Published in final edited form as:
AIDS. 2010 June 19; 24(10): 1509–1517. doi:10.1097/QAD.0b013e32833ad914.

Traditional risk factors and D-dimer predict incident cardiovascular disease events in chronic HIV infection

Emily S FORD[1,2], **Jamieson H GREENWALD**[1], **Aaron G RICHTERMAN**[1], **Adam RUPERT**[3], **Lauren DUTCHER**[1], **Yunden BADRALMAA**[3], **Ven NATARAJAN**[3], **Catherine REHM**[1], **Colleen HADIGAN**[1,*], and **Irini SERETI**[1,4,5,6,7,*]

[1] National Institute of Allergy and Infectious Diseases, National Institutes of Health, 10 Center Drive, Bethesda, MD 20892, USA.

[2] E.S.F. was a 2008-2009 participant in the Clinical Research Training Program, a public–private partnership supported jointly by the NIH and Pfizer Inc. via a grant to the Foundation for NIH from Pfizer Inc.

[3] AIDS Monitoring Laboratories, Science Applications International Corporation, Frederick, MD 21702, USA.

Abstract

Objective: Cardiovascular disease (CVD) contributes significantly to HIV-related morbidity and mortality. Chronic immune activation and inflammation are thought to augment the progression of atherosclerotic disease. In this retrospective, case-control study of HIV-infected subjects, we investigated the association of traditional cardiac risk factors, HIV-related disease and inflammation with CVD events.

Methods: HIV-infected subjects who experienced an incident CVD event while enrolled in National Institutes of Health clinical protocols from 1995-2009 were matched 2:1 to HIV-infected subjects without known CVD. Markers of inflammation and cell activation were measured in serum or plasma using ELISA-based assays and peripheral mononuclear cells by four-color flow cytometry.

Results: Fifty-two patients experienced an incident CVD event. Events were related to smoking, dyslipidemia, hyperglycemia and family history as well as D-dimer, sVCAM-1, TIMP-1, and soluble tissue factor but not hsCRP. No significant differences in antiviral therapy, CD4+ T-cell count or CD38 and HLA-DR expression were identified between cases and controls. In multivariable analysis, smoking, family history, D-dimer, and glucose were independently related to CVD risk.

[4] To whom correspondence should be addressed: Irini Sereti, MD, MHS, Address: National Institutes of Health, 10 Center Drive, Building 10, Room 11B07A, Bethesda MD 20892 isereti@niaid.nih.gov, Phone: +1 301 496 5533, Fax: +1 301 480 9978.

[5] The authors report no conflicts of interest

[6] This research was supported in part by the Intramural Program of the NIH, NIAID and Critical Care Medicine Department. Additionally, this project has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

[7] These data have been presented in part at Keystone HIV Immunobiology, Keystone, CO, April 2009, abstract number 220, and at the 17th Conference on Retroviruses and Opportunistic Infections, San Francisco, CA, February 2010, abstract number 713.

[*] CH and IS contributed equally to this work.

Conclusion: In this cohort, CVD risk was related to traditional CVD risk factors and markers of thrombosis and endothelial damage but not to hsCRP or markers of T-cell activation such as CD38 +/HLA-DR coexpression. D-dimer may help identify HIV-infected patients at elevated CVD risk.

Keywords

HIV; cardiovascular disease; myocardial infarction; smoking; D-dimer; tissue factor; VCAM-1

Introduction

Non-infection-related morbidity and mortality has become increasingly significant in the care and management of HIV-infected patients treated with combination antiretroviral therapy (ART). An increased risk of myocardial infarction (MI) in HIV-infected patients was described in 2007 by Triant et al. [1]. In this large cohort, the relative risk of MI was 1.4 fold greater in men with HIV and 3.0 fold greater in women with HIV. HIV infection is associated with known cardiovascular risk factors such as increased total cholesterol, decreased HDL cholesterol, lipodystrophy and the metabolic syndrome [1,2]. Furthermore, the DAD study group showed that the risk of MI and cardiac death increased with duration of ARV therapy [3]. While much of the increased risk associated with protease inhibitor use is attributable to related dyslipidemia, the nucleoside reverse transcriptase inhibitors abacavir and, to a lesser degree, didanosine, have also been linked to increased CVD risk in some cohorts [4,5].

Contrary to expectations that reduced exposure to ARVs would decrease CVD risk in HIV infected patients, the SMART study of CD4+ T cell count-guided antiretroviral (ARV) therapy demonstrated that CVD events increased in the treatment interruption arm [HR 1.57 (95% CI 1.00-2.46, $p = 0.05$)] over the continuous viral suppression arm [6,7]. This observation suggests that HIV infection itself is an important contributor to cardiovascular disease. HIV infection is also associated with greater carotid artery intimal medial thickness [8] and reduced arterial elasticity [9,10], both surrogate markers of vascular disease.

In a subsequent analysis of the SMART data, elevated levels of D-dimer and the proinflammatory cytokine IL-6 were associated with HIV-RNA viremia and related to all-cause mortality, supporting a mechanism by which HIV infection contributes to a pro-inflammatory, pro-thrombotic state [11]. D-dimer, hsCRP, ICAM-1, and IL-6 are all associated with risk of CVD in non-HIV-infected subjects [12-14]. Much of HIV-related pathology is attributed to HIV-induced immune activation and dysregulation [15]. T-cell markers of activation and exhaustion such as CD38, HLA-DR, Ki-67 and PD-1 are all up-regulated [16-18]. T-cell activation and chronic inflammation are known to participate in CVD, as is evidenced by the increased incidence of atherosclerotic heart disease in populations with autoimmune inflammatory conditions [19]. Innate immunity also likely plays a role in CVD; increased circulating CD14+ monocytes have been linked to CVD risk [20]. In HIV-infected subjects the CD14++CD16+ subset has been linked to the progression of CVD [21,22] and HIV [23].

