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Biomarkers of Vascular Dysfunction in Children Infected with Human Immunodeficiency Virus-1

Tracie L. Miller, MD, **Gabriel Somarriba, DPT**, **E. John Orav, PhD**, **Armando J. Mendez, PhD**, **Daniela Neri, RD**, **Natasha Schaefer**, **Lourdes Forster, MD**, **Ronald Goldberg, MD**, **Gwendolyn B. Scott, MD**, and **Steven E. Lipshultz, MD**
Divisions of Pediatric Clinical Research (TLM, GS, DN, NS, SEL), General Pediatrics (LF), Infectious Diseases and Immunology (GBS), Department of Pediatrics; Diabetes Research Institute (AM, RG), Miller School of Medicine, University of Miami, Miami, FL and the Department of Epidemiology and Biostatistics, Harvard School of Public Health, Boston, MA (EJO)

Abstract

Background—We compared biomarkers of vascular dysfunction among HIV-infected children to a demographically-similar group of uninfected children and determined factors associated with these biomarkers.

Methods and Results—We measured several biomarkers of vascular dysfunction: C-Reactive Protein (CRP), interleukin-6 (IL-6), and monocyte chemoattractant protein -1 (MCP1) [inflammation]; fibrinogen and P-selectin [coagulant dysfunction]; soluble intracellular cell adhesion molecule-1 (sICAM), soluble vascular cell adhesion molecule-1 (sVCAM), and E-selectin [endothelial dysfunction]; and leptin [metabolic dysfunction]. Anthropometry, body composition, CD4%, HIV viral load, and antiretroviral therapy were recorded. Mean age was 14.8y [106 HIV-infected children] and 12.3y [55 control children]. Sex and body mass index Z-scores were similar. Infected children had higher sICAM, sVCAM, MCP1, IL-6, and fibrinogen levels. E-selectin (p=0.07), and CRP (p=0.08) trended to be greater in the HIV group, yet leptin, and P-selectin were similar. In multivariable analyses in the HIV-infected children alone, each 1-standard-deviation increase in waist:hip ratio was associated with increases in sICAM (17%), MCP1 (19%), IL6 (18%), and CRP (59%). CD4% was inversely associated with sVCAM, MCP1, IL6, fibrinogen, and CRP.

Conclusion—HIV-infected children have higher levels of biomarkers of vascular dysfunction than healthy children. Risk factors associated with these biomarkers include higher waist:hip ratios and HIV disease severity.

Keywords

Children; HIV/AIDS; vascular dysfunction; cardiovascular risk factors; biomarkers

INTRODUCTION

Children with the human immunodeficiency virus (HIV) living in developed countries can now expect to live with their disease for many years. Advances in early diagnosis and treatment, as

Please address all correspondence to: Tracie L. Miller, MD, Division of Pediatric Clinical Research, Department of Pediatrics (D820), Miller School of Medicine at the University of Miami, Batchelor Children's Research Institute, PO Box 016820, Miami, FL 33101, Phone (305)-243-1423, Facsimile (305)-243-8475, tracie.miller@miami.edu.

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DISCLOSURES
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well as improved therapeutics against HIV, are largely responsible for this longer life expectancy.[1] However, improved survival has brought other medical risks associated with this chronic viral illness, and often, a life-long exposure to antiretroviral medications has its own toxicities.

Abnormal metabolic conditions associated with HIV infection and its treatments are some of the most prevalent problems in both adults and children. These conditions include hyperlipidemia, insulin resistance, type 2 diabetes mellitus, and body fat redistribution.[2] As patients live longer with HIV, these conditions in adults are now contributing to more serious, life-threatening disorders that are associated with atherosclerotic cardiovascular risk, such as myocardial infarction and stroke.[3] Although HIV-infected children have risk factors for atherosclerotic cardiovascular disease,[4] the prevalence of end-stage events (e.g., overt cardiovascular disease) is not yet apparent, in part because of insufficient follow-up, the overall protective effects of youth, and fewer exposures to life-style risk factors (alcohol, smoking, physical inactivity).

The pathogenesis of atherosclerotic cardiovascular disease has been largely linked to a proinflammatory response in the arteries.[5] Higher levels of C-reactive protein (CRP), indicating a proinflammatory state, have been associated with cardiovascular risk in adults without HIV infection and has been proposed for clinical use. However, several other biomarkers have also been associated with atherosclerotic cardiovascular risk, including biomarkers associated with inflammation, procoagulation, endothelial dysfunction, and metabolic dysregulation.[6] Changes in any of these pathways may increase atherosclerotic cardiovascular risk, and emerging evidence indicates abnormalities in these pathways in HIV-infected adults. Whether these relationships are also found in children who have been exposed to a lifetime of HIV infection and its therapies is unknown.

