Clin. Cardiol. 28, 149–153 (2005)

Viral Load of the Human Immunodeficiency Virus Could Be an Independent Risk Factor for Endothelial Dysfunction

ARNON BLUM, M.D., VERED HADAS, R.N.,* MICHAEL BURKE, M.D.,* ISRAEL YUST, M.D.,* ADA KESSLER, M.D.†

Department of Internal Medicine A, Poria Medical Center; *Clinical Immunology Unit; †Department of Roentgenology, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel

Summary

Background: Recent reports of myocardial infarction in young persons infected with human immunodeficiency virus (HIV) who are receiving protease inhibitor therapy have raised concerns about premature coronary artery disease in this population. However, endothelial dysfunction, hypercoagulability, hypertriglyceridemia, and abnormal coronary artery pathology have been observed in association with HIV infection prior to the availability of protease inhibitor therapy.

Hypothesis: The study was undertaken to determine the association between endothelial function, viral load, CD4+ count, and other well-established risk factors for atherosclerosis.

Methods: This prospective, case-controlled study compared viral (HIV) load and the CD4+ T-lymphocyte count and endothelial function in 24 HIV-positive carriers. Brachial artery diameter, HIV viral load, and CD4 count were measured.

Results: We found that viral load correlated inversely with endothelial function; the higher the viral load, the worse the endothelial dysfunction ($p < 0.005$).

Conclusion: High viral load appears to be associated with endothelial dysfunction in patients with HIV. This preliminary observation supports the infectious theory that viruses may play an important role in the pathogenesis of atherosclerosis.

Address for reprints:

Arnon Blum, M.D.
Department of Internal Medicine A
Poria Medical Center
Lower Galilee 15208, Israel
e-mail: navablum@hotmail.com

Received: July 29, 2004
Accepted with revision: November 16, 2004

Key words: human immunodeficiency virus, endothelial dysfunction, atherosclerosis, viral load

Introduction

Endothelial dysfunction, hypercoagulability, hypertriglyceridemia, and abnormal coronary artery pathology have been described in association with human immunodeficiency virus (HIV) infection prior to the availability of protease inhibitor therapy.[1] Endothelial cell products playing a role in hemostasis were studied in 125 HIV-positive patients. Antigenic von Willebrand factor increased significantly with disease progression and correlated closely with CD4+ cell counts and beta 2-microglobulin levels. Mean total protein S levels were lower in HIV-positive patients, and were associated with a decrease in free protein S levels in 27.2% of the patients.[2]

Cytomegalovirus (CMV) DNA has been demonstrated in atherosclerotic coronary arteries in restenotic lesions, and prior infection with CMV could be a strong independent risk factor for restenosis after coronary atherectomy.[3] We have shown that patients who had a high anti-CMV titer had a higher prevalence of coronary artery disease and a higher restenosis rate than CMV-seropositive patients with low antibody titers.[4]

These and other data indicate a possible etiological link between viruses and atherosclerosis. An established hypothesis is that chronic infection with *Chlamydia pneumonia*, cytomegalovirus, hepatitis A, and *Helicobacter pylori* may cause long-standing inflammation that leads to endothelial dysfunction and eventually clinical atherosclerosis.[5]

In this preliminary study, the associations between endothelial function, viral load (VL), CD4+ count, as well as other well-established risk factors for atherosclerosis were studied in four young patients with HIV.

Methods

This is a prospective case-controlled pilot study, in which 24 HIV-positive carriers were studied, among them 4 patients who were followed for 3 months. Endothelial function, VL,

lipid profile, CD4 count, and antibodies against CMV (IgG type) were measured. The study was approved by the Internal Review Board of the hospital, and the 24 participants signed an informed consent form before entering the study.

