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Serum IL-6 Levels are Associated with Significant Coronary Stenosis in Cardiovascularly Asymptomatic Inner-City Black Adults in the US

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Abstract

Objectives and design—The objective of this study was to explore whether increased levels of inflammatory cytokines are associated with the risk of clinically silent coronary artery disease.

Subjects—Three-hundred-fifty-six black adults aged 25–54 residing in inner city of Baltimore, Maryland, United States were included in this study.

Methods—Sociodemographics were assessed as were lipid profiles, IL-6, tumor necrosis factor- α (TNF- α), soluble intercellular adhesion molecule-1 (sICAM-1), and high-sensitivity C-reactive protein (hs-CRP) levels. Computed tomography (CT) coronary angiography was performed.

Results—Coronary calcification was identified in 22.5% participants and 14% had significant ($\geq 50\%$) coronary stenosis. Multiple logistic regression analyses suggested that IL-6 levels were independently associated with the presence of coronary calcification and significant coronary stenosis, while TNF- α , sICAM-1 and hs-CRP levels were not.

Conclusions—This study underscores a critical role for IL-6 in atherosclerosis and suggests that IL-6 may be a marker for significant coronary stenosis in cardiovascularly asymptomatic individuals.

Keywords

interleukin-6 (IL-6); significant coronary stenosis; CT coronary angiography; marker for clinically silent CAD; black race

Introduction

Atherosclerotic coronary artery disease (CAD) is characterized by the accumulation of atheromatous plaques within the walls of the coronary arteries. In the development of coronary atherosclerosis, as an indicator of atherosclerotic disease, calcium precipitates in the coronary artery wall [1]. Black adults have the highest CAD mortality rate of any ethnic group in the United States [2,3]. Young and middle-aged blacks have a disproportionately high cardiovascular morbidity and mortality [4–9]. Although the risk factors for CAD have been extensively investigated in general [10], the factors that influence CAD in blacks, especially

black adults residing in inner-city with lower socioeconomic status, are poorly documented. In addition to traditional risk factors, novel risk markers, such as, IL-6 and high-sensitivity C-reactive protein (hs-CRP), may provide greater insight into the arterial insult and underlying risk burden. The objectives of this study were (1) to estimate the prevalence of coronary calcification, coronary plaques, and coronary stenosis, including significant ($\geq 50\%$) coronary stenosis in cardiovascularly asymptomatic blacks residing in inner-city neighborhoods in Baltimore, Maryland; and (2) to explore whether increased levels of inflammatory cytokines, IL-6, TNF-alpha and sICAM-1, are independently associated with the risk of clinically silent CAD in this population. Clinically silent (asymptomatic and undiagnosed) CAD was examined with coronary CT angiography and the presence of coronary calcification, plaques, and stenosis was presented.

Subjects and Methods

Study Subjects

Between May 2003 and March 2007, 377 black adults aged between 25 and 54 years from Baltimore, Maryland, were enrolled in an observational study investigating the factors that are associated with subclinical atherosclerosis. Interviews about sociodemographics and drug-use behaviors, clinical examinations, electrocardiograms (ECGs), echocardiographic examinations, lipid profiles, high sensitivity C-reactive protein tests, cytokines assays for IL-6, TNF-alpha, and sICAM-1 and spiral computed tomographies (CTs) for coronary calcium (CAC) were performed.

The participants included subjects with HIV infection (40%), subjects with cocaine use (77%), subjects with both (31%), and subjects with neither (14%). Inclusion criteria were age between 25 and 54 years and black race. Exclusion criteria were 1) any evidence of hypertension or ischemic heart disease, on the basis of clinical history, previous hospitalization for myocardial infarction, angina pectoris, or ECG and/or echocardiographic evidence of previous myocardial damage by ischemic heart disease; 2) any symptoms believed to be related to cardiovascular disease; and 3) pregnancy. Information about sociodemographics, medical history, medication use, and cocaine-use behaviors was obtained by interviewer-administered questionnaires. The Committee on Human Research at the Johns Hopkins Bloomberg School of Public Health approved the study protocol, and all study participants provided written informed consent. All procedures used in this study were in accordance with institutional guidelines. Although the overall investigation is a cohort study, the data presented here are cross-sectional only.