Identification of markers of increased CVD risk in HIV-infected patients is important and may inform the implementation of preventative measures by early identification of high-risk patients who may be candidates for intervention. Additionally, in the HIV-infected population, current methods of CVD risk stratification such as the Framingham Risk Calculator may underestimate the actual CVD risk [24]. In this retrospective, case-control study of HIV-infected adults enrolled in National Institutes of Health (NIH) intramural clinical protocols, we hypothesized that inflammation and immune activation, which have both been associated with HIV infection and HIV-related mortality, may contribute to an increased risk of cardiovascular disease in HIV-infected patients.

Methods

Case Identification

HIV-infected patients who experienced an incident CVD event while participating in National Institute of Allergy and Infectious Diseases (NIAID), NIH intramural clinical protocols between January 1995 and March 2009 were identified from electronic medical records. Seven categories of CVD events were defined; 1) Acute myocardial infarction (AMI) – acute clinical coronary syndrome associated with elevated cardiac enzymes or EKG documenting acute ischemia or with evidence of recent blockage by invasive imaging (N=25); 2) Silent MI (SMI) – the appearance of EKG changes indicating history of ischemia with new Q waves in two contiguous leads, dated by discovery of EKG changes (N=4); 3) Coronary Revascularization (CRv) – angioplasty with stent placement or coronary artery bypass grafting without preceding AMI or SMI (N=14); 4) Acute Coronary Syndrome (ACS) – clinical signs and symptoms of ACS including chest pain at rest lasting longer than 30 minutes associated with changes on EKG or elevated cardiac enzymes, but not considered to be sufficient for AMI by clinical judgment at the time (N=2); 5) Cerebrovascular accident (CVA) – focal neurologic deficit lasting longer than 24h and determined to be of ischemic origin (N=4); 6) Lower Extremity Revascularization – procedure to restore arterial blood supply (N=1); or 7) Sudden Cardiovascular Death – death related to CVD as determined by treating facility or autopsy (N=2). (End points modeled after those defined by the PROactive Study [25]).

Events were confirmed through detailed chart review including records submitted from outside healthcare facilities when available. Subjects were excluded if they had experienced a CVD event prior to enrollment in NIAID/NIH clinical protocols (N=17) or the acquisition of HIV (N=1). If a patient experienced multiple events while enrolled only the first was considered.

Matching

Case patients were matched 2:1 to HIV-infected individuals by sex, age (+/− 2.5 years), and enrollment date (+/− 3 years) in NIAID intramural protocols. Controls were considered eligible if they had not experienced any of the above-defined CVD events and had continuing participation in NIAID protocol(s) that included sample storage at the time of the matching event.

Clinical Data

Hypertension was defined as the use of antihypertensive medications at the event or a documented blood pressure reading >140 mmHg systolic or >90 mmHg diastolic on two or more occasions prior to the event (PTE). Dyslipidemia was defined as the use of lipid-lowering therapy or the presence of any of the following: total cholesterol >240 mg/dl, LDL >160 mg/dl, HDL <40 mg/dl or triglycerides >250 mg/dl at the visit most closely preceding the event date [26]. Diagnosis of diabetes was confirmed by a random glucose level >200mg/dl or fasting glucose >126mg/dl on two occasions PTE or use of anti-diabetic medications. Family history of premature MI was defined as MI in a male <55y or female <65y first-degree relative as documented PTE. History of smoking was positive if a patient reported having smoked >100 cigarettes; smoking was considered to be current if the patient reported on-going use or quitting less than 1 month PTE. Patient deaths were confirmed through the online social security death index (SSDI) database when possible. Cumulative exposure to ARVs, ARV regimen at event and use of risk-related medications were determined by prescribing information and history in the medical record. Standard clinical and laboratory values were collected from patient records at approximately 4 months and 2 years prior to the event (PTE).

Measurement of soluble biomarkers

Cryopreserved stored samples from approximately 4 months (4.5±0.1 months) and 2 years (21.6±0.2 months) prior to the matching event were tested. CMV DNA was quantitated by realtime PCR as described by Yun et al [27]. Plasma samples were analyzed by ELFA (Enzyme Linked Fluorescent Assay) on a VIDAS instrument for D-Dimer (bioMerieux Inc., Durham, NC) and by ELISA for HMGB-1 (Shino-Test Corp., Tokyo, Japan), soluble CD14 and soluble Tissue Factor (R&D Systems, Minneapolis, MN). Serum samples were analyzed by ELFA on the VIDAS for NT-pro BNP (bioMerieux Inc., Durham, NC) and ELISA using a 9-plex Pro-inflammatory kit, a 9-plex Chemokine kit, a 4-plex Vascular injury II kit, and four single-plex kits (Meso Scale Discovery, Gaithersburg, MD) for the detection of the following cytokines: GM-CSF, IFNγ, IL-1β, IL-10, IL-12p70, IL-2, IL-6, IL-8, TNFα, Eotaxin, Eotaxin-3, IP-10, MCP-1, MCP-4, MDC, MIP-1b, TARC, Serum Amyloid A, VCAM-1, CRP, ICAM-1, MPO, TIMP, TNFR-II, and Adiponectin. All of the listed tests were performed according to the manufacturers' instructions. Reference values from pooled and individual healthy donors as provided by the manufacturers (mean and standard deviation), and the NIAID clinical laboratories as available are as follows: D-dimer <0.5ug/ml, CRP <3.0mg/ml, GM-CSF (1.1 ±0.4 pg/ml), IFNγ (3.1±3.8 pg/ml), IL-1β (1.3±0.9 pg/ml), IL-10 (8.2±24.4 pg/ml), IL-12p70 (17.8±59.1 pg/ml), IL-2 (1.0±0.3 pg/ml), IL-6 (3.2±9.3 pg/ml), IL-8 (20.6±14.0 pg/ml), TNFα ((8.2±2.0 pg/ml), Eotaxin (1516±226 pg/ml), Eotaxin-3 (12.3±5.3 pg/ml), IP-10 (86±29 pg/ml), MCP-1 (502±57 pg/ml), MCP-4 (1043±137 pg/ml), MDC (3230±790 pg/ml), MIP-1b (151±19 pg/ml), TARC (753±111 pg/ml), Serum Amyloid A (1485±189 pg/ml), VCAM-1 (419±127 pg/ml), ICAM-1 (250±36 pg/ml), MPO (383±84 pg/ml), TIMP-1 (308±43 ng/ml), TNFR-II (1490±354 pg/ml), and Adiponectin (8460±189 ng/ml).