The Laboratory Methods: the Vascular Studies

These studies were performed in the vascular laboratory, using the technique of Celermajer.[6] All subjects lay supine, and an imaging study of the left brachial artery was performed using high-resolution ultrasound (Vivid 5 7.5 MHz linear-array transducer, GE Vingmed, Atlanta, Ga., USA) following 10 min of rest. Baseline images and measurements of the brachial artery proximal to the antecubital fossa were obtained. After finding the clearest view, the skin was marked and the arm remained in the same position throughout the study. Baseline measurements included brachial artery diameter and flow velocity, measured by pulse-Doppler at approximately 70° to the vessel. Endothelium-dependent vasodilation was assessed by measuring the maximum increase in diameter of the brachial artery during reactive hyperemia created by an inflated cuff (250 mmHg for 5 min) on the forearm. After cuff deflation, flow velocity was measured for the first 15 s, then the artery lumen was recorded continually for the next 90 s of hyperemia. Arterial diameter was measured in millimeters from the intima–blood interface on both the anterior and posterior walls, coincident with the R wave on the ECG. Fifteen min later a repeat baseline measurement of diameter and flow velocity was recorded, followed by a nitroglycerin tablet (0.4 mg) given sublingually, to assess endothelium-independent vasodilation. Three min later, diameter and flow velocity measurements were recorded. The vascular measurements were performed on subjects who were identified only by a nurse. The vascular study operators were blinded to the patients' identity and clinical condition.

The viral load: Plasma HIV ribonucleic acid (RNA) levels were performed using the RT-PCR NASBA kit (Nuclisens, Organon-Teknika, Boxtel, The Netherlands). The limit of detection was 50 copies per ml.

The CD4 count: Lymphocyte subpopulations were characterized by the monoclonal antibodies against CD3/CD4 and CD3/CD8, measured by the fluorescent activated cell scanner (FACS) (FACS Caliber [Becton Dickinson], San Jose, Calif., USA).

The cytomegalovirus antibodies: The antibody titer was measured by enzyme-linked immunosorbent assay (ELISA) methods (Organon-Teknika).

Statistical Methods

The two-tailed paired Student's *t*-test was used for analysis. A p value of < 0.05 was considered statistically significant.

Results

Twenty four HIV-positive carriers (mean age 30 ± 14 years, all men) took part in the study. Of these, 17 had low VLs (a range of 30–5100 HIV RNA copies/ml, mean ± standard deviation (SD) = 694.21 ± 1428.25), and 7 had high VLs (15,000–46,000 HIV RNA copies/ml, mean ± SD = 25,666.67 ± 17,616.28). The results showed that those who had low VLs had normal endothelium-dependent vasodilation (mean ± SD of 10.958 ± 3.789 mm), while those who had high VLs had poor endothelium-dependent vasodilation (3.619 ± 1.488 mm), with a significant difference between them ($p < 0.005$) (Table I). There was no difference between the two groups of patients (with the high and low VLs) in relation to endothelium-independent vasodilation: 13.186 ± 5.175 mm for patients with low VL, and 13.323 ± 5.270 mm for patients with high VL (p = NS) (Table I).

Four patients (of the 24 enrolled patients), aged 27, 29, 35, and 44 years, were followed for 3 months. The first patient was treated with zidovudine (AZT) 300 mg b.i.d., lamuvidine (3TC) 150 mg b.i.d., and efavirenz (Stocrin®, Bristol-Myers Squibb, Princeton, N.J., USA) 600 mg once daily. The second one was treated with zidovudine (AZT) 300 mg b.i.d., lamuvidine (3TC) 150 mg b.i.d., and nevirapin 200 mg b.i.d. The third patient was not treated. The fourth patient was treated with stavudine (D4T) 40 mg b.i.d., nevirapine 200 mg b.i.d., and amprenavir 1200 mg b.i.d.

The first patient had a very high plasma VL at the beginning of the study (260,000 HIV RNA copies per ml), which de-

TABLE I Brachial artery measurements (mean ± standard deviation) in relation to the viral load of the 20 human immunodeficiency virus (HIV)-positive patients whose viral load was measured only once

	Low VL (< 10,000)	High VL (> 10,000)	Low VL (< 10,000)	High VL (> 10,000)
	EDD	EDD	EID	EID
Basal (mm)	4.71 ± 0.64	5.06 ± 0.87	4.77 ± 0.61	5.00 ± 0.80
Hyperemia (mm)	5.21 ± 0.63	5.29 ± 0.81	5.40 ± 0.68	5.60 ± 0.55
FMD (%)	10.96 ± 3.78	3.62 ± 1.48 [a]	13.18 ± 5.17	13.19 ± 6.95

[a] $p < 0.05$.

