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Leukocyte Count is Associated with Increased Platelet Reactivity and Diminished Response to Aspirin in Healthy Individuals with a Family History of Coronary Artery Disease

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Abstract

Background—Markers of systemic inflammation, including blood leukocyte count, are associated with increased cardiovascular risk, but the mechanisms underlying this association are unclear. Leukocytes may promote platelet reactivity and thrombus formation, providing a basis for increased risk, but a relation between leukocyte count and platelet function has not been studied.

Methods—We evaluated the relation of blood leukocyte count, C-reactive protein (CRP), and interleukin-6 (IL-6) to platelet aggregation to collagen, ADP and arachidonic acid, and to urinary excretion of 11-dehydro thromboxane B2. Studies were conducted in 1600 individuals (45.0 ± 12.9 years, 42.7% male) at risk for coronary artery disease (CAD) before and after low dose aspirin.

Results—At baseline, platelet reactivity increased with increasing quartile of leukocyte count (median counts for each quartile were normal) for all measures of platelet function ($P<0.0001$). These relations were unchanged by aspirin. The relation between leukocyte count and each measure of platelet reactivity remained significant ($P<0.05$) after multivariable adjustment for CRP, IL-6, cardiac risk factors, hematologic variables, and platelet thromboxane production. CRP and IL-6 were independently associated with few measures of platelet reactivity.

Conclusions—Increasing quartile of leukocyte count, even within the normal range, is associated with increasing platelet reactivity in individuals at risk for CAD. This relationship is not altered by

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Conflicts of Interest

NF- U01 HL72518, patent filed on novel antithrombotic agents and methods of use thereof; LRY- none; DV- none; BK- none; RQ- none; JEH-G- none; TFM- none; DMB- U01 HL72518, McNeil Consumer and Specialty Pharmaceuticals and AspirinWorks; LCB- U01 HL72518, McNeil Consumer and Specialty Pharmaceuticals, and AspirinWorks.

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aspirin and is independent of inflammatory markers and platelet thromboxane production. Additional studies are needed to determine the mechanism(s) for this association and therapies to reduce cardiovascular risk in patients with elevated leukocyte counts.

Keywords

coronary disease; leukocytes; myocardial infarction; platelets; thrombosis

Systemic levels of inflammatory markers are associated with increased cardiovascular risk in primary and secondary prevention populations. This association is reported for blood leukocyte count[1, 2], C-reactive protein (CRP)[3–5] and interleukin-6 (IL-6)[6, 7]. In general, this association has been attributed to inflammation and atherosclerosis occurring in the arterial wall[8]. Alternatively, leukocytes may promote vascular occlusion by modifying platelet reactivity and promoting thrombus formation[9]; however, the clinical relevance of these proposed leukocyte functions is uncertain. A relation between the numbers of leukocytes in peripheral blood and platelet reactivity has not been investigated.

Platelet activation plays a critical role in myocardial infarction (MI) and unstable angina[10]. Low dose aspirin therapy is standard of care for primary[11] and secondary cardiovascular disease prevention[12, 13], and its cardioprotection is attributed to suppression of platelet function through its inhibitory effect on cyclooxygenase-1 (COX-1) and reduction of thromboxane formation[14]. Aspirin-treated individuals who display higher levels of platelet reactivity in vitro are reported to be at increased risk for MI, stroke, and cardiovascular death[15–19]. Some authors have suggested that platelet-leukocyte interactions may play a role in diminishing aspirin's antiplatelet action[20–22]; however, such an effect has not been verified and remains mechanistically ill-defined. Indeed, an association between leukocyte count and aspirin's ability to suppress platelet activation, independent of other inflammatory mediators, has not been determined.

The purpose of this study was to determine the relation between blood leukocyte count and platelet reactivity by assessing platelet function at baseline and after low dose aspirin therapy in a primary prevention cohort. We examined whether leukocyte count, even within the normal range, is associated with increased platelet reactivity in vitro and in vivo, and whether this relationship is independent of other inflammatory markers, including CRP and IL-6, and platelet thromboxane formation.

Methods

Participants and Study Design

Subjects were recruited from GeneSTAR (Genetic Study of Aspirin Responsiveness), an ongoing study designed to identify genetic determinants of platelet responsiveness to low dose aspirin. Details on the study population and design have been previously reported[23]. Briefly, subjects were recruited from Caucasian and African American families with a history of premature coronary artery disease (CAD, onset < age 60). Subjects were eligible if they were free of overt manifestations of CAD and had no serious comorbidity. Subjects were excluded if they had a history of aspirin allergy or intolerance, or for baseline platelet count <100,000 or >500,000, hematocrit <30%, or white blood cell count >20,000. Aspirin and nonsteroidal anti-inflammatory drug use were prohibited for 10 days prior to the baseline assessment and throughout the study interval. Subjects were given a supply of 81 mg aspirin tablets and instructed to take one pill each day for 14 days. Platelet function was assessed before and after aspirin treatment using a series of assays that evaluated platelet reactivity directly and indirectly related to thromboxane formation. The study was approved by the Johns Hopkins Institutional Review Board and all subjects provided written informed consent.