We determined whether the levels of selected biomarkers associated with vascular inflammation pathways in HIV-infected children differed from those in a similar group of non-infected children. Furthermore, we determined whether there were any disease-specific or treatment-specific factors associated with higher levels of these biomarkers.

METHODS

Patients

For this case-control study, cases were identified from all subjects with HIV infection who were enrolled in a National Heart, Lung, and Blood Institute-supported cohort study on cardiovascular risk in children with HIV infection being conducted at the University of Miami, Miami, Florida from December, 2006 through March, 2008. HIV infection was confirmed by chart review, documenting repeatedly positive serum enzyme-linked immunosorbent assays confirmed by western blot assays, repeatedly positive HIV RNA or DNA polymerase chain reaction (PCR) assays, or by HIV culture. At the time of study, HIV-infected children did not have acute infectious illnesses.

A convenience sample of controls were identified from either the siblings of the HIV-infected children and youth or from an urban general pediatric outpatient program of the University of Miami that cares for children and youth of similar socioeconomic backgrounds. None of the controls were known to be HIV-infected. Controls were excluded if they had any chronic illness or an acute infectious process.

The Institutional Review Board at the University of Miami approved the research protocol, and informed consent from the parent or guardian and assent from the patient (when appropriate) were obtained.

Data Collection

Clinical data for all children and youth (HIV-infected and controls) at the time of the study visit included age, sex, race, weight, height, and body mass index (BMI: calculated as weight (kg)/height^2 (m^2)). Weight, height, and BMI were expressed as Z-scores.[7] For HIV-infected children, we recorded Center for Disease Control (CDC) pediatric HIV disease stage,[8] percentage of CD4 % T-lymphocyte cells and plasma HIV-1 RNA concentration by quantitative HIV-1 RNA PCR (Amplicor HIV-1 Monitor test, Roche Diagnostic Systems, Branchburg, NJ). Duration of antiretroviral (ART) drug therapy with nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PIs) was documented as the absolute amount of time the subject was exposed to each class of ART. Infected children also underwent dual X-ray absorptiometry testing (DXA) to quantify body fat and its regionalization (GE/Lunar Prodigy, Madison, WI; enCORE 2006 software version 10.50.086) using standard methods.[9] Waist and hip circumferences were measured using a non-stretchable plastic tape measure. Waist circumference was measured at the navel at the end of gentle exhalation. Hip circumference was measured at the maximal extension of the buttocks according to standard methods.[10]

Biomarkers of Vascular Dysfunction

Fibrinogen and C-reactive protein (CRP) were measured by nephelometry on a Dade-Behring (Deerfield, Illinois) auto-analyzer using manufacturer's reagents and instructions. Intra-and interassay coefficients of variation were 2.6% and 2.7%, respectively, for fibrinogen and 4.4% and 5.7%, respectively, for CRP. Leptin was measured by a double antibody radioimmunoassay (Linco Research, St. Charles, Missouri); intra- and inter-assay coefficients of variation were both less than 5%.

Interleukin-6 (IL-6), monocyte chemoattractant protein -1 (MCP-1), soluble vascular cell adhesion molecule-1 (sVCAM), soluble intracellular cell adhesion molecule-1 (sICAM), soluble E-selectin and soluble P-Selectin were measured by ELISA using reagents manufactured by R&D Systems (Minneapolis, MN). Intra- and interassay coefficients of variation were, respectively; IL-6, 6.8% and 9.4%; MCP-1 4.0% and less than 7.5%; sVCAM-1, 5.9% and 10.2%; sICAM-1, 4.8% and 10.1%; E-Selectin, 5.0% and 8.8%; and P-Selectin, 4.2% and 9.8%

Statistical Methods

Demographic and anthropometric characteristics of the HIV-infected children and controls were summarized using means and standard deviations or percentages, as appropriate, and compared using *t*-tests and Fisher exact tests, respectively. Biomarkers for the two study groups were log-transformed because of right-skewed distributions and the log-transformed values were used for all analyses.

Log-transformed biomarkers were compared between HIV-infected and control children using repeated measures linear regression. The regression model allowed for exchangeable correlation between siblings, and was adjusted for sex, race/ethnicity (black, Hispanic, white/other), quartiles of age, and quartiles of BMI z-scores. For interpretability on the natural measured scale, the effect estimate from the regression model was exponentiated in order to produce a geometric mean. The geometric mean is similar to an ordinary mean, but is less influenced by outliers. After adjustment for the regression covariates, the geometric mean represents the typical value of the inflammatory marker for an average child.

