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White blood cell count in young adulthood and coronary artery calcification in early middle age: coronary artery risk development in young adults (CARDIA) study

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Conflict of interest

None.

Supplementary material

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Abstract

White blood cell (WBC) count is associated with incident coronary heart disease (CHD). Data are sparse regarding its association in young adults with future coronary artery calcification (CAC). Our study was conducted among coronary artery risk development in young adults (CARDIA) participants ($n = 3,094$). We examined the association between baseline (Y0) WBC counts and CHD risk factors using linear regression models. We further assessed prospective associations between Y0 WBC and inflammatory biomarkers during the follow-up, and the presence of CAC 15 and 20 years later. In total, 272 and 566 subjects had CAC scores > 0 at year (Y) 15 and Y20, respectively. Baseline total WBC counts were cross-sectionally associated with SBP, BMI, and smoking, or HDL-cholesterol ($p = 0.01$) at Y0, and prospectively associated with C-reactive protein at Y7, Y15, and Y20, and fibrinogen at Y5 and Y20 ($p < 0.01$). After adjustment for potential confounding factors, baseline neutrophil count was borderline associated with CAC presence 15 years later (OR = 1.18 per unit, 95 % CI 1.00–1.44) and total WBC (OR = 1.07, 95 % CI 0.96–1.19) or eosinophil (OR = 1.12, 95 % CI 1.00–1.25) was borderline associated with CAC presence at Y20. Baseline total WBC counts in young adults was associated prospectively with CAC presence 20 years later after adjusting for age, sex, and race. Results are attenuated when other risk factors are accounted for. Our results suggest the possible early involvement of WBC, particularly eosinophils, in the early stages of atherosclerosis.

Keywords

White blood cell count; Coronary artery calcification; Atherosclerosis

Introduction

Cardiovascular disease (CVD) is the leading cause of death and a major cause of disability in the United States [1]. Epidemiological evidence has illustrated the association between long-term exposure to CVD risk factors and increased incidence of future subclinical and clinical CVD [2–4]. Baseline and long-term risk burden over 15 years in 18- to 30-year-old young adults from the coronary artery risk development in young adults (CARDIA) is associated with CAC presence at ages 33–45 years [5]. These data indicate that risk burden at young age is associated with the development of atherosclerosis over years and decades.

Atherosclerosis is a multistep disease that involves chronic inflammation at every stage [6–8]. White blood cells (WBC) play an important role in the inflammatory responses

involved in the initiation and propagation of atherosclerosis [9]. Extensive data have shown that total WBC count is associated independently with risk for future clinical atherosclerotic CVD events [10-12]. Counts of WBC subpopulations, such as neutrophils, monocytes, lymphocytes, and eosinophils, have also been associated with atherosclerosis and CHD, respectively [13-16].

Noncontrast cardiac CT can be used to measure coronary artery calcification (CAC) non-invasively. CAC identifies individuals with advanced coronary atheroma and is a good surrogate measure of the overall burden of coronary atherosclerosis [17-19]. Asymptomatic individuals with CAC have a substantially increased likelihood of future CHD and other CVD events. In the Multi-Ethnic Study of Atherosclerosis (MESA), individuals with Agatston CAC scores > 100 had 7-fold greater adjusted hazards for major coronary events over 3.8 years compared with those with CAC = 0, even after adjustment for traditional risk factors [20]. Therefore, preventing the development of subclinical atherosclerosis, as manifested by CAC, may lower CHD incidence. Study of the biomarkers involved in the early stages of atherosclerotic lesion formation may provide important insights into CHD prevention.

Data are sparse regarding the prospective association of WBC count and risk of subclinical atherosclerosis over a 20-year period in young and middle-aged adults. To better understand the role of WBC and inflammation in the early stage of atherosclerosis development, we examined whether baseline WBC counts are associated with future risk for the development and progression of subclinical atherosclerosis as measured by CAC 20 years later.

Methods

CARDIA cohort

The coronary artery risk development in young adults (CARDIA) is a multi-center longitudinal cohort study of risk factors for CHD development in young adults free of CVD (n = 5,115) aged 18–30 years at baseline (1985–1986). Participants have undergone eight examinations to date, including a baseline examination (Y0) and follow-up examinations at Y2, 5, 7, 10, 15, 20, and 25, with a 70 % examination rate at Y20 (2005–2006). A detailed description of study design, sampling, and response rates was published previously [21]. Institutional review boards at each study site reviewed the protocol and procedures and approved the research. All participants provided written informed consent. Our analyses for the present study included participants (n = 3,094) who had information on WBC count at baseline and participated in CAC measurement at Y15 or Y20.