Blood and Urine Sample Collection

Blood and urine were sampled at the same time of day before and after aspirin to reduce the effect of circadian rhythm on platelet function. Blood was collected via venipuncture into vacutainer tubes containing EDTA, 3.2% sodium citrate, or serum separator as appropriate. A complete blood count (CBC), including total leukocyte count and differential, hematocrit, and platelet count, was determined by automated cell counter (ACT-Diff, Beckman-Coulter, Miami, FL). Mean platelet volume, a measure of platelet size that is related to platelet reactivity in vitro and cardiovascular disease events in vivo[24–26], was also determined by automated cell counter. CBC and platelet functional studies were completed within 2 hours of blood drawing. Plasma, serum, and urine were stored at −80C until analyzed.

Assessment of platelet function

In vitro platelet reactivity was assessed in whole blood by impedance aggregometry and in platelet rich plasma (PRP, 2×10^5 platelets/μl) by optical aggregometry; and, by platelet production of thromboxane B2 (Tx-B2). (Leukocytes remaining in PRP were $<0.1 \times 10^3$ cells/μl.) In vivo platelet reactivity was assessed by urinary excretion of the thromboxane B2 metabolite 11-dehydro thromboxane B2 (Tx-M). Platelet aggregation to arachidonic acid (0.5 mM for whole blood and 1.6 mM for PRP), collagen (1 μg/ml), and ADP (10 μM) was measured as previously described[23]. Platelet thromboxane B2 (Tx-B2) production was determined from collagen-stimulated whole blood samples as previously described using commercially available enzyme-linked immunosorbent assay (ELISA, Assay Designs, Inc, Ann Arbor, MI) [23]. Tx-M was also quantified by commercially available ELISA (Cayman Chemical Co., Ann Arbor, MI) and normalized to urine creatinine.

Assessment of plasma inflammatory markers

CRP (Dako, Inc., Carpinteria, CA) and IL-6 (Pierce, Woburn, MA) were quantified from plasma using commercially available high sensitivity ELISA's. Fibrinogen was measured by the Johns Hopkins Hospital Clinical Coagulation Laboratory using an automated optical clot detection device (Behring Coagulation System; Dade-Behring, Newark, DE).

Assessment of cardiac risk factors

Demographics, hypertension, diabetes, and cigarette smoking were determined as previously described[23]. Height and weight were measured and body mass index (BMI) calculated (kg/m^2). A fasting lipid profile was determined from serum samples using a Cholestech LDX analyzer (Cholestech Corporation, Hayward, CA).

Statistical Analysis

Data were analyzed using SAS (version 9.1, 2002–2003, SAS Institute, Inc., Cary, NC) and SUDAAN (version 9.0.1, 2005, Research Triangle Institute, Research Triangle Park, NC). Means (± 1 SD) of continuous variables were calculated. Variables that were non-normal were transformed and normality confirmed by the Kolmogorov-Smirnov test. Aggregation to arachidonic acid after aspirin treatment was dichotomized into zero response or >0 response because this variable could not be normally transformed. Measurements before and after aspirin treatment were compared using paired t-tests or chi-squared tests (for aggregation to arachidonic acid). The relation between quartile of leukocyte count and each measure of platelet reactivity, before and after aspirin, was determined by analysis of variance. The relation between quartile of leukocyte of count and sample characteristics was determined by analysis of variance for continuous variables and chi square for categorical variables. The relation of leukocyte count and measured variables to platelet reactivity was determined using linear regression, unadjusted and adjusted for each of the following covariates: age, sex, race, hypertension, diabetes, cigarette smoking, total cholesterol, BMI, CRP, IL-6, fibrinogen,

hematocrit, platelet count, mean platelet volume, and platelet Tx-B2 release in vitro. The relation between leukocyte count and arachidonic acid aggregation after aspirin was determined by logistic regression, unadjusted and adjusted for the same covariates. All regressions were adjusted using the general estimating equation to account for intra-familial clustering.

Results

One thousand six hundred subjects were evaluated. Mean age was 45.0 ± 12.9 years, 42.7% were male, and 37.3% were African American. Hypertension, diabetes, and current cigarette smoking were common and on average subjects were overweight (table 1). On average, total leukocyte counts were normal and CRP and IL-6 tended to be moderate to high based on previously published risk strata for these inflammatory markers[7, 27]. There were no differences in leukocyte counts (6.3 ± 2.0 vs. 6.3 ± 2.1 × 10^3/μl), CRP levels (2.68 ± 3.10 vs. 2.66 ± 3.17 ng/L), or IL-6 levels (6.60 ± 13.5 vs. 6.62 ± 13.8 ng/L) before and after two weeks of aspirin treatment.