VL = viral load; HIV RNA copies/ml.

Abbreviations: FMD = flow-mediated diameter, EDD = endothelium-dependent diameter, EID = endothelium-independent diameter, RNA = ribonucleic acid.

TABLE II Viral load, CD4 count, and the lipid profile of the four human immunodeficiency virus (HIV)-positive carriers whose viral load was changed with time

	Low VL	High VL	Low VL	High VL	Low VL	High VL	Low VL	High VL
Patient No.	1		2		3		4	
Age (years)	27		29		35		44	
CD4 (cells/mm^3)	602	759	1,131	862	775	491	138	137
CD4 (%)	40	34	48	40	29	29	9	6
VL (copies/ml)	1,000	260,000	< 50	160,000	8,500	24,000	9,600	16,000
CMV titer (IgG)	1:600		Neg		1:600		Neg	
Cholesterol (mg%)	188	190	163	157	153	123	365	370
LDL-C (mg%)	106	95	102	93	97	66	250	255
HDL-C (mg%)	34	34	47	40	35	32	46	50
TG (mg%)	238	303	72	120	104	125	347	498

VL = viral load; HIV RNA copies/ml.
Low VL = < 10,000 HIV RNA copies/ml.
High VL = > 10,000 HIV RNA copies/ml.
Abbreviations: CMV = cytomegalovirus, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, TG = triglyceride. Other abbreviations as in Table I.

creased to 1000 HIV RNA copies per ml after 3 months. The second patient initially had 160,000 plasma HIV RNA copies per ml that declined to < 50 copies per ml after 3 months. The third patient had a VL of 8,500 HIV RNA copies per ml at the start, and this was increased to 24,000 HIV RNA copies per ml 3 months later. The fourth patient had 16,000 HIV RNA copies at entry to the study, and the VL decreased to 9,600 after 3 months (Table II).

The CD4 count was above 500 cells/mm^3 in three patients at the start and after 3 months. The fourth patient had low CD4 counts—138 and 137 cells/mm^3, respectively. Anti cytomegalovirus antibody (IgG) levels were 1:600, negative, 1:600, and negative, respectively (Table II).

The lipid profile was unchanged during the follow-up period, and the low-density lipoprotein (LDL) cholesterol levels were all approximately 100 mg/dl in three patients, with no change in the two visits. The fourth patient had high total cholesterol (365 and 370 mg%) and LDL cholesterol (250 and 255 mg%). The first and fourth patients had high triglyceride (TG) levels, which did not change with time. The other two patients had low TG levels (Table II).

The vascular studies demonstrated a statistically significant difference in endothelium-dependent dilation (EDD) between the two periods: at a low VL (< 10,000 copies per ml), the mean diameter of the brachial artery increased from 4.88 ± 0.48 mm at baseline to 5.53 ± 0.56 mm (EDD of 13.37 ± 4.65%) after hyperemia; at a high VL (> 10,000 copies per ml), the baseline brachial artery diameter was 4.68 ± 0.23 and 4.68 ± 0.52 mm (EDD of −0.14 ± 7.6%) after hyperemia (Table III). Measurements of the brachial artery after nitroglycerin (endothelium-independent diameter [EID]) demonstrated a change from 4.91 ± 0.31 mm at baseline to 5.90 ± 0.36 (after nitroglycerin) when the patients were at low VLs (EID 20.11 ± 5.53%). At higher VLs, the baseline diameter rose from 4.75 ± 0.2 to 5.50 ± 0.25 mm after nitroglycerin (EID of 15.88 ± 4.39%) (Table III). A significantly worse

TABLE III Brachial artery measurements (mean ± standard deviation) in relation to the viral load of the four human immunodeficiency virus (HIV)-positive patients whose viral load was changed with time