CAC assessment

Coronary artery calcification examinations were conducted by electron beam or multi-detector computed tomography (CT) to detect the presence and extent of CAC at Y15 and Y20. Details were as described previously [22]. Briefly, calcifications located along a coronary artery that measured 4 adjacent pixels or more (approximately 2 mm²) with a computed tomographic number higher than 130 Hounsfield were counted. CAC lesions of this size have been found to be reproducible [23, 24]. To ensure comparability of the

measures between the Y15 and Y20 CT exams, the Y15 participants with any CAC and a random sample of those with zero scores were re-analyzed using the same readers and workstations as at the Y20 exam. The calcium score was calculated for each scan multiplying the area of the focus in mm² by a coefficient based on the peak computed tomography number of the focus, as described by Agatston using a commercially available and FDA approved workstation (TeraRecon Aquarius Workstation, San Mateo, CA) [25]. Individuals with CAC scores > 0 were considered CAC (+). 566 total participants had CAC scores greater than zero during the 20 years of follow-up, including 272 participants at Y15 and 294 additional participants at Y20. Participants who have information on WBC count at baseline and CAC measurement at Y20 were included for CAC-associated analysis. CAC progression between Y15 and Y20 was examined only among those with CAC score >0 at Y15, defined as either an annualized change of 10 Agatston units among participants with 0 < CAC < 100 at Y15 or an annualized percent change (annualized change in CAC score divided by the baseline CAC score) > 10% among participants with CAC ≥ 100 at Y15 [20]. Those with CAC = 0 at Y15 and CAC > 0 at Y20 were not included in the progression-related analysis.

Statistical analyses

Baseline characteristics of demographics and traditional CVD risk factors as well as inflammatory biomarkers during follow-up were compared by baseline total WBC counts using general linear models for continuous variables and cross-tabulations for categorical variables. We used multivariable linear regression to examine the cross-sectional associations of baseline WBC (total and subtype) count and known CVD risk factors including systolic blood pressure (SBP), body mass index (BMI), smoking status, total cholesterol, and HDL cholesterol. We used multivariable linear regression models to examine the relation of baseline WBC counts to circulating biomarkers of inflammation measured at Y20. To assess the prospective associations of WBC count and CAC presence at Y15 and Y20, and CAC progression between Y15 and Y20, respectively, we used multiple logistic regressions to calculate adjusted odds ratios (OR) and 95 % confidence intervals (CI) with standardized estimates provided. Because of a skewed distribution, WBC counts were natural log transformed to approximate a normal distribution when used as a continuous variable in analyses. Statistical significance was determined by two-sided $p < 0.05$. All analyses were performed using SAS 9.2 (Cary, NC).

Results

The study sample consisted of 3,094 subjects, of whom 43.2 % were men with mean (SD) age of 25.1 (3.6) at baseline. The prevalence of CAC at follow-up was 11.1 % at Y15 and 18.3 % at Y20. Participants with higher total WBC counts were more likely to be non-Hispanic white females and had higher systolic blood pressure and BMI but lower high density cholesterol levels ($p < 0.01$). They were also more likely to be smokers. Those with higher baseline total WBC had higher plasma levels of inflammatory biomarkers during the follow-up, including C-reactive protein CRP (Y7, 15, and 20), IL6 (Y20), and fibrinogen (Y5 and Y20) (Table 1).

Table 2 shows associations of baseline major CVD risk factors and WBC counts using multivariate linear regression. After adjusting for demographic covariates (i.e., age, sex, and race) and other baseline known risk factors, smoking was positively associated with total WBC and lymphocyte, neutrophil, monocyte, eosinophil, and basophil counts. A similar pattern was observed for BMI, except for eosinophil and basophil counts. SBP was associated with WBC total and neutrophil counts. Total cholesterol level was not associated with any WBC count, and HDL cholesterol level was inversely and minimally associated with total WBC count and lymphocytes.

Table 3 shows associations of baseline WBC count and follow-up inflammatory markers using multivariate linear regression. In CARDIA, data is available on Y7, Y15, and Y20 CRP; Y5 and Y20 fibrinogen; and Y20 IL6. Our analyses show that baseline total WBC count was associated with CRP at Y7, Y15, and Y20, and fibrinogen at Y5 and Y20, respectively.

Total WBC (OR = 1.23 per unit, 95 % CI 1.12–1.36), neutrophils (OR = 1.12, 95 % CI 0.99–1.26), lymphocytes (OR = 1.16, 95 % CI 1.06–1.29), and eosinophils (OR = 1.16, 95 % CI 1.05–1.28) were associated with CAC presence at Y20 when adjusted for sex, age, and race (Table 4). When further adjusted for known CVD risk factors at baseline, the association between total WBC count and Y20 CAC presence was attenuated (OR = 1.07, 95 % CI 0.96–1.19), whereas the association between eosinophil count and Y20 CAC was only minimally attenuated (OR = 1.12, 95 % CI 1.00–1.25). After adjusting for total WBC count, the association between eosinophil count and CAC presence at Y20 remained. CAC scores ≥ 100 could be false positive. Our further examination by excluding individuals with CAC scores ≥ 100 or ≥ 10 at Y20 showed that total and subtype WBC counts were significantly associated with CAC presence at Y20. Similar patterns for total WBC (OR = 1.27, 95 % CI 1.11–1.46), neutrophils (OR = 1.31, 95 % CI 1.10–1.57), lymphocytes (OR = 1.15, 95 % CI 1.01–1.31) and basophils (OR = 1.21, 95 % CI 1.00–1.46) were observed with CAC presence at Y15 after adjusting for age, sex, and race. These associations were no longer statistically significant after further adjustment for known CVD risk factors except for neutrophil count (OR = 1.18, 95 % CI 1.00–1.44). Baseline total WBC was not associated with CAC progression from Y15 to Y20 (OR = 0.84, 95 % CI 0.61–1.15), nor were any subtype counts associated with progression. There are 96 participants with WBC counts of >10 or $<2.5 \times 10^9/L$. The analysis by excluding these subjects produced similar results. We further performed the analysis stratified by sex, and the results for men and women were comparable (Supplemental Table 1). In addition, 35 study subjects developed cardiovascular disease prior to Y20 CAC examination. We also examined the association of baseline WBC and CAC presence at Y20 by excluding these subjects, and the result remained similar (Supplemental Table 2).