Flow Cytometry

To investigate contribution of cellular immune activation, T-cell and monocyte markers of activation were measured by four-color flow cytometry in cryopreserved peripheral blood mononuclear cells (PBMCs) from time points matching those used for the soluble markers. The fluorochrome-conjugated antibodies used were: anti-CD3 Pacific Blue, anti-CD3 APC-Cy7, anti-CD25 PE-Cy7, anti-CD36 FITC, anti-CD38 APC, anti-CD56 PB, anti-CD142 PE, anti-Ki-67 FITC, anti-HLA-DR PE-Cy7, anti-CCR5 PE and anti-HLA-DR APC-Cy7 from BD-Biosciences, anti-PD-1 PerCP-Cy5.5 from BioLegend, anti-CD3 PE, anti-CD4 Qdot605, anti-CD8 PB, anti-CD14 Qdot605, anti-CD16 PerCP, anti-CD20 PB and Live/Dead Fixable Blue Dead Cell Stain Kit with UV excitation from Invitrogen, and anti-CD11c PE-Cy7, anti-CD39 FITC and anti-FoxP3 PE-Cy5 from eBioscience. Samples were acquired on an LSR-II flow cytometer (BD) and data were analyzed using FlowJo software version 8.8.6 (Treestar Inc, Ashland, OR).

Statistical methods

The prevalence of clinical risk factors was compared by chi square analysis with Fisher's exact test. Continuous variables were compared by Student's t test. Non-normally distributed variables were $\log_{10}$-transformed for analyses to approximate a normal distribution. Multivariable analysis was performed using a forward stepwise regression model including all significant variables as defined by univariate logistic regression ($p < 0.05$). All statistical analyses were performed with JMP software (JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007).

Results

Fifty-two patients who experienced incident CVD events were identified from a pool of 1,709 HIV-infected subjects enrolled in NIAID/NIH clinical protocols between 1995 and 2009 (5.1 events/1000 patient years). One hundred and four HIV-infected subjects without a history of

CVD events were matched as described above to serve as controls. Cardiac events represented 90% of the case population. Both cases of sudden cardiac death were determined to be due to acute myocardial infarction, one by the receiving emergency care facility and the second by autopsy. All cases of silent MI with Q wave changes in successive EKGs had been confirmed by invasive imaging techniques.

Clinical Risk Factors

There were no significant differences in race, nadir CD4+ count, peak plasma HIV-RNA, mode of acquisition, or duration of HIV infection between cases and controls (Table 1).

The prevalence of dyslipidemia was greater in cases than controls (87.0% vs. 71.9%, p=0.05). The prevalence of hypertension in this population was relatively high, but no greater in cases than controls (72.5% vs. 67.3%, p=0.57). Cases were significantly more likely to be active smokers at the time of the event (49.0% vs. 25.0%, p=0.004). A positive family history of premature MI was also significantly more likely in the cases than controls (29.8% vs. 10.9%, p=0.003). There were no differences in the prevalence of diabetes or in mean BMI between groups (Table 1), or in history of substance abuse or known cocaine use (data not shown).

There were no statistically significant differences between cases and controls in cumulative months of exposure to all antiretroviral agents or classes of antiretroviral agents (Table 1). In addition, there was no difference in the prevalence of PI or NNRTI use at the time of the event, nor were there differences in the subjects' recent or past exposure to IL-2, didanosine or abacavir (data not shown).

Total and LDL cholesterol were both elevated in the cases; this difference was significant at 4 months PTE (p=0.002 and p=0.04, respectively). HDL and triglycerides were not significantly different at either time point. Serum glucose was significantly higher in the cases at 2 years PTE (115 vs. 102, p=0.03). The number of circulating CD14+ monocytes was significantly higher in the cases 4 months PTE (p=0.04). The prothrombin time (PT) was significantly lower in cases than controls at 4 months PTE. Plasma HIV-RNA was significantly lower in the cases than the controls at 4 months PTE (2540 vs. 13860 copies/ml, p=0.04). No differences were seen in CD4+ or CD8+ T cell count at either time point (Table 2). The prevalence of CMV viremia (at 4 months PTE) was 8% in controls and 6% in cases; neither prevalence nor CMV viral load was significantly different between the groups (data not shown).

No differences in prevalence of febrile, neoplastic, or inflammatory diseases were observed between cases or controls, nor did the analysis change significantly when these patients were excluded (data not shown).

Biomarkers

Two biomarkers were elevated in the cases at both tested time points PTE; D-dimer (p=0.003 at 4 months and p=0.04 at 2 years) and VCAM-1 (p=0.02 at 4 months and p=0.03 at 2 years). Soluble tissue factor and TIMP-1 were elevated in the cases at 4 months PTE (p=0.02 in both). Serum amyloid A and myeloperoxidase (MPO) were elevated in the cases compared to controls at 2 years PTE (p=0.03 and p=0.005, respectively) (Table 3 and Figure 1). IL-6, TNFα, MDC and NT-proBNP tended to be higher in the cases than controls, but these differences were not significant (Table 3). Serum levels of hsCRP, adiponectin, IL-1β, IL-2, IL-8, IL-10, IL-12p70, TARC, Eotaxin, MIP-1β, GM-CSF, IFNγ, MCP-1, MCP-4, sICAM-1, and TNF-RII did not differ significantly between cases and controls.

Cell Surface Markers

There were no significant differences in the expression of CD38/HLA-DR, CCR5 or PD-1 on CD4+ and CD8+ T cells or in the relative percentages of T-regulatory cells as measured by CD25/FoxP3 co-expression, though cases tended to have lower PD-1 expression on CD8+ T cells at 4 months PTE (18.8% vs. 22.4%, $p = 0.15$) and lower co-expression of CD11c and CD36 on monocytes at 2 years PTE (40.5% vs. 46.0%, $p = 0.06$) (Table 3). There were no significant differences in the expression of CD16, CD142 (Tissue Factor), CD11c, co-expression of CD11c with CD16, or in the relative sizes of monocyte CD14+/CD16+ subsets of cases and controls (data not shown).

