic for shortness of breath on exertion showed a long calcific plaque in the LAD with a stenosis> 50% of the vessel lumen (A) . ICA confermed a severe stenosis (90%) of the mid-LAD (B), treated with percutaneous coronary intervention with shockwave in our hospital with optimal angiographic result (C).

DISCUSSION

The HIV infection alone appears to confer a 61% increased risk for cardiovascular disease compared with the general population [16,46]. Furthermore, the prolongation of life expectancy in the past two decades obtained with the introduction of cART means that HIV-infected people have been affected by more comorbidities and age-related non-communicable diseases. The pathogenic mechanisms underlying CVD in HIV-infected patients are still debated and risk stratification tools to predict the risk in this population are lacking[74]. The current study shows a surprisingly high prevalence of CVD in HIV-infected patients > 40 years old with at least one CV risk factor. Of the 117 patients enrolled in our study, 45% showed a cardiovascular disease. Of note, 10% of the cohort examined required hospitalization to perform ICA in the suspicion of critical CAD, which was confirmed in 8 patients out of 11.

Importantly, none of these patients presented with typical chest pain, and half of them were completely asymptomatic. They are highlighting the importance of performing a comprehensive cardiac evaluation in all HIV patients at increased cardiovascular risk, even if they do not complain of typical symptoms for CAD, to unmask silent but potentially life-threatening cardiac conditions.

The algorithm that we applied for the diagnosis of CCS followed the current European guidelines[271] recommending either non-invasive anatomical imaging using CCTA or functional imaging as the initial test to rule out or establish the diagnosis of CCS in high-risk patients. In non-HIV patients, CCTA demonstrated strong prognostic value in patients with suspected CAD. The Prospective Studies PROMISE and SCOT-HEART have shown the incremental value of CCTA over the conventional practice in investigating patients with suspected angina[275]. Moreover, technological developments have also led to a marked reduction in radiation dose. Imaging with most recent dedicated CT scanners can be performed with low (3–5 mSv) and ultra-low (<1 mSv) radiation doses[276]. Non-invasive functional imaging such as stress echocardiography or stress CMR is an alternative to CCTA in the diagnosis of myocardial ischemia, and it is recommended if coronary CTA has shown CAD of uncertain functional significance or is not diagnostic. Although exercise-ECG was the most widely used provocative test in clinical practice, recent guidelines have downgraded its indication due to its low sensitivity, moderate specificity, and frequent equivocal results. For these reasons, exercise ECG was avoided in a population at high risk of CV events as the HIV one due to its low accuracy. In contrast, non-invasive advanced imaging modalities such as CCTA were extensively applied in patients with suspicion of CCS. The benefit is not only to diagnose flow-limiting CAD but also to detect non-obstructive CAD and thus to identify patients who could benefit from aggressive preventive medications (i.e., aspirin or statins) and lifestyle interventions. This

allowed us to limit invasive angiography to a small minority of patients, with angiography confirming in most of them severe coronary artery disease.

Non-Ischemic heart disease was frequent in our population, with an overall prevalence of 40.1%, and included a heterogeneous cluster of conditions, with hypertensive heart disease being the most frequent.

Similarly to the general population, hypertension appears to play an essential role in the development of CVD also for our HIV patients. It is of clinical relevance that an SBP mean value overcoming slightly the target for treatment established by the EACS guidelines (SBP<140) [277], was found both in the group with evidence of CVD (140±17; p<0,001) and in the sub-group with CAD (144±18; p=0,007). As for Diastolic blood pressure (DBP), the mean values remained under the target for both groups (DBP<90). There is no tailored guideline for hypertension treatment in HIV patients, forcing physicians to adopt the same approach applied to the general population. However, this data suggests that BP targets may be lowered in HIV patients such as in other high-risk patients such as those with diabetes, starting lifestyle modifications and drug interventions as soon as BP exceeds the upper range of normal.

Patients with CVD, and particularly those with CAD, showed an increased prevalence of glucose metabolism disorders. Conversely, we did not find any significant association between CVD and the alteration of the lipid profile. In fact, despite a high prevalence of dyslipidaemia in the whole study group (51.9%), higher (58,8%) among those who had CVD, these findings were not supported by statistical significance.

Our study is in line with previous reports concerning the current smoking habit, which appeared strongly associated with the development of CAD. These results confirmed the importance of counselling for quitting the smoking habit in this high-risk group of patients.

The population we examined was well treated for their HIV infection, presenting a detectable viremia (>20 copies/μL) only in 9.6% of patients. The same prevalence was found in the group with CVD, regardless of the time of infection and ART treatment. This data is similar to other studies[39], where it has already been clarified the emerging role of inflammation and immune activation as drivers of comorbidities, despite an optimal control of viremia and CD4+ T cells level [78].