Discussion

Leukocyte recruitment and expression of pro-inflammatory cytokines characterize all steps of atherogenesis [26]. In the present investigation, baseline CVD risk factors, such as smoking, BMI, HDL cholesterol, or SBP, were associated with baseline WBC total and/or subtype counts. WBC counts were prospectively associated with circulating CRP and

fibrinogen. Total WBC and eosinophils were borderline or significantly positively associated with the probability of having CAC 20 years later.

Exposure to cardiovascular risk factors can cause persistent, low-grade systemic inflammation and subsequent inflammatory responses, including increased WBC count and the release of inflammatory mediators [27, 28, 29, 30, 31, 32, 33, 34]. Our finding regarding correlation of total WBC counts with risk factors is in line with previous studies. Total WBC has been shown to correlate positively with these known risk factors, including cigarette smoking, serum total cholesterol, clotting factors, fasting glucose levels, and diastolic blood pressure [10, 35, 36, 37, 38]. The circulating cytokines produced by blood leukocytes can affect the biological function of vascular endothelium as well as adipocytes and hepatocytes, to generate a spectrum of pro-atherogenic changes that include CRP and fibrinogen, insulin resistance, dyslipidemia, pro-thrombotic effects, pro-oxidative stress, and endothelial dysfunction [39]. We observed that total WBC count is positively associated with CRP at Y7, Y15, and Y20 and fibrinogen at Y5 and Y20. We also observed that lymphocyte count is associated with fasting insulin levels at Y20 (Supplemental Table 3). Further adjustment for diabetic treatment at the time that the measurements were conducted did not change the results meaningfully. Taken together, WBC and their produced inflammatory mediators may serve as the key element of the biological pathways linking cardiovascular risk factors with atherosclerosis.

Previous studies have shown that WBC count is independently associated with the risk for future CVD events in those with and without pre-existing CVD [10, 11, 12]. Blood monocytes, and other subtypes of WBC with relatively low numbers in local atherosclerotic plaque, such as neutrophils, lymphocytes, and eosinophils, have also previously been shown to be associated with atherosclerosis and CHD [13, 14, 15, 16]. Among CARDIA participants, we also found that total WBC count was associated with CAC presence 20 years later, although with some attenuation after adjusting for CVD risk factors. Attenuated associations may be due to the direct involvement of WBC in the pathways between CVD risk factors and CAC, and further suggest the important role of WBC in atherosclerosis [40]. Total WBC counts may represent the immune system in its true context, i.e., with all cellular components represented and interacting together, thus providing a realistic reflection of in vivo events.

Although monocytes are the most prevalent inflammatory cells in the atherosclerotic lesion and play a pivotal role in plaque formation [7], we did not observe a significant association between baseline monocyte count and future CAC presence. However, WBC subtypes that are not prevalent in atherosclerotic plaque, such as neutrophils, lymphocytes, and eosinophils, were associated with CAC presence at Y20 when adjusted for age, sex, and race. Neutrophils are known to be involved in atherogenesis by causing endothelial damage and interacting with endothelium to release monocyte chemo-attractant protein-1, thus promoting monocyte recruitment [41, 42, 43]. Lymphocytes appear to affect atherosclerosis primarily through cytokine secretion and immunoglobulin production.

In the present study, we found that baseline eosinophil count was significantly associated with the risk of CAC 20 year later, independent of CVD risk factors. This observation is

consistent with the results from prospective studies that have shown an association between eosinophil count and increased risk for future cardiovascular events [12, 44]. Several plausible mechanisms may explain how increased eosinophil count may promote atherosclerosis. It was demonstrated by Marone et al. [45] that when activated, eosinophils secrete several proteins, including eosinophil cationic protein (ECP), a new biomarker of coronary atherosclerosis. ECP up-regulates ICAM-1 expression in endothelial cells [46], allowing monocyte adhesion on endothelium, which is known to be a fundamental step in atherogenesis. ECP has also been shown to modulate fibroblast activity, leading to increased collagen release [47], which might have a stabilizing effect on plaque growth. One study has shown that that ECP is associated with coronary atherosclerosis and, when added to main cardiovascular risk factors, improves the classification performance for the diagnosis of angiographically detectable coronary atherosclerosis among patients undergoing coronary angiography due to chest pain [48].