Measurement of Lipids

Venous blood samples were obtained after an overnight fast from a large antecubital vein. Serum was separated by centrifugation 2000 g for 15 minutes at 4 °C and stored at -75 °C until assayed. Serum lipid parameters, including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were directly determined with the use of a Roche Hitachi 747 analyzer (Englewood, NJ).

Cytokine Analysis

Blood was collected, centrifuged at 2000 g for 15 minutes at 4 °C, aliquoted and stored in a -70 freezer until analyzed. Serum levels of IL-6, TNF-alpha and sICAM-1 were determined using commercially available enzyme-linked immunosorbent assay (EIA) kits (R&D Systems, Minneapolis, MN). Serum samples were tested in duplicate according to the manufacture's instructions. The plates were read immediately after application of the stop solution. The optical density of each sample was determined using VERSAmax tunable microplate reader (Molecular Devices, Sunnyvale, CA). Results were calculated from a standard curve and reported accordingly in picograms or nanograms per milliliter. The minimum detectable dose

for each cytokine measured is: IL-6 is < 0.7 pg /ml: TNF-alpha is 1.6 pg /ml and sICAM-1 is <0.33 ng /ml.

Blood Pressure Measurement

Systolic and diastolic sitting blood pressure (BP) was measured twice with a standard mercury sphygmomanometer. A nurse at the clinic measured the study participant's arm circumference and applied a correctly sized cuff. The participant sat quietly for 5 minutes, and then the nurse obtained the systolic and diastolic blood pressure measurements; a second measurement was made 3 minutes later. The average of the two readings was reported.

Coronary CT angiography with a 64 slice Siemens MDCT scanner

A noncontrast MDCT scan was performed on a Sensation 64 Cardiac Siemens Medical Solutions scanner (Erlangen, Germany) to determine the coronary artery calcium score with a sequential scan of 3-mm slices with prospective ECG triggering, 30 Å~ 0.6-mm detector collimation, and tube current 135 mAs at 120 kV. Subsequently, coronary CT angiography (CTA) was performed with the same equipment using 80 mL of isosmolar contrast agent (320 mg iodine/mL) injected at 4–5 mL/s. Imaging was performed with retrospective ECG-gating, 32 Å~ 0.6-mm detector collimation with flying focal spot to give effective detector collimation of 64 × 0.6 mm, 330 ms gantry rotation, 850 mAs and 120 kV. Subsequently, 0.75-mm thick axial slices were reconstructed at 0.4-mm intervals with B25 kernel using a half-scan reconstruction algorithm with resulting temporal resolution of 165–185 ms. Ten reconstructions were done through the cardiac cycle at 10% increments in the R-R interval. If needed, patients were premedicated with metoprolol prior to the scan to achieve a heart rate < 65 beats per minute.

Coronary artery calcium score, volume, and mass were measured on a workstation (Leonardo, Syngo, Siemens Medical Solutions, Malvern, PA). Regions of interest were placed over each of the coronary arteries with the threshold for pixels greater than 130 HU for determining calcified plaque. Coronary arteries were assessed for patency and stenosis using 3D visualization tools after the axial images were reviewed for determination of anatomy, quality of the study, and appearance of the vessels.

Two reviewers (H.P and E.K.F) independently evaluated the contrast-enhanced MDCT scans by reviewing the axial slices, curved multiplanar reformations, and thin-slab maximum intensity projections. The coronary artery tree was segmented according to the modified American Heart Association classification and the segments were investigated for plaque and luminal narrowing. Vessel plaque and diameter were assessed on 2D multiplanar reconstruction (MPR) and 2D thin slab maximum intensity projection (MIP) images. 3D volume rendered (VR) images were used to display long segments of the vessels and their branches. The coronary arteries were divided into proximal, mid, and distal segments, with each segment investigated for luminal narrowing. Plaques were classified as calcified or noncalcified and the degree of stenosis was classified as less than or greater than 50% diameter stenosis. Noncalcified atherosclerotic plaque was defined as a soft tissue attenuation structure in the coronary artery wall with a CT density that was less than that of calcified plaque as well as less than that of the contrast in the lumen but was greater than the adjacent epicardial fat. For determining the mean attenuation of noncalcified plaques, the vessel segment was displayed in the short axis plane orthogonal to the vessel or the long axis plane of the vessel on 0.75 mm thick slices in multiplanar reformation. The plane that demonstrated the thickest width of the plaque was selected for measuring the attenuation. The largest possible region of interest was then manually placed in the plaque avoiding overlap with adjacent tissue or vessel lumen. Window width and level were interactively adjusted by the reader for each case to

