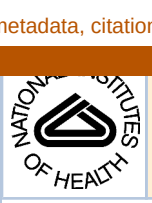

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Risk factor correlates of platelet and leukocyte markers assessed by flow cytometry in a population-based sample

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Abstract

Background—Platelet and leukocyte products are involved in atherothrombosis. However, the determinants of of platelet and leukocyte markers assessed by flow cytometry have not been documented in a population-based sample.

Methods and results—We performed flow cytometry on blood from participants (*n*=1,894) in the Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study. Cellular aggregates and multiple platelet and leukocyte markers, such as myeloperoxidase in granulocytes and toll-like receptor-4, CD14, and CD45 in monocytes, were quantified. Their cross-sectional associations with demographic and risk factors were assessed using multiple linear regression. Mean values of most cellular markers and aggregates were considerably higher in blacks than whites ($p<0.01$). There were some differences in cellular markers between men and women, but little association with age. LDL-cholesterol was associated positively with several markers (toll-like receptor-4 and myeloperoxidase in granulocytes and CD162 in lymphocytes). Lipid lowering therapy tended to show opposite associations. Smokers had much higher granulocyte myeloperoxidase than nonsmokers. However, most other correlations between risk factors and cellular markers were nonsignificant.

Conclusions—Race/ethnicity, sex, and to a lesser degree LDL-cholesterol and lipid-lowering therapy, but few other risk factors, were correlated with markers of cellular activation in this population-based study.

Keywords

Blood platelets; Flow cytometry; Leukocytes; Monocytes; Risk factors

1. Introduction

Activated platelets and leukocytes play important roles in various physiological and pathological conditions. Platelets play a major role in the hemostatic process, and increased platelet activation and aggregation are central to the pathophysiology of arterial thrombosis.

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In addition, recent studies have provided insight into platelet functions in inflammation and atherosclerosis [1,2]. Several platelet derived factors, both membrane bound and soluble, may be involved in the inflammatory interaction between platelets, leukocytes, and endothelial cells [3]. Monocyte-derived macrophages play an important role in the development of macrovascular disease and atheromatous lesions. Increased numbers of circulating proinflammatory monocytes, circulating activated platelets, and/or circulating leukocyte-platelet aggregates have been observed in patients with inflammatory and infectious diseases, sepsis, acquired immune deficiency syndrome, diabetes mellitus, acute ischemic cardiovascular syndromes, pre-eclampsia, renal disease, malignant and myeloproliferative disorders, and autoimmune, allergic, and neurodegenerative diseases [4-10]. Cell-specific (cellular) markers help identify components of the pathological process and play an important role in the diagnosis, prognostic assessment, and management of patients with suspected syndromes.

Flow cytometry, an established fluorescence-based technology used to quantify antigenically-defined cell populations, can accurately assess markers of platelet and leukocyte activation and cellular aggregation in whole blood. It characterizes cells by the identification of cell surface or intracellular antigens, utilizing antibody reagents that recognize specific cell-associated molecules. Because flow cytometry is expensive and labor intensive, and requires highly skilled personnel, few large-scale studies have employed this technique. Little information is therefore available on the distribution and determinants of these cellular activation markers and aggregates in the general population. We used data from a large epidemiologic study to characterize these associations in a cross-sectional sample of adults.

2. Methods

The Atherosclerosis Risk in Communities (ARIC) Study recruited a cohort of 15,792, blacks and whites, men and women, in 1987-89 from four U.S. communities: Forsyth County, NC; Jackson, MS; suburban Minneapolis, MN; and Washington County, MD [11]. Participants underwent up to four examinations including carotid ultrasound between 1987 and 1998. In 2005-2006 we re-examined a stratified sample of 2,066 surviving participants as part of an ARIC Carotid MRI study [12]. The sampling goal was to recruit (a) 1200 participants with high values of maximum carotid intima-media thickness (IMT) (maximum over six sites: left and right, common, bifurcation, internal) at their last ARIC ultrasound examination in the 1990s, and (b) 800 individuals randomly sampled from the remainder of the carotid IMT distribution. Field-center-specific cutpoints of carotid IMT were used to approximately achieve this goal, with 100% sampling above the cutpoint, and a sampling fraction below the cutpoint to achieve the desired n=800. The IMT cutpoints were 1.14, 1.00, 1.28, and 1.22 mm at Forsyth County, Jackson, Minneapolis suburbs, and Washington County, respectively, representing the 73^{rd}, 69^{th}, 73^{rd}, and 68^{th} percentiles of maximal IMT from Exam 4. Participants were considered ineligible for the MRI exam if they had implanted metallic devices; carotid revascularization on either side for the low IMT group or on the side selected for imaging for the high IMT group; weight greater than 320 pounds; or difficulties in understanding questions or in completing the informed consent. This analysis is based on the Carotid MRI cross-sectional data, whose protocol was approved by the institutional review boards of each participating center.