For the biomarkers that we found to be higher in the HIV-infected children than in controls, we wanted to further examine whether the extent of elevation was related to the extent of disease or to the extent of anthropometric changes linked to HIV. These analyses were carried out in

the HIV children only and, since there were no siblings within the HIV cohort, simple correlation coefficients were initially used to measure the relationships between the log-transformed biomarker and each of the disease and anthropometric measures. From these initial results, we found 3 disease measures (CD4%; log-transformed viral load; and duration of HAART therapy) and 4 anthropometric measures (waist-to-hip ratio; DXA body fat %; trunk fat %; and height z-score) that were considered for inclusion in multivariable models. For each log-transformed biomarker marker, a linear regression model was run that included sex, race/ethnicity, age, and the most significant disease and anthropometric measures. The effect estimates from the regression model were exponentiated in order to express the relative change in the biomarker that could be expected due to differences in the predictors.

RESULTS

Demographic and Clinical Characteristics

We enrolled 106 HIV-infected children and 55 normal control children (25 siblings of the cases and 30 unrelated) (Table 1). HIV infection was acquired perinatally in 101 subjects and horizontally in 5 subjects. Infected children were significantly older than controls (14.8 vs 12.3 y). Sex, weight, and BMI were similar between the groups, although the HIV group had slightly more Black non-Hispanics and were shorter than controls. Among children with HIV, 73% were CDC stage B/C and had a median viral load of 882 copies/mL [IQR=50, 11500]; 86% were receiving highly active antiretroviral therapy (HAART) for a mean of 6 years.

Differences in Biomarkers of Vascular Dysfunction

Table 2 shows the comparison of vascular biomarkers between the HIV-infected group and controls. After adjustment for sex, race, age, and BMI, two biomarkers of endothelial dysfunction, sICAM and sVCAM, were greater in the HIV group than in the controls. There were also differences in measures of inflammation where IL-6, MCP-1, and CRP were all higher in the HIV group compared to controls, although the difference for CRP was not statistically significant (p=.08). Fibrinogen (a measure of coagulant dysfunction, but also an acute phase reactant) was also significantly greater in the children with HIV infection.

Correlates of Elevated Biomarkers of Vascular Dysfunction

For the HIV-infected children only, we analyzed clinical correlates of five of the nine biomarkers that differed significantly between groups (Table 3). Not only were there no significant differences between HIV and control children with respect to P-selectin, E-selectin, and leptin, but within the cohort of HIV-infected children, there was no significant relationship to either % CD4 or viral load. We also analyzed CRP because the difference was almost significant and because it is easily measured in clinical settings. Results of the unadjusted correlations are shown in Table 3. Higher levels of sVCAM, a measure of endothelial dysfunction, correlated with greater disease severity (as defined by CD4 percent and viral load). Measures of inflammation (MCP-1, CRP) had greater correlations with anthropometric and body fat measures. For example, higher levels of MCP-1 correlated with greater waist:hip ratio and CRP correlated with higher body fat. Furthermore, higher measures of inflammation (MCP-1, CRP and IL-6) also correlated with greater disease severity (lower CD4 percent), as examples. Higher levels of the procoagulant fibrinogen were associated with adiposity (waist:hip ratios and body fat percentage) and disease severity (CD4 percent). No significant relationships were found for age, weight, BMI, waist size, hip size, or any specific antiretroviral therapy (PI, NRTI, or NNRTI).

Each of the 6 biomarkers of vascular dysfunction was then considered as an outcome in a multivariate analysis (Table 4). In general, there were few associations between any of these biomarkers and demographic variables, including age, sex, and race, with the exception the

girls had 31% lower levels of sICAM than boys. Considering all anthropometric and body composition measures, waist:hip ratio was most consistently associated with higher levels of biomarkers for inflammation and endothelial dysfunction. For example, for each increase of 1 standard deviation in waist:hip ratio, sICAM increased by 17% and CRP by 59%. CD4% level, as a measure of disease severity, was most consistently associated with inflammation and procoagulation. Each increase of 1 standard deviation in CD4 percent was correlated with anywhere from an 18% (MCP-1) to 34% (CRP) lower level of inflammation. A higher viral load was more predictive of higher sVCAM (22% increase per 1 SD increase in viral load; $P<0.001$).

DISCUSSION

Our study shows that HIV-infected children have higher levels of the biomarkers of vascular dysfunction than do an urban cohort of otherwise healthy children. These biomarkers have been associated with obesity, insulin resistance and atherosclerotic cardiovascular risk in adults.[6] In particular, biomarkers associated with inflammation, endothelial dysfunction, and procoagulation were higher in infected children than in controls. Clinical factors associated with these biomarkers of vascular dysfunction were more related to HIV disease severity (low CD4 counts and higher viral load) than to antiretroviral drug exposure. Furthermore, abdominal adiposity, as reflected by higher waist:hip ratio, was the best anthropometric correlate of these biomarkers.