Platelet aggregation in whole blood increased significantly with increasing quartile of total leukocyte count in activation pathways both directly (arachidonic acid aggregation) and indirectly (collagen and ADP aggregation) related to thromboxane formation (table 2). Urinary excretion of Tx-M also increased with increasing quartile of leukocyte count. Although platelet reactivity was significantly less, as expected, after aspirin treatment ($P < 0.0001$ vs. before aspirin, except for ADP aggregation), residual reactivity increased significantly with increasing quartile of total leukocyte count (table 2). Platelet release of Tx-B2 in vitro was markedly reduced after low dose aspirin treatment (66.2 ± 117 vs. 0.93 ± 3.68 ng/10^8 platelets, $P < 0.0001$); however, there was no relation between quartile of total leukocyte count and platelet Tx-B2 release either before or after aspirin. In contrast to the whole blood environment, there was no relation between total leukocyte count and PRP aggregation to any of the agonists tested either before or after aspirin (data not shown).

Analysis of leukocyte subsets demonstrated similar results to those observed with total leukocyte count: Platelet aggregation in whole blood and Tx-M excretion increased significantly with increasing quartile of neutrophil count and lymphocyte count both before and after aspirin treatment. For example, aggregation to collagen before aspirin was 18.0 ± 4.4, 19.8 ± 4.7, 21.7 ± 5.2, and 23.5 ± 5.8 ohms for neutrophil quartiles 1 through 4, respectively ($P < 0.0001$); and after aspirin, collagen aggregation was 6.4 ± 5.2, 6.4 ± 5.1, 7.3 ± 5.3, and 7.6 ± 6.0 ohms for neutrophil quartiles 1 through 4, respectively ($P = 0.0018$). Similar relations between neutrophil quartile and the other reactivity measures were seen and between lymphocyte quartiles and reactivity measures (data not shown). Because total leukocyte count, neutrophil count, and lymphocyte count were highly correlated, additional analyses were restricted to total leukocyte count.

The relation of sample characteristics to quartile of total leukocyte count is shown in table 3. Many of the characteristics showed differences across leukocyte quartiles. In univariable regression analyses, total leukocyte count was a significant positive predictor of all whole blood aggregation measures and urine Tx-M excretion, both before and after aspirin treatment (tables 4–7). CRP and IL-6 were also associated with many of the platelet reactivity measures in univariable analyses, as were age, sex, BMI, current smoking, fibrinogen, hematocrit, platelet count, and mean platelet volume. After multivariable adjustment, only total leukocyte count remained a significant positive predictor of all platelet reactivity measures directly and indirectly related to thromboxane formation, in vitro and in vivo, both before and after aspirin (tables 4–7). This effect was independent of the other inflammatory markers, cardiac risk factors, and hematologic variables. After multivariable adjustment, CRP was only related to

ADP aggregation before aspirin and IL-6 was only related to Tx-M before and after aspirin. Similarly, cardiac risk factor and hematologic covariates were independently associated with some but not all platelet reactivity measures in adjusted analyses. Platelet production of Tx-B2 in vitro was associated with aggregation in vitro and Tx-M excretion in vivo. However, this association was independent of the relation between leukocyte count and platelet reactivity in vitro and in vivo (see tables 4–7, multivariable analyses).

Discussion

This study demonstrates a strong association between blood leukocyte count and increased platelet reactivity in vitro and in vivo, which is independent of other inflammatory markers, cardiac risk factors, hematologic variables, and platelet thromboxane production. This relation exists under baseline conditions and persists despite treatment with aspirin, even though the absolute magnitude of platelet reactivity is suppressed by aspirin therapy. Furthermore, our data demonstrate that an association between leukocyte count and platelet reactivity is present in the whole blood milieu, but not in PRP depleted of leukocytes. Importantly, we demonstrate that this association is not mediated by common soluble inflammatory markers or platelet thromboxane formation. Rather, the close link between leukocyte count and platelet reactivity suggests that the number of circulating leukocytes is either a direct modifier of platelet reactivity both before and after aspirin, or a marker for an as yet unidentified inflammation-related substance that modifies platelet reactivity.

This is the first study to demonstrate a direct association between peripheral blood leukocyte count and platelet reactivity in vitro and in vivo. This relation was observed through a normal range of leukocyte counts and was present in each of several distinct platelet activation pathways, which are directly and indirectly related to thromboxane formation. The relation between leukocyte count and platelet reactivity was independent of CRP, IL-6, and fibrinogen, and, indeed, the independent relation of these other inflammatory markers to platelet reactivity was quite limited. The relation between leukocyte count and platelet reactivity was also independent of key hematologic variables, such as hematocrit, platelet count, and mean platelet volume, which have previously been reported to modify platelet aggregation responses in vitro[24, 28]. Our findings in 1600 individuals at risk for CAD are consistent with smaller studies that failed to show a relation between CRP and platelet reactivity[29–31] but contrast with two other studies in which higher CRP levels were associated with increased platelet reactivity and diminished response to aspirin[32, 33]. However, in these other studies, the influence of leukocyte count on platelet reactivity was not considered.