After informed consent, a fasting whole blood sample was obtained into Cyto-Chex® BCT vacutainer tubes (Streck, Omaha, NE) by a standardized protocol [13]. These tubes contain a combination of EDTA and a blood cell membrane stabilizer. The BCT tubes were inverted 8 times, stored briefly at room temperature, and shipped overnight in temperature-stabilizing packages to the flow cytometry laboratory. The containers had sufficient insulating capacity to maintain the transportation temperatures of the specimens. Immediately upon receipt, the

laboratory prepared and analyzed samples within 24 hours of blood drawing using a Coulter® Epics™XL™ flow cytometer (Beckman Coulter, Inc., Miami, FL). Before initiation of the Carotid MRI study, we reviewed and adopted recommendations addressing flow cytometry analysis in multicenter studies [14-18]. In addition, we carried out multiple protocol-development studies and validated the stability of the flow cytometry measurements when the samples were shipped and received within 24 hours. Specifically, we tested blood cell preservation and fixation, and levels of markers at different time points. The time points included 0h, 24h, 48h and 72h after blood collection within single center. Although it was impossible to mimic shipping conditions exactly, we conducted a "simulation" of blood shipping by keeping specimens in a Styrofoam container with refrigerant packs and lightly shaking them every few hours. In addition, we conducted a repeatability study using 14 volunteers from two field centers. These samples were sent to the laboratory within 24h of blood draw, run the same day (within 24h), and at 48h and 72h, to assess repeatability over time. The within-run repeatability was good and within the bounds anticipated based on similar assays, but the average values did change somewhat after 24h. Our pilot study results showed that although some of the markers were stable at 48h, the best preservation of cell attributes was achieved when cells were analyzed within 24h of blood collection. In general, the intracellular markers were stable at 48h, but heterotypic cellular aggregates were significantly elevated when samples were analyzed at later time points.

The detailed protocols, antibodies, and the reproducibility of the methods are described in detail elsewhere [13]. The variables of interest included the following markers of potential relevance to atherosclerosis: platelet glycoprotein IIb (GPIIb, CD41); platelet glycoprotein IIIa (GPIIIa, CD61); platelet P-selectin (CD62P); platelet CD40 ligand (CD154); myeloperoxidase (MPO) in monocytes or granulocytes; monocyte toll-like receptors (TLR) 2 and 4; monocyte CD14 (lipopolysaccharide (LPS) receptor); P-selectin glycoprotein ligand-1 (PSGL-1, CD162) in monocytes, granulocytes and lymphocytes; pan-leukocyte marker (CD-45); and platelet-leukocyte aggregates. Variables are expressed as percent of positive cells and/or median fluorescence intensity (MFI). Results are classically given as both the percentage of positive cells and fluorescence intensity. Antigen-negative controls are used to set the threshold between positive and negative cell populations. For each positive population, the percentage of positive cells (% positive) and the median fluorescence intensity (MFI) values were determined. Fluorescent intensity represents a measurement of the amount of fluorochrome bound to a cell, and under appropriate conditions, it can be related to the number of binding sites a cell has for a particular fluorochrome-conjugated reagent. For most analytes, the laboratory CV was <10% (mean 3.6%, range 0%-14.5%) and split specimen reliability was excellent (75% of analytes had $R > 0.90$). Reliability coefficients based on replicate blood samples taken 4-8 weeks apart in 55 people indicated substantial to high repeatability ($R > 0.60$) for CD14, TLR-2, CD162, CD61, CD41, CD62P, CD154, and platelet-leukocyte aggregates. In contrast, TLR-4, CD45, and MPO had slight to moderate repeat visit reliability [13].