optimize display of the lumen and surrounding soft tissues. A percent diameter stenosis $\geq 50\%$ was defined as significant coronary stenosis.

Statistical Analysis

Statistical analysis was performed with SAS (Statistical Analysis System, Cary, North Carolina, USA). All continuous parameters were described by means and standard deviations (SDs), and all categorical parameters were summarized as proportions. **To examine the associations of demographic, clinical characteristics and levels of cytokines with the presence of significant coronary stenosis, we used Student *t* test for continuous variables and chi-square test for categorical variables.** Univariate logistic regression analysis was performed to examine crude associations between the presence of significant coronary stenoses and each of covariates, including demographic factors, cigarette smoking, alcohol use, body mass index (BMI), systolic and diastolic blood pressures, total cholesterol, HDL, LDL, triglycerides, HCV seropositivity, and elevated IL-6 (>1 pg/ml), individually. Those variables that exhibited statistical significance at the 0.10 or lower levels in univariate logistic regression models were jointly entered into a multiple logistic regression model and those that ceased to be significant were deleted, yielding the final model. All *P* values reported are 2-sided.

Results

General Characteristics

Demographic and clinical characteristics of the study participants are presented in Table 1. Of the 377 participants in this study, 356 included in this study since the data on cytokines were not complete in 21 participants. Of these 356, 122 (34.3%) were women. The mean age ($\pm$ SD) was 42.8 ± 6.3 years. Eighty-seven percent of the study participants were cigarette smokers, 21% had family history of heart attack. The mean cholesterol level was 172.5 ± 36.7 mg/dl, mean systolic blood pressure was 113 ± 12 mmHg, and mean diastolic blood pressure was 75 ± 9 mmHg. Forty percent were HIV-infected, and 77% were chronic cocaine users. Approximately 50% were HCV antibody-positive. The Framingham risk score was 4.1 ± 3.1 . Coronary plaques have been identified in 28% of the total participants studied and 14% of total subjects had significant ($\geq 50\%$) coronary stenosis. Among those who had coronary plaques, 75% had calcified plaques and 25% had noncalcified plaques. Among those who had significant coronary stenosis, 81% had calcified plaques and 19% had noncalcified plaques.

Association between Significant Coronary Calcification and IL-6 Levels

IL-6 levels were marginally associated with the presence of coronary calcification ($p=0.11$) by univariate analysis. After controlling for age, HDL, LDL, IL-6 levels were significantly associated with the presence of coronary calcification ($p=0.024$). In contrast, CRP levels were not associated with the presence of coronary calcification ($p=0.92$). Furthermore, neither TNF- α nor sICAM-1 were associated with presence of coronary calcification (Table 2).

Association between Significant Coronary Stenosis and IL-6 Levels

According to univariate logistic regression analyses, age ($p<0.0001$), female gender ($p=0.01$), family history of heart attack ($p=0.097$), LDL ($p=0.014$), HDL ($p=0.017$), total cholesterol ($p=0.0003$), diastolic blood pressure ($p=0.037$), and IL-6 ($p=0.10$) were significantly associated with the presence of significant coronary stenosis (Table 3). Multiple logistic regression analysis revealed that age ($p<0.0001$), diastolic blood pressure ($p=0.004$), LDL ($p=0.014$), and IL-6 level ($p=0.019$) were independently associated with the presence of significant coronary stenosis (Table 3).