The relation between leukocyte count and increased platelet reactivity was observed for total leukocyte count, total neutrophil count, and total lymphocyte count. Both neutrophil count ($r > 0.9$) and lymphocyte count ($r > 0.6$) showed a strong positive correlation with total leukocyte count in this group of individuals without acute illness, precluding a definitive determination of the role of leukocyte subsets in platelet reactivity. In observational studies of cardiovascular disease outcome, higher total leukocyte and neutrophil counts were associated with increased future risk of MI and cardiovascular death; however, there was an inverse relation noted between lymphocyte count and cardiovascular risk[34, 35]. Based on these observational data and the known interactions between neutrophils and platelets[36–41], the neutrophil sub-population appears more likely to explain our findings than lymphocytes. Additional in vitro studies are needed to determine the relation between specific leukocyte subtypes and platelet reactivity.

One mechanism to explain the association between higher leukocyte count and greater platelet reactivity is through a leukocyte-mediated increase in platelet thromboxane formation. Leukocytes and platelets are capable of cooperative synthesis and transfer of arachidonic acid

and its derivatives [36, 37], and some authors have suggested that platelet-leukocyte interactions may mitigate aspirin's suppressive effect on platelet function by enhancing thromboxane formation independent of platelet COX-1[20–22]. The association between blood leukocyte count and urinary Tx-M excretion we observed suggests that such a phenomenon may occur in vivo. However, several of our other findings dissociate the relation among leukocytes, platelet activation, and thromboxane production: First, we found no relation between quartile of leukocyte count and platelet thromboxane production in vitro before or after aspirin. Second, the relation between quartile of leukocyte count and platelet reactivity was equally strong before and after aspirin, despite >50-fold reduction in thromboxane production after aspirin treatment. Third, the association between leukocyte count and platelet reactivity persisted for all reactivity measures even after adjustment for platelet thromboxane production in vitro. Thus, the data suggest that enhanced thromboxane production cannot fully account for the association between leukocyte count and platelet reactivity.

Leukocytes and platelets are capable of interacting in several other ways that might lead to enhanced platelet activation including: engagement of leukocyte P-selectin glycoprotein ligand-1 by platelet P-selectin[38], leukocyte release of platelet activating proteases[39, 40], and leukocyte release of reactive oxygen species[41]. However, the physiologic relevance of these mechanisms to vascular thrombosis in human health and disease has not been determined. This is the first study to demonstrate a direct relation between increasing leukocyte count and platelet reactivity in a large human population using both in vitro and in vivo metrics. Furthermore, we show that low dose aspirin, which is standard therapy in both primary and secondary cardiovascular prevention, does not alter the relation between leukocyte count and platelet reactivity. Thus, the mechanisms that link leukocyte count and platelet reactivity appear distinct from COX-1-dependent thromboxane formation- the molecular pathway targeted by aspirin. Findings from this study support a prothrombotic role for leukocytes[9], which has been proposed to explain the strong relation between leukocyte count and atherothrombotic morbidity observed in primary and secondary prevention cohorts[1, 2]. Although the mechanisms linking leukocyte count and platelet reactivity are not clear, data from this and previous studies suggest that aspirin may have limited ability to inhibit leukocyte-related platelet activation[42].

Previous studies have demonstrated a higher incidence of MI, stroke and cardiovascular death in patients with known CAD who demonstrate increased platelet aggregability in vitro and urinary excretion of thromboxane in vivo[15, 16, 18, 19]. Although we also found increased platelet aggregability and thromboxane excretion for subjects in our cohort with higher leukocyte counts, this report is limited by the absence of manifest CAD in the subject population and lack of clinical outcome data. Thus, we cannot determine if the association between leukocyte count and platelet reactivity we observed is related to clinical thrombotic morbidity in vivo. Additional research is required to identify the mechanism(s) that explains the association between leukocyte count and increased platelet reactivity and its relation to CAD expression. Such studies have potential to identify specific therapies to prevent and manage MI and stroke in patients with elevated leukocyte counts.

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Abbreviations

ADP

adenosine diphosphate

BMI
body mass index

CAD
coronary artery disease

COX
cyclooxygenase

CRP
C-reactive protein

ELISA
enzyme-linked immunosorbent assay

IL-6
interleukin-6

MI
myocardial infarction

PRP
platelet rich plasma

Tx-B2
thromboxane B2

Tx-M
urinary 11-dehydro thromboxane B2

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Table 1

Baseline Sample Characteristics (n = 1600)

Age, mean ± SD, years	45.0 ± 12.9
Male sex, %	42.7
African American race, %	37.3
Hypertension, %	32.8
Diabetes, %	8.3
Current smoking, %	25.3
Total cholesterol, mean ± SD, mg/dL	198 ± 41
Body Mass Index, mean ± SD, kg/m^2	29.5 ± 6.9
Leukocyte count, mean ± SD, $\times 10^3$ cells/μL	6.3 ± 2.0
C-reactive protein, mean ± SD, mg/L	2.68 ± 3.10
Interleukin-6, mean ± SD, ng/L	6.60 ± 13.5
Fibrinogen, mean ± SD, mg/dL	391 ± 120
Hematocrit, mean ± SD, percent	41.2 ± 3.9
Platelet count, mean ± SD, $\times 10^3/\mu l$	260 ± 62
Mean platelet volume, mean ± SD, fl	7.61 ± 0.83

Table 2

Effect of quartile of leukocyte count on platelet reactivity before and after aspirin (ASA) treatment*