Coronary heart disease patients have also been shown to have higher levels of eotaxin, a specific eosinophilic chemokine, as compared to controls [49], and high levels of eotaxin mRNA and protein were reported in human atherosclerotic plaques [50]. An association between the number of diseased coronary arteries and circulating levels of eotaxin has also been demonstrated [49]. Moreover, the enzyme arachidonate 15-lipoxygenase is expressed in significant quantities in eosinophils [51]. This enzyme has been shown to be involved in oxidative modification of LDL in the early phase of atherogenesis, thus contributing to the development of arteriosclerotic lesions. Taken together, it is biologically plausible that eosinophils have a role in determining coronary atherosclerotic burden and coronary artery calcification.

Studies have shown that exposure to cardiovascular risk factors in young adulthood, or even earlier, is associated with future presence and extent of CAC [3, 4]. In CARDIA, risk burden over 15 years in 18- to 30-year-old young adults was significantly associated with premature CAC presence at ages 33–45 years [5]. We have demonstrated, in the present study, the associations between baseline total or subtype WBC counts and cardiovascular risk factors, as well as baseline neutrophil count and CAC presence 15 years later and total WBC or eosinophil counts and CAC presence 20 years later. These pieces of data suggest the hypothesis that CRFs in young adults may induce systemic inflammation that may persist over time and influence the development of local atherosclerotic lesions.

The limitations of our study warrant discussion. First, our study included participants with other inflammatory conditions, which may limit the applicability of our observations. Second, CARDIA has data on WBC at baseline but did not measure WBC count during the 20-year follow-up, limiting our ability to understand the longitudinal association of WBC and changes in WBC and WBC subtypes with CAC. Third, we did not measure other inflammation markers, such as CRP, fibrinogen, and IL-6 at baseline, which are associated with cardiovascular disease. Lastly, a large number of statistical analyses were performed in our present study, which may lead to possible chance findings. However, our primary analysis focuses on baseline total WBC count and CAC presence 20 years later and other analyses related to subtype WBC counts are secondary for further understanding of the

specific role of subtypes. Thus, the possibility of chance findings for total WBC is minimal although it is likely that some of our results on WBC subtypes are chance findings.

In conclusion, we demonstrated that total WBC counts in young adults is associated prospectively with subclinical atherosclerosis measured by CAC 20 years later into middle age, suggesting total WBC and eosinophil counts may be a marker of early stage atherosclerosis. Our conclusion is based on the age and sex adjusted models and the results are attenuated (no longer statistically significant) when other cardiovascular risk factors are accounted for.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, et al. Heart disease and stroke statistics—2012 update: a report from the American Heart Association. *Circulation*. 2012;125(1):e2–220. doi: CIR.0b013e31823ac046. [PubMed: 22179539]
2. Lloyd-Jones DM. Short-term versus long-term risk for coronary artery disease: implications for lipid guidelines. *Curr Opin Lipidol*. 2006;17(6):619–25. doi: 10.1097/MOL.0b013e3280108740. [PubMed: 17095905]
3. Mahoney LT, Burns TL, Stanford W, Thompson BH, Witt JD, Rost CA, et al. Coronary risk factors measured in childhood and young adult life are associated with coronary artery calcification in young adults: the Muscatine Study. *J Am Coll Cardiol*. 1996;27(2):277–84. doi: 0735-1097(95)00461-0. [PubMed: 8557894]
4. Klag MJ, Ford DE, Mead LA, He J, Whelton PK, Liang KY, et al. Serum cholesterol in young men and subsequent cardiovascular disease. *N Engl J Med*. 1993;328(5):313–8. doi: 10.1056/NEJM199302043280504. [PubMed: 8419817]
5. Loria CM, Liu K, Lewis CE, Hulley SB, Sidney S, Schreiner PJ, et al. Early adult risk factor levels and subsequent coronary artery calcification: the CARDIA Study. *J Am Coll Cardiol*. 2007;49(20):2013–20. doi: S0735-1097(07)00832-7. [PubMed: 17512357]
6. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105(9):1135–43. [PubMed: 11877368]
7. Ross R Atherosclerosis—an inflammatory disease. *N Engl J Med*. 1999;340(2):115–26. doi: 10.1056/NEJM199901143400207. [PubMed: 9887164]
8. Prentice RL, Szatrowski TP, Fujikura T, Kato H, Mason MW, Hamilton HH. Leukocyte counts and coronary heart disease in a Japanese cohort. *Am J Epidemiol*. 1982;116(3):496–509. [PubMed: 7124717]
9. Sen S, Hinderliter A, Sen PK, Simmons J, LeGrys VA, Beck J, et al. Association of leukocyte count with progression of aortic atheroma in stroke/transient ischemic attack patients. *Stroke*. 2007;38(11):2900–5. doi: STROKEAHA.107.489658. [PubMed: 17901385]
10. Madjid M, Awan I, Willerson JT, Casscells SW. Leukocyte count and coronary heart disease: implications for risk assessment. *J Am Coll Cardiol*. 2004;44(10):1945–56. doi: S0735-1097(04)01707-3. [PubMed: 15542275]