Multivariate Analysis

After determining variables for entry into multivariate analysis by univariate regression ($p < 0.05$), a stepwise multivariate model was constructed for each of the two time points. At 2 years prior to the event, family history of premature MI ($p = 0.03$), plasma D-dimer ($p = 0.006$), and serum glucose ($p = 0.001$) contributed independently to CVD event risk. At 4 months prior to the event, plasma D-dimer ($p = 0.02$), family history ($p = 0.006$), current smoking ($p = 0.004$), and total cholesterol ($p = 0.0005$) contributed independently to CVD event risk.

Discussion

In this study, traditional risk factors and markers of inflammation and cell activation were evaluated in HIV-infected individuals prior to an incident CVD event to investigate the potential shared and unique CVD risk factors that exist within the HIV-infected population. While the strongest contributors were traditional CVD risk factors such as smoking and high cholesterol, markers of innate immune activation, endothelial cell dysfunction, and thrombosis were also related to CVD events.

The two clinical factors most strongly associated with future CVD events were family history of premature myocardial infarction and active smoking at the time of the event. The overall prevalence of a history of smoking was relatively high in both groups, but at the time of the event was nearly twice as high in cases as controls, demonstrating the known influence of active smoking on CVD risk. It also highlights the importance of smoking cessation counseling in the HIV clinical setting.

The high frequency of dyslipidemia in both cases and controls was likely due to a combination of genetic predisposition, HIV infection and ARV therapy. Although there were no differences in HDL or triglycerides, which are frequently abnormal and directly related to HIV infection, cases had higher total and LDL cholesterol compared to controls. The prevalence of diabetes was 13% (compared to 7.8% in the US population (http://diabetes.niddk.nih.gov/)) and the average BMI was above the normal range but non-obese (25.6 kg/m^2). One indication of the relation of hyperglycemia with CVD risk in this study, however, was that cases had significantly higher blood glucose at two years PTE. This effect was maintained in multivariable analysis, highlighting a long-term risk of hyperglycemia. It was not possible to determine the prevalence of the metabolic syndrome, as data on abdominal girth were not available.

Large multicenter cohorts such as the DAD study group [3] have identified an increased risk of MI with increased years of ARV exposure compared to HIV-infected individuals who are ARV naïve. Our data did not support a connection between specific antiretroviral drugs, drug classes, or duration of ARV exposure and incidence of cardiovascular disease, although this may be due to the small number of cases and inclusion of only two ARV naïve subjects (both controls).

Plasma HIV-RNA viremia, which is known to cause immune activation, was not associated with increased CVD risk in our cohort. Instead, prior to the event the cases were more likely to have lower plasma HIV-RNA, which correlated with a trend toward lower PD-1 expression in CD8+ T cells, a marker of immune dysregulation that is strongly related to viremia [28]. The difference in plasma HIV-RNA between the groups was not associated with significant differences in duration of therapy or treatment regimen.

HIV also causes activation and dysregulation in the innate immune system [29,30] that may negatively influence cardiovascular health. Activated CD14+ monocytes are associated with increased CVD risk in the general population [20,31]. Additionally, monocyte-associated tissue factor (CD142) is up-regulated in HIV infection and related to viremia, potentially indicating an induced hypercoagulable state [32,33]. Although the number of CD14+ cells was significantly higher in the cases, this was not associated with elevated expression of other markers of monocyte activation such as HLA-DR and CD142. The detection of these monocyte markers may have been limited by cryopreservation. Importantly, increased monocyte activation in the cases was demonstrated by significantly higher levels of soluble tissue factor and MPO and a trend toward higher TNFα, MDC and IL-6. Soluble CD14 (sCD14), a marker of monocyte activation by LPS that is elevated in HIV-infected individuals and is suspected to contribute to chronic inflammation and cardiovascular risk [32,34], was not different between cases and controls.

D-dimer, a soluble product of fibrinogen breakdown, is a marker of thrombosis that is elevated in patients with premature cardiovascular disease [35] and was associated with mortality in the SMART study [11]. In our cohort, D-dimer was significantly elevated in the cases at both time points and independently related to CVD risk in multivariate analysis. In contrast, there was no difference in hsCRP between case and control subjects, although hsCRP is related to CVD event risk and associated risk factors such as the metabolic syndrome in the non-HIV population [36-38]. The capacity for CRP to serve as a discriminatory marker of CVD risk in the HIV-infected population may be confounded by multiple, distinct factors influencing CRP including significant underlying disease.

Five markers of endothelial activation and damage were measured: sVCAM-1, sICAM-1, TIMP-1 and Eotaxins 1 and 3. TIMP-1 is an inhibitor of matrix metalloproteinases postulated to indicate vascular damage or oxidative stress [39] and Eotaxin-3 paradoxically is lower in patients with elevated CVD risk [40]. In our study TIMP-1 was significantly elevated in the cases 4 months PTE, and Eotaxin-3 tended to be lower in the cases 2 years PTE. Vascular cellular adhesion molecule (VCAM-1) is expressed by vascular endothelial cells in response to pro-inflammatory cytokines TNFα and IL-1 as well as shearing or wall stress [41]. In the HIV-infected population VCAM-1 expression is elevated in early HIV infection and with viremia, reported to return to baseline after two years of consistent ART therapy [42] and is associated with the metabolic syndrome [43]. Here VCAM-1 was significantly elevated in the cases over controls at both time points, indicating its potential use in risk stratification in the HIV-infected population. Subgroup analysis excluding the five cases with non-cardiac CVD-related events did not significantly change this analysis.