Association between IL-6 Levels and HIV infection

Although HIV infection was not significantly associated with significant stenosis, both HIV infection (OR=2.36, 95%CI: 1.41–3.95) and CRP $\geq$ 2.0 mg/dl (OR: 2.01, 95%CI: 1.20, 3.37) were independently associated with elevated IL-6. This suggests that HIV infection may indirectly promote premature atherosclerosis. In contrast to the IL-6 data, serum levels of TNF-alpha and sICAM-1 did not correlate with the development of coronary stenosis.

Discussion

Recent studies have implicated inflammatory processes and the generation of IL-6 in the early development of atherosclerosis [11,12]. Earlier studies have established that coronary calcification is an indicator of atherosclerosis [13]. In this study asymptomatic subjects were evaluated for silent CAD using coronary CT angiography to determine the presence of calcification, plaques and stenosis. We found that in young to middle aged black adults from the inner city there is an independent, positive association between IL-6 levels and the presence of coronary calcification. More importantly, our data also suggests that elevated IL-6 levels, not CRP (p=0.8), were a strong and independent predictor of clinically silent CAD.

Over the past decade inflammation has been identified as a critical component in cardiovascular pathology [14–16]. Furthermore, increasing evidence supports the involvement of inflammation in the various stages of the atherosclerotic process [14]. IL-6, in particular, a potent inflammatory cytokine that is produced by several cell types, including activated macrophages, endothelial cells and smooth muscle cells, has been recognized as a potential marker linked to cardiovascular events.

IL-6 regulates the inflammatory system in a variety of ways, for example, it plays an essential role in mediating the acute phase response by up-regulating the hepatic synthesis of acute phase reactants, such as, CRP. Moreover, IL-6 also influences diverse cellular actions, including, effects on platelets, endothelium, factors of metabolism and coagulation [17,18]. Numerous investigations have reported that increased levels of IL-6 and CRP are associated with increased cardiovascular disease, atherosclerosis and myocardial infarction (MI) [19–21]. In addition, enhanced levels of IL-6 in the circulation have been observed in unstable angina, acute MI and chronic heart failure [21–23]. Moreover, IL-6 has been shown to be an independent risk factor for future MI, is associated with impaired short- and long- term prognosis in patients with acute coronary syndromes and has been found in atherosclerotic plaques [24–26]. In addition, studies have revealed that IL-6 levels are associated with subclinical atherosclerotic lesions independent of traditional risk factors [27]. More recently, Noto et al reported that in patients with clinical CAD, increased levels of CRP, sICAM-1, 8-isoprostone and IL-6 were good predictors of critical coronary stenosis and that these plasma biomarker levels increased with the number of diseased vessels [28]. Furthermore, they show in this population of patients, that IL-6 levels were the best predictor of critical coronary stenosis with the highest sensitivity and specificity. Our findings herein extend this data and show that in pre-clinical (asymptomatic) CAD individuals, IL-6 levels correlate with significant coronary stenosis.

In this study 40% of our participants were HIV positive. It is known that IL-6 is up-regulated in variety of clinical conditions, such as, injury, trauma, and during infections [29]. Previously, it has been reported that HIV individuals have elevated levels of IL-6 [30]. Our data demonstrate that HIV infected individuals were 3.4 times more likely to have elevated IL-6 (OR=3.4, 95%CL (1.9, 6.1)) than non-infected individuals. However, HIV infection alone was not associated with significant stenosis but was independently associated with increased IL-6.

Inflammation can be observed in three stages of the atherosclerotic process, at early development of atherosclerosis, at progression of the atherosclerotic plaque, and at acute plaque

rupture [11,31]. Immune cells, including macrophages, and T-lymphocytes, have been found in human fatty streaks, the earliest stage of the disease process [32,33] suggesting that immune reactivity may play an initiating or early role in the development of the lesion. Numerous cytokines, such as TNF-alpha and IL-6, and growth factors have also been found within atheromatous lesions at all stages [31,34,35]. Plaque rupture also involves inflammatory mechanisms. Rupture occurs at sites of the fibrous plaque where activated macrophages enter [31,36] and have been reported to release proteolytic enzymes, including metalloproteinases and collagenases[37].