	Low VL (< 10,000)	High VL (> 10,000)	Low VL (< 10,000)	High VL (> 10,000)
	EDD	EDD	EID	EID
Basal (mm)	4.88 ± 0.48	4.68 ± 0.23	4.91 ± 0.31	4.75 ± 0.2
Hyperemia (mm)	5.53 ± 0.56	4.68 ± 0.52	5.90 ± 0.36	5.50 ± 0.25
FMD (%)	13.37 ± 4.65 [a]	−0.14 ± 7.6	20.11 ± 5.53	15.88 ± 4.39

[a] $p < 0.005$.
VL = viral load; HIV RNA copies/ml.
Abbreviations as in Table I.

EDD was demonstrated when the VL was high (> 10,000 HIV RNA copies per ml). There was no change in EID at the two visits (whether the patient had a high or a low VL) (Table III).

Discussion

In this study we used a noninvasive method for studying EDD (as a bioassay for nitric oxide bioavailability) and EID (as a bioassay for the vascular smooth muscle activity) in patients with HIV. Our results demonstrated that in the four patients whom we followed, the VL load correlated inversely with endothelial function, without any relation to antiretroviral drugs, CD4 count, or the lipid profile. It is interesting to mention that a change in VL was associated with a change in endothelial function; thus, when the VL was increased, endothelial function became worse, and vice versa. The same trend was observed whether the high VL was detected in the first measurement and decreased later on, or whether a low VL was observed first. The change in endothelial function correlated inversely with the VL, and the change was observed within 3 months of follow-up.

The other 20 patients in whom only the first VL measurement was taken also demonstrated an inverse correlation with VL.

As the survival of HIV-infected patients is increasing, vascular complications may be expected to become more prevalent.[7, 8] Therefore, an improved understanding of the mechanisms of endothelial function and dysfunction is needed. It is still controversial whether the antiretroviral therapy (especially protease inhibitors)[9] is responsible for the hyperlipoproteinemia and the accelerated atherosclerosis in HIV-positive patients,[7, 8] or maybe it is the natural history of the disease and the longer survival of these patients.

An alternate explanation is a hypercoagulable state which has been demonstrated in patients with HIV, involving an increase in von Willebrand factor (vWf) and in plasminogen-activating inhibitor 1 (PAI-1) and a decrease in mean protein S levels.[10] It is suggested that vascular endothelial cell injury is associated with disease progression and might result from several distinct mechanisms of HIV pathogenesis. In this condition, endothelial damage may contribute to the occurrence of more severe vascular complications, such as venous and/or arterial thrombosis.

The Infectious Theory

The infectious theory of atherosclerosis was suggested in the past.[11] Both antigens and nucleic acid sequences of CMV were detected in smooth muscle cells from carotid artery plaques,[12] and polymerase chain reactions demonstrated CMV DNA in arterial samples from patients with atherosclerosis who underwent vascular surgery more often than control subjects.[13] It has been demonstrated that patients with atherosclerosis had higher antibody titer against CMV[14] and that one-third of the atherosclerotic lesions obtained by coronary atherectomy contained CMV DNA sequences.[4] Smooth muscle cells that were grown from such lesions expressed IE84 (one of the immediate early proteins of the virus that binds and inhibits p53).[4] These effects may enhance the proliferation of smooth muscle cells or inhibit apoptosis, either of which may contribute to accelerated atherosclerosis. Prior infection with CMV has been suggested as a strong independent risk factor for restenosis after coronary atherectomy,[15] and there is a graded relation between the odds of intimal-medial thickening and levels of CMV antibodies that remained significant after adjustment for the main cardiovascular risk factors.[16, 17]

Other viruses have been "accused" as well, for example, the hepatitis A (HAV) virus.[18] An association of herpesvirus and atherosclerosis has been suggested by seroepidemiologic studies and detection of the virus in arterial tissues.[19]

