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Platelet and leukocyte activation, atherosclerosis and inflammation in European and South Asian men

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Summary

Background—Increased platelet activation occurs in ischemic heart disease (IHD), but increased platelet activation is also seen in cerebrovascular atherosclerosis and peripheral artery disease. It is not clear therefore whether platelet activation is an indicator of IHD or a marker of generalized atherosclerosis and inflammation. South Asian subjects are at high risk of IHD, but little is known regarding differences in platelet and leukocyte function between European and South Asian subjects.

Methods—Fifty-four male subjects (age 49-79 years) had coronary artery calcification measured by multislice computed tomography (CT), aortic atherosclerosis assessed by measurement of carotid-femoral pulse wave velocity (aortic PWV), and femoral and carotid atherosclerosis measured by B-mode ultrasound. Platelet and leukocyte activation was assessed by flow cytometry of platelet-monocyte complexes (PMC), platelet expression of PAC-1 binding site and CD62P, and expression of L-selectin on leukocytes.

Results—Elevated circulating PMC correlated significantly with elevated aortic PWV and PMC were higher in subjects with femoral plaques. In contrast PMC did not differ by increasing coronary artery calcification category or presence of carotid plaques. Higher numbers of PMC were independently related to elevated levels of C-reactive protein (CRP), higher aortic PWV, hypertension and smoking in a multivariate model. Markers of platelet and leukocyte activation did not differ significantly by ethnicity.

Conclusions—Increased PMC are related to the extent of aortic and femoral atherosclerosis rather than coronary or carotid atherosclerosis. The association between elevated CRP and increased PMC suggests that inflammation in relation to generalized atherosclerosis may play an important role in PMC activation.

Keywords

atherosclerosis; monocyte; platelet; pulse wave velocity

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Disclosure of Conflict of Interests
The authors state that they have no conflict of interest.

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Introduction

Activation of platelets is thought to play a key role in the development, progression and stability of atherosclerosis [1,2]. Previous studies have observed increased platelet activation in patients with coronary artery disease or acute myocardial infarction [3-6]. However, some studies have also reported increased platelet activation in association with carotid atherosclerosis [7,8] and peripheral arterial disease [9,10] and it is not clear whether platelet activation is a consequence of atherosclerosis at a particular location (e.g. the coronary artery) or is due to the impact of generalized atherosclerosis and inflammation.

Despite the evidence linking platelet activation to atherosclerosis, there is limited evidence regarding ethnic differences in platelet activation and its relationship to atherosclerosis, and what evidence exists is inconsistent [11-14]. This relative lack of information is surprising because there are marked differences in risk of cardiovascular disease between different ethnic groups. For example, people of South Asian origin are at markedly elevated risk of ischemic heart disease (IHD) and stroke compared with individuals of European origin in the UK [15] and USA [16].

This study was undertaken to test two hypotheses: (i) that platelet/leukocyte activation would be more closely associated with aortic/femoral atherosclerosis than carotid or coronary atherosclerosis; and (ii) that platelet/leukocyte activation would be increased in subjects of South Asian origin compared with those of European ethnicity across the spectrum of atherosclerotic disease.

Methods

Study design and patient recruitment

The study was conducted as a nested sub-study within the Peripheral ARtery disease in South Asian and European men with Coronary artery disease (PARSEC) study, which was designed to assess relationships between peripheral arterial disease and coronary artery disease in people of European and South Asian descent [17]. The study population was recruited from two sources: the first consisted of a male population sample drawn from a family practice register in north-west London. Over 97% of individuals are registered with such family practices [18]. Men aged 45 years and above (European, $n = 41$, and South Asian, $n = 43$), were invited to participate. As levels of atherosclerotic disease were anticipated to be low in a sample drawn from the population, this group was enriched with men who had known coronary artery disease established by angiography. These individuals (European, $n = 42$, and South Asian, $n = 41$) were recruited from cardiology clinics at St Mary's Hospital and Ealing Hospital in London, UK (the referral centres for the participating family practices). Men with coronary stents or atrial fibrillation were excluded, as these interfere with assessment of coronary calcification. Individuals with unstable angina, or other severe co-morbidity that restricted full participation, were also excluded from the study.

From the main study, a total of 64 men agreed to participate in a sub-study examining platelet and leukocyte function. Of these, 10 were excluded from the main analyses as they were receiving clopidogrel at the time of the study and this agent interferes with the measures of platelet activation used in this study ([19] and unpublished data). The data presented therefore pertain to the remaining 54 subjects (30 Europeans and 24 South Asians) whose characteristics are shown in Table 1. Both PARSEC and the sub-study were approved by the St Mary's Hospital Local Research Ethics committee and all participants gave informed consent to both studies.