In addition to evaluating IL-6 in this study, we also measured TNF-alpha and sICAM-1. Both of these molecules have been previously associated with cardiovascular disease [27,38]. However, in this study, neither TNF-alpha nor sICAM-1 was associated with significant coronary stenosis. In fact, unlike IL-6, TNF-alpha was detected in a limited number of individuals. One explanation may be the fact that TNF-alpha signals locally and has a short half-life in the circulation. Furthermore, released TNF-alpha binds rapidly to its cell surface receptor and this may make serum levels difficult to measure [39]. Likewise, sICAM-1, an adhesion molecule up-regulated during inflammation also did not correlate with significant stenosis. Further analysis of our data revealed that increased levels of IL-6 were significantly associated with elevated TNF-alpha (OR=14.0, 95% CL (5.4, 36.3)). This is not surprising in view of the fact that these two pro-inflammatory cytokines are often co-expressed in a number of conditions. Nevertheless, TNF-alpha was detected in fewer individuals than IL-6 and again this could be due to the rapid clearance of this molecule.

Of note was the observation that age was significantly associated with increased IL-6 (OR=1.05, 95%CL (1.01, 1.10)). In this report, our mean age was 42.8 ± 6.3 and our range was 25–54 years of age. A number of groups have analyzed IL-6 in healthy young and old subjects [40]. Some studies revealed that increased IL-6 levels are more frequently detected in the older populations. However, this has not been a consistent observation and additional studies will be needed to confirm these findings.

This study has several limitations. First, the study participants were not a random sample of the inner-city black populations. Thus, the results must be interpreted with caution. Second, the study was a cross-sectional investigation in nature and the findings should be confirmed by longitudinal studies. Third, although non-invasive CTA provide an excellent diagnostic tool, revealing the atherosclerotic burden, 10 to 14% of coronary arterial segments visualized by CT angiography are not assessable [41]. Nevertheless, recent studies have reported excellent diagnostic accuracy for 64-slice MDCT in the detection of significant stenosis in smaller coronary artery segments and side branches (sensitivity of 86% to 94%; specificity of 93% to 97%) [42–45]. Despite these limitations, this is the first study, which has reported that IL-6 levels are independently associated with clinically silent CAD in a cardiovascularly asymptomatic black population and the associated risk factors. Since our study population was in black individuals only, future studies with more diverse populations will be necessary to determine if this is a generalized finding or specifically associated with the black population. This study underscores a critical role for IL-6 in atherosclerosis and suggests that IL-6 may be a marker for significant coronary stenosis.