At the Carotid MRI visit, ARIC staff measured blood pressure three times in the right arm of rested, seated participants using a random-zero sphygmomanometer. The mean of the last two was used as the measure of blood pressure. A central laboratory measured plasma total and HDL cholesterol and triglycerides by enzymatic methods, and LDL-cholesterol was calculated. High-sensitivity C-reactive protein (hs-CRP) was measured using a latex particle enhanced immunoturbidimetric assay (Equal Diagnostics, Exton, PA) on an Olympus AU400e automated chemistry analyzer. This method has been validated to the Dade Behring method [19]. Diabetes was defined as a fasting glucose ≥126 mg/dL, nonfasting glucose ≥200 mg/dL, a self-reported physician diagnosis of diabetes, or current use of hypoglycemic agents. Participants provided all of their medications for transcription, including statins, antihypertensives, and anti-platelet drugs. Physical activity at sports and leisure was assessed

by the Baecke questionnaire [20]. Current ethanol use was estimated from the frequency of drinking of various alcoholic beverages. Body mass index (BMI) and waist (umbilical)/hip (maximum) were obtained. Race/ethnicity was self-reported.

A total of 4,306 ARIC cohort members were contacted for study, of whom 1,403 refused participation, 837 were ineligible to undergo MRI for medical reasons, and 2,066 were examined. Reasons given by the 1,403 refusers were: fearful of MRI (26%), not interested (36%), too far to travel (12%), too busy (14%), or other (12%). Risk factor levels at ARIC Visit 4 (1996-1998) tended to be lower in ARIC MRI participants than nonparticipants (Table 1). From the sample of 2,066 participants, we excluded those taking chemotherapy or steroids (n = 73 total), or totally missing flow cytometry data (n=99), leaving 1,894 for analysis. Individual cell marker variables were missing on 15 to 91 of the 1,894 subjects (Table 2).

The analysis was cross-sectional, with cellular markers and risk factors measured at the same visit. Mean flow cytometry variables were compared by age (60-69, 70-74, and 75+), race (white, black), and sex groups using linear regression. Linear regression was used to calculate age-, race-, and sex-adjusted differences in flow cytometry variables per interquartile difference for continuous cardiovascular risk factors, and yes versus no for dichotomous risk factors. Because of the large number of associations examined, a p-value of <0.01 was used to indicate statistical significance. All analyses were based on methods appropriate for stratified random sample methods, with weighting by the inverse of the sampling fractions in the eight sampling strata (4 field centers by 2 IMT groups). The sampling fractions were based on those persons actually screened for participation. Those who actually participated (non-refusing eligibles) were analyzed as a sub-population, or domain [21], in calculating variances and confidence intervals of estimators. Analysis used SAS version 9.1 for descriptive statistics, but since domain analysis is not yet available in SAS, SUDAAN regression was used for modeling. Finite population correction factors were not applied. Tests of difference in weighted means between groups were derived from a weighted linear regression model that accounted for the sampling design.

3. Results

Participant characteristics are described in Table 1. Since ARIC Visit 4, the sample now had aged 9 years, were more likely to be taking antihypertensive and antilipidemic medications, and were more likely to have diabetes.

Mean values of the cell marker variables varied considerably by race/ethnicity (Table 2). In fact, the only markers that did not (p>0.01) were platelet CD40 ligand, and platelet-granulocyte complexes. Blacks had higher values than whites for most markers measured on granulocytes, lymphocytes, and platelets. Blacks also had more cell aggregates. In contrast, monocyte markers were sometimes higher in whites and sometimes higher in blacks. The size of the race/ethnic differences in leukocyte markers was large – (S.D.). For example, the MPO difference of 96 units is more than half of the approximate S.D. of 160 (Table 2). Adjustment for education level had no impact on these race/ethnic associations, because education was not, itself, related to cellular marker levels.

Markers varied little across age groups of 60-69, 70-74, and 75+ (data not shown), but varied to some degree by sex (Table 3). Some markers were higher in men, others in women, and the magnitudes of significant differences were typically 0.3 to 0.5 of an S.D. The race and sex differences shown in Tables 2 and 3 virtually all persisted in a regression model that simultaneously included race, sex, and age (continuous). Similarly, adjustment for other cardiovascular risk factors had little impact on the results in Tables 2 or 3.