Study investigations

Angina symptoms were ascertained by patients' self-completion of the Rose angina questionnaire [20]. Measures of height, weight and other anthropomorphic parameters were performed as previously described [17]. A resting ECG was also performed and sitting brachial systolic and diastolic blood pressure and heart rate (HR) were measured as the average of three readings made using a validated automatic BP device (Omron 705CP; Omron Healthcare Inc., Vernon Hills, IL, USA). Hypertension was defined as blood pressure > 140/90 mmHg or use of antihypertensive agents. Fasting bloods were also taken for quantification of serum glucose, total and high density lipoprotein (HDL) cholesterol, triglycerides and C-reactive protein (CRP). CRP was measured with a highly sensitive sandwich enzyme immunoassay, using rabbit anti-human CRP immunoglobulin as a catching and detecting antibody (Dako, Copenhagen, Denmark).

Assessment of atherosclerosis

Coronary calcification—All subjects underwent coronary computed tomography (CT) scanning using either a Philips M ×8000 4-detector multislice CT scanner, or a Philips M ×8000 IDT 16-detector multislice CT scanner. To ensure similar imaging between machines, identical scanning parameters were used in all subjects. Contiguous 3-mm sections covering the whole heart were acquired using prospective ECG gating during suspended inspiration. Scans were acquired with 120 kV, 140 mAs, field of view of 250 mm, and display matrix 512 × 512. Coronary artery calcification was quantified using proprietary software on a Philips MxView computer workstation, and calcification was defined as an area > $1mm^2$ of density > 130 Hounsfield Units (HU). The coronary artery calcification score was calculated as the sum of all lesion scores. Coronary calcification scores are highly skewed and therefore for the purposes of analysis subjects were subdivided into tertiles of coronary calcification score using cut-off points of 0-49, 50-400 and > 400 HU. A single observer (ARW) made all coronary calcification measurements. A repeatability study of 20 scans showed a mean difference ± SD of difference between cycles of 1 ± 10 HU.

Ultrasound investigations—B mode, color and pulsed Doppler measurements were performed in all subjects using an HDI 5000 ultrasound machine (Philips, Bothell, WA, USA) equipped with a linear array broad-band 7-4 MHz transducer. Atherosclerotic plaques (defined as focal thickenings of the intima-media > 1.3mm or a distinct area with an IMT > 50% thicker than the adjacent region) [21] were identified in the carotid and femoral artery using B mode ultrasound. Color Doppler and pulsed Doppler were also used to assist in confirmation of possible plaques.

Aortic PWV, an indicator of aortic atherosclerosis, was measured using ECG-gated pulsed Doppler, as previously described [22]. The reproducibility of PWV was studied in six subjects and the mean ± SD intra-observer difference was 0.39 ± 0.9 m s^{-1}.

Assessment of platelet and leukocyte activation

Peripheral venous blood was collected into siliconized Vacutainer tubes containing trisodium citrate (Becton Dickinson, Oxford, UK) by clear venepuncture with minimal stasis according to the European Working Group on Clinical Cell Analysis: Consensus Protocol for the Flow Cytometric Characterization of Platelet Function [23].

Platelet activation was assessed by measurement of platelet-monocyte complexes (PMC) and the platelet activation markers, PAC-1 binding and p-selectin (CD62P) in unstimulated whole blood. PAC-1 binding and CD62P were also measured following activation with adenosine diphosphate (10 μ_M; ADP) for 2 min. Measurement of L-selectin was performed only on unstimulated samples.

For measurement of PAC-1 binding and CD62P, samples of citrated whole blood were incubated with the following monoclonal antibodies (mAb): peridinin chlorophyll protein (PerCP)-conjugated anti-CD61, fluorescein isothiocyanate (FITC)-conjugated PAC-1 and phycoerythrin (PE)-conjugated anti-CD62P at room temperature for 15 min according to the manufacturers' recommendations. IgG_1-PE and IgG_1-FITC were utilized for non-specific binding isotype controls and the specific inhibitor of PAC-1 binding to the platelet surface, Arg-Gly-Asp-Ser peptide (RGDS; 10 mg mL^{-1}) was included in controls for PAC-1. Samples for platelet activation tests were then fixed with 0.1% paraformaldehyde (CellFIX, BD Biosciences, Oxford, UK) and kept in the dark at 4°C prior to analysis.

Samples for PMC were incubated with PE-conjugated anti-CD14 + FITC-conjugated anti-CD42a and samples for leukocyte activation studies were incubated with PE-conjugated anti-CD62L. PE-CD14 + FITC-IgG_1 and IgG_1-PE acted as controls for PMC studies and leukocyte studies, respectively. After incubation samples were treated with erythrocyte lysis solution (FACS Lysing Solution, Becton Dickinson) at room temperature for 15 min and then centrifuged (900 × *g* for 4 min) and washed with Cell Wash (BD Biosciences) twice. After the second wash the pellet was resuspended in 0.5 mL of Cell Wash. All samples were stored in the dark at 4°C until analysis, which was performed within 60 min of blood withdrawal.