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References

1. Stanford W. The role of coronary artery calcification in coronary artery disease. *Int J of Cardiovasc Imaging* 2001;17:473–474. [PubMed: 12365549]
2. Gillum RF. The epidemiology of cardiovascular disease in black Americans. *New Engl J Med* 1996;335:1597–1599. [PubMed: 8900095]
3. Ferdinand KC. Coronary heart disease and lipid-modifying treatment in African American patients. *Am Heart J* 2004;147:774–782. [PubMed: 15131530]
4. U.S. Department of Health and Human Services, Task Force on Black & Minority Health. Report of the Secretary's Task Force on Black & Minority Health. Washington, D.C: 1985.
5. U.S. Department of Health and Human Services. Healthy People 2000: National Health Promotion and Disease Prevention Objectives: full report, with commentary. Washington, D.C: Government Printing Office; 1990. (DHHS publication no. (PHS) 91-50212.)
6. House JS, Kessler RC, Herzog AR. Age, socioeconomic status, and health. *Milbank Q* 1990;68:383–411. [PubMed: 2266924]
7. Geronimus AT. The weathering hypothesis and the health of African-American women and infants: evidence and speculations. *Ethn Dis* 1992;2:207–221. [PubMed: 1467758]
8. Sorlie PD, Backlund E, Keller JB. US mortality by economic, demographic, and social characteristics: the National Longitudinal Mortality Study. *Am J Public Health* 1995;85:949–956. [PubMed: 7604919]
9. Pappas G, Queen S, Hadden W, Fisher G. The increasing disparity in mortality between socioeconomic groups in the United States, 1960 and 1986. *N Engl J Med* 1993;329:103–109. [PubMed: 8510686]
10. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998;97:1837–1847. [PubMed: 9603539]
11. Ross R. Atherosclerosis-an inflammatory disease. *N Engl J Med* 1999;340:115–126. [PubMed: 9887164]
12. Lee WY, Allison MA, Kim DJ, Song CH, Barrett-Connor E. Association of interleukin-6 and C-reactive protein with subclinical carotid atherosclerosis (the Rancho Bernado Study). *Am J Cardiol* 2007;99:99–102. [PubMed: 17196470]
13. Bild DE, Folsom AR, Lowe LP, Sidney S, Kiefe C, Westfall AO, et al. Prevalence and correlates of coronary calcification in black and white young adults The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Arterioscler Thromb Vasc Biol* 2001;21:852–857. [PubMed: 11348886]
14. Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868–874. [PubMed: 12490960]
15. Hoffmeister A, Rothenbacher D, Kunze M, Brenner H, Koenig W. Prognostic value of Inflammatory markers alone and in combination with blood lipids in patients with stable coronary artery disease. *Eur J Intern Med* 2005;16:47–52. [PubMed: 15733822]
16. 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* 2008;199:3–11. [PubMed: 18378239]
17. Woods A, Brull DJ, Humphries SE, Montgomery HE. Genetics of inflammation and risk of coronary artery disease: the central role of interleukin-6. *Eur Heart J* 2000;21:1574–1583. [PubMed: 10988009]
18. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 2000;148:209–214. [PubMed: 10657556]
19. Erren M, Reinecke H, Junker R, Fobker M, Schulte H, Schurek JO, et al. Systemic inflammatory parameters in patients with atherosclerosis of the coronary and peripheral arteries. *Arterioscler Thromb Vasc Biol* 1999;19:2355–2363. [PubMed: 10521364]
20. Volpato S, Guralnik JM, Ferrucci L, Balfour J, Chaves P, Fried LP, et al. Cardiovascular disease, interleukin-6, and risk of mortality in older women: the women's health and aging study. *Circulation* 2001;103:947–953. [PubMed: 11181468]

21. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000;101:1767–1772. [PubMed: 10769275]
22. Biasucci LM, Vitelli A, Liuzzo G, Altamura S, Caligiuri G, Monaco C, et al. Elevated levels of interleukin-6 in unstable angina. *Circulation* 1996;94:874–877. [PubMed: 8790019]
23. McCarthy MJ, Loftus IM, Thompson MM, Jones L, London NJ, Bell PR, et al. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. *J Vasc Surg* 1999;30:261–268. [PubMed: 10436445]
24. Fisman EZ, Benderly M, Esper RJ, Behar S, Boyko V, Adler Y, et al. Interleukin-6 and the risk of future cardiovascular events in patients with angina pectoris and/or healed myocardial infarction. *Am J Cardiol* 2006;98:14–18. [PubMed: 16784912]
25. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. *N Engl J Med* 2000;343:1139–1147. [PubMed: 11036119]
26. Rus HG, Vlaicu R, Niculescu F. Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. *Atherosclerosis* 1996;127:263–271. [PubMed: 9125317]
27. Amar J, Fauvel J, Drouet L, Ruidavets JB, Perret B, Chamontin B, et al. Interleukin 6 is associated with subclinical atherosclerosis: a link with soluble intercellular adhesion molecule 1. *J Hypertens* 2006;24:1083–1088. [PubMed: 16685208]
28. Noto D, Cottone S, Cefalu AB, Vadala A, Barbagallo CM, Rizzo M, et al. Interleukin 6 plasma levels predict with high sensitivity and specificity coronary stenosis detected by coronary angiography. *Thromb Haemost* 2007;98:1362–1367. [PubMed: 18064337]
29. Mogensen TH, Paludan SR. Molecular pathways in virus-induced cytokine production. *Microbiol Mol Biol Rev* 2001;65:131–150. [PubMed: 11238989]
30. Sanders CM, Cruse JM, Lewis RE. Toll-like receptors, cytokines and HIV-1. *Exp Mol Pathol* 2008;84:31–36. [PubMed: 17997401]
31. Elkind MS. Inflammation, atherosclerosis, and stroke. *Neurologist* 2006;12:140–148. [PubMed: 16688015]
32. Munro JM, van der Walt JD, Munro CS, Chalmers JA, Cox EL. An immunohistochemical analysis of human aortic fatty streaks. *Hum Pathol* 1987;18:375–380. [PubMed: 3549534]
33. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr, Rosenfeld ME. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1994;89:2462–2478. [PubMed: 8181179]
34. van der Wal AC, Das PK, Bentz van de Berg D, van der Loos CM, Becker AE. Atherosclerotic lesions in humans: in situ immunophenotypic analysis suggesting an immune mediated response. *Lab Invest* 1989;61:166–170. [PubMed: 2787872]
35. Nilsson J. Cytokines and smooth muscle cells in atherosclerosis. *Cardiovasc Res* 1993;27:1184–1190. [PubMed: 8252576]
36. Lee RT, Libby P. The unstable atheroma. *Arterioscler Thromb Vasc Biol* 1997;17:1859–1867. [PubMed: 9351346]
37. Schönbeck U, Mach F, Sukhova GK, Murphy C, Bonnefoy JY, Fabunmi RP, et al. Regulation of matrix metalloproteinase expression in human vascular smooth muscle cells by T lymphocytes: a role for CD40 signaling in plaque rupture? *Circ Res* 1997;81:448–454. [PubMed: 9285647]
38. Reilly MP, Rohatgi A, McMahon K, Wolfe ML, Pinto SC, Rhodes T, et al. Plasma cytokines, metabolic syndrome, and atherosclerosis in humans. *J Investig Med* 2007;55:26–35.
39. Munoz C, Misset B, Fitting C, Bleriot JP, Carlet J, Cavaillon JM. Dissociation between plasma and monocyte-associated cytokines during sepsis. *Eur J Immunol* 1991;21:2177–2184. [PubMed: 1889462]
40. Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, Glaser R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc Natl Acad Sci USA* 2003;100:9090–9095. [PubMed: 12840146]

41. Pannu HK, Jacobs JE, Lai S, Fishman EK, Coronary CT. angiography with 64-MDCT: assessment of vessel visibility. *AJR Am J Roentgenol* 2006;187:119–126. [PubMed: 16794165]
42. Raff GL, Gallagher MJ, O'Neill WW, Goldstein JA. Diagnostic accuracy of noninvasive coronary angiography using 64-slice spiral computed tomography. *J Am Coll Cardiol* 2005;46:552–557. [PubMed: 16053973]
43. Leber AW, Knez A, von Ziegler F, Becker A, Nikolaou K, Paul S, et al. Quantification of obstructive and nonobstructive coronary lesions by 64-slice computed tomography: a comparative study with quantitative coronary angiography and intravascular ultrasound. *J Am Coll Cardiol* 2005;46:147–154. [PubMed: 15992649]
44. Leschka S, Alkadhi H, Plass A, Desbiolles L, Grünenfelder J, Marincek B, et al. Accuracy of MSCT coronary angiography with 64-slice technology: first experience. *Eur Heart J* 2005;26:1482–1487. [PubMed: 15840624]
45. Mollet NR, Cademartiri F, van Mieghem CA, Runza G, McFadden EP, Baks T, et al. High-resolution spiral computed tomography coronary angiography in patients referred for diagnostic conventional coronary angiography. *Circulation* 2005;112:2318–2323. [PubMed: 16203914]

Table 1
Demographic and Clinical Characteristics of Study Participants by the Presence of Significant Coronary Stenosis