Since the introduction of highly active antiretroviral therapy (HAART), HIV disease in developed nations has transitioned from an almost uniformly fatal illness to a disease in which therapies are now targeted toward indefinite viral suppression.[11] Although certain cardiovascular risk factors, such as hyperlipidemia, diabetes and endothelial dysfunction, were present before the advent of HAART,[12, 13] they have increased both in prevalence and severity.[14] These cardiovascular risks and metabolic complications have been better described in adults,[2, 3, 14] more limited data are available on children.[15–19] Potential contributors to increased cardiovascular risk in adults include HIV alone, specific types of HAART therapy, sex, life style habits (exercise, smoking), and successful viral suppression.[3, 20, 21] In children, PI therapy is associated with several cardiovascular risk factors.[4]

For HIV-infected children, the true impact of these CVD risk factors can only be appreciated after years of follow-up: most have not aged sufficiently to reach the associated endpoints. However, the multinational Data Collection on Adverse Events of Anti-HIV Drugs (DAD) Study Group in adults with HIV infection found an incidence of coronary heart disease (CHD) of 3.5/1000 person-years and 11% of all recorded patient deaths caused by myocardial infarction, stroke, or other cardiovascular events.[3] Relative risks up to 25% higher than those in the general population have been cited[3, 22] and substantiated.[23, 24] Several studies of subjects without HIV infection found that biomarkers of vascular dysfunction predict adverse cardiovascular events.[25, 26] Increasing evidence indicates the importance of vascular inflammation or dysfunction in the pathogenesis of cardiovascular disease in HIV-infected individuals.[27] Vascular dysfunction may be a result of the direct cytopathic effects of the virus on the endothelial cell or of the inflammation and oxidative stress[28, 29] associated with chronic immune activation induced by the virus.[30] Further, dysfunction may be induced by the metabolic consequences of the disease and its therapies, or by a combination of both.

Several studies in adults report associations between biomarkers of endothelial dysfunction such as ICAM, VCAM, E-selectin, P-selectin, and VWF, and the platelet factor, P-selectin, among others, with HIV disease severity.[31, 32] These findings suggest that HIV itself may cause immune activation and be an important mechanism for cardiovascular risk[31] and effective antiretroviral therapy decreases these vascular inflammatory biomarkers.[32] Finally,

the HIV *tat* and *nef* proteins induce VCAM-1, ICAM-1, MCP-1 and other inflammatory chemokines that disrupt the vascular endothelium in coronary vessels.[13, 33, 34] In addition MCP-1 has been proposed to activate viral infection.[35]

Alternatively, HIV-infected patients have metabolic conditions often related to antiretroviral therapy, including dyslipidemia, insulin resistance, and abnormal fat distribution, that can contribute to the activation or injury of the endothelium.[27] Certain antiretroviral agents also appear to damage endothelial mitochondrial DNA and are toxic to the endothelial cell itself, including the ability to induce apoptosis.[36–38] Protease inhibitors can increase mitochondrial production of reactive oxygen species,[38] increase endothelial cell permeability[39] and leukocyte adhesion[40] in cell culture. Johnson[41] reported higher levels of proinflammatory cytokines in the subcutaneous adipose tissue of HIV-infected individuals with lipodystrophy, when compared to those without. Others have found associations between these biomarkers, HAART, and metabolic dysfunction.[42] Thus, antiretroviral therapy could directly or indirectly (through changes in the metabolic profile) increase levels of these biomarkers.

Studies on vascular inflammatory pathways and vascular dysfunction (ie, vessel compliance, distensibility and structure) in HIV-infected children have been limited.[43–46] McComsey showed greater carotid intima media thickness and higher levels of certain biomarkers among 31 HIV-infected children.[46] A study of 49 children found that vascular dysfunction (stiffness) was greater in HIV-infected children than in controls, independent of known CV risk factors and antiretroviral therapy.[43] However, other studies show carotid intima thicknesses are similar to controls.[46] Charakida found among 83 HIV-infected children,[45] carotid intima thickness was greater than it was in controls, as were the abnormalities in flow-mediated vasodilation. These differences were more pronounced in children receiving PIs and lipid abnormalities did not account for these differences. Interestingly, some pre-HAART studies in children showed increased coronary artery calcifications,[47] suggesting the contribution of baseline immune activation to cardiovascular risk.

Limitations of the Study

As with many studies of cardiovascular risk in children, finding risk may not always equate to adverse outcomes. Only continued longitudinal follow-up of children will definitively confirm that our findings translate to cardiovascular disease events. Although we tried to limit the number of outcomes to those that differed between cases and controls and the number of predictors that were linked to progressive HIV disease, we cannot rule out false positive findings secondary to multiple statistical testing. Confirmatory studies are needed. We have not evaluated the association of other metabolic parameters (lipids, insulin resistance) or other known risk factors such as family history, smoking exposure and alcohol intake on our outcomes because of incomplete data on the entire sample. These metabolic factors and health behaviors need to be critically evaluated as potential causative factors in the future. However, early data in another study[48] suggest there are minimal relationships. Finally, our control group was slightly unbalanced in age and race. However, all of our analyses were adjusted for these demographic factors.

Conclusions

We evaluated early mechanistic factors for cardiovascular disease in children with HIV infection. Elevations of the biomarkers of vascular dysfunction in HIV-infected children provide strong evidence of ongoing cardiovascular risk in this population. Disease severity and truncal adiposity were both independently associated with higher levels of these biomarkers of vascular dysfunction, suggesting that these biomarkers and other cardiovascular risk factors should be closely monitored in children with those characteristics. There were no associations

with antiretroviral therapy. Long-term evaluation of cardiovascular risk, incorporating complete metabolic screens, as well as interventions to modify this risk, should be considered.

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References

1. Laufer M, Scott GB. Medical management of HIV disease in children. Pediatr Clin North Am Feb; 2000 47(1):127–153. [PubMed: 10697645]
2. Carr A, Samaras K, Chisholm DJ, Cooper DA. Abnormal fat distribution and use of protease inhibitors. Lancet Jun 6;1998 351(9117):1736. [PubMed: 9734916]
3. Friis-Moller N, Sabin CA, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. N Engl J Med Nov 20;2003 349(21):1993–2003. [PubMed: 14627784]
4. Miller TL, Orav EJ, Lipshultz SE, et al. Risk factors for cardiovascular disease in children infected with human immunodeficiency virus-1. J Pediatr Oct;2008 153(4):491–497. [PubMed: 18538789]
5. Libby P. Inflammation in atherosclerosis. Nature Dec 19–26;2002 420(6917):868–874. [PubMed: 12490960]
6. Ikonomidis I, Stamatelopoulos K, Lekakis J, Vamvakou GD, Kremastinos DT. Inflammatory and non-invasive vascular markers: the multimarker approach for risk stratification in coronary artery disease. Atherosclerosis Jul;2008 199(1):3–11. [PubMed: 18378239]
7. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. Adv Data Jun 8;2000 (314):1–27. [PubMed: 11183293]
8. Caldwell MB, Oxtoby MJ, Simonds RJ, Lindegren ML, Rogers MF. 1994 Revised Classification System for Human Immunodeficiency Virus Infection in Children Less Than 13 Years of Age. MMWR CDC Surveill Summ 1994;43:1–10.
9. Margulies L, Horlick M, Thornton JC, Wang J, Ioannidou E, Heymsfield SB. Reproducibility of pediatric whole body bone and body composition measures by dual-energy X-ray absorptiometry using the GE Lunar Prodigy. J Clin Densitom Fall;2005 8(3):298–304. [PubMed: 16055960]
10. National Health and Nutrition Examination Survey III Body Measurements (Anthropometry). [Accessed May 1, 2009]. 2009 http://www.cdc.gov/nchs/data/nhanes/nhanes3/cdrom/nchs/manuals/anthro.pdf
11. Powderly WG, Landay A, Lederman MM. Recovery of the immune system with antiretroviral therapy: the end of opportunism? JAMA Jul 1;1998 280(1):72–77. [PubMed: 9660367]
12. Teitel JM, Shore A, Read SE, Schiavone A. Immune function of vascular endothelial cells is impaired by HIV. J Infect Dis Sep;1989 160(3):551–552. [PubMed: 2788201]
13. Dhawan S, Puri RK, Kumar A, Duplan H, Masson JM, Aggarwal BB. Human immunodeficiency virus-1-tat protein induces the cell surface expression of endothelial leukocyte adhesion molecule-1, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 in human endothelial cells. Blood Aug 15;1997 90(4):1535–1544. [PubMed: 9269771]
14. Fisher SD, Miller TL, Lipshultz SE. Impact of HIV and highly active antiretroviral therapy on leukocyte adhesion molecules, arterial inflammation, dyslipidemia, and atherosclerosis. Atherosclerosis Mar;2006 185(1):1–11. [PubMed: 16297390]
15. Bitnun A, Sochett E, Dick PT, et al. Insulin sensitivity and beta-cell function in protease inhibitor-treated and -naive human immunodeficiency virus-infected children. J Clin Endocrinol Metab Jan; 2005 90(1):168–174. [PubMed: 15483082]
16. Miller TL. Nutritional aspects of HIV-infected children receiving highly active antiretroviral therapy. AIDS Apr;2003 17(Suppl 1):S130–140. [PubMed: 12870539]
17. Arpadi SM, Bethel J, Horlick M, et al. Longitudinal changes in regional fat content in HIV-infected children and adolescents. AIDS. Jun 20;2009