Flow cytometric analysis

Samples were analyzed in a Becton Dickinson FACScan flow cytometer with CELLQUEST software (Becton Dickinson Immunocytometry Systems, Oxford, UK) by ARW (OD) masked to patient identity. The flow cytometer was calibrated regularly using BD FACSCOMP software with BD CaliBRITE beads. Platelets were identified on the basis of their light scatter characteristics and positive staining for the GPIIIa subunit of the GPIIb/IIIa complex (PerCP-CD61). Gating was set so that only fluorescence positive events were analyzed and platelets positive for PAC-1 binding and CD62P expression were calculated as the percentage of the CD61-positive population. PMC formation was quantified within the monocyte population as the percentage of events positive for both a platelet-specific marker, CD42a, and a monocyte marker, CD14. L-selectin expression, a marker of neutrophil activation, was quantified as the average mean fluorescence intensity (MFI). More than 5000 activation-independent platelet events were acquired for each assay and the coefficients of intra- and inter-assay variability were < 15%.

Reagents

ADP was purchased from Roche Applied Science (Indiana-polis, IN, USA); all mAb were purchased from BD Biosciences; and RGDS and other reagents were purchased from Sigma (Poole, UK).

Statistical analysis

Statistical power calculations were conducted using estimates of effect size based on previously published data [24,25], with a significance level of 5% and 80% power. On this basis, in order to detect a correlation ($r \geq 0.4$) between aortic PWV and PMC, CD62P or PAC-1 binding, a minimum total of 46 subjects was required. To detect a difference of ≥ 25% in PMC, CD46P and PAC-1 binding between subjects with and without carotid and femoral plaque, at least 34 subjects were required, and to detect an increase ≥ 15% in PMC, CD62P or PAC-1 binding at each level of coronary artery calcification category, a minimum of 45 subjects were required. All analyses were conducted using STATA (version 9.2, Stata Corp LP, College Station, TX, USA). Subject characteristics are presented as means ± SD and results as means (95% CI). Skewed data were log transformed prior to statistical analysis and are presented as medians (interquartile range). Statistical comparisons were

made using analysis of covariance with age as a prespecified covariate. Additional exploratory analyses were undertaken using multiple linear regression. A *P*-value of < 0.05 was considered statistically significant.

Results

Platelet/leukocyte activation and regional atherosclerosis

Circulating PMC correlated positively and significantly with aortic PWV (Fig. 1) and this relationship was also evident after statistical adjustment for age (Table 2). There were no significant relationships between PAC-1 binding, CD62P or L-selectin and aortic PWV (Table 2).

PMC were also significantly elevated in subjects with femoral plaques compared with subjects without femoral plaques (Table 3). PAC-1 binding, CD62P and L-selectin did not differ significantly between subjects with and without femoral atherosclerosis.

PMC and other markers of platelet activation did not differ with increasing coronary artery calcification category or with respect to presence of plaques in the carotid artery (Tables 3 and 4). L-selectin did not differ by coronary artery calcification category, but was reduced in subjects with carotid plaques.

Platelet/leukocyte measures and ethnicity—Subjects of South Asian origin had lower body mass index (BMI), increased HbA1c and were less likely to smoke, otherwise the two groups did not differ significantly (Table 1). Platelet/leukocyte measures did not differ significantly between the two ethnic groups (Table 5), although L-selectin tended to be higher in subjects of South Asian origin. Interethnic differences remained non-significant after further statistical adjustment for BMI, HbA1c and smoking (data not shown).

Predictors of elevated PMC

CRP was closely associated with increased PMC ($r = 0.44$, $P < 0.001$). Hypertension was also associated with an increase in PMC (normotensive = 28.6 ± 2.6, hypertensive = 39.0 ± 3.4, $P = 0.019$). Stepwise multivariate regression modeling (including age, total cholesterol, HDL-cholesterol, triglycerides, creatinine, fasting glucose, HbA1c, BMI, CRP, presence of femoral or carotid plaques, coronary artery calcification category, carotid and femoral IMT, known IHD, hypertension and diabetes status) showed that CRP, PWV, hypertension and smoking were independent significant predictors of PMC (Table 6), and after adjustment these accounted for 51% of the variance in PMC.

Discussion

This study shows that increased PMC, a sensitive measure of platelet activation *in vivo*, is closely associated with increased aortic and femoral atherosclerosis, but is not significantly related to the extent of carotid and coronary atherosclerosis. We also observed that increased CRP (along with aortic PWV, hypertension and smoking) was an independent predictor of elevated circulating PMC in multivariate analyses, suggesting that enhanced PMC formation could be related to increased inflammation in association with atