

REVIEW

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# What happens to cardiovascular system behind the undetectable level of HIV viremia?

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## Abstract

Despite the combined antiretroviral therapy has improved the length and quality of life of HIV infected patients, the survival of these patients is always decreased compared with the general population. This is the consequence of non-infectious illnesses including cardio vascular diseases. In fact large studies have indicated an increased risk of coronary atherosclerotic disease, myocardial infarction even in HIV patients on cART. In HIV infected patients several factors may contribute to the pathogenesis of cardiovascular problems: life-style, metabolic parameters, genetic predisposition, viral factors, immune activation, chronic inflammation and side effects of antiretroviral therapy. The same factors may also contribute to complicate the clinical management of these patients. Therefore, treatment of these non-infectious illnesses in HIV infected population is an emerging challenge for physicians. The purpose of this review is to focus on the new insights in non AIDS-related cardiovascular diseases in patients with suppressed HIV viremia.

**Keywords:** Cardiovascular diseases, cARV, HIV, Premature aging

## A change of perspectives in the management of HIV infection: from an infectious disease to an internal medicine illness

The introduction of potent combined antiretroviral therapy (cART) has dramatically increased the survival of HIV infected patients and has changed their causes of morbidity and mortality from AIDS-related opportunistic infections to serious non AIDS events (SNAEs) [1].

Currently achieving undetectable viral load has become the minimum objective achievable with antiretroviral therapy; otherwise the safety of the therapy, the reduction of residual viremia, the eradication of the virus from the reservoirs and the reduction of the process of immune activation and chronic inflammation are still challenges for scientific research.

In particular to date is not well cleared the mechanisms for the establishment and maintenance of chronic immune activation. All available data suppose the presence of multiple and complex mechanisms

acting synergistically and may contribute to the pathogenesis of SNAEs: viral factors, microbial translocation (MT), immune activation, systemic inflammation and side effects of therapy. Residual viremia, defined as the continuous persistence of the virus also at low levels despite effective cART, could be responsible of the chronic generalized inflammation and of the activation of the coagulation system. In addition the damage of mucosal barrier in the gastrointestinal tract (GI) increases MT into the circulating blood and increase the risk of systemic complication such as immune activation.

The deleterious effect of the status of immune activation on the host immune system is still unclear. Undoubtedly an important consequence of the general status of inflammation is the ability of HIV to infect and kill activated CD4 T-helper cells with the impaired homeostasis of CD4 T cells and memory B cells by the fibrosis of lymphoid tissue; all that may contribute to the consequent inability of the host to control a wide range of potential pathogens. In fact the persistent immune activation makes CD4 T cells more susceptible to infection and causes a vicious cycle increasing the production of soluble inflammatory markers such as IFN- $\gamma$ , inflammatory

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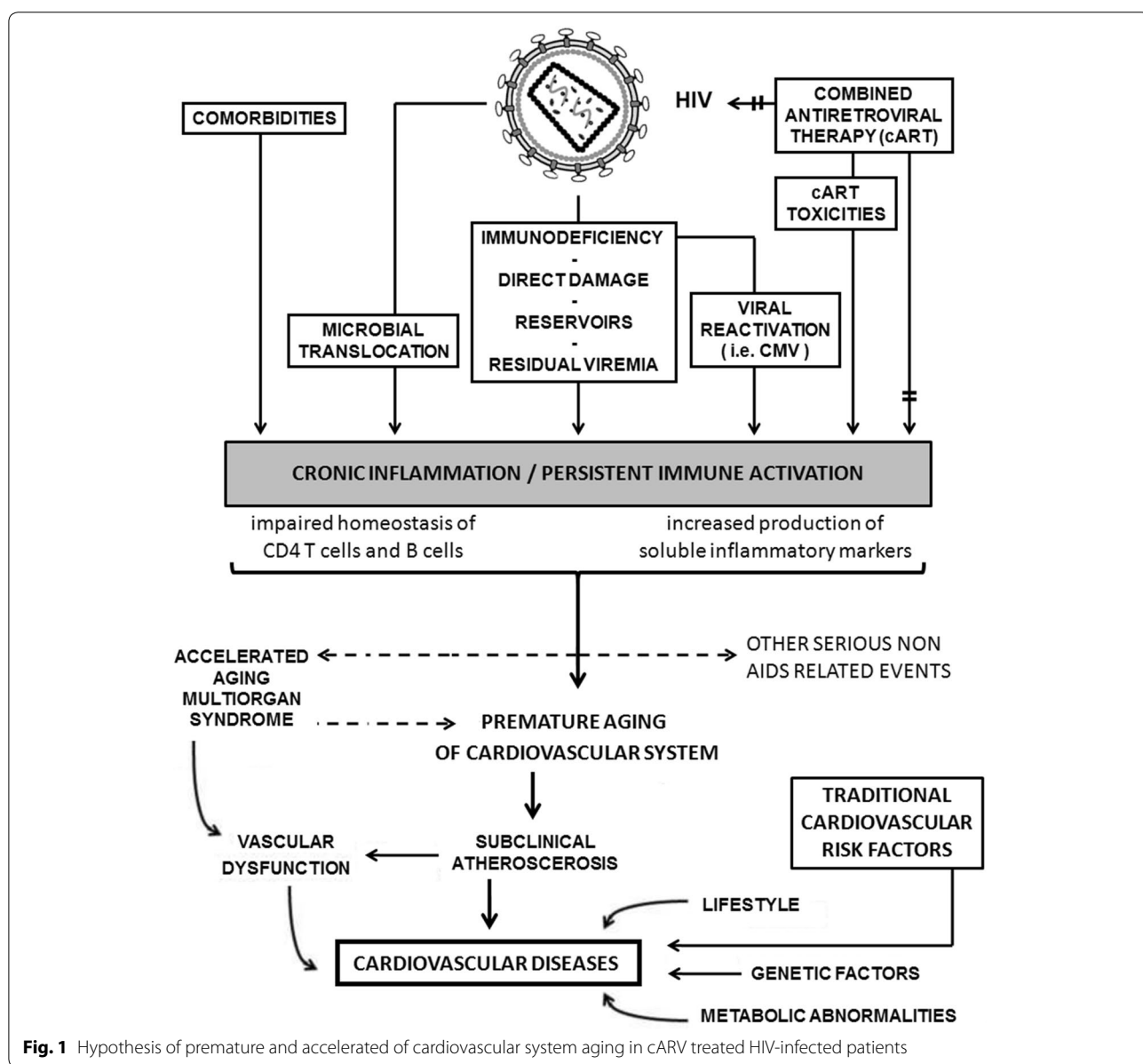
cytokines (i.e. IL-6) and indoleamine 2,3-dioxygenase (IDO) [2–7].

The excellent results now routinely achieved by cART have made the occurrence of opportunistic infections increasingly less frequent; at this time however it is common to observe cases of patients with undetectable blood levels of HIV-RNA, significant recovery of immunological status and SNAEs including cardiovascular diseases (Fig. 1). For this reasons it becomes always more intriguing to know the pathogenesis and clinical characteristics of SNAEs during HIV infection to cure them.

### The cardiovascular disease: a challenge for the cure of HIV patients

#### Epidemiology of cardiovascular diseases in HIV positive patients

A large body of evidences in scientific literature supports the epidemiological observation of an increase of morbidity and mortality related to clinical and sub-clinical cardiovascular diseases (CVD) in HIV patients in the era of cART. A significantly higher risk of acute myocardial infarction (AMI) was observed in HIV infected patients compared with uninfected in veterans aging cohort study virtual cohort (VACS-VC) that analyzed data collected



**Fig. 1** Hypothesis of premature and accelerated of cardiovascular system aging in cART treated HIV-infected patients

in 82,459 veterans followed for an average of 5.9 years; unexpectedly the highest risk was found in patients in cART and with HIV-RNA less than 500 copies/ml [8]. Also Silverberg et al. [9] reported an higher risk of AMI among HIV+ patients with a low recent or nadir CD4 cells (<200) compared with HIV-subject in a cohort study population included 22,081 HIV positive and 230,069 HIV negative subjects.

In contrast Klein et al. [10] reported a decline of CVD in HIV patients as observed by the analysis of the data of Kaiser Permanente Southern California and Northern California health plans including 24,768 HIV positive patients and 257,600 controls. By these data the adjusted relative risk of AMI in HIV+ patients declined from 1.8 in 1996–1999 to 1.0 in 2010–2011 possibly due to the better control of the traditional CVD risk factors, the lower incidence of severe immunodeficiency, and the use of more lipid-friendly cART in the patients enrolled in this study.

Although the data reported by Klein et al. probably influenced by the specific characteristics of their cohort, epidemiological studies show a significant overall impact of HIV on CVD. Since the size of the problem (although decreased after the introduction of lipid free antiretroviral drugs) remains very important, the actual aim of HIV medical practitioners is to better understand the kind of cardiovascular (CV) prevention to be applied to HIV population.

Recent retrospective and prospective studies evaluating the epidemiology of CVD in HIV positive patients were reported in Table 1.

#### **The premature aging of cardiovascular system driven by chronic inflammation and immune activation**

Many factors may contribute to the increase of incidence of CVD in HIV infected patients: the traditional determinants of disease (cigarette smoking, diabetes mellitus, dyslipidemia, obesity, systemic hypertension, sedentary lifestyle, stress and family history of coronary artery or vascular disease) appear to be more common than in HIV negative population [11–13]. Moreover alterations of regulation of glucose homeostasis and lipid metabolism and metabolic syndrome are frequently related with cART and are higher in patients previous treated with older antiretroviral drugs [14].

Anyway the excess risk of CVD observed in course of HIV infection cannot be explained only by the factors mentioned above. Several studies have also observed that co-morbidities, like CVD, which are normally witnessed later on in life as a result of natural aging, were increasingly prominent among the HIV-infected population [15, 16]. These observations led to the hypothesis that the cART treated HIV-infected patients experienced a

premature and accelerated aging probably driven by side effects of antiretroviral drugs, chronic inflammation and persistent immune activation (Fig. 1).

Undoubtedly a pivotal role in the development of accelerated aging of cardiovascular system and in the pathogenesis of CVD is attributed to the status of immune activation. In fact in HIV patients the levels of pro-inflammatory cytokines and biomarkers associated to endothelial dysfunction are always higher and they can accelerated the atherosclerosis process. For example monocyte activation caused by microbial translocation drives the releases of soluble CD14 and CD163: the first is associated to increased death and the second with the risk of coronary artery progression and atherosclerosis [17–20]. High levels of these inflammatory markers and of factors of hypercoagulation (i.e. D-dimers, fibrinogen) are linked to systemic clotting and chronic inflammatory damage of vascular endothelium [21]. Also HIV itself is considered responsible of persistent immune activation and endothelial dysfunction in fact it can penetrate into endothelial cells by receptor of CD4-T cells or chemokine receptors pathway. Moreover some HIV viral proteins, such as Gp120 and tumor necrosis-alpha (TNF- $\alpha$ ), induce important consequences on the vascular tone, on the adhesion and aggregation of the platelet by the decrease of nitric oxide (NO) levels in the endothelial cell [22–24]. The residual viremia may be the source of harm linked directly to the presence of the virus in course of effective cART, but may also be linked to the maintenance of the process of chronic inflammation and persistent immune activation.

Also Cytomegalovirus (CMV) infection appears to have a role in the premature aging of CV system: in a large Italian cohort CMV/HIV co-infection was associated with the risk of non-AIDS events including CV events independently of other prognostic factors. The potential role of CMV infection in CV disorders could depend by the complex interplay between viral and immunological activation, culminating in cyclical growth, damage and repair of endothelial cells. In fact from a pathogenic point of view, CMV can promote abnormal growth of endothelial cells and pathogenesis of atherosclerosis enhanced by proangiogenic factors including IL-6 and granulocyte macrophage colony stimulating factor [25, 26].

For this reason, the research's interest is to understand the hidden inflammatory causes of CVD in HIV patients and the first objective is to control the levels of persistent immune activation. In fact, to date, immunological therapeutic strategies and virological interventions are investigated with the aim of controlling the levels of immune activation and then reduce the impact of CVD promoted by chronic inflammation. Table 2 shows the studies, registered on <https://www.clinicaltrials.gov> and classified as

**Table 1 Recent retrospective and prospective studies evaluating the epidemiology of cardiovascular diseases in HIV positive patients**

Author, year [Ref.]	Cohort	Type of study	Population	Time of follow up	Aims	Results
Tseng et al. [96]	HIV+ patients enrolled in a public HIV Clinic in San Francisco in 10 years	Retrospective	2860 HIV+	3.7 years	To determine the incidence of SCD in HIV+ patients	Of 230 deaths, 13 % met SCD criteria SCDs accounted for 86 % of all cardiac deaths (30 of 35) Mean SCD rate: 2.6 per 1000 person-years (95 % CI 1.8–3.8), 4.5-fold higher than expected
Esser et al. [97]	HIV+ outpatients (ClinicalTrials.gov NCT01119729)	Prospective observational	803 HIV+	N/A	To elucidate CVD prevalence in HIV+ outpatients by standardized non-invasive CV screening	Prevalence of CVD: 10.1 % (95 % CI 8.0–12.2 %) Aging HIV-infected patients (≥45 years) exhibited significantly increased rates of CVD; CAD (7.5 vs. 1.8 %, $p < 0.001$ ) MI (6.0 vs. 1.8 %, $p = 0.002$ ) PAD (4.6 vs. 1.5 %, $p < 0.017$ ) Significantly associated with the prevalence of CVD in multivariate analyses: Age (OR 2.05 <i>xd</i> , 95 % CI 1.64–2.56) Smoking (OR 5.96 <i>xd</i> , 95 % CI 2.31–15.38) Advanced symptomatic HIV infection (OR 2.60 <i>xd</i> , 95 % CI 1.31–5.15)
Freiberg et al. [8]	Veterans aging cohort study virtual cohort (VACS-VC)	Prospective observational	55,109 HIV+ 27,350 HIV–	5.9 years	To investigate whether HIV is associated with an increased risk of MI	The mean MI events per 1000 person-years significantly higher ( $p < 0.05$ for all) for HIV-positive compared with uninfected veterans: Age 40–49 years, 2.0 (1.6–2.4) vs. 1.5 (1.3–1.7) Age 50–59 years, 3.9 (3.3–4.5) vs. 2.2 (1.9–2.5) Age 60–69 years, 5.0 (3.8–6.7) vs. 3.3 (2.6–4.2) After adjusting for Framingham risk factors, comorbidities, and substance use, HIV-positive veterans had an increased risk of incident MI compared with uninfected veterans (hazard ratio, 1.48; 95 % CI, 1.27–1.72)
Silverberg et al. [9]	Kaiser Permanente California	Retrospective	22,081 HIV+ 230,069 HIV–	13 years	To evaluate association of HIV infection and immune-deficiency on MI risk	MI incidence rate per 100,000 person-years: 283 for HIV+ subjects [RR of 1.4 (95 % CI 1.3–1.6)] Nadir CD4: associated with MIs (RR per 100 cells = 0.88; 95 % CI 0.81–0.96) Recent CD4, HIV-RNA, prior ART use, duration of PI and NNRTI: not associated with MIs Advanced clinical and immunological stages:
Esser et al. [98]	HIV HEART (HIVH) study	Prospective observational	1481 HIV+	7.5 years	To assess the frequency and clinical course of CVE in HIV+ patients by standardized non-invasive CV screening	Significantly ( $p < 0.001$ ) associated with higher incidences of CVE (A 17.7 %; B 33.1 %; C 49.2 % and I 3.1 %; II 32.3 %; III 64.6 %) No associated with the duration of HIV-infection (per year: HR: 0.91 [0.88–0.94]) and ART (per year: HR: 0.81 [0.79–0.84])

**Table 1 continued**

Author, year [Ref.]	Cohort	Type of study	Population	Time of follow up	Aims	Results
Petoumenos et al. [99]	Data collection on adverse events of anti-HIV Drugs (D:A:D)	Retrospective	24,323 HIV+ men	N/A	To statistically model the relative increased risk of MI, CAD and CVD per year older	Crude MI, CAD and CVD event rates per 1000 person-years increased from 2.29, 3.11 and 3.65 in those aged 40–45 years to 6.53, 11.91 and 15.89 in those aged 60–65 years, respectively
Carballo et al. [100]	Acute myocardial infarction in Switzerland (AMIS) registry plus Swiss HIV cohort study (SHCS) (aggregated data)	Retrospective	Patients who survived an incident MI occurring on or after 1/1/2005: 133 HIV+, 5328 HIV–	1 year	To determine whether HIV infection is a risk factor for worse outcomes in patients who survived an incident MI:	HIV infection associated with a significantly increased risk of all-cause mortality 1 year after incident MI No significant differences in recurrent MI (4 [3.0 %] HIV+ and 146 [3.0 %] HIV– individuals, or 1.16, 95 % CI 0.41–3.27)
Klein et al. [10]	Kaiser Permanente California	Retrospective	24,768 HIV+ 257,600 HIV–	15 years	To evaluate changes of MI risk from 1996 to 2011 by HIV status	The adjusted MI RR for HIV status declined from 1.8 in 1996–1999 to 1.0 in 2010–2011

CAD coronary artery disease, CI confidence interval, CV cardiovascular, CVD cardiovascular diseases, CVE cardiovascular event, *xd* per decade, HR Hazard ratio, MI myocardial infarction, N/A not applicable, NNRTI non-nucleoside reverse transcriptase inhibitors, OR odds ratio, PAD peripheral arterial diseases, PI protease inhibitors, RR rate ratio, SCD sudden cardiac death

“open,” and provides highlights for the current and future research topics in this area.

#### **Current trends and challenges in evaluation of CV risk in HIV positive patients**

As previous described, an increase of morbidity and mortality related to clinical and sub-clinical CVD in HIV patients are reported. An important paradigm in the comprehension of this increased incidence is to understand the role of traditional risk factors. Evaluating the HIV cohorts, numerous data suggest that the prevalence of “traditional” risk factors is higher in HIV subjects if compared with general population. Triant et al. [27] observed greater incidence of hypertension (HIV: 21.2 vs. non-HIV: 15.9 %;  $p < 0.001$ ), diabetes (HIV: 11.5 vs. non-HIV: 6.6 %;  $p < 0.0001$ ), and dyslipidemia (HIV: 23.3 vs. non-HIV: 17.6 %;  $p < 0.0001$ ) in HIV positive patients than in the controls. In particular, the serum triglyceride levels are higher in HIV population respect to controls [28]. Moreover, triglycerides work in a synergic way with both infection and cART: in fact, triglycerides levels increase when signs and symptoms of AIDS appear but also their serum levels are raised by protease inhibitors therapy [29].

However the high prevalence of “traditional” risk factors do not seem to be sufficient to explain the increased cardiovascular risk observed in the HIV positive population and the cardiovascular risk assessment based on conventional risk prediction models (i.e. Framingham system, PROCAM, SCORE) does not offer a good predictive value for the HIV-positive population [30]. In fact cardiovascular risk scores were built for general population, and were not validated in the HIV positive population. Probably the factors that most affect the failure in estimating the cardiovascular risk in HIV-positive patients are HIV infection itself and the premature aging of CV system driven by chronic inflammation and immune activation: in this sense the virus may be considered “the missing factor” essential for a correct evaluation of the overall risk [31].

Given the inadequacy of the risk estimation algorithms available, recently new models of risk prediction, specific for HIV-positive patients, were designed: for example Friis-Møller et al. [31] proposed the Data Collection on Adverse Effects of Anti-HIV Drugs (D:A:D) study equation in which age, sex, systolic blood pressure, serum cholesterol total and HDL-cholesterol level, diabetes, smoking status, family history of CVD, current use of abacavir, indinavir, or lopinavir; and the number of years on indinavir or lopinavir were the variables included. This cardiac risk model was created based upon data from the large cohort of HIV-infected patients who were followed longitudinally for cardiac events and performed

better than the Framingham risk score among patients in that cohort. Moreover increased C-reactive protein (CRP) levels, uncontrolled HIV viral load at time of CV event and slower immunologic response were found to be associated with increased CVD risk in D:A:D: cohort [32]. Anyway a recent study of Thompson-Paul et al. [33] evaluating the D:A:D score and comparing with traditional algorithm for CV risk evaluation in HIV negative patients, highlighted that also the use of this algorithm was associated with the underestimation of CV risk. The authors concluded underlying that to better estimate CV risk in HIV-infected persons, additional risk factors, such as immunologic or virologic status may need to be considered.

The failure of traditional methods of CV risk assessment has made it necessary to evaluate new methods to better understand the risk of CVD in HIV population.

Among the inflammatory biomarkers of CV risk, several have been proposed for diagnostic use, including IL-6, D-dimer, MMP-9, and high-sensitivity CRP (hsCRP). In HIV seronegative patients, hsCRP has emerged as the most useful because it has been proven to add risk to the other factors described within the Framingham cohort. Anyway for the time being, the meaning of hsCRP cannot be relied upon in the same manner in patients with HIV infection, in fact the results of studies in this setting are conflicting [34, 35].

In the field of diagnostic imaging, carotid intima-media thickness (cIMT), brachial ankle pulse wave velocity and coronary artery calcium (CAC) score are innovative indicators for assessment of subclinical atherosclerosis and could give information about the development of CVD in patient without history of atherosclerotic diseases. These cardiovascular disease risk surrogate markers, are currently relegated to a secondary role, anyway their results should be considered complementary, and not alternative, to the information provided by CVD risk scores. In fact CVD risk assessment algorithms were not developed to predict coronary or carotid atherosclerosis, but rather cardiovascular events depending on the algorithm. For example baseline levels and change in levels of sub-clinical atherosclerosis, assessed by cIMT, could provide additional informations to determine how intensively to intervene on lifestyle and whether medication in correspondence with individual's cardiovascular risk factors is indicated [36–40].

Therefore in the last years the search for an optimal algorithm for estimating cardiovascular risk has been partly replaced by the search for exams increasingly able to estimate directly the presence of subclinical atherosclerotic lesions. The prevention is therefore increasingly moving towards the ability to diagnose the presence of silent atherosclerotic lesions able to progress and to



**Table 2 Clinical studies registered on <https://www.clinicaltrials.gov> classified as “open”, and matched search queries with the following keywords: “HIV”, “cardiovascular”, “inflammation” and/or “immune-activation” (last accession date 19 Dec 2015)**

Official title of the study ClinicalTrials.gov Id. Study type and design sponsor	Purpose and description	Primary outcome measures	Secondary outcome measures
A comparison of endothelial function between HIV-infected subjects not receiving anti-retroviral therapy and matched hiv-uninfected con-trol subjects NCT00919724 Observational, case control, prospective Indiana University	The purpose of this study is to determine whether people infected with HIV have worse blood vessel function than people without HIV infection. Specifically, inflammation, immune activation, endothelial activation, and metabolic measures will be compared This study will involve two groups of participants. The first group will consist of people with HIV who are enrolling in two other separate HIV studies (NCT00864916 and NCT00796822), one lasting 8 weeks and the other lasting 48 weeks. The second group will consist of people without HIV who are similar to the first group in terms of age, sex, smoking status, and height	Brachial artery reactivity: the maximum change in brachial artery diameter after induction of reactive hyperemia post-release of vascular occlusion	Inflammatory/endothelial activation markers: (MCP-1, sVCAM-1, IL-6, TNF-α, IP-10, MMP-9, TIMP-1, PAI-1 active, hsCRP) Peripheral blood immune activation: (percentage of CD8 +/CD38 +/HLA-DR+ T cells) Metabolic parameters: (fasting lipoprotein fractions/triglycerides, HOMA-IR)
Biomarkers of inflammation, coagulation, and endothelial function in HIV-infected adults NCT00776412 Observational, prospective NIAID	This study will collect information about markers of inflammation, blood clotting and blood vessel function in HIV-infected adults and healthy volunteers Initially, the study will recruit HIV-infected adults with HIV viremia who are not taking ART and compare their clinical histories and biomarker findings with those from a group of HIV-infected adults with controlled HIV viremia who are receiving ART, and with those from a control group of HIV-negative healthy subjects. Additionally a cohort of HIV-infected adults with poor CD4 + cell recovery despite effective ART, will be enrolled (immunologic non-responder cohort) and for comparison, a control group with similar nadir CD4 counts but with good CD4+ cell recovery on ART	Not provided	Not provided
Open-label, randomized, 24-week pilot study of metformin vs observation for persistent immune activation in chronic HIV infection NCT02383563 Interventional, randomized University of Hawaii	This proposal seeks to assess the impact of 24 weeks of metformin on non-calcified plaques and calcified plaques assessed by coronary CT angiography, and on whether these changes can be explained by metformin-induced phenotypic and secretory changes of monocytes	Coronary plaques by CT angiography change in total numbers of atherosclerotic plaques detected in the coronary arteries	Change in numbers of each monocyte subset Change in monocyte secretory function Change in sub-types of coronary plaques by CT angiography (N° of calcified, non-calcified, mixed atherosclerotic coronary plaques)
Imaging companion study To ACTG A5314: effect of reducing inflammation with low dose methotrexate on inflammatory markers and endothelial function in treated and suppressed hiv infection NCT02312219 Interventional, randomized Massachusetts general hosp	The investigators propose to conduct a time sensitive ancillary imaging study whose overall goal is to determine if treating virologically suppressed, HIV-infected individuals with low-dose methotrexate will reduce inflammation within the arterial wall. arterial FDG uptake provides a measure of inflammation in the artery wall; in fact atherosclerotic inflammation can be non-invasively and reproducibly measured with fluorodeoxyglucose (FDG)-PET/CT imaging, a well-validated quantitative technique that can sensitively detect changes in atherosclerotic inflammation and which has been employed in several multi-center trials to measure changes in arterial inflammation in response to anti-inflammatory treatments	Change in arterial FDG uptake	Change in splenic FDG uptake