The Pathogen Burden Theory

Immunoglobin G antibodies to CMV, HAV, herpes simplex virus type 1 (HSV1), HSV type 2 (HSV2), *Chlamydia pneumonia*, and *Helicobacter pylori* levels were tested in baseline blood samples from 890 patients who had significant coronary artery disease on angiography. The mean follow-up was 3 years. The baseline prevalence of antibodies directed against CMV, HSV1, or HSV2, but not *C. pneumonia* and *H. pylori*, was significantly higher among patients who subsequently developed myocardial infarction (MI) or death than among control subjects. Increasing pathogen burden was significantly associated with increasing risk of MI or death in a dose-response fashion.[20]

Of 375 patients undergoing coronary angiography, 218 had assessment of endothelial function using intracoronary acetylcholine (ACH). Immunoglobulin G antibody titers to CMV, *C. pneumonia*, *H. pylori*, HAV, and HSV-1 were measured. Although positive serology to individual pathogen tended to be associated with increased incidence of coronary arteriosclerosis (CAD), the pathogen burden correlated with the presence of CAD even after adjustment for risk factors. The severity of CAD was independently associated with the pathogen burden, which was an independent predictor of endothelial dysfunction. The Ig-G antibody response to multiple pathogens (pathogen burden) is an independent risk factor for endothelial dysfunction and the presence and severity of CAD. Endothelial dysfunction provides the crucial link by which pathogens may contribute to atherogenesis.[21]

The Human Immunodeficiency Virus

A postmortem study on 15 patients with HIV revealed thickening of the intima at the proximal coronary networks, caused by a proliferation of secreting cells, phenotypically identified as smooth muscle cells, with exaggerated production of elastic fibers and in association with an increase in the expression of tumor necrosis factor-alpha and interleukin-1 alpha.[22] The lesions were intermediate between the lesions observed during common coronary atherosclerosis and atherosclerosis associated with chronic rejection of cardiac transplants.

The basic mechanism that could explain the endothelial damage caused by viruses such as the sendai virus, influenza, and HIV could be attributed to oxidative stress. Vascular oxidative stress is due to inflammatory and immune responses of vascular cells and to reperfusion after recanalization of blocked arteries. Studies of CMV interactions with smooth muscle and endothelial cells could be used as a model for viral-endothelial/smooth muscle cell interactions. Infection causes generation of intracellular reactive oxygen species (ROS), which activate nuclear factor kappa B (NFκB), a cellular transcription factor.[22] Nuclear factor kappa B mediates expression of the CMV promotor and of genes involved in the immune and inflammatory responses.[22]

Cytomegalovirus is a common coinfecting organism in patients with HIV infection, and we cannot exclude its role in the vasculopathy of these patients. However, the marked change in HIV VL that was inversely associated with EDD may suggest a role for HIV itself.

Our hypothesis is that the human immunodeficiency virus has at least some of these capabilities and shares some of the basic virus–endothelial/smooth muscle cell interactions that have been demonstrated with CMV.

Conclusion

We conclude from our study that high viral load is associated with endothelial dysfunction in patients with HIV. This observation supports the viral-infectious theory of atherosclerosis, suggesting that viruses play a role in the pathogenesis of atherosclerosis and demonstrating an inverse correlation between HIV VL and endothelial function. Studies with a large cohort of patients over a prolonged time period are needed to confirm and validate our observation.