In conclusion, in this cohort of HIV-infected patients who experienced cardiovascular events, the factors most related to a subject's status as a case or control included active smoking, family history of premature myocardial infarction, elevated total cholesterol, glucose, plasma D-dimer and soluble tissue factor. Unexpectedly, in this cohort of patients with long-term HIV infection who are well controlled on HAART, HIV-related characteristics such as ARV therapy or regimen and CD4+ T-cell count were not related. Additionally, while markers of innate immune activation, thrombosis, and endothelial cell damage including D-dimer, soluble tissue factor, VCAM-1, MPO, and serum amyloid A were associated with CVD events, these

elevations were not accompanied by higher expression of markers of T-cell activation that are characteristic of HIV infection. It is possible that other important markers, such as NT-proBNP, IL-6, MDC, and GM-CSF did not reach statistical significance due to the small sample size. Our findings support an aggressive approach in identifying significant family history and targeting traditional cardiac risk factors for therapeutic intervention such as management of dyslipidemia, smoking cessation and the potential addition of biomarkers such as D-dimer in further stratification of high-risk patients.

Acknowledgments

This research was supported in part by the Intramural Program of the NIH, NIAID and Critical Care Medicine Department. Additionally, this project has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

The authors would like to thank all study participants and the staff of outpatient clinic 8 at the National Institute of Allergy and Infectious Disease (NIAID) of the NIH clinical center. Thanks also to William Thompson for technical assistance and Dr. Douglas Rosing for his review of study participant EKGs.

IS and CH designed the study, identified case subjects and assisted in data analysis and in writing the manuscript. EF identified case and control subjects, gathered clinical data, and assisted in performance of lab studies, data analysis, and in writing the manuscript. JG and AR assisted in the performance of lab studies and data analysis. AR, YB, and VN assisted in the performance of lab studies. LD assisted in the matching of control subjects. CR assisted in the identification of subjects and patient samples. All authors assisted in reviewing the manuscript and approved the final version of the manuscript.

References

1. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab 2007;92:2506–2512. [PubMed: 17456578]
2. Grinspoon SK, Grunfeld C, Kotler DP, Currier JS, Lundgren JD, Dube MP, et al. State of the Science Conference: Initiative to Decrease Cardiovascular Risk and Increase Quality of Care for Patients Living With HIV/AIDS: Executive Summary. Circulation 2008;118:198–210. [PubMed: 18566320]
3. Friis-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio Monforte A, et al. Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. AIDS 2003;17:1179–1193. [PubMed: 12819520]
4. The DAD Study Group. Class of Antiretroviral Drugs and the Risk of Myocardial Infarction. N Engl J Med 2007;356:1723–1735. [PubMed: 17460226]
5. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. The Lancet 2008;371:1417–1426.
6. El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med 2006;355:2283–2296. [PubMed: 17135583]
7. Phillips AN, Carr A, Neuhaus J, Visnegarwala F, Prineas R, Burman WJ, et al. Interruption of antiretroviral therapy and risk of cardiovascular disease in persons with HIV-1 infection: exploratory analyses from the SMART trial. Antivir Ther 2008;13:177–187. [PubMed: 18505169]
8. Grunfeld C, Delaney JA, Wanke C, Currier JS, Scherzer R, Biggs ML, et al. Preclinical atherosclerosis due to HIV infection: carotid intima-medial thickness measurements from the FRAM study. AIDS 2009;23:1841–1849. [PubMed: 19455012]
9. van Vonderen MG, Smulders YM, Stehouwer CD, Danner SA, Gundy CM, Vos F, et al. Carotid intima-media thickness and arterial stiffness in HIV-infected patients: the role of HIV, antiretroviral therapy, and lipodystrophy. J Acquir Immune Defic Syndr 2009;50:153–161. [PubMed: 19131894]
10. Baker JV, Duprez D, Rapkin J, Hullsiek KH, Quick H, Grimm R, et al. Untreated HIV infection and large and small artery elasticity. J Acquir Immune Defic Syndr 2009;52:25–31. [PubMed: 19731451]

11. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med 2008;5:e203. [PubMed: 18942885]
12. Ridker P, Hennekens C, Cerskus A, Stampfer M. Plasma concentration of cross-linked fibrin degradation product (D- dimer) and the risk of future myocardial infarction among apparently healthy men. Circulation 1994;90:2236–2240. [PubMed: 7955179]
13. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation 1998;98:731–733. [PubMed: 9727541]
14. Empana JP, Canoui-Poitrine F, Luc G, Juhan-Vague I, Morange P, Arveiler D, et al. Contribution of novel biomarkers to incident stable angina and acute coronary syndrome: the PRIME Study. Eur Heart J 2008;29:1966–1974. [PubMed: 18621771]
15. Ford ES, Puronen CE, Sereti I. Immunopathogenesis of asymptomatic chronic HIV Infection: the calm before the storm. Curr Opin HIV AIDS 2009;4:206–214. [PubMed: 19532052]
16. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 2006;443:350–354. [PubMed: 16921384]
17. Giorgi JV, Detels R. T-cell subset alterations in HIV-infected homosexual men: NIAID Multicenter AIDS cohort study. Clin Immunol Immunopathol 1989;52:10–18. [PubMed: 2656013]
18. Sachsenberg N, Perelson AS, Yerly S, Schockmel GA, Leduc D, Hirschel B, Perrin L. Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen. J Exp Med 1998;187:1295–1303. [PubMed: 9547340]
19. Haque S, Gordon C, Isenberg D, Rahman A, Lanyon P, Bell A, et al. Risk Factors for Clinical Coronary Heart Disease in Systemic Lupus Erythematosus: The Lupus and Atherosclerosis Evaluation of Risk (LASER) Study. J Rheumatol. 2009
20. Hubacek JA, Pit'ha J, Skodova Z, Poledne R. C(−260)->T Polymorphism in the Promoter of the CD14 Monocyte Receptor Gene as a Risk Factor for Myocardial Infarction. Circulation 1999;99:3218–3220. [PubMed: 10385492]
21. Heine GH, Ulrich C, Seibert E, Seiler S, Marell J, Reichart B, et al. CD14(++)CD16+ monocytes but not total monocyte numbers predict cardiovascular events in dialysis patients. Kidney Int 2008;73:622–629. [PubMed: 18160960]
22. Crowe SM, Westhorpe CL, Mukhamedova N, Jaworowski A, Sviridov D, Bukrinsky M. The macrophage: the intersection between HIV infection and atherosclerosis. J Leukoc Biol. 2009
23. Han J, Wang B, Han N, Zhao Y, Song C, Feng X, et al. CD14(high)CD16(+) rather than CD14(low) CD16(+) monocytes correlate with disease progression in chronic HIV-infected patients. J Acquir Immune Defic Syndr 2009;52:553–559. [PubMed: 19950429]
24. Rossi R, Nuzzo A, Guaraldi G, Orlando G, Squillace N, Ligabue G, et al. The role of the Framingham risk score to predict the presence of subclinical coronary atherosclerosis in patients with HIV infection. J Acquir Immune Defic Syndr 2009;52:303–304. [PubMed: 20118681]
25. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. Lancet 2005;366:1279–1289. [PubMed: 16214598]
26. Grundy SM, Cleeman JI, Merz CNB, Brewer HB Jr, Clark LT, Hunninghake DB, et al. Implications of Recent Clinical Trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. Circulation 2004;110:227–239. [PubMed: 15249516]
27. Yun Z, Lewensohn-Fuchs I, Ljungman P, Ringholm L, Jonsson J, Albert J. A real-time TaqMan PCR for routine quantitation of cytomegalovirus DNA in crude leukocyte lysates from stem cell transplant patients. J Virol Methods 2003;110:73–79. [PubMed: 12757923]
28. Salisch NC, Kaufmann DE, Awad AS, Reeves RK, Tighe DP, Li Y, et al. Inhibitory TCR Coreceptor PD-1 Is a Sensitive Indicator of Low-Level Replication of SIV and HIV-1. J Immunol 2010;184:476–487. [PubMed: 19949078]