18. Miller TL, Grant YT, Almeida DN, Sharma T, Lipshultz SE. Cardiometabolic disease in human immunodeficiency virus-infected children. J Cardiometab Syndr Spring;2008 3(2):98–105. [PubMed: 18453810]
19. Miller TL, Mawn BE, Orav EJ, et al. The effect of protease inhibitor therapy on growth and body composition in human immunodeficiency virus type 1-infected children. Pediatrics May;2001 107 (5):E77. [PubMed: 11331727]
20. Hadigan C, Meigs JB, Corcoran C, et al. Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy. Clin Infect Dis Jan;2001 32(1):130–139. [PubMed: 11118392]
21. Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. N Engl J Med Jan 6;2005 352(1):48–62. [PubMed: 15635112]
22. Friis-Moller N, Reiss P, Sabin CA, et al. Class of antiretroviral drugs and the risk of myocardial infarction. N Engl J Med Apr 26;2007 356(17):1723–1735. [PubMed: 17460226]
23. Martinez E, Larrousse M, Gatell JM. Cardiovascular disease and HIV infection: host, virus, or drugs? Curr Opin Infect Dis Feb;2009 22(1):28–34. [PubMed: 19532078]
24. 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 Jul;2007 92(7):2506–2512. [PubMed: 17456578]
25. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med Mar 23;2000 342(12):836–843. [PubMed: 10733371]
26. Hwang SJ, Ballantyne CM, Sharrett AR, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation Dec 16;1997 96(12):4219–4225. [PubMed: 9416885]
27. Grinspoon SK, Grunfeld C, Kotler DP, 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 Jul 8;2008 118(2):198–210. [PubMed: 18566320]
28. Pace GW, Leaf CD. The role of oxidative stress in HIV disease. Free Radic Biol Med Oct;1995 19 (4):523–528. [PubMed: 7590404]
29. Lo J, Grinspoon S. Cardiovascular disease in HIV-infected patients: does HIV infection in and of itself increase cardiovascular risk? Curr Opin HIV AIDS May;2008 3(3):207–213. [PubMed: 19372968]
30. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. J Acquir Immune Defic Syndr Jul 1;2009 51(3):268–273. [PubMed: 19387353]
31. de Larranaga GF, Bocassi AR, Puga LM, Alonso BS, Benetucci JA. Endothelial markers and HIV infection in the era of highly active antiretroviral treatment. Thromb Res May 1;2003 110(2–3):93–98. [PubMed: 12893023]
32. Wolf K, Tsakiris DA, Weber R, Erb P, Battegay M. Antiretroviral therapy reduces markers of endothelial and coagulation activation in patients infected with human immunodeficiency virus type 1. J Infect Dis Feb 15;2002 185(4):456–462. [PubMed: 11865397]
33. Swingler S, Mann A, Jacque J, et al. HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. Nat Med Sep;1999 5(9):997–103. [PubMed: 10470075]
34. Cota-Gomez A, Flores NC, Cruz C, et al. The human immunodeficiency virus-1 Tat protein activates human umbilical vein endothelial cell E-selectin expression via an NF-kappa B-dependent mechanism. J Biol Chem Apr 26;2002 277(17):14390–14399. [PubMed: 11827962]
35. Vicenzi E, Alfano M, Ghezzi S, et al. Divergent regulation of HIV-1 replication in PBMC of infected individuals by CC chemokines: suppression by RANTES, MIP-1alpha, and MCP-3, and enhancement by MCP-1. J Leukoc Biol Sep;2000 68(3):405–412. [PubMed: 10985258]
36. Zhong DS, Lu XH, Conklin BS, et al. HIV protease inhibitor ritonavir induces cytotoxicity of human endothelial cells. Arterioscler Thromb Vasc Biol Oct 1;2002 22(10):1560–1566. [PubMed: 12377730]