We next examined the age, race, and sex-adjusted relation of each cell marker with the following risk factors: LDL-cholesterol, HDL-cholesterol, triglycerides, CRP, systolic blood pressure, ethanol intake, sports activity score, BMI, waist/hip, diabetes status, smoking status, and use of cholesterol-lowering medication, antihypertensive medication, aspirin, or other antiplatelet agents. Two of the more striking associations with cell markers were for LDL-cholesterol and cholesterol-lowering medications (Table 4). Per 46 mg/dL increment of LDL-cholesterol (the interquartile range), there was a 0.12 unit greater monocyte TRL-4 value a 20.2 unit higher granulocyte MPO value; and a 0.93 unit higher lymphocyte CD162 value. In contrast, users of lipid lowering medications had 0.2 unit lower monocyte TLR-4 and 28.6 unit lower granulocyte MPO and 2.67 unit higher platelet GPIIIa level. These differences tended to be in the range of 0.1 to 0.2 of an S.D.

The other risk factors, adjusted for age, race, and sex, were associated with cell markers ($p<0.01$) in the following ways: HDL cholesterol negatively with monocyte CD45 and positively with granulocyte MPO; triglycerides positively with monocyte CD14 and CD45 and negatively with granulocyte CD162; CRP negatively with granulocyte MPO, and platelet P-selectin; ethanol positively with granulocyte MPO; sport activity positively with monocyte TLR-4 and negatively with monocyte PSGL-1; BMI positively with monocyte PSGL-1; waist/hip ratio positively with monocyte CD14; diabetes negatively with monocyte TLR-4 and positively with monocyte CD45; smoking positively with monocyte CD14 and negatively with monocyte and granulocyte MPO; antihypertensive medication use negatively with monocyte CD14 and lymphocyte CD162 and positively with monocyte CD45; aspirin use positively with platelet GPIIb and platelet-lymphocyte aggregates; and use of other antiplatelet agents negatively with platelet CD40L. Of these, statistical evidence was strongest ($p<0.001$) for smokers having 75 units (approximately 0.5 S.D.) lower age, race, and sex-adjusted granulocyte MPO than nonsmokers; a 6.7 kg/m^2 greater BMI being associated with a 2.7 (approximately 0.2 S.D.) unit higher monocyte PSGL-1; a 3.2 mg/L higher CRP value being associated with a 0.26 unit (approximately 0.05 S.D.) lower P-selectin value; and a 90 mg/dL higher triglyceride level being associated with a 1.2 unit (approximately 0.1 S.D.) higher CD45 value.

Considering the results in different way, most cell markers had zero to three risk factor correlates beyond age, race/ethnicity, and sex at $p<0.01$. Those with four or more risk factor correlates were granulocyte MPO (correlated positively with LDL cholesterol, HDL cholesterol, and ethanol intake, and negatively with CRP, smoking, and cholesterol-lowering medication); monocyte TRL-4 (correlated positively with LDL cholesterol and sports activity, and negatively with diabetes and cholesterol-lowering medication); monocyte CD14 (correlated positively with triglycerides, waist/hip, and smoking and negatively with antihypertensive medication); and monocyte CD45 (correlated positively with triglycerides, diabetes, and antihypertensive medications, and negatively with HDL cholesterol). The amount of variance in these four cell markers explained by all risk factors (r-square value) was low, ranging from 8-12%, and explained mostly by race/ethnicity and sex.

4. Discussion

This large cross-sectional study found differences between blacks and whites in platelet and leukocyte markers assessed by flow cytometry. We also observed relations of some markers with sex and cardiovascular risk factors. In particular, LDL-cholesterol and lipid-lowering therapy had opposite associations with several leukocyte markers. Although there is no epidemiologic evidence that the cellular markers studied predict future risk of cardiovascular disease, there is much basic science support for their role in vascular disease.

Generally, compared with whites, blacks had higher levels of markers on granulocytes, lymphocytes, and platelets. The reasons for these strong racial/ethnic differences are unknown. Racial/ethnic differences in health tend to be more related to sociocultural factors than to biologic or genetic factors [22,23]. However, adjustment for education level did not change the findings. Thus, given that most other risk factors were only weakly related to cellular markers, a race-based genetic contribution to these cellular markers seems possible and should be explored. It might be speculated that these findings are consistent with the higher levels of plasma inflammatory markers and higher cardiovascular disease risk in blacks than whites [24,25]. On the other hand, most African Americans were in one ARIC center (Jackson), while all whites were in the other three ARIC centers. Thus, the putative racial/ethnic differences observed here may be partly due to geographic differences in sample collection or other unidentified factors.

Platelet P-selectin was negatively associated with CRP. CRP has various biological functions; its effect on platelets has been reported to be both inhibitory and stimulatory. A recent report showed that CRP promotes formation of platelet-monocyte aggregates in a P-selectin dependent manner [26]. Our results indicate that high CRP was associated with low P-selectin, presumably because P-selectin was engaged in platelet-leukocyte formation.