**Table 2 continued**

Official title of the study ClinicalTrials.gov Id. Study type and design sponsor	Purpose and description	Primary outcome measures	Secondary outcome measures
Immunologic and inflamm-matory factors and cardio-vascular risk in patients with HIV infection or autoimmune diseases NCT01519141 Observational, prospective University of California, San Francisco	The investigators plan to obtain measurement of carotid artery intima media thickness (IMT) using high resolution ultrasound as a noninvasive means for tracking atherosclerotic progression. The investigators will also measure lipid and lipoprotein levels, inflammatory markers, markers of cytomegalovirus (CMV) infection, thrombotic markers, atherogenic lipoproteins, and markers of immune function. immunophenotyping will be performed on freshly collected blood and analyzed by flow cytometry to identify activated T-cells, T-cell turnover, proportions of T-cells, and CMV function. HIV-infected patients will have CD4 count and HIV viral load measured in addition. Patients will also go assessment of endothelial function, endothelial progenitor cells, arterial stiffness as measured using pulse wave tonometry	Increased carotid intima-media thickness (mm) Decreased brachial artery flow-mediated dilatation (%) Increased D-dimer levels (mcg/mL)	Not provided
Effect of reducing inflammation with low dose methotrexate on inflammatory markers and endothelial function in treated and suppressed HIV infection NCT01949116 Interventional, Randomized NIAID and NHLBI	People with HIV infection who are taking antiretroviral therapy may be at risk for cardiovascular disease, which can be caused by inflammation. methotrexate is a medication used to treat inflammation in people with rheumatoid arthritis. This study will evaluate the safety and effectiveness of low-dose methotrexate (LDMTX) at reducing inflammation in HIV-infected adults	Change from baseline to week 24 in brachial artery flow-mediated vasodilation (FMD) (%) (Defined as the maximum FMD (%) calculated from reactive hyperemia (RH) 60 and RH 90 relative to resting artery diameter at baseline) (Other immune-virological and safety endpoints)	Change from baseline to week 12 in brachial artery FMD and brachial artery diameter Change from baseline to week 24 in brachial artery diameter, brachial artery hyperemic flow velocity, levels of high-sensitivity C-reactive protein, IL-6, sCD163, D-dimer, monocyte levels, adhesion and activation indices, and CX3CR1 expression
Effect of IL-1 $\beta$ inhibition on inflammation and cardio-vascular risk NCT02272946 Interventional, randomized University of California, San Francisco	The purpose of this study is to evaluate the effects of IL-1 $\beta$ inhibition on safety, measures of systemic and vascular inflammation and endothelial function (all indicators of cardiovascular risk) in treated and suppressed HIV infected individuals. This study will assess the safety and effects of canakinumab on endothelial function (assessed by flow-mediated vasodilation [FMD] of the brachial artery), vascular inflammation (assessed by FDG-PET/CT scanning), key inflammatory markers of cardiovascular disease (CVD) risk (high-sensitivity C-reactive protein [hsCRP]), interleukin-6 (IL-6), soluble CD163 (sCD163), D-dimer, T-cell and monocyte activation in the blood, and size of the HIV reservoir. 20 individuals will receive a single dose of 150 mg canakinumab with follow-up for 18 weeks	Number of adverse events at week 1, 2, 4, 8, 12, 18 as a measure of safety	Change in brachial artery FMD: brachial artery FMD is calculated as the percentage increase in brachial artery diameter with hyperemia induced relative to the resting brachial artery diameter). Change from baseline in FDG uptake assessed by FDG-PET/CT as a measure of vascular inflammation, assessed by FDG-PET/CT scanning Rate of change in inflammatory markers of CVD risk: hsCRP, IL-6, sCD163, D-dimer, T-cell and monocyte activation in the blood, and size of the HIV reservoir



**Table 2 continued**

Official title of the study ClinicalTrials.gov Id. Study type and design sponsor	Purpose and description	Primary outcome measures	Secondary outcome measures
Does rosuvastatin delay progression of atherosclerosis in people with hiv infection at moderate cardiovascular risk? a multicentre randomised, double-blind placebo-controlled trial NCT01813357 Interventional, randomized Bayside health	This study is a randomised double blind placebo controlled trial comparing Rosuvastatin with placebo in HIV positive people who are at intermediate cardiovascular risk. It is possible that HIV positive people will receive a greater benefit from statins because of their higher baseline levels of inflammation and this study aims to determine what benefit HIV infected people will receive from starting statin therapy earlier than currently recommended. Participants will undergo blood tests and ultrasounds of the arteries of the neck (carotid intima media thickness) prior to starting Rosuvastatin and then after 1 and 2 years on the drug to determine what effect it has on markers of inflammation, cholesterol levels and thickness of blood vessels.	Progression of carotid intima media thickness. (carotid intima media thickness will be measured by ultrasonography and the change from baseline at 1 and 2 years calculated)	Rates of adverse events (Number of participants with adverse events in total and also the number of participants with adverse events thought secondary to the study medication)
The effects of statin therapy on coronary flow reserve and inflammatory markers in hiv-positive patients NCT02234492 Interventional, efficacy study Ottawa Heart Institute Research Corporation	The purpose of this study is to determine whether the use of rosuvastatin in human immunodeficiency virus (HIV) infected individuals lowers inflammation in blood vessels and improves blood circulation in the small arteries that provide nutrients to the heart muscle.	Correlation between coronary flow reserve (CFR) and maximum target to background ratio (TBR max). At baseline, correlation between CFR by MCE and vascular inflammation (TBR max) by FDG-PET/CT will be assessed	Changes in CFR as measured by MCE will be evaluated over 6 months Changes in vascular inflammation (TBRmax) as measured by FDG-PET/CT
Myocardial adipose inflammation and pericardial adipose volume as markers for coronary artery disease in HIV positive patients NCT02399384 Observational, case control University of Cincinnati	The investigators propose to correlate 1) cardiac MRI pericardial adipose volume, 2) the presence of pericardial monocytes and 3) circulating immune biomarkers in persons with and without CHD and HIV infection compared to seronegative controls with known CHD. The investigators aim to test the hypothesis that higher amounts of pericardial fat deposition and increased presence of monocytes within this adipose tissue are associated with underlying coronary artery disease in persons with HIV infection as measured by cardiac MRI	Pericardial adipose tissue volume	Adipose spin spin relaxivity as measured by T2 star time