	Quartile (Q) of Leukocyte Count: Median [Interquartile Range]			
	Q1: 4.2 [0.8] ×10^3 cells/μL	**Q2: 5.4 [0.6] ×10^3 cells/μL**	**Q3: 6.6 [0.8] ×10^3 cells/μL**	**Q4: 8.6 [1.8] ×10^3 cells/μL**
Collagen aggregation pre-ASA (ohms) Collagen aggregation post-ASA (ohms)	18.1 ± 4.4 5.9 ± 5.1	20.0 ± 4.8 6.3 ± 5.1	21.5 ± 5.1 6.5 ± 5.4	23.4 ± 6.1† 7.6 ± 5.8†
ADP aggregation pre-ASA (ohms) ADP aggregation post-ASA (ohms)	11.5 ± 5.2 11.1 ± 5.2	12.6 ± 5.3 12.0 ± 5.6	13.3 ± 5.7 12.7 ± 6.2	14.9 ± 6.6† 15.6 ± 6.8†
Arachidonic acid aggregation pre-ASA (ohms) Arachidonic acid aggregation post-ASA (% >0 aggregation)	14.7 ± 5.4 4.4	15.8 ± 5.9 3.8	17.2 ± 6.6 3.4	19.7 ± 6.9† 9.2†
Tx-M pre-ASA (ng/mmol creatinine) Tx-M post-ASA (ng/mmol creatinine)	194 ± 430 35.0 ± 40.1	170 ± 396 40.4 ± 55.1	326 ± 1118 52.4 ± 113	446 ± 2122† 77.5 ± 414†

*Data are presented as mean ± SD except Arachidonic acid aggregation post-ASA, which is presented as percent.

ASA = aspirin; Tx-M = urinary 11-dehydro thromboxane B2

†P< 0.0001 for effect of quartile by analysis of variance

Table 3

Relation of sample characteristics to quartile of leukocyte count

	Leukocyte Quartile 1	Leukocyte Quartile 2	Leukocyte Quartile 3	Leukocyte Quartile 4	p-value
Age, mean ± SD, years	46.0±13.1	46.1±12.6	44.4±13.1	43.5±12.7	0.0097
Male sex, %	45.0	43.4	43.6	38.6	0.2949
African American race, %	54.2	34.1	33.6	28.2	<0.0001
Hypertension, %	33.2	29.0	33.3	35.5	0.2738
Diabetes, %	7.6	7.8	7.5	10.4	0.4186
Current smoking, %	13.7	18.4	26.7	42.2	<0.0001
Total cholesterol, mean ± SD, mg/dL	194±42	198±41	197±40	203±44	0.0137
Body Mass Index, mean ± SD, kg/m^2	28.8±6.4	28.6±5.9	29.7±7.2	30.9±7.7	<0.0001
C-reactive protein, mean ± SD, mg/L*	2.12±2.7	2.02±2.7	2.62±2.9	3.96±3.6	<0.0001
Interleukin-6, mean ± SD, ng/L*	5.15±7.9	6.45±17.1	6.53±12.2	8.29±15.0	<0.0001
Fibrinogen, mean ± SD, mg/dL	371±112	375±112	397±117	421±133	<0.0001
Hematocrit, mean ± SD, percent	40.6±3.9	40.8±3.7	41.5±3.9	41.7±4.0	<0.0001
Platelet count, mean ± SD, $\times 10^3/\mu L$	239±57	253±59	264±60	284±65	<0.0001
Mean platelet volume, mean ± SD, fl	7.64±0.84	7.52±0.82	7.61±0.82	7.65±0.83	0.1808

*Transformed variable

Table 4

Association of leukocyte count, other inflammatory markers, and other variables with platelet aggregation to collagen before and after aspirin.