References

1. Passalaris JD, Sepkowitz KA, Glesby MJ: Coronary artery human immunodeficiency virus infection. *Clin Infect Dis* 2000;31(3):787–797
2. Zandotti C, Quilichini R, Aubert L, Tamalet C, Juhan-Vague I, Gastaut JA: Endothelial cell dysfunction in HIV infection. *J Acq Immune Defic Syndr* 1992;5(2):127–131
3. Speir E, Modali R, Huang ES, Leon MB, Shawl F, Finkel T, Epstein SE: Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. *Science* 1994;265(5170):391–394
4. Blum A, Giladi M, Weinberg M, Kaplan G, Pasternack H, Laniado S, Miller H: High anti-cytomegalovirus (CMV) IgG antibody titer is associated with coronary artery disease and may predict post-coronary balloon angioplasty restenosis. *Am J Cardiol* 1998;81(7):866–888
5. Shah F: Link between infection and atherosclerosis: Who are the culprits: Viruses, bacteria, both, or neither? *Circulation* 2001;103(1):5–6
6. Celermajer DS, Sorensen KE, Gooch VM: Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111–1115
7. Behrens G, Schmidt H, Meyer D, Stoll M, Schmidt RE: Vascular complications associated with use of HIV protease inhibitors (comment). *Lancet* 1998;351:1958
8. Henry K, Melroe H, Huebsch J, Hermundson J, Levine C, Swensen JD, Daley J: Severe premature coronary artery disease with protease inhibitors. *Lancet* 1998;351:1328
9. Stein JH, Klein MA, Bellehumeur JL, Aeschlimann SE, McBride PE, Sosman JM: Use of human immunodeficiency virus-1 protease inhibitors is associated with endothelial dysfunction (letter). *American Heart Association* 2000;II-7:3812
10. Lafeuillade A, Alessi MC, Poizot-Martin I, Boyer-Neumann C, Zandotti C, Quilichini R, Aubert L, Tamalet C, Juhan-Vague I, Gastaut JA: Endothelial cell dysfunction in HIV infection. *J Acq Immune Defic Syndr* 1991;5(2):127–131
11. Fabricant CG, Fabricant J, Litrenta MM, Minick CR: Virus induced atherosclerosis. *J Exp Med* 1978;148:335–340
12. Melnick JL, Petrie BL, Dreesman GR, Burek J, McCollum CH, DeBakey ME: Cytomegalovirus antigen within human arterial smooth muscle cells. *Lancet* 1983;2(8351):644–647
13. Hendrix MGR, Salimans MMM, van Boven CRA, Bruggeman CA: High prevalence of latently present cytomegalovirus in arterial walls of patients suffering from grade III atherosclerosis. *Am J Pathol* 1990;136:23–28
14. Adam E, Melnick JL, Probtsfield JL, Petrie BL, Burek J, Bailey KR, McCollum CH, DeBakey ME: High level of cytomegalovirus antibody in patients requiring vascular surgery for atherosclerosis. *Lancet* 1987;2:291–293
15. Zhou YF, Leon MB, Waclawin MA, Popma JJ, Yu ZX, Finkel T, Epstein SE: Association between prior cytomegalovirus infection and the risk of restenosis after coronary atherectomy. *N Engl J Med* 1996;335:624–630
16. Nieto FJ, Adam E, Sorlie P, Farzadegan H, Melnick JL, Comstock GW, Szklo M: Cohort study of cytomegalovirus infection as a risk factor for carotid intimal-medial thickening, a measure of subclinical atherosclerosis. *Circulation* 1996;94:922–927
17. Sorlie PD, Nieto FJ, Adam E, Folsom AR, Shahar E, Massing M: A prospective study of cytomegalovirus, herpes simplex virus 1, and coronary artery disease: The atherosclerosis Risk In Communities (ARIC) study. *Arch Intern Med* 2000;160(13):2027–2032
18. Zhu J, Quyyumi AA, Norman JE, Costello R, Csako G, Epstein SE: The possible role of hepatitis A virus in the pathogenesis of atherosclerosis. *J Infect Dis* 2000;182(6):1583–1587
19. Lin TM, Jiang MJ, Eng HL, Shi GY, Lai LC, Huang BJ, Huang KY, Wu HL: Experimental infection with bovine herpesvirus-4 enhances atherosclerotic process in rabbits. *Lab Invest* 2000;80(1):3–11
20. Zhu J, Nieto J, Horne BD, Anderson JL, Muhlestein JB, Epstein SE: Prospective study of pathogen burden and risk of myocardial infarction or death. *Circulation* 2001;103:45–57
21. Prasad A, Zhu J, Halcox JPJ, Waclawiw MA, Epstein SE, Quyyumi AA: Predisposition to atherosclerosis by infections. Role of endothelial dysfunction. *Circulation* 2002;106:184–197
22. Tabib A, Leroux C, Mornex JF, Loire R: Accelerated coronary atherosclerosis and arteriosclerosis in young human-immunodeficiency-virus-positive patients. *Coron Artery Dis* 2000;11(1):41–46