29. Mandl JN, Barry AP, Vanderford TH, Kozyr N, Chavan R, Klucking S, et al. Divergent TLR7 and TLR9 signaling and type I interferon production distinguish pathogenic and nonpathogenic AIDS virus infections. Nat Med 2008;14:1077–1087. [PubMed: 18806803]
30. Boasso A, Shearer GM. Chronic innate immune activation as a cause of HIV-1 immunopathogenesis. Clin Immunol 2008;126:235–242. [PubMed: 17916442]
31. Jude B, Agraou B, McFadden EP, Susen S, Bauters C, Lepelley P, et al. Evidence for time-dependent activation of monocytes in the systemic circulation in unstable angina but not in acute myocardial infarction or in stable angina. Circulation 1994;90:1662–1668. [PubMed: 7923650]
32. Funderburg NT, Mayne E, Sieg SF, Asaad R, Jiang W, Kalinowska M, et al. Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. Blood. 2009
33. Schecter AD, Berman AB, Yi L, Mosoian A, McManus CM, Berman JW, et al. HIV envelope gp120 activates human arterial smooth muscle cells. Proc Natl Acad Sci U S A 2001;98:10142–10147. [PubMed: 11504923]
34. Lien E, Aukrust P, Sundan A, Muller F, Froland SS, Espevik T. Elevated Levels of Serum-Soluble CD14 in Human Immunodeficiency Virus Type 1†(HIV-1) Infection: Correlation to Disease Progression and Clinical Events. Blood 1998;92:2084–2092. [PubMed: 9731066]
35. Mills JD, Mansfield MW, Grant PJ. Tissue plasminogen activator, fibrin D-dimer, and insulin resistance in the relatives of patients with premature coronary artery disease. Arterioscler Thromb Vasc Biol 2002;22:704–709. [PubMed: 11950714]
36. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO III, Criqui M, et al. Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: A Statement for Healthcare Professionals From the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499–511. [PubMed: 12551878]
37. Festa A, D'Agostino R Jr. Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000;102:42–47. [PubMed: 10880413]
38. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation 1998;97:2007–2011. [PubMed: 9610529]
39. Moore R, Hawley A, Sigler R, Farris D, Wrobleski S, Ramacciotti E, Myers D. Tissue inhibitor of metalloproteinase-1 is an early marker of acute endothelial dysfunction in a rodent model of venous oxidative injury. Ann Vasc Surg 2009;23:498–505. [PubMed: 19467832]
40. Falcone C, Minoretti P, D'Angelo A, Buzzi MP, Coen E, Emanuele E, et al. Markers of eosinophilic inflammation and risk prediction in patients with coronary artery disease. European Journal of Clinical Investigation 2006;36:211–217. [PubMed: 16620281]
41. O'Keeffe LM, Muir G, Piterina AV, McGloughlin T. Vascular cell adhesion molecule-1 expression in endothelial cells exposed to physiological coronary wall shear stresses. J Biomech Eng 2009;131:081003. [PubMed: 19604015]
42. Francisci D, Giannini S, Baldelli F, Leone M, Belfiori B, Guglielmini G, et al. HIV type 1 infection, and not short-term HAART, induces endothelial dysfunction. AIDS 2009;23:589–596. [PubMed: 19177019]
43. Jacobs M, van Greevenbroek MM, van der Kallen CJ, Ferreira I, Blaak EE, Feskens EJ, et al. Low-grade inflammation can partly explain the association between the metabolic syndrome and either coronary artery disease or severity of peripheral arterial disease: the CODAM study. Eur J Clin Invest 2009;39:437–444. [PubMed: 19397692]