Variable	Before Aspirin Beta ± SE (P-value)		After Aspirin Beta ± SE (P-value)	
	univariable	**multivariable**	**univariable**	**multivariable**
Total leukocyte count (×10^3 cells/μL)	**1.0372 ± 0.0703 (<0.0001)**	**1.0744 ± 0.0859 (<0.0001)**	**0.0484 ± 0.0094 (<0.0001)**	**0.0513 ± 0.0098 (<0.0001)**
Age (years)	−0.0108 ±0.0108 (0.3173)	0.0121 ± 0.0127 (0.3415)	**0.0040 ± 0.0016 (0.0103)**	**0.0078 ± 0.0019 (0.0001)**
Male sex	**−0.9358 ±0.2458 (0.0002)**	**0.7459 ± 0.3642 (0.0411)**	−0.1536 ±0.0398 (0.0001)	−0.0596 ±0.0529 (0.2606)
African American race	−0.2971 ±0.3250 (0.3610)	−0.5010 ±0.3701 (0.1766)	0.1225 ± 0.0452 (0.0070)	0.0635 ± 0.0472 (0.1789)
Hypertension	0.2730 ± 0.2950 (0.3551)	0.1091 ± 0.3407 (0.7489)	0.0580 ± 0.0382 (0.1299)	−0.0340 ±0.0481 (0.4804)
Diabetes	−0.2614 ±0.5340 (0.6247)	−0.5975 ±0.5179 (0.2492)	0.0590 ± 0.0612 (0.3362)	−0.0041 ±0.0675 (0.9514)
Current smoking	0.7441 ± 0.3296 (0.0244)	−0.0451 ±0.3621 (0.9009)	0.0842 ± 0.0409 (0.0402)	0.0562 ± 0.0424 (0.1857)
Total cholesterol (mg/dl)	−0.0002 ±0.0035 (0.9531)	−0.0048 ±0.0034 (0.1602)	0.0007 ± 0.0005 (0.1703)	−0.00004 ± 0.0005 (0.9267)
Body mass index (kg/m2)	0.0847 ± 0.0222 (0.0001)	0.0450 ± 0.0264 (0.0885)	0.0065 ± 0.0026 (0.0131)	0.0002 ±0.0033 (0.9642)
C-reactive protein (mg/L*)	0.5226 ± 0.0943 (<0.0001)	0.1957 ± 0.1186 (0.0995)	0.0209 ± 0.0129 (0.1072)	−0.0049± 0.0163 (0.7636)
Interleukin-6 (ng/L*)	0.2941 ± 0.1323 (0.0268)	−0.2166 ±0.1305 (0.0978)	0.0237±0.0183 (0.1973)	−0.0382 ±0.0189 (0.0432)
Fibrinogen (mg/dl)	**0.0027 ± 0.0012 (0.0231)**	**−0.0038 ±0.0014 (0.0066)**	0.0006 ± 0.0002 (0.0006)	0.0001± 0.0002 (0.6339)
Hematocrit (%)	**−0.1675 ±0.0323 (<0.0001)**	**−0.2754 ±0.0434 (<0.0001)**	**−0.0268 ±0.0048 (<0.0001)**	**−0.0192 ±0.0065 (0.0034)**
Platelet count (×10^3 cells/μL)	**0.0194±0.0021 (<0.0001)**	**0.0096 ±0.0028 (0.0007)**	0.0002 ±0.0003 (0.5932)	0.0006 ±0.0004 (0.1322)
Mean platelet volume (fl)	−0.0677 ±0.1833 (0.7122)	−0.0176 ±0.2110 (0.9336)	**0.1224 ± 0.0238 (<0.0001)**	**0.1217 ± 0.0279 (<0.0001)**
Thromboxane-B2 (ng/10^8 platelets*)	**0.7423 ± 0.1806 (<0.0001)**	**0.9911 ± 0.2281 (<0.0001)**	**0.1381 ± 0.0177 (<0.0001)**	**0.1430 ± 0.0195 (<0.0001)**

Values in bold type denote significance in both univariable (unadjusted) and multivariable (adjusted for all variables) linear regression models.

*Transformed variable

Table 5

Association of leukocyte count, other inflammatory markers, and other variables with platelet aggregation to ADP before and after aspirin

Variable	Before Aspirin Beta ± SE (P-value)		After Aspirin Beta ± SE (P-value)	
	univariable	**multivariable**	**univariable**	**multivariable**
Total leukocyte count (×10^3 cells/μL)	**0.7071 ± 0.0839 (<0.0001)**	**0.5785 ± 0.0905 (<0.0001)**	**0.8380 ± 0.0883 (<0.0001)**	**0.6285 ± 0.1038 (<0.0001)**
Age (years)	0.0105 ± 0.0111 (0.3440)	0.0264 ± 0.0129 (0.0417)	0.0133 ± 0.0120 (0.2681)	0.0309 ± 0.0132 (0.0195)
Male sex	**−3.6425 ±0.2903 (<0.0001)**	**−1.1800 ±0.4194 (0.0051)**	**−3.6593 ±0.2888 (<0.0001)**	**−1.2605 ±0.4294 (0.0035)**
African American race	1.3570 ± 0.3348 (0.0001)	0.4583 ± 0.3570 (0.1999)	**1.9451 ± 0.3785 (<0.0001)**	**1.3101 ± 0.3736 (0.0005)**
Hypertension	0.4078 ± 0.3005 (0.1754)	−0.2383 ±0.3114 (0.4444)	0.6339 ± 0.3368 (0.0605)	−0.2267± 0.3599 (0.8563)
Diabetes	0.0445 ± 0.5544 (0.9361)	−0.6983 ±0.5286 (0.1871)	0.2745 ± 0.6116 (0.6538)	−1.0585 ±0.5432 (0.0519)
Current smoking	0.2522 ± 0.3657 (0.4908)	0.0292 ±0.3840 (0.9395)	0.2181 ± 0.3871 (0.5734)	−0.1366 ±0.3830 (0.7216)
Total cholesterol (mg/dl)	0.0019 ± 0.0037 (0.6075)	−0.0048 ±0.0032 (0.1420)	0.0095 ± 0.0043 (0.0268)	0.0024 ± 0.0039 (0.5366)
Body mass index (kg/m2)	0.0759 ± 0.0201 (0.0002)	0.0041 ± 0.0212 (0.8487)	0.1226 ± 0.0247 (<0.0001)	0.0272 ± 0.0255 (0.2864)
C-reactive protein (mg/L*)	**0.4005 ± 0.1012 (0.0001)**	**−0.3015 ±0.1114 (0.0071)**	0.5960 ± 0.1000 (<0.0001)	−0.0895 ±0.1303 (0.4928)
Interleukin-6 (ng/L*)	0.4531 ± 0.1453 (0.0019)	−0.1379 ±0.1320 (0.2968)	0.6048 ± 0.1478 (0.0001)	−0.0563± 0.1359 (0.6787)
Fibrinogen (mg/dl)	0.0083 ± 0.0013 (<0.0001)	0.0014 ± 0.0015 (0.3579)	0.0087 ± 0.0014 (<0.0001)	−0.0016± 0.0017 (0.3460)
Hematocrit (%)	**−0.4367 ±0.0355 (<0.0001)**	**−0.2366 ±0.0551 (<0.0001)**	**−0.3830 ±0.0331 (<0.0001)**	**−0.1450 ±0.0500 (0.0039)**
Platelet count (×10^3 cells/μL)	**0.0334 ±0.0022 (<0.0001)**	**0.0326 ±0.0026 (<0.0001)**	**0.0351 ±0.0026 (<0.0001)**	**0.0357 ±0.0031 (<0.0001)**
Mean platelet volume (fl)	**1.0665 ± 0.1970 (<0.0001)**	**1.8617 ± 0.2070 (<0.0001)**	**1.6325 ± 0.1893 (<0.0001)**	**2.3377 ± 0.1822 (<0.0001)**
Thromboxane-B2 (ng/10^8 platelets*)	0.0070 ± 0.1457 (0.9618)	−0.1576 ±0.1699 (0.3541)	**−0.3591 ±0.1208 (0.0031)**	**−0.2250 ±0.1098 (0.0410)**