The Framingham score was unable to stratify the cardiovascular risk in our population, confirming, as previous studies stated [122], whereas DAD score appears to be superior in discriminating patients with CAD.

In our study several non-traditional risk factors for cardiovascular disease have been evaluated: in particular, we explored various patterns of inflammation linked to HIV infection. IL-6 is a pleiotropic

cytokine produced by several cell types including monocytes, fibroblasts and endothelial cells which appears to have a central role in the development of atherosclerosis is general population and is currently under investigation as a potential therapeutic target [278].
In our study IL-6 was found to be higher in HIV patients with CAD (28% vs 11,3%; P value 0,04) . On the contrary, hs-CRP elevation, even if greater in CAD group than in HIV patients without CAD, was not significantly associated with coronary disease in our analysis (p value 0,52). Thus, in our study IL-6 has appeared superior to hs-CRP in predicting CVD and CAD. Hs-CRP seems, therefore, to be a less specific epiphenomenon of inflammatory states. In fact, hs-CRP is one of the acute-phase proteins produced by the liver in response to inflammation, and less specific for inflammation associated to a significant atherosclerotic burden in our patients.

In the sub-analysis of HIV patients who underwent CCTA exams, IL-6 confirmed to be positively strongly correlated with the presence, the degree and the burden of CAD in HIV patients, assessed visually with semiquantitative methods. In particular, levels of IL-6 > 5 pg/ml strongly correlated with obstructive disease (p=0.003), with the coronary segments affected (p=0.01), with the severity of coronary segments with atherosclerosis (p=0.03)and with the burden of mixed plaques, whereas no association was found between IL-6 and soft or calcific plaques, likely due to the exiguity of our sample examined. Furthermore, ultrasonographic measurement of carotid IMT was found to be increased in patients with obstructive CAD, suggesting a potential role of this non-invasive, cost-effective and widely available method to detect early atherosclerosis in risk stratification of HIV patients.

These findings suggest that inflammation and chronic activation are complex and important drivers of atherosclerosis in HIV positive patients and open the perspective for new therapeutic strategies targeting inflammation, through the inhibition of IL-6 or its receptor, to reduce cardiovascular risk in this subset of patients.

Furthermore, these results suggest the urgency to elaborate new risk stratification algorithms tailored for the HIV patients that should include non-conventional cardiovascular risk factors such as the biomarkers of inflammation. In particular, our study suggests a potential role of IL-6 in identifying patients at high-risk for coronary artery disease, superior than the conventional cardiovascular risk factors adopted for general population.

The main limitation of this study regards the small dimension of the investigation as well as the lack of longitudinal follow-up data, which could evaluate the significant benefit in terms of survival and reduction of major adverse cardiovascular events caused by the application of the diagnostic and therapeutic algorithms applied in this study. Moreover, a match with an HIV-negative control group is welcome to evaluate differences in the prevalence of CVD in comparable cohorts.

CONCLUSION

A comprehensive cardiovascular diagnostic work-up, including new advanced multimodality diagnostic imaging modalities, demonstrated to be a useful tool to ensure early diagnosis and better management of CVD in HIV patients.

This study confirmed the relevance of chronic inflammation on the accelerate development of atherosclerosis. Increased blood levels of IL-6, a marker of inflammation, emerged as being significantly associated to the development of CVD and CAD. Further analysis of expanded samples are necessary to confirm it, but the results of this study suggest that IL-6 holds promise to represent a novel therapeutic target to reduce cardiovascular risk in HIV positive patients and a good risk stratification tool to identify patients at high risk of cardiovascular events.

We believe that the application of tailored cardiovascular diagnostic pathways and adequate risk stratification could lead to the early diagnosis of CVD and, together with a the strict control of cardiovascular risk factors and prompt management of the disease, can modify the natural course of cardiovascular disease in HIV patients.

FUTURE PERSPECTIVES

- Comparison of our population with a group of controls negative for HIV infection and with at least one cardiovascular risk factor with the purpose to evaluate differences about prevalence of CVD and plaque characteristics at coronary imaging.
- Further investigation about the weight of each cardiovascular risk factor in the development of CVD. Showing strong evidences would permit the production of clear and tailored guidelines for the risk reduction of HIV-infected patients.

- Creation of a new cardiovascular risk tools tailored for the HIV-infected patients that should include biomarkers of inflammation and immune activation, particularly focusing on the role of IL-6 in predicting CAD in HIV patients.
- Comparing and screening the cardiovascular profile of naïve-patients vs experienced ones. This would help the comprehension of pathogenic mechanisms without confounding factors derived from ART and aging.
- HIV Team: a team of physicians who mixed their different expertise in the evaluation and management of all the comorbidities of HIV patients, which with the progressive increase of aging are meant to increase

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