37. Jiang B, Hebert VY, Li Y, Mathis JM, Alexander JS, Dugas TR. HIV antiretroviral drug combination induces endothelial mitochondrial dysfunction and reactive oxygen species production, but not apoptosis. Toxicol Appl Pharmacol Oct 1;2007 224(1):60–71. [PubMed: 17669453]
38. Hebert VY, Crenshaw BL, Romanoff RL, Ekshyyan VP, Dugas TR. Effects of HIV drug combinations on endothelin-1 and vascular cell proliferation. Cardiovasc Toxicol 2004;4(2):117–131. [PubMed: 15371629]
39. Chen C, Lu XH, Yan S, Chai H, Yao Q. HIV protease inhibitor ritonavir increases endothelial monolayer permeability. Biochem Biophys Res Commun Sep 30;2005 335(3):874–882. [PubMed: 16105660]
40. Mondal D, Pradhan L, Ali M, Agrawal KC. HAART drugs induce oxidative stress in human endothelial cells and increase endothelial recruitment of mononuclear cells: exacerbation by inflammatory cytokines and amelioration by antioxidants. Cardiovasc Toxicol 2004;4(3):287–302. [PubMed: 15470276]
41. Johnson JA, Albu JB, Engelson ES, et al. Increased systemic and adipose tissue cytokines in patients with HIV-associated lipodystrophy. Am J Physiol Endocrinol Metab Feb;2004 286(2):E261–271. [PubMed: 14532165]
42. de Gaetano Donati K, Rabagliati R, Tumbarello M, et al. Increased soluble markers of endothelial dysfunction in HIV-positive patients under highly active antiretroviral therapy. AIDS Mar 28;2003 17(5):765–768. [PubMed: 12646805]
43. Bonnet D, Aggoun Y, Szezepanski I, Bellal N, Blanche S. Arterial stiffness and endothelial dysfunction in HIV-infected children. AIDS Apr 30;2004 18(7):1037–1041. [PubMed: 15096807]
44. Giuliano Ide C, de Freitas SF, de Souza M, Caramelli B. Subclinic atherosclerosis and cardiovascular risk factors in HIV-infected children: PERI study. Coron Artery Dis May;2008 19(3):167–172. [PubMed: 18418233]
45. Charakida M, Donald AE, Green H, et al. Early structural and functional changes of the vasculature in HIV-infected children: impact of disease and antiretroviral therapy. Circulation Jul 5;2005 112 (1):103–109. [PubMed: 15983247]
46. McComsey GA, O'Riordan M, Hazen SL, et al. Increased carotid intima media thickness and cardiac biomarkers in HIV infected children. AIDS May 11;2007 21(8):921–927. [PubMed: 17457085]
47. Perez-Atayde AR, Kearney DI, Bricker JT, et al. Cardiac, aortic, and pulmonary arteriopathy in HIV-infected children: the Prospective P2C2 HIV Multicenter Study. Pediatr Dev Pathol Jan–Feb;2004 7(1):61–70. [PubMed: 15255036]
48. Miller, T.; Jacobson, D.; Mendez, A., et al. Biomarkers of Vascular Dysfunction in HIV-Infected Children With and Without Hyperlipidemia. 16th Conference on Retroviruses and Opportunistic Infections (CROI 2009); Montreal, Canada. February 8–11, 2009; 2009. (Abstract #917)

Table 1

Clinical Characteristics of Children with and Without HIV Infection in a Case-Control Study of Biomarkers of Vascular Dysfunction

Variable	Controls (n=55)	HIV + (n=106)	P
Mean (SD) Age, y	12.3 (3.8)	14.8 (4.3)	<0.001
Percent female	43	56	0.14
Race, %			0.04
Black	78	62	
Hispanic	18	24	
White/other	4	15	
Mean (SD) Weight z-score	0.55 (1.2)	0.26 (1.3)	0.17
Mean (SD) Height z-score	0.19 (.98)	−0.46 (1.2)	<0.001
Mean (SD) BMI z-score	0.51 (1.2)	0.51 (1.1)	0.99
Mean (SD) Body fat, %	…	24 (13)	
Mean (SD) Trunk fat, %	…	24 (14)	
Mean (SD) Waist circumference, cm	…	76 (15)	
Mean (SD) Hip circumference, cm	…	86 (15)	
Mean (SD) Waist:hip ratio	…	0.89 (0.07)	
Disease Characteristics			
CDC Stage, %			
Stage A	…	22	
Stage B	…	34	
Stage C	…	39	
Stage N	…	6	
Mean (SD) CD4, %	…	27 (13)	
Median [IQR] Viral load	…	882 [50, 11500]	
% Receiving HAART	…	86	
Mean (SD) duration of HAART, y	…	6.0 (3.1)	
% Receiving Protease Inhibitors	…	80	
Mean (SD) duration of PI therapy, y	…	5.0 (3.3)	
% Receiving NRTI	…	90	
Mean (SD) duration of NRTI, y	…	9.2 (4.6)	
% Receiving NNRTI	…	11	
Mean (SD) duration of NNRTI therapy, y	…	2.3 (2.6)	

IQR = interquartile range; HAART = highly active antiretroviral therapy; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor

Table 2

Values of Biomarkers of Vascular Dysfunction among 160 Children, by HIV Status.

	Adjusted Geometric Mean [95% CI]		
Log-transformed biomarker	**Control**	**HIV**	**P-value**
Inflammation			
CRP (mg/L)**	0.54 [0.34, 0.86]	0.92 [0.64, 1.34]	0.08
IL-6 (pg/mL)	0.88 [0.68, 1.14]	1.26 [1.06, 1.51]	0.029
MCP-1 (pg/mL)	106 [92, 123]	154 [139, 171]	<0.001
Coagulant Dysfunction			
Fibrinogen (mg/dL)	324 [299, 350]	376 [356, 399]	0.006
P-selectin (ng/mL)	28.5 [25.0, 32.5]	30.9 [28.2, 33.8]	0.29
Endothelial Dysfunction			
sICAM (ng/mL)	185 [154, 222]	240 [210, 271]	0.032
sVCAM (ng/mL)	685 [589, 793]	1141 [1026, 1263]	<0.001
E-selectin (ng/mL)	25.0 [21.6, 29.0]	29.7 [26.7, 32.7]	0.07
Metabolic Dysfunction			
Leptin (ng/mL)	6.6 [5.2, 8.2]	5.3 [4.5, 6.2]	0.12

CRP = C-reactive protein

IL-6 = interleukin-6

MCP-1 = monocyte chemoattractant protein-1

sICAM = soluble intracellular cell adhesion molecule-1

sVCAM = soluble vascular cell adhesion molecule-1

* Means and p-values are adjusted for correlation between siblings, as well as for sex, race, age in quartiles and BMI z-scores in quartiles. Data were analyzed on the log scale.