Values in bold type denote significance in both univariable (unadjusted) and multivariable (adjusted for all variables) linear regression models.

*Transformed variable

Table 6

Association of leukocyte count, other inflammatory markers, and other variables with platelet aggregation to arachidonic acid before and after aspirin

Variable	Before Aspirin Beta ± SE (P-value)		After Aspirin Beta ± SE (P-value)	
	univariable	**multivariable**	**univariable**	**multivariable**
Total leukocyte count (×10^3 cells/μL)	**1.0060 ± 0.0872 (<0.0001)**	**0.9632 ± 0.0943 (<0.0001)**	**0.1496 ± 0.0439 (0.0007)**	**0.1665 ± 0.0559 (0.0031)**
Age (years)	**−0.0311 ±0.0124 (0.0125)**	**−0.0342± 0.0135 (0.0116)**	**−0.0349 ±0.0096 (0.0003)**	**−0.0300 ±0.0129 (0.0207)**
Male sex	−2.0509 ±0.3251 (<0.0001)	0.0783 ±0.4418 (0.8595)	−0.0111 ±0.2290 (0.9613)	−0.5412 ±0.5803 (0.3515)
African American race	0.6954 ± 0.3945 (0.0786)	0.6401 ± 0.4481 (0.1538)	0.5210 ± 0.2242 (0.0206)	−0.0178± 0.3018 (0.9531)
Hypertension	0.4384 ± 0.3471 (0.2072)	0.0479 ±0.3909 (0.9026)	−0.3181 ±0.2664 (0.2330)	−0.0498 ±0.3026 (0.8693)
Diabetes	−0.2654 ±0.5382 (0.6221)	−0.9636 ±0.4967 (0.0530)	0.3084 ± 0.3680 (0.4025)	0.4439 ± 0.5111 (0.3855)
Current smoking	1.5254 ± 0.3899 (0.0001)	0.4016 ± 0.4057 (0.3227)	0.3725 ± 0.2394 (0.1204)	−0.1919 ±0.3233 (0.5530)
Total cholesterol (mg/dl)	0.0074 ± 0.0043 (0.0833)	0.0025 ± 0.0040 (0.5387)	−0.0020 ±0.0000 (<0.0001)	0.0006 ± 0.0027 (0.8229)
Body mass index (kg/m2)	0.1381 ± 0.0238 (<0.0001)	0.0538 ±0.0302 (0.0755)	0.0320 ± 0.0148 (0.0316)	0.0098 ±0.0224 (0.6624)
C-reactive protein (mg/L*)	0.7159 ± 0.1107 (<0.0001)	0.1656 ± 0.1337 (0.2163)	0.1460 ± 0.0846 (0.0852)	−0.0064± 0.1233 (0.9588)
Interleukin-6 (ng/L*)	0.7265 ± 0.1628 (<0.0001)	0.0579 ±0.1724 (0.7372)	0.0920 ± 0.1080 (0.3946)	0.0291 ±0.1237 (0.8140)
Fibrinogen (mg/dl)	0.0087 ± 0.0014 (<0.0001)	0.0025± 0.0017 (0.1337)	0.0010 ± 0.0009 (0.2373)	0.0009 ± 0.0011 (0.3793)
Hematocrit (%)	**−0.2858 ±0.0412 (<0.0001)**	**−0.2754 ±0.0596 (<0.0001)**	0.0230 ± 0.0000 (<0.0001)	0.0214 ± 0.0821 (0.7941)
Mean platelet volume (fl)	0.4562 ± 0.2028 (0.0249)	0.1533 ± 0.2065 (0.4584)	**0.4283 ± 0.1353 (0.0017)**	**0.2687 ± 0.0000 (<0.0001)**
Thromboxane-B2 (ng/10^8 platelets*)	0.1449 ± 0.1817 (0.4257)	−0.1924± 0.2224 (0.3876)	**1.0247 ± 0.1082 (<0.0001)**	**1.0598 ± 0.1661 (<0.0001)**