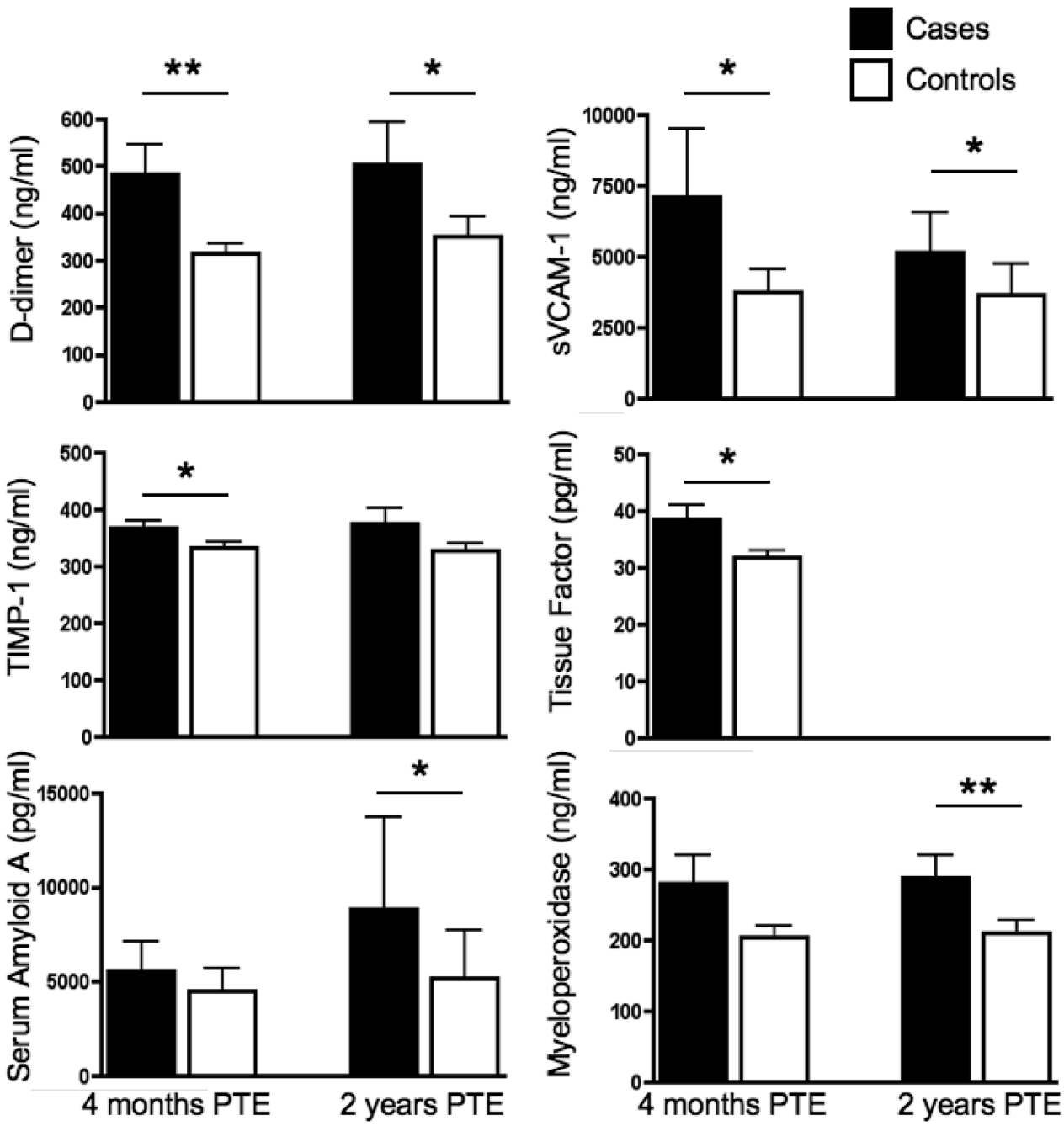

Figure 1.

D-dimer, soluble vascular cell adhesion molecule-1 (sVCAM-1), tissue inhibitor of metalloproteinases-1 (TIMP-1), soluble tissue factor (sTF), serum amyloid A (SAA) and myeloperoxidase (MPO) in cases and controls at 4 months and 2 years prior to the matching event (PTE). D-dimer and sVCAM-1 were significantly elevated in cases at both time points, TIMP-1 and sTF at 4 months PTE, and SAA and MPO at 2 years PTE.

Table 1

Demographics, HIV-infection related characteristics and prevalence of traditional risk factors at the time of the matching event[8]

	Cases (N=52)	Controls (N=104)	P
Age	50.8 ± 1.0 (32.2 – 62.8)	50.8 ± 0.7 (31.1 – 65.1)	0.97
% Male	98.0%	98.0%	1.0
% African American	19.2%	14.4%	0.48
Exposure (% MSM)	87.5%	92.9%	0.35
Years of follow up	8.2 ± 0.7 (0.5 – 19.5)	8.6 ± 0.5 (1.0 – 20.1)	0.61
Years of HIV infection	13.4 ± 0.7 (2.1 – 24.2)	14.0 ± 0.5 (2.6 – 24.5)	0.50
Nadir CD4+ T cells/uL	209 ± 21 (4 – 623)	229 ± 15 (0 – 693)	0.42
Peak plasma HIV-RNA copies/mL	$4.3\times10^5 \pm 1.5\times10^5$	$5.4\times10^5 \pm 1.4\times10^5$	0.40
Years of ARV exposure	8.9 ± 0.5	8.4 ± 0.4	0.67
Years of PI exposure	3.7 ± 0.4	4.0 ± 0.3	0.61
% HCV co-infection	13.5%	13.5%	1.0
Dyslipidemia*	87.0%	71.9%	**0.05**
Use of lipid-lowering therapy	34.6%	23.1%	0.13
Hypertension**	72.5%	67.3%	0.57
Use of anti-hypertensive therapy	38.5%	24.0%	*0.06*
History of ever smoking	58.8%	52.5%	0.46
Current smoking at event	49.0%	25.0%	**0.004**
Diabetes	15.4%	12.7%	0.62
Family history of premature MI	29.8%	10.9%	**0.003**
BMI 4mo PTE (kg/m^2)	25.6 ± 0.5	25.6 ± 0.4	1.0

[8]Shown are means and standard error with ranges in parentheses when appropriate.

Abbreviations used: MSM – men who have sex with men, ARV – antiretroviral, PI – protease inhibitor, HCV – hepatitis C virus, and PTE - prior to the event.