11. Giugliano G, Brevetti G, Lanero S, Schiano V, Laurenzano E, Chiariello M. Leukocyte count in peripheral arterial disease: a simple, reliable, inexpensive approach to cardiovascular risk prediction. *Atherosclerosis*. 2010;210(1):288–93. [PubMed: 19963213]
12. Margolis KL, Manson JE, Greenland P, Rodabough RJ, Bray PF, Safford M, et al. Leukocyte count as a predictor of cardiovascular events and mortality in postmenopausal women: the Women's Health Initiative Observational Study. *Arch Intern Med*. 2005;165(5):500–8. doi: 165/5/500. [PubMed: 15767524]
13. Sweetnam PM, Thomas HF, Yarnell JW, Baker IA, Elwood PC. Total and differential leukocyte counts as predictors of ischemic heart disease: the Caerphilly and Speedwell studies. *Am J Epidemiol*. 1997;145(5):416–21. [PubMed: 9048515]
14. Huang ZS, Chien KL, Yang CY, Wang CH, Chang TC, Chen CJ. Peripheral differential leukocyte counts and subsequent mortality from all diseases, cancers, and cardiovascular diseases in Taiwanese. *J Formos Med Assoc*. 2003;102(11):775–81. [PubMed: 14724723]
15. Kawaguchi H, Mori T, Kawano T, Kono S, Sasaki J, Arakawa K. Band neutrophil count and the presence and severity of coronary atherosclerosis. *Am Heart J*. 1996;132(1 Pt 1):9–12. [PubMed: 8701881]
16. Olivares R, Ducimetiere P, Claude JR. Monocyte count: a risk factor for coronary heart disease? *Am J Epidemiol*. 1993;137(1):49–53. [PubMed: 8434572]
17. O'Malley PG, Taylor AJ, Jackson JL, Doherty TM, Detrano RC. Prognostic value of coronary electron-beam computed tomography for coronary heart disease events in asymptomatic populations. *Am J Cardiol*. 2000;85(8):945–8. doi: S0002-9149(99)00906-6. [PubMed: 10760331]
18. Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA, et al. Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. *J Am Coll Cardiol*. 1998;31(1):126–33. doi: S0735-1097(97)00443-9. [PubMed: 9426030]
19. Arad Y, Spadaro LA, Goodman K, Lledo-Perez A, Sherman S, Lerner G, et al. Predictive value of electron beam computed tomography of the coronary arteries. 19-month follow-up of 1,173 asymptomatic subjects. *Circulation*. 1996;93(11):1951–3. [PubMed: 8640967]
20. Berry JD, Liu K, Folsom AR, Lewis CE, Carr JJ, Polak JF, et al. Prevalence and progression of subclinical atherosclerosis in younger adults with low short-term but high lifetime estimated risk for cardiovascular disease: the coronary artery risk development in young adults study and multi-ethnic study of atherosclerosis. *Circulation*. 2009;119(3):382–9. doi: CIRCULATIONAHA.108.800235. [PubMed: 19139385]
21. Friedman GD, Cutter GR, Donahue RP, Hughes GH, Hulley SB, Jacobs DR Jr, et al. CARDIA: study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol*. 1988;41(11):1105–16. doi: 0895-4356(88)90080-7. [PubMed: 3204420]
22. Iribarren C, Sidney S, Bild DE, Liu K, Markovitz JH, Roseman JM, et al. Association of hostility with coronary artery calcification in young adults: the CARDIA study. *Coronary Artery Risk Development in Young Adults*. *JAMA*. 2000;283(19):2546–51. [PubMed: 10815118]
23. Kaufmann RB, Sheedy PF 2nd, Breen JF, Kelzenberg JR, Kruger BL, Schwartz RS, et al. Detection of heart calcification with electron beam CT: interobserver and intraobserver reliability for scoring quantification. *Radiology*. 1994;190(2):347–52. [PubMed: 8284380]
24. Bielak LF, Kaufmann RB, Moll PP, McCollough CH, Schwartz RS, Sheedy PF 2nd. Small lesions in the heart identified at electron beam CT: calcification or noise? *Radiology*. 1994;192(3):631–6. [PubMed: 8058926]
25. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15(4):827–32. doi: 0735-1097(90)90282-T. [PubMed: 2407762]
26. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation*. 2005;111(25):3481–8. doi: 111/25/3481. [PubMed: 15983262]
27. Asthana A, Johnson HM, Piper ME, Fiore MC, Baker TB, Stein JH. Effects of smoking intensity and cessation on inflammatory markers in a large cohort of active smokers. *Am Heart J*. 2010;160(3):458–63. [PubMed: 20826253]