Values in bold type denote significance in both univariable (unadjusted) and multivariable (adjusted for all variables) logistic regression models.

*Transformed variable

Table 7

Association of leukocyte count, other inflammatory markers, and other variables with urinary 11-dehydro thromboxane B2 before and after aspirin

Variable	Before Aspirin Beta ± SE (P-value)		After Aspirin Beta ± SE y(P-value)	
	univariable	multivariable	univariable	multivariable
Total leukocyte count (×10^3 cells/μL)	**0.1051 ± 0.0174 (<0.0001)**	**0.0686 ± 0.0241 (0.0046)**	**0.0726 ± 0.0133 (<0.0001)**	**0.0375 ± 0.0187 (0.0462)**
Age (years)	**0.0101 ± 0.0024 (<0.0001)**	**0.0096 ± 0.0033 (0.0041)**	**0.0090 ± 0.0022 (<0.0001)**	**0.0059 ± 0028 (0.0343)**
Male sex	−0.1425 ±0.0598 (0.0176)	−0.0380 ±0.0846 (0.6530)	−0.0798 ±0.0517 (0.1231)	−0.0834 ±0.0796 (0.2951)
African American race	0.0291 ± 0.0705 (0.6802)	−0.1081 ±0.0903 (0.2320)	−0.0218 ±0.0632 (0.7307)	−0.0993 ±0.0800 (0.2153)
Hypertension	0.1502 ± 0.0727 (0.0392)	0.0524 ± 0.0956 (0.5838)	0.2466 ± 0.0586 (<0.0001)	0.1320 ± 0.0800 (0.0999)
Diabetes	0.2737 ± 0.1274 (0.0322)	0.1066 ± 0.1367 (0.4359)	0.2280 ± 0.0914 (0.0126)	0.0796 ± 0.1013 (0.4320)
Current smoking	**0.3850 ± 0.0756 (<0.0001)**	**0.3478 ± 0.0872 (0.0001)**	**0.2480 ± 0.0637 (0.0001)**	**0.1990 ± 0.0783 (0.0113)**
Total cholesterol (mg/dl)	0.0005 ± 0.0007 (0.4812)	−0.0010 ±0.0008 (0.1771)	0.0001± 0.0007 (0.9204)	−0.0008 ±0.0007 (0.2724)
Body mass index (kg/m2)	0.0156 ± 0.0042 (0.0002)	0.0059 ± 0.0054 (0.2746)	0.0128 ± 0.1123 (0.0003)	0.0001 ± 0045 (0.9900)
C-reactive protein (mg/L*)	0.1022 ± 0.0219 (<0.0001)	0.0420 ± 0.0303 (0.1662)	0.0682 ± 0.0179 (0.0002)	0.0142 ± 0.0252 (0.5723)
Interleukin-6 (ng/L*)	**0.1664 ± 0.0302 (<0.0001)**	**0.1061 ± 0.0340 (0.0019)**	**0.1140 ± 0.0222 (<0.0001)**	**0.0798 ± 0.0245 (0.0012)**
Fibrinogen (mg/dl)	0.0009 ± 0.0003 (0.0011)	−0.0002 ±0.0004 (0.5026)	0.0006 ± 0.0003 (0.0133)	0.00004±0.0003 (0.9117)
Hematocrit (%)	−0.0038 ±0.0092 (0.6772)	−0.0047 ±0.0122 (0.7008)	−0.0002 ±0.0079 (0.9773)	−0.0016 ±0.0120 (0.8910)
Platelet count (×10^3 cells/μL)	0.0011 ±0.0005 (0.0463)	−0.00003 ±0.0007 (0.9616)	0.0007 ±0.0005 (0.1204)	0.0003 ±0.0007 (0.6096)
Mean platelet volume (fl)	0.0284 ± 0.0421 (0.5005)	−0.0061± 0.0406 (0.8813)	−0.0052 ±0.0369 (0.8875)	−0.0228 ±0.0438 (0.6032)
Thromboxane-B2 (ng/10^8 platelets*)	**0.0860 ± 0.0348 (0.0137)**	**0.1453 ± 0.0378 (0.0001)**	**0.1186 ± 0.0269 (<0.0001)**	**0.1450 ± 0.0317 (<0.0001)**

Values in bold type denote significance in both univariable (unadjusted) and multivariable (adjusted for all variables) linear regression models.

*Transformed variable