The principal sources of blood MPO are neutrophils and monocytes. MPO has proinflammatory and proatherogenic roles in vascular disease. It contributes to formation of dysfunctional HDL and oxidized LDL. Our MPO results can be compared with data of the EPIC-Norfolk study [27], although they measured serum levels of MPO, not intracellular. They found a positive association of serum MPO with CRP and smoking, whereas we found negative association of intracellular MPO with CRP and smoking. We hypothesize that our findings and EPIC's findings can be reconciled if the MPO was released from leukocytes into the blood upon their activation by CRP and smoking. Similarly, we found a positive association with HDL-cholesterol, whereas they found a negative association with HDL-cholesterol. There were no associations of MPO with other risk factors in the EPIC study, but we found a positive association of MPO with LDL-cholesterol and ethanol intake. Blood levels of MPO were not associated with LDL-cholesterol in another study [28]. Our study also showed negative associations of a cellular MPO and use of statins, in line with published reports indicating that statins downregulate MPO gene expression and activity [29,30].

CD14 is a monocyte receptor for LPS and has a critical role in regulation of the inflammatory response. When stimulated by LPS via the CD14 receptor, monocytes express a large variety of inflammatory cytokines. CD14 signaling and activity occur together with TLR4. In our study cellular CD14 was associated positively with triglycerides, waist/hip, and smoking, and negatively with antihypertensive medications. Soluble CD14 was reported in other studies to be associated with various inflammatory markers and lipid parameters, including triglycerides [31] but prior findings were not consistent; CD14 has been more extensively studied in relation to its polymorphisms [32-34]. Similarly, monocyte expression of TLR-4 has been studied mainly in relation to polymorphisms. In our study TLR4 was associated positively with LDL-cholesterol and sport activity, and negatively with diabetes and statin use. Pasini et al [35] also showed a significant positive correlation between oxidized LDL and CD14 and TLR4, indicating that oxidized LDL may be implicated in their expression levels.

CD45, the leukocyte common antigen, is best known for its crucial role in immunity, for efficient T and B lymphocyte function. Altered CD45 expression or different isoforms have been reported to be associated with some autoimmune and infectious diseases and hematological malignancies. Recently, it was found that CD45 negatively regulates cytokine and interferon receptors suggesting this protein might have roles in glucose and lipid metabolism [36,37]. Our results showing significant positive associations of CD45 with

triglycerides and diabetes and negative association with HDL-cholesterol need further exploration.

Limitations of our study warrant consideration. First, the study refusal rate was relatively high and those who participated tended to have lower risk factors. Although this may somewhat limit the generalizability of our results, it seems unlikely that nonparticipation would lead to bias in observed associations with physiological cell markers. Second, a lot of markers and risk factors were studied; some associations could be due to chance due to multiple testing. Third, the findings of this cross-sectional study may not reflect cause and effect relations, which would need to be established by experiments or clinical trials, where feasible. Fourth, although many of the associations make sense, the biology for several findings is unclear. Particularly, the reasons for the apparent strong racial/ethnic differences in cellular markers need further investigation. As noted above, these may not be biologic, but rather partly due to socioeconomic factors or to geographic field center differences resulting from the ARIC study design. Finally, whether the observed associations contribute to future risk of cardiovascular disease will require prospective follow-up.

In conclusion, race/ethnicity, sex, and to a lesser degree LDL-cholesterol and lipid-lowering therapy, but few other risk factors, were correlated with markers of cellular activation in this population-based study.

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Table 1

Mean (SD) or prevalence (%) of major risk factors at ARIC Visit 4 (1996-1998) for Carotid MRI Study nonparticipants and participants, and current risk factors (2005-2006) of participants with cell marker data