28. Yasue H, Hirai N, Mizuno Y, Harada E, Itoh T, Yoshimura M, et al. Low-grade inflammation, thrombogenicity, and atherogenic lipid profile in cigarette smokers. *Circ J*. 2006;70(1):8–13. [PubMed: 16377917]
29. Laurson KR, McCann DA, Senchina DS. Age, sex, and ethnicity may modify the influence of obesity on inflammation. *J Investig Med*. 2011;59(1):27–31. doi: 10.231/JIM.0b013e318200151a.
30. Ohshita K, Yamane K, Hanafusa M, Mori H, Mito K, Okubo M, et al. Elevated white blood cell count in subjects with impaired glucose tolerance. *Diabetes Care*. 2004;27(2):491–6. [PubMed: 14747234]
31. Alexander RW. Theodore cooper memorial lecture. Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of arterial inflammatory response: a new perspective. *Hypertension*. 1995;25(2):155–61. [PubMed: 7843763]
32. Ritchie SA, Connell JM. The link between abdominal obesity, metabolic syndrome and cardiovascular disease. *Nutr Metab Cardiovasc Dis*. 2007;17(4):319–26. doi: S0939-4753(06)00150-5. [PubMed: 17110092]
33. Pauletto P, Rattazzi M. Inflammation and hypertension: the search for a link. *Nephrol Dial Transplant*. 2006;21(4):850–3. doi: gfl019. [PubMed: 16464884]
34. Libby P Inflammation in atherosclerosis. *Nature*. 2002;420(6917):868–74. doi: 10.1038/nature01323. [PubMed: 12490960]
35. Bovill EG, Bild DE, Heiss G, Kuller LH, Lee MH, Rock R, et al. White blood cell counts in persons aged 65 years or more from the Cardiovascular Health Study. Correlations with baseline clinical and demographic characteristics. *Am J Epidemiol*. 1996;143(11):1107–15. [PubMed: 8633599]
36. Ogawa Y, Imaki M, Yoshida Y, Shibakawa M, Tanada S. An epidemiological study on the association between the total leukocyte and neutrophil counts, and risk factors of ischemic heart disease by smoking status in Japanese factory workers. *Appl Human Sci*. 1998;17(6):239–47.
37. Ford ES, Galuska DA, Gillespie C, Will JC, Giles WH, Dietz WH. C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988–1994. *J Pediatr*. 2001;138(4):486–92. doi: S0022-3476(01)30378-5. [PubMed: 11295710]
38. Capuano V, Lamaida N, De Martino M, Mazzotta G. Association between white blood cell count and risk factors of coronary artery disease. *G Ital Cardiol*. 1995;25(9):1145–52. [PubMed: 8529851]
39. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how “high-grade” systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation*. 2003;108(24):2957–63. doi: 10.1161/01.CIR.0000099844.31524.05108/24/2957. [PubMed: 14676136]
40. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107(3):499–511. [PubMed: 12551878]
41. Schratzberger P, Dunzendorfer S, Reinisch N, Kahler CM, Herold M, Wiedermann CJ. Release of chemoattractants for human monocytes from endothelial cells by interaction with neutrophils. *Cardiovasc Res*. 1998;38(2):516–21. doi: S0008-6363(98)00014-5. [PubMed: 9709414]
42. Blann AD, Seigneur M, Adams RA, McCollum CN. Neutrophil elastase, von Willebrand factor, soluble thrombomodulin and percutaneous oxygen in peripheral atherosclerosis. *Eur J Vasc Endovasc Surg*. 1996;12(2):218–22. [PubMed: 8760986]
43. Mohacsi A, Fulop T Jr, Kozlovsky B, Hauck M, Kiss I, Leovey A. Sera and leukocyte elastase-type protease and antiprotease activity in healthy and atherosclerotic subjects of various ages. *J Gerontol*. 1992;47(5):B154–8. [PubMed: 1380968]
44. Lee CD, Folsom AR, Nieto FJ, Chambless LE, Shahar E, Wolfe DA. White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and White men and women: atherosclerosis risk in communities study. *Am J Epidemiol*. 2001;154(8):758–64. [PubMed: 11590089]
45. Marone G Asthma: recent advances. *Immunol Today*. 1998;19(1):5–9. doi: S0167569997011870. [PubMed: 9465481]

46. Chihara J, Yamamoto T, Kurachi D, Kakazu T, Higashimoto I, Nakajima S. Possible release of eosinophil granule proteins in response to signaling from intercellular adhesion molecule-1 and its ligands. *Int Arch Allergy Immunol.* 1995;108(Suppl 1):52–4. [PubMed: 7549525]
47. Hernnas J, Sarnstrand B, Lindroth P, Peterson CG, Venge P, Malmstrom A. Eosinophil cationic protein alters proteoglycan metabolism in human lung fibroblast cultures. *Eur J Cell Biol.* 1992;59(2):352–63. [PubMed: 1493801]
48. Niccoli G, Ferrante G, Cosentino N, Conte M, Belloni F, Marino M, et al. Eosinophil cationic protein: a new biomarker of coronary atherosclerosis. *Atherosclerosis.* 2010;211(2):606–11. [PubMed: 20307883]
49. Emanuele E, Falcone C, D'Angelo A, Minoretti P, Buzzi MP, Bertona M, et al. Association of plasma eotaxin levels with the presence and extent of angiographic coronary artery disease. *Atherosclerosis.* 2006;186(1):140–5. [PubMed: 16084515]
50. Haley KJ, Lilly CM, Yang JH, Feng Y, Kennedy SP, Turi TG, et al. Overexpression of eotaxin and the CCR3 receptor in human atherosclerosis: using genomic technology to identify a potential novel pathway of vascular inflammation. *Circulation.* 2000;102(18):2185–9. [PubMed: 11056090]
51. Sigal E, Sloane DL, Conrad DJ. Human 15-lipoxygenase: induction by interleukin-4 and insights into positional specificity. *J Lipid Mediat.* 1993;6(1–3):75–88. [PubMed: 8358018]

Table 1

Baseline characteristics of demographics and traditional CVD risk factors, and inflammatory biomarkers during follow-up by baseline total WBC counts

Baseline characteristic	Tertiles of total WBC counts, 10 ⁹ /L			<i>p</i> value *
	<5.1	5.1–6.5	>6.5	
Age, years	25.2 ± 3.6	25.2 ± 3.6	25.1 ± 3.6	0.55
Race, Black (%)	51.5	41.2	41.4	<0.01
Sex, Male (%)	48.8	44.6	35.8	<0.01
Education, years	14.1 ± 2.27	14.1 ± 2.2	14.1 ± 2.6	0.61
Systolic BP (mmHg)	109 ± 10	110 ± 11	110 ± 11	0.05
Diastolic BP (mmHg)	68 ± 9	69 ± 9	68 ± 10	0.25
BMI (kg/m ²)	23.6 ± 3.9	24.2 ± 4.4	24.9 ± 5.2	<0.01
Total Cholesterol (mg/dL)	177.7 ± 33.3	176.4 ± 32.0	178.4 ± 33.3	0.38
HDL Cholesterol (mg/dL)	54.4 ± 12.9	53.6 ± 13.1	52.3 ± 12.5	<0.01
Smoking (%)	16.4	23.7	38.0	<0.01
Anti-HTN Tx (%)	1.87	2.27	2.38	0.70
Diabetes (%)	0.56	0.39	0.99	0.21
CRP at Y7, ug/ml	2.07 ± 3.91	2.56 ± 4.07	3.36 ± 4.07	<0.01
CRP at Y15, ug/ml	2.65 ± 5.16	2.84 ± 3.81	3.90 ± 5.90	<0.01
CRP at Y20, ug/ml	2.31 ± 4.62	2.58 ± 4.07	3.31 ± 5.54	<0.01
Fibrinogen at Y5, mg/dl	251.6 ± 52.7	262.5 ± 55.5	271.6 ± 61.0	<0.01
Fibrinogen at Y20, mg/dl	393.4 ± 84.3	404.3 ± 90.7	415.4 ± 95.6	<0.01
IL-6 at Y20, pg/ml	2.48 ± 3.10	2.50 ± 2.90	2.77 ± 2.90	0.05

* *p* value for the difference among WBC tertile groups

Table 2

Associations^a of baseline CVD risk factors and WBC total and subtype count^b

Risk Factors ^c	Total WBC		Lymphocytes		Neutrophils		Monocytes		Eosinophils		Basophils	
	β (SE)	p value	β (SE)	p value	β (SE)	p value	β (SE)	p value	β (SE)	p value	β (SE)	p value
SBP, mmHg	0.03 (0.01)	<0.01	0.004 (0.006)	0.50	0.05 (0.01)	<0.01	0.02 (0.01)	0.06	0.01 (0.01)	0.62	0.04 (0.01)	<0.01
Smoking status	0.16 (0.01)	<0.01	0.10 (0.01)	<0.01	0.17 (0.02)	<0.01	0.15 (0.03)	<0.01	0.16 (0.03)	<0.01	0.13 (0.02)	<0.01
BMI, kg/m ²	0.03 (0.005)	<0.01	0.03 (0.006)	<0.01	0.02 (0.01)	0.02	0.05 (0.01)	<0.01	0.02 (0.01)	0.16	0.01 (0.01)	0.25
Total cholesterol, mg/dL	-0.003 (0.005)	0.50	0.006 (0.006)	0.28	-0.01 (0.01)	0.45	-0.004 (0.01)	0.72	0.004 (0.01)	0.77	-0.003 (0.01)	0.78
HDL cholesterol, mg/dL	-0.01 (0.005)	0.01	-0.03 (0.006)	<0.01	-0.02 (0.01)	0.10	-0.01 (0.01)	0.51	-0.01 (0.01)	0.43	-0.01 (0.01)	0.19

^aRisk factors were fitted simultaneously in addition to age, race, and sex adjustments

^bAll cell count variables are on the logarithmic (log) scale; standardized parameter estimation per SD unit