Table 2

Laboratory measurements[9]

	4 months PTE			2 years PTE		
	Cases (N = 52)	**Controls (N = 104)**	**P**	**Cases (N = 52)**	**Controls (N = 104)**	**P**
Total Cholesterol (mg/dL)	212 ± 5.4	188 ± 4.2	**0.002**	206 ± 6.5	196 ± 4.6	0.29
LDL Cholesterol (mg/dL)	130 ± 5.0	116 ± 3.2	**0.04**	127 ± 5.0	123 ± 3.8	0.64
HDL Cholesterol (mg/dL)	41 ± 1.6	39 ± 1.1	0.47	38 ± 1.6	42 ± 1.2	0.11
Triglycerides (mg/dL)	333 ± 34	281 ± 22	0.25	374 ± 57	279 ± 25	0.12
Glucose (mg/dL)	116 ± 5.1	107 ± 2.6	0.16	115 ± 5.2	102 ± 1.9	**0.03**
White blood cells/mL	6611 ± 462	5633 ± 189	*0.06*	6420 ± 430	5790 ± 180	0.20
Platelets (/mL)	231 ± 10	218 ± 6	0.25	213 ± 11	216 ± 6	0.53
CD14+ cells/mL	1200 ± 112	825 ± 78	**0.04**	1090 ± 104	866 ± 44	*0.06*
CD4+ T cells/mL	646 ± 45	625 ± 32	0.70	647 ± 59	616 ± 31	0.67
CD8+ T cells/mL	1112 ± 68	1065 ± 51	0.60	1106 ± 81	1099 ± 47	0.94
Prothrombin time (seconds)	12.6 ± 0.1	13.0 ± 0.08	**0.04**	12.8 ± 0.1	12.7 ± 0.08	0.53
% with macroproteinuria	46.8%	33.7%	0.13	51.3%	37.6%	0.14
HIV-RNA (copies/mL)	2540 ± 1570	13860 ± 3790	**0.04**	20560 ± 10740	15390 ± 3540	0.34
% undetectable HIV-RNA	59.2%	54.8%	0.61	63.0%	45.6%	**0.05**
Systolic BP (mmHg)	127 ± 2	128 ± 2	0.41	130 ± 3	127 ± 2	0.10
Diastolic BP (mmHg)	77 ± 1	76 ± 1	0.38	78 ± 1	76 ± 1	*0.06*

[9]Abbreviations used: PTE – prior to the event, BP – blood pressure

AIDS. Author manuscript; available in PMC 2011 June 19.

AIDS. Author manuscript; available in PMC 2011 June 19.

Table 3

Serum and plasma markers of inflammation, endothelial insult, and thrombosis and cellular markers of T cell and monocyte activation[10]

	4 months PTE			2 years PTE		
	Cases (N=40)	**Controls (N=87)**	**P**	**Cases (N=40)**	**Controls (N=86)**	**P**
MDC (pg/mL)	3470 ± 290	2880 ± 160	0.11	3360 ± 240	3000 ± 160	*0.10*
Serum Amyloid A (ng/mL)	5560 ± 1610	4490 ± 1250	*0.06*	8880 ± 4850	5220 ± 2500	**0.03**
ICAM-1 (ng/mL)	309 ± 15	306 ± 17	0.23	304 ± 20	296 ± 14	0.27
VCAM-1 (ng/mL)	7130 ± 2370	3770 ± 770	**0.02**	5170 ± 1380	3650 ± 1120	**0.03**
hsCRP (ng/mL)	570 ± 41	570 ± 26	0.60	570 ± 40	570 ± 26	0.47
MPO (ng/mL)	281 ± 39	205 ± 15	*0.07*	289 ± 32	210 ± 19	**0.005**
D-dimer (ng/mL)	482 ± 65	315 ± 20	**0.003**	505 ± 88	350 ± 44	**0.04**
TIMP-1 (ng/mL)	369 ± 12	332 ± 12	**0.02**	376 ± 28	327 ± 14	0.16
NT-proBNP (pg/mL)	48 ± 14	26 ± 3	*0.08*	60 ± 21	25 ± 2	*0.09*
Eotaxin-3 (pg/mL)	13.4 ± 1.4	12.9 ± 0.9	0.95	16 ± 1.3	18 ± 4.1	*0.08*
IL-2 (pg/mL)	1.5 ± 0.3	1.0 ± 0.1	0.21	1.6 ± 0.6	1.6 ± 0.6	0.87
IL-6 (pg/mL)	5.1 ± 1.2	3.4 ± 0.4	*0.09*	31 ± 27	3.8 ± 0.5	*0.10*
IL-10 (pg/mL)	65 ± 63	3.4 ± 0.4	0.88	163 ± 150	5.0 ± 2.0	0.27
sCD14 (pg/mL)	2.2 ± 0.07	2.2 ± 0.04	0.52			
sTF (pg/mL)	38.5 ± 2.5	31.9 ± 1.2	**0.02**			
TNFa (pg/mL)	20.9 ± 3.5	18.1 ± 2.3	0.29	26.1 ± 6.0	21.5 ± 2.8	*0.08*
IFNy (pg/mL)	1.7 ± 0.3	1.3 ± 0.1	0.83	1.7 ± 0.3	1.5 ± 0.2	0.55
CD11c/CD36+ monocytes	43.2 ±17.6%	46.2 ±14.5%	0.39	40.5 ±14.5%	46.0 ±15.5%	*0.06*
CD8/PD-1+ T cells	23.2 ±10.4%	23.6 ±11.9%	0.85	18.8 ± 6.6%	22.4 ± 1.5%	0.15
CD4/CD38/HLADR+ T cells	11.4 ± 2.2%	10.0 ± 0.9%	0.93	8.3 ± 0.8%	10.0 ± 0.8%	0.29
CD8/CD38/HLADR+ T cells	32.1 ± 2.3%	30.7 ± 1.6%	0.48	26.6 ± 2.1%	29.8 ± 1.6%	0.27

[10] Abbreviations used – PTE – prior to the event; MDC – macrophage-derived chemokine (CCL22); ICAM-1 – intercellular adhesion molecule 1 (CD54); VCAM-1 – vascular cell adhesion molecule 1 (CD106); hsCRP – high sensitivity C-reactive protein; MPO – myeloperoxidase; TIMP-1 – tissue inhibitor of metalloproteinase 1; NT-proBNP – N-terminal fragment of brain natriuretic peptide; IL – interleukin; sCD14 – soluble CD14; sTF – soluble tissue factor; TNFα – tumor necrosis factor alpha; IFNγ – interferon gamma