** Sample sizes for CRP are 49 in controls and 70 in HIV-infected children.

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Table 3

Unadjusted Correlations Between Elevated Biomarkers of Vascular Dysfunction, Anthropometry Measures, and Disease Severity in 105 Children with HIV*

	Biomarker: Correlation Coefficient (P-value)**					
	Inflammation			Coagulant dysfunction	Endothelial dysfunction	
Correlate	**CRP r (P)**	**IL-6 r (P)**	**MCP-1 r (P)**	**Fibrinogen r (P)**	**sICAM r (P)**	**sVCAM r (P)**
Anthropometry						
Waist:hip	0.17 (0.19)	0.19 (0.06)	0.39 (<0.001)	0.20 (0.052)	0.22 (0.02)	0.05 (0.65)
% Body fat	0.24 (0.05)	0.19 (0.06)	0.08 (0.46)	0.28 (0.01)	−0.001 (0.99)	−0.08 (0.43)
% Trunk fat	0.22 (0.07)	0.18 (0.07)	0.10 (0.33)	0.24 (0.02)	0.03 (0.80)	−0.07 (0.47)
Height Z-score	−0.18 (0.17)	0.01 (0.94)	−0.08 (0.44)	−0.07 (0.53)	−0.15 (0.15)	−0.20 (0.05)
HIV disease severity						
CD4 percent	−0.28 (0.02)	−0.24 (0.01)	−0.30 (0.002)	−0.21 (0.04)	−0.09 (0.37)	−0.24 (0.01)
Viral load***	0.21 (0.09)	0.14 (0.14)	0.24 (0.01)	0.06 (0.59)	0.12 (0.23)	0.39 (<0.001)
HAART duration	−0.12 (0.32)	−0.07 (0.45)	0.11 (0.25)	−0.05 (0.64)	−0.17 (0.08)	0.05 (0.60)

* In addition to the measures shown here, we also considered but found no relationship with age, weight z-score, BMI z-score, waist and hip circumference, current NRTI therapy, current NNRTI therapy, current PI therapy, or current HAART therapy.

** All biomarkers of vascular dysfunction were analyzed after log-transformation.

*** Viral load was log-transformed.

CRP = C-reactive protein

IL-6 = interleukin-6

MCP-1 = monocyte chemoattractant protein-1

sICAM = soluble intracellular cell adhesion molecule-1

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Table 4

Multivariable Predictors of Biomarkers of Vascular Dysfunction

	Biomarker: Effect Estimate* (P-value)					
		Inflammation		Coagulant dysfunction	Endothelial dysfunction	
Predictor	**CRP % (P)**	**IL-6 % (P)**	**MCP-1 % (P)**	**Fibrinogen % (P)**	**sICAM % (P)**	**sVCAM % (P)**
Demographic**						
Female (vs male)	4% (0.91)	31% (0.09)	5% (0.58)	4% (0.41)	−31% (0.002)	−7% (0.45)
Hispanic (vs Black)	−49% (0.10)	−10% (0.61)	6% (0.65)	−4% (0.41)	15% (0.35)	7% (0.61)
White/other (vs Black)	−8% (0.92)	30% (0.53)	78% (0.02)	−6% (0.58)	−6% (0.84)	32% (0.29)
Anthropometric						
Waist:hip (per 1 SD increase)	59% (0.01)	18% (0.04)	19% (<0.001)		17% (0.01)	
% Body fat (per 1 SD increase)				6% (0.03)		
Height z-score (per 1 SD increase)						−8% (0.10)
HIV disease severity						
CD4 percent (per 1 SD increase)	−34% (0.02)	−22% (0.004)	−18% (<0.001)	−5% (0.03)		
HAART duration (per year)	−10% (0.046)				−6% (0.002)	
Viral load (log) (per 1 SD increase)						22% (<0.001)

*Biomarkers of vascular dysfunction were log-transformed before regression analyses, so that effect estimates were exponentiated and compared to 1.00 in order to represent percent change on the raw scale.

**In addition to the predictors shown, all models were adjusted for age. Age was not significantly related to any of the vascular biomarkers (p>.15 for every marker).

CRP = C-reactive protein

IL-6 = interleukin-6

MCP-1 = monocyte chemoattractant protein-1

sICAM = soluble intracellular cell adhesion molecule-1

sVCAM = soluble vascular cell adhesion molecule-1