**Table 2 continued**

Official title of the study ClinicalTrials.gov Id. Study type and design sponsor	Purpose and description	Primary outcome measures	Secondary outcome measures
Evaluating the use of pitavastatin to reduce the risk of cardiovascular disease in HIV-infected adults (REPRIEVE) NCT02344290 Interventional National Institute of Allergy and Infectious Diseases (NIAID)	This study will evaluate the use of pitavastatin to reduce the risk of CVD in adults infected with HIV who are on antiretroviral therapy (ART). This study will enroll adults infected with HIV who are on any ART regimen (ART is not provided by the study) for at least 6 months before study entry considered low-to-moderate risk using the 2013 American College of Cardiology (ACC)/American Heart Association (AHA) guideline thresholds for recommended statin initiation. Total study duration will be approximately 72 months from the time the first participant is enrolled. Participants will be randomly assigned to receive 4 mg of pitavastatin or placebo once a day for the entire time they are enrolled in the study. Some participants will have the option of enrolling in a substudy (effects of pitavastatin on coronary artery disease and inflammatory biomarkers: mechanistic substudy of REPRIEVE [A5333 s]). The substudy will evaluate the effect of pitavastatin on the progression of non-calcified coronary atherosclerotic plaque (NCP) and inflammatory biomarkers in adults infected with HIV. Participants in the substudy will attend study visits at study entry and months 4 and 24. The visits will include questionnaires and assessments, a blood collection, and a coronary computed tomography angiography (CCTA)	Time to the first event of a composite of major cardiovascular events (includes atherosclerotic or other CVD death, nonfatal myocardial infarction, unstable angina hospitalization, coronary or peripheral arterial revascularization, nonfatal stroke or transient ischemic attack (TIA), urgent peripheral arterial disease (PAD) ischemic event)	Time to the first of each individual component of the primary endpoint Time to death (all-cause mortality) Time to death (all-cause mortality) and/or major adverse cardiovascular events (MACE) Time to any (composite) or each (individual) of the following clinical diagnoses (Non AIDS-defining cancers; AIDS-defining events; initiation of dialysis or renal transplantation; cirrhosis, or hepatic decompensation requiring hospitalization) Calculated fasting LDL and HDL cholesterol level Time to any of the following adverse events (Serious adverse event, incident diabetes mellitus (DM), grade 3 or 4 ALT, or grade 3 or 4 myopathy)

ART antiretroviral therapy, NIAID National Institute of Allergy and Infectious Diseases, NHLBI National Heart, Lung, and Blood Institute

determine a significant symptoms in asymptomatic subjects. On the basis of the above considerations the diagnostic applications of molecular imaging techniques becomes always more important in the detection of atherosclerotic plaques. The imaging by 18-fluoredeoxyglucose positron emission tomography (PET) and single photon emission computed tomography (SPECT) are considered important to identify activated macrophages infiltrating the arterial wall. Moreover both PET and SPECT provide information about the risk of plaque rupture and furthermore evaluate the vulnerability of itself. Recently Knudsen et al. conducted a prospective cross sectional study in which 56 HIV positive patients and 25 controls were scanned using 82Rb PET to obtain the stress flow (MFR). The results obtained were stratified in: low <1.5, borderline >1.5–2 or normal >2 and the Author did not observe difference between HIV positive and negative controls [41]. The data about the use of PET and SPECT in HIV population are not enough to conclude the useful of that in the real life. On the other hand the utility of imaging techniques in the detection of subclinical atherosclerotic plaques was reported in a cross-sectional study using dual-source CT (MDCT) coronary angiography conducted in HIV-infected subjects with low CV risk: MDCT showed an unexpected, age-associated high rate of significant coronary stenosis requiring coronary angiography in 29.1 % of asymptomatic HIV positive subjects enrolled. On the basis of these findings authors suggested that MDCT may be appropriate for CVD screening programs in HIV positive population [42].

Detection of subclinical atherosclerosis by noninvasive tests such as CAC score, cIMT, MDCT, and PET may improve risk prediction above that of established risk scoring models. Moreover interesting results are expected by promising innovative techniques, such as 3D quantification of carotid plaque by ultrasound (as in the Bioimage study), which are still waiting to be properly tested in HIV-positive population.

However, despite the interesting results obtained, more studies are need to investigate if diagnostic imaging exams can be used to guide patient screening, management and therapy in HIV setting.

#### **Clinical impact of inflammation on cardiovascular system: from subclinical atherosclerosis to complications of coronary revascularization**

As previously reported, HIV-infected patients receiving combination antiretroviral therapy may experience metabolic complications (i.e. dyslipidemia, impaired glucose metabolism and abnormal body fat distribution), potentially increasing their risk of cardiovascular diseases. In fact HIV infection is associated with a marked rise in the

frequency of coronary heart disease and with accelerated coronary atherosclerosis and vasculopathy.

The progression from subclinical atherosclerosis to symptomatic disease is estimated to be higher and earlier in HIV patients than in general population. In fact HIV patients have a high burden of subclinical atherosclerosis including an increased amount of non-calcified coronary plaque compared with HIV-uninfected individuals independently by cardiovascular risk.

On these basis, recently, an increasing number of HIV patients undergo invasive cardiovascular procedures as a result of acute cardiovascular events or severe atherosclerotic disease. Although percutaneous coronary intervention (PCI) frequently used to treat coronary artery disease in HIV, little is known regarding the outcomes and the effects of immune activation on the this procedure.