^cDependent variables: total WBC and subtype count

Table 3

Associations^a of baseline WBC counts and inflammatory biomarkers during 20-year follow-up^b

Inflammatory Markers ^c	Total WBC	Neutrophils	Monocytes	Lymphocytes	Eosinophils	Basophils
	β (SE) p value	β (SE) p value	β (SE) p value	β (SE) p value	β (SE) p value	β (SE) p value
CRP at Y7, ug/ml	0.46 (0.09) <0.01	0.36 (0.10) <0.01	0.12 (0.09) 0.19	0.30 (0.09) <0.01	0.09 (0.09) 0.33	0.09 (0.13) 0.48
CRP at Y15, ug/ml	0.31 (0.10) <0.01	0.21 (0.11) 0.05	0.07 (0.10) 0.42	0.28 (0.09) <0.01	-0.14 (0.10) 0.16	-0.04 (0.13) 0.78
CRP at Y20, ug/ml	0.24 (0.08) <0.01	0.25 (0.10) 0.01	0.10 (0.08) 0.25	0.08 (0.08) 0.31	0.07 (0.09) 0.42	-0.03 (0.14) 0.86
Fibrinogen at Y5, mg/dl	4.46 (1.00) <0.01	3.68 (1.17) <0.01	2.70 (0.97) <0.01	1.74 (0.97) 0.07	-0.19 (1.04) 0.85	0.90 (1.37) 0.51
Fibrinogen at Y20, mg/dl	4.71 (1.60) <0.01	3.52 (1.92) 0.07	2.97 (1.56) 0.06	2.08 (1.57) 0.18	2.34 (1.65) 0.15	0.16 (2.32) 0.94
IL-6 at Y20, pg/ml	0.03 (0.06) 0.58	0.05 (0.06) 0.43	-0.04 (0.05) 0.46	0.08 (0.06) 0.12	-0.03 (0.06) 0.64	-0.02 (0.08) 0.81

^a Adjusted for age, sex, race, SBP, BMI, total cholesterol, HDL, and smoking status

^b All cell variables are in standard deviation units on the logarithmic scale

^c Dependent variables: inflammatory biomarkers

Table 4

Associations of WBC counts at baseline and CAC presence and progression during 20-year follow-up

	Model 1	Model 2	Model 3
<i>CAC > 0 at Y15 (272/2456)</i>			
WBC	1.27 (1.11–1.46)	1.09 (0.94–1.27)	–
Monocyte	1.11 (0.97–1.27)	1.01 (0.88–1.15)	0.97 (0.83–1.13)
Neutrophil	1.31 (1.10–1.57)	1.18 (1.00–1.44)	1.07 (0.73–1.57)
Lymphocyte	1.15 (1.01–1.31)	1.03 (0.89–1.18)	0.99 (0.85–1.15)
Eosinophil	1.05 (0.91–1.20)	1.01 (0.87–1.16)	0.98 (0.84–1.14)
Basophil	1.21 (1.00–1.46)	1.10 (0.89–1.35)	0.96 (0.71–1.29)
<i>CAC > 0 at Y20 (566/3094)</i>			
WBC	1.23 (1.12–1.36)	1.07 (0.96–1.19)	–
Monocyte	1.01 (0.92–1.12)	0.94 (0.85–1.04)	0.90 (0.80–1.01)
Neutrophil	1.12 (0.99–1.26)	1.01 (0.89–1.16)	0.88 (0.68–1.12)
Lymphocyte	1.16 (1.06–1.29)	1.03 (0.93–1.14)	1.00 (0.89–1.12)
Eosinophil	1.16 (1.05–1.28)	1.12 (1.00–1.25)	1.11 (0.99–1.24)
Basophil	1.10 (0.96–1.27)	1.02 (0.88–1.19)	0.90 (0.73–1.11)
<i>CAC progression (102/272)[†]</i>			
WBC	1.00 (0.77–1.31)	0.84 (0.61–1.15)	–
Monocyte	1.01 (0.79–1.29)	0.91 (0.70–1.19)	0.97 (0.72–1.30)
Neutrophil	1.20 (0.85–1.70)	1.07 (0.72–1.59)	1.08 (0.49–2.39)
Lymphocyte	1.05 (0.81–1.37)	0.95 (0.71–1.27)	1.04 (0.74–1.46)
Eosinophil	0.98 (0.75–1.27)	0.99 (0.75–1.30)	1.03 (0.76–1.37)
Basophil	0.76 (0.47–1.22)	0.70 (0.41–1.22)	0.64 (0.30–1.35)

All cell variables are in standard deviation units on the logarithmic scale

Model 1: adjusted for age, sex, and race

Model 2: Model 1 plus education, SBP, BMI, total cholesterol, HDL, smoking status, and blood-pressure-lowering medication at Y0

Model 3: Model 2 plus total WBC count

[†]CAC progression was defined as either an annualized change of 10 Agatston units among participants with 0 < CAC < 100 at Y15 or an annualized percent change ≥ 10 % among participants with CAC ≥ 100 at Y15