	ARIC Visit 4 (1996-1998)		ARIC MRI Visit (2005-2006)
	MRI Non-Participants (*N* = 1956)	MRI Participants (*N* = 2011)	Participants with Cell Marker Data (*N* = 1894)
Age	63.1 (5.6)	61.7 (5.4)*	70.3 (5.5)
Gender (Male)	39.1%	43.2%	44.1%
Race (Black)	22.2%	21.1%	20.9%
LDL-Cholesterol (mg/dL)	122 (34)	122 (32)	114 (37)
HDL-Cholesterol (mg/dL)	50.7 (16.9)	51.4 (17.7)	49.9 (14.8)
Triglycerides (mg/dL)	146 (92)	137 (81)	151 (85)
Cholesterol Medication	14.6%	12.0%	44.5%
Systolic Blood Pressure (mmHg)	128 (19)	126 (18)*	126 (9)
Hypertension Medication	38.1%	31.0%*	58.2%
Diabetes	18.2%	12.3%*	24.5%
Smoking	15.4%	12.0%	7.8%
BMI (kg/m^2)	29.1 (5.9)	28.4 (5.2)*	29.1 (5.4)
Waist to Hip Ratio	0.95 (0.08)	0.94 (0.07)*	0.94 (0.08)
CRP (mg/L)	NA	NA	3.6 (5.1)
Aspirin Use	NA	NA	66.7%

NA = Not available

* $p<0.01$ for difference from MRI nonparticipants

Table 2

Weighted means (standard deviations) for the cell marker variables by race/ethnicity, ARIC

	Alternate Name	Units	Missing (*n*)[a]	White (*n* = 1432)	Black (*n* = 462)	P-diff
Leukocyte Markers						
Monocytes						
CD14+	Lipopolysaccharide (LPS) receptor	MFI	29	109.4 (20.0)	120.3 (22.1)	<0.001
CD14+ / TLR-2+	Toll-like receptor-2	%	29	64.0 (11.6)	56.2 (10.3)	<0.001
		MFI	29	14.1 (1.5)	13.5 (0.8)	<0.001
CD14+ / TLR-4+	Toll-like receptor-4	%	31	65.4 (4.2)	64.2 (3.3)	<0.001
		MFI	31	16.4 (1.0)	16.7 (0.9)	<0.001
CD14+ / CD162+	P-selectin glycoprotein ligand-1 (PSGL-1)	MFI	29	111.5 (15.6)	114.4 (14.3)	0.002
CD45+	Pan-leukocyte marker	MFI	91	76.3 (10.0)	70.7 (9.7)	<0.001
CD45+ / MPO+	Myeloperoxidase	MFI	37	89.6 (23.1)	103.1 (28.4)	<0.001
Granulocytes						
CD162+		MFI	29	76.7 (14.3)	88.4 (14.2)	<0.001
MPO+	Myeloperoxidase	MFI	37	816 (154)	912 (166)	<0.001
Lymphocytes						
CD162+		MFI	29	51.8 (9.9)	59.4 (10.8)	<0.001
Platelet Markers						
CD61+	GPIIIa receptor (β_3)	MFI	15	59.2 (17.6)	68.2 (17.4)	<0.001
CD61+ / CD62P+	P-selectin	%	15	27.5 (13.3)	31.6 (16.2)	<0.001
		MFI	15	21.5 (5.1)	23.2 (7.5)	<0.001
CD41+	GPIIb (α_{IIb})	MFI	50	77.9 (12.2)	82.3 (13.5)	<0.001
CD41+ / CD154+	CD40 ligand	%	50	2.9 (2.6)	2.8 (2.0)	0.45
		MFI	50	12.6 (2.5)	12.4 (1.6)	0.03
Cell Aggregates						
Platelet-monocyte aggregates		%	31	16.6 (4.1)	17.9 (4.3)	<0.001
		MFI	31	47.1 (6.1)	49.0 (6.7)	<0.001
Platelet-granulocyte aggregates		%	31	17.3 (4.3)	18.5 (4.2)	<0.001
		MFI	31	49.1 (6.3)	51.2 (13.5)	0.01
Platelet-lymphocyte aggregates		%	31	16.5 (4.2)	18.2 (4.2)	<0.001
		MFI	31	47.6 (7.5)	50.0 (8.4)	<0.001

ARIC = Atherosclerosis Risk in Communities, % = percentage of positive events gated, MFI = median fluorescence intensity

[a] Although the maximum sample sizes with marker data was 1,894, this column indicates the number missing for any individual marker.