Characteristic	Total (N=356)	Significant Coronary Stenosis		p-value
		No (N=306)	Yes (N=50)	
Age (year)	42.8±6.3	42.2±6.2	46.5±5.1	<0.0001
Female sex (%)	34.3	41.2	22.0	0.01
Cigarette smoking (%)	87.1	86.9	88.0	0.83
Alcohol use (%)	89.3	89.2	90.0	0.87
Family history of heart disease (%)	21.1	19.6	30.0	0.09
HIV infection (%)	40.0	39.6	40.0	0.95
Cocaine use (%)	77.0	75.9	84.0	0.20
BMI (kg/m ²)	26.0±9.1	26.1±9.6	24.9±4.7	0.15
SBP (mm Hg)	113±12	113±12	115±10	0.28
DBP (mm Hg)	75±9	75±9	78±9	0.03
HDL (mg/dl)	55.0±18.2	54.1±16.3	60.8±26.7	0.09
LDL (mg/dl)	96.5±32.5	94.7±32.3	107.1±32.5	0.01
Fasting glucose (mg/dl)	88.6±26.8	88.9±28.4	87.2±12.6	0.49
Total cholesterol (mg/dl)	172.5±36.7	169.5±36.5	190.2±33.2	0.0002
Triglycerides (mg/dl)	104.8±57.0	103.7±54.2	111.7±72.1	0.46
HCV infection (%)	52.4	50.0	64.3	0.16
hsCRP (mg/l)	4.6±10.7	4.6±11.3	3.4±5.1	0.22
IL6 (pg/ml)	5.7±25.5	4.5±17.6	12.3±51.4	0.30
sICAM-1 (ng/ml)	298.4±210.4	299.7±200.0	290.7±265.9	0.82
TNF-alpha (pg/ml)	8.6±25.0	7.8±22.8	13.0±34.9	0.33
Coronary calcification (%)	22.5	13.1	80.0	<0.0001
Framingham Risk Score	4.13±3.11	3.81±2.70	6.14±4.45	0.0008

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high sensitivity C-reactive protein.

Table 2

Associations Between Cytokines and the Presence of Coronary Calcification

Variable	Univariate	Multivariate	
	OR (95%CI)	OR (95% CI) [*]	p-value
hs CRP (mg/l)	1.00 (0.97, 1.02)	1.00 (0.97, 1.03)	0.92
IL-6 (pg/ml)	1.01 (1.00, 1.02)	1.01 (1.01, 1.02)	0.024
sICAM-1 (ng/ml)	1.00 (0.99, 1.00)	1.00 (0.99, 1.00)	0.35
TNF-alpha (pg/ml)	1.01 (1.00, 1.01)	1.01 (1.00, 1.02)	0.08

* Adjusted for age, HDL (high-density lipoprotein) and LDL (low-density lipoprotein); hsCRP, high sensitivity C-reactive protein; OR, odds ratio.

Table 3

Factors Associated with the Presence of Significant Coronary Stenosis

Variable	Univariate	Multivariate	
	OR (95%CI)	OR (95% CI)	p-value
Age (year)	1.37 (1.07, 1.21)	1.14 (1.07, 1.22)	<0.0001
Female sex	0.40 (0.20, 0.82)		
Cigarette smoking	1.10 (0.44, 2.76)		
Alcohol use	1.09 (0.40, 2.93)		
Family history of heart disease	1.76 (0.90, 3.43)		
HIV infection	1.07 (0.55, 1.87)		
Cocaine use	1.67 (0.75, 3.71)		
BMI (kg/m ²)	0.97 (0.91, 1.03)		
SBP (mm Hg)	1.01, 0.99, 1.04)		
DBP (mm Hg)	1.04 (1.00, 1.08)	1.03 (1.01, 1.04)	
HDL (mg/dl)	1.02 (1.00, 1.03)		0.004
LDL (mg/dl)	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)	
Fasting glucose (mg/dl)	0.99 (0.98, 1.01)		
Total cholesterol (mg/dl)	1.02 (1.01, 1.02)		
Triglycerides (mg/dl)	1.00 (0.99, 1.01)		
HCV infection (%)	1.80 (0.77, 4.17)		
hs CRP (mg/l)	0.98 (0.94, 1.03)		
IL-6 (pg/ml)	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)	
sICAM-1 (ng/ml)	1.00 (0.99, 1.01)		
TNF-alpha (pg/ml)	1.01 (0.99, 1.02)		
Framingham Risk Score	1.24 (1.13, 1.35)		0.019

OR, odds ratio; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein, hsCRP, high sensitivity C-reactive protein.