In literature an observational study on 50 HIV infected patients undergoing PCI found that this procedure is a safe treatment strategy of coronary revascularization in HIV positive patients without significant differences in terms of clinical restenosis from the control population [43].

On the other hand Martín-Reyes et al. [44] reported that in-hospital prognosis in patients with HIV, undergoing PCI, was worse than in control subjects: in fact HIV-positive patients presented a lower rate of PCI success (75 vs. 85 %) respect HIV-negative patients. Moreover Segev et al. [45] analyzing the long-term outcome of PCI in 12 HIV-infected patients, observed that 58.3 % of subjects suffered from severe clinical and/or angiographic restenosis requiring additional interventions or causing severe angina pectoris. They concluded that HIV-infected patients should be considered as high risk group and treated routinely with drug-eluting stents but they did not given any pathogenic interpretation of their observations. Although HIV-infected patients have a higher incidence of post-PCI ischemic events, restenosis, and stent thrombosis, Matetzky underlined that the intermediate-term mortality is low [46].

At this moment few data are available regarding the pathogenesis of restenosis after coronary revascularization in HIV positive patients. It is possible that the concomitant presence of traditional risk factors, assumption of antiretroviral therapy and his metabolic complications contribute to the restenosis events after PCI in HIV-positive population. However, although the presence of these well-known reasons of atherogenesis, the rapidity of progression of atherosclerosis and restenosis process seems linked principally to chronic inflammation and excess immune activation in HIV-infected patients [47–49]. In fact chronic HIV infection is characterized by multifaceted systemic immune activation, including increased

frequencies of activated T-cells and increased turnover of T-cells that correlate directly with disease progression [50]. Moreover a previous study have described other conditions (coronary allograft vasculopathy, native atherosclerosis) associated with an inappropriate inflammatory reactivity that may predispose to re-stenosis after PCI [51]. For these reasons therapies that reduce immune activation may be of benefit, particularly for such individuals [50, 52]; moreover a strictly monitoring of coronary atherosclerosis is required in management of HIV-infected patients with concomitant non-traditional risk factors for intense immune-activation.

In summary contrasting results are reported in the literature on the outcome of coronary revascularization; moreover the coronary revascularization with PCI and stents seems to be influenced by the excess of HIV related inflammation and immune activation [53]. Large clinical studies are needed to clarify aspects of the matter is not yet resolved.

#### **New therapeutic insights in minimizing the risk of cardiovascular disease**

As previously highlighted, HIV-infected patients experienced a premature and accelerated aging probably driven by chronic inflammation and persistent immune activation. For this reason currently immunological therapeutic strategies and virological interventions are investigated with the aim of controlling the levels of immune activation and then reducing the impact of CVD promoted by chronic inflammation.

An innovative immunological/pharmacological intervention, granted by experience in HIV negative populations, is based on use of statins. These class of drugs, widely used for primary prevention of atherosclerotic CVD, are known to decrease cholesterol level as well as inflammation in HIV seronegative patients. For example in the JUPITER trial rosuvastatin significantly reduced all-cause mortality and in particular the incidence of major cardiovascular events in healthy persons without hyperlipidemia but with elevated hsCRP levels, a well-known marker of inflammation [54]. Statins will emerge also as potential components of the therapeutic armamentarium for chronic inflammatory and autoimmune pathologies which share some pathogenetic aspects concerning chronic inflammation with HIV infection [55]. In fact statin therapy has been shown to reduce disease severity in patients with rheumatoid arthritis (RA) and to improve endothelium-dependent vasodilation in patients with RA and systemic lupus erythematosus (SLE) [56–60]. In systemic inflammatory diseases (such as RA, SLE, inflammatory bowel diseases, psoriasis, spondyloarthritis and others) multiple factors, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), circulating inflammatory cytokines,

reactive oxygen species, autoantibodies, and oxidized low density lipoprotein (LDL) directly and indirectly activate endothelial cells, increased endothelial permeability, increased leukocyte adhesion and generation of a pro-thrombotic state. In this case statins could have a potential role in modifying initiation and amplification of immune inflammatory responses and their anti-inflammatory activity is linked to different mechanisms including (but not limited) the decrease in chemotaxis of monocytes and macrophages, the lipopolysaccharide (LPS) mediated release of TNF- $\alpha$ , and the activation of NO synthase [61–64].

The efficacy and safety of different statins for HIV-infected individuals in the primary prevention setting remain to be established. Also the effect of statins use on overall mortality in HIVpositive patients remains controversial [65, 66].

A recent meta-analysis, including 18 clinical studies with 736 HIV-positive patients under cART treated with statins in the primary prevention, showed that this therapy significantly lowers plasma total cholesterol (TC) and low density lipoprotein (LDL) levels. In particular rosuvastatin 10 mg and atorvastatin 10 mg provided the largest reduction in TC levels while atorvastatin 80 mg and simvastatin 20 mg had the largest reduction in LDL [67].

Moreover atorvastatin and rosuvastatin appear to reduce non-calcified coronary plaque volume and slow progression of carotid intima-media thickness in HIV positive patients under cART [68].

Conversely the effects of statins on inflammation and are less conclusive at the moment: a recent study, evaluating the effect of rosuvastatin on markers of immune activation in treatment-naïve HIV-patients, showed that this molecule had a small but significant positive effect on CD4/CD8 T cell ratio, but not influenced other activation markers (neopterin, soluble Toll-like receptor (TLR) 2, sTLR4, interleukin (IL)-6, IL-1Ra, IL-18, D-dimer, hsCRP) [69]. Interesting the protective effect of statins on CV system is related not only to their anti-inflammatory activity but also to a control of oxidative stress: in fact oxidative stress plays a significant role in atherosclerosis development and is also elevated in HIV+ patients due to excessive production of free radicals that play a major role in oxidative modification in LDL particle. Serum oxidized LDL levels may contribute to monocyte activation, are associated with clinical manifestations of atherosclerosis, decreases in response to statin therapy and relates independently to reductions in coronary plaque in patients with HIV. Statins may reduce oxidative stress and subsequently subclinical vascular disease in HIV but further study in the context of HIV disease is warranted [70–72].

Considered the importance of the topic and the still existing doubts, the US National Institutes of Health

(NIH) recently launched the REPRIEVE trial to definitively assess the efficacy of statins as a primary prevention strategy for CVD in this at-risk population. This clinical study launched in 2015 is the largest ongoing trial of HIV-related CVD to date (see Table 2) [73].

Some concerns are the potential side effects described for these class of drugs. i.e. statin use was found associated with a modestly increased risk of incident diabetes mellitus in HIV Outpatient Study (HOPS) participants [74]. Also severe rhabdomyolysis, hepatotoxicity and acute renal failure were described in HIV positive population [75–77]. Concerns are also the potential numerous drug–drug interactions with cART dependent on statins' pharmacokinetic profile: i.e. simvastatin and lovastatin, metabolized through cytochrome P450 (CYP) 3A, have the significant potency for drug–drug interaction with potent CYP3A inhibitors such as ritonavir- or cobicistat-boosted HIV-protease inhibitors (PIs). Less potent drug–drug interactions have been reported for atorvastatin although it is also a CYP3A substrate. Non-CYP3A-dependent statin concentrations are also affected when co-administered with PIs, mainly through interaction with OATP1B1 [78, 79].

Interesting pitavastatin, a newer statin that does not have substantial interactions with antiretroviral drugs, will be tested in the REPRIEVE trial. At the present time, however, many aspects of potential drug interactions remain to be clarified. Despite all the warnings described, the overall use of statins in HIV population is associated with low rates of adverse events and the mean discontinuation rate reported in meta-analysis and attributable to these problems was 0.12 per 100 person-years [95 % CI (0.05, 0.20)] [67].

The possibility that residual immune activation (and the consequent inflammation) is related to persistent virus replication has suggested that more potent antiretroviral regimen (i.e., ART “intensification”) may be more effective in reducing the HIV-associated immune activation. In one study intensification was carried out for 10 weeks with cART regimens (atazanavir/ritonavir, lopinavir/ritonavir, efavirenz) consisting of drugs that were not used previously in the enrolled patients. Since the median levels of viremia were not significantly different between pre-intensification and post-intensification period, the authors concluded that the levels of residual virus replication, and thus immune activation, may depend on the size of stable reservoir compartments that are established prior to the initiation of cART [80]. Another study of Llibre et al. [81] assigned randomly 69 patients with undetectable levels of viraemia for more than 1 year to treatment groups in which 45 intensify therapy with raltegravir and 24 continue the same therapy for 48 weeks. The author observed after 24 weeks in

the group of intensification therapy a reduction of the size of the reservoirs and of the levels of immune activation (measured as fraction of CD8+ T-cells expressing CD38+ and/or HLA-DR). Further studies on the effects of cART intensification on the residual immune activation will be needed to ascertain whether a complete recovery of the “ideal” pre-HIV infection immune system function can be achieved through virological interventions alone. In absence of conclusive results in this research field, the alternative possibilities, i.e. specific immune-based interventions, will be necessary to try to protect HIV infected individuals by damages of immune activation. In this sense we know that the most important source of the status of generalized inflammation is attributed to the breakdown in the integrity of the gut mucosa with the increase of microbial translocation and the transition of the microbial products in the systemic circulation. For this reason different trials were conducted to explore the possibility of intervention on this source of chronic inflammation. In a recent study in order to enhance GI tract immunity, we recruited and treated 20 HIV-infected humans with cART supplemented with probiotics and followed inflammation and immunological parameters. We observe that cART did not normalize the levels of immune activation in HIV positive patients, anyway inflammation and markers of microbial translocation were significantly reduced with probiotic supplementation. Patients enrolled showed a clear and statistically significant reduction in the levels of immune activation on CD4 T-lymphocytes, for both markers CD38 and HLA-DR and their simultaneous expression, LBP and hsCRP plasma levels after probiotic diet supplementation. In conclusion this study, like other recent reports [82–85], evidenced that supplementing cART with probiotics in HIV-infected individuals may improve GI tract immunity and thereby mitigate inflammatory sequelae, ultimately improving prognosis [86].

Another potentially interesting therapeutic concept for the reduction of high levels of inflammation and to control CVD is to use non pharmacological tools of intervention such as physical activity. Several published studies indicate that physical activity could represent a beneficial non-pharmacological intervention to reduce chronic inflammation. Currently available data are limited, nonetheless increasing evidence suggests that the introduction of regular physical exercise in the clinical management of HIV-infected individuals may have a significant therapeutic impact in reducing inflammation and CVD. For example Longo et al. [87] conducted a longitudinal study on 50 ART-treated HIV-infected patients with sedentary life style to evaluate the effect of 12 weeks of moderate intensity exercise on parameters of immune activation and metabolic profile. At the enrollment and at 12 weeks

all participants were assessed by a 6-min walking test and 1-RM, DEXA, metabolic and immune-virologic parameters, and markers of immune activation (IL-6, d-Dimer, sCD14 and IL-18). The authors found a significant improvement of both fitness and immune activation at the week-12 time point.

On the basis of these evidences, several authors proposed that regular physical exercise should be further studied as a potential anti-inflammatory, non-pharmacological approach to be used to treat HIV residual disease and non-AIDS-defining illnesses in cART-treated HIV-infected individuals [88–94].

Likewise, lifestyle interventions are another non pharmacological tool reputed able to reduce cardiovascular risk in general population: nevertheless a recent randomized pilot study with a follow-up 36 months evidenced that a multidisciplinary lifestyle intervention resulted in a slight improvement in some cardiovascular risk factors and did not prevent cIMT progression in HIV-infected patients with Framingham scores >10 % [95]. Further studies are required to confirm the importance of all these data.

## Conclusions

In cART treated HIV-infected individuals with undetectable viremia the level of immune activation is dramatically reduced compared to baseline (i.e., pre-treatment) but rarely goes back to normal levels. This immune activation is associated with long-term sequelae of HIV infection such as accelerated atherosclerosis, neurological disease, and several other conditions that may modify the quality of life of HIV-infected subjects. In addition, the residual viral replication appears to be a uncontrolled determinant of the levels of immune activation in HIV patients treated with cART.

The goal of practitioners is changed in the last years: in fact if initially it was to reach undetectable plasma levels of HIV viremia, now it is to prevent the premature aging and improve the quality of life of all patients. For this reason it becomes necessary to control the hidden effects of HIV, to develop safe antiretroviral regimen and discover new scores to predict the non AIDS related manifestations.

## Authors' contributions

GdE and GC contributed equally to this paper, conceived and wrote the manuscript. PP, PV, IS, GDG,SS and NG wrote paragraphs of manuscript and collected references. SS and NG contributed to first revisions. VV critically revised all manuscript steps. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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