Table 3

Weighted means (standard deviations) for the cell marker variables by sex, ARIC

	Units	Women (*N* = 965)	Men (*N* = 929)	P-diff
Leukocyte Markers				
Monocytes				
CD14+	MFI	109.4 (21.5)	114.5 (20.0)	<0.001
CD14+ / TLR-2+	% MFI	62.7 (11.4) 13.9 (1.0)	61.9 (12.2) 14.0 (1.8)	0.29 0.17
CD14+ / TLR-4+	% MFI	64.9 (3.9) 16.3 (1.0)	65.4 (4.3) 16.6 (1.0)	0.08 <0.001
CD14+ / CD162+	MFI	111.6 (14.9)	112.8 (15.9)	0.20
CD45+	MFI	74.4 (10.3)	76.0 (10.1)	0.006
CD45+ / MPO+	MFI	95.7 (25.8)	88.3 (23.1)	<0.001
Granulocytes				
CD162+	MFI	78.2 (14.7)	80.4 (15.4)	0.009
MPO+	MFI	838 (166)	834 (157)	0.65
Lymphocytes				
CD162+	MFI	51.1 (10.0)	56.4 (10.6)	<0.001
Platelet Markers				
CD61+	MFI	62.1 (17.5)	59.8 (18.4)	0.03
CD61+ / CD62P+	% MFI	29.0 (14.3) 21.8 (5.4)	27.6 (13.7) 21.8 (6.1)	0.08 0.96
CD41+	MFI	79.5 (12.6)	78.0 (12.6)	0.05
CD41+ / CD154+	% MFI	3.0 (2.7) 12.5 (2.5)	2.7 (2.1) 12.6 (2.1)	0.04 0.36
Cell Aggregates				
Platelet-monocyte aggregates	% MFI	17.9 (4.2) 47.6 (6.2)	15.5 (3.8) 47.5 (6.4)	<0.001 0.75
Platelet-granulocyte aggregates	% MFI	18.7 (4.3) 49.4 (6.3)	16.2 (3.8) 49.6 (10.4)	<0.001 0.77
Platelet-lymphocyte aggregates	% MFI	18.0 (4.3) 48.5 (8.2)	15.4 (3.7) 47.5 (7.0)	<0.001 0.03

ARIC = Atherosclerosis Risk in Communities, % = percentage of positive events gated, MFI = median fluorescence intensity

Table 4

Age, race, sex-adjusted difference (p-value) in cell marker variables per inter-quartile increment[a] of LDL-cholesterol or use of cholesterol-lowering medication, ARIC

	Units	LDL-Cholesterol	Cholesterol lowering medication
Leukocyte Markers			
Monocytes			
CD14+	MFI	1.32 (0.06)	-2.51 (0.04)
CD14+ / TLR-2+	% MFI	0.27 (0.49) 0.08 (0.12)	-0.07 (0.92) -0.07 (0.41)
CD14+ / TLR-4+	% MFI	-0.04 (0.78) 0.12 (0.007)[b]	0.23 (0.33) -0.20 (0.001)[b]
CD14+ / CD162+	MFI	0.53 (0.42)	0.47 (0.60)
CD45+	MFI	-0.35 (0.37)	0.19 (0.75)
CD45+ / MPO+	MFI	2.23 (0.02)	-2.74 (0.05)
Granulocytes			
CD162+	MFI	0.96 (0.06)	-0.82 (0.32)
MPO+	MFI	20.2 (0.001)[b]	-28.6 (0.002)[b]
Lymphocytes			
CD162+	MFI	0.93 (0.008)[b]	-0.42 (0.45)
Platelet Markers			
CD61+	MFI	-0.88 (0.17)	2.67 (0.009)[b]
CD61+ / CD62P+	% MFI	0.70 (0.14) 0.34 (0.12)	0.05 (0.95) -0.48 (0.15)
CD41+	MFI	0.31 (0.51)	0.32 (0.66)
CD41+ / CD154+	% MFI	0.16 (0.07) -0.04 (0.61)	-0.06 (0.67) -0.19 (0.14)
Cell Aggregates			
Platelet-monocyte aggregates	% gated MFI	0.37 (0.01) 0.08 (0.74)	0.05 (0.84) -0.22 (0.55)
Platelet-granulocyte aggregates	% gated MFI	0.37 (0.01) -0.15 (0.60)	0.00 (0.99) 0.35 (0.51)
Platelet-lymphocyte aggregates	% gated MFI	0.38 (0.01) 0.04 (0.90)	-0.01 (0.96) 0.42 (0.36)

LDL-cholesterol and cholesterol-lowering medications were in separate models and adjusted for age, race, and sex.

ARIC = Atherosclerosis Risk in Communities, % = percentage of positive events gated, MFI = median fluorescence intensity

[a] 46 mg/dL for LDL-C

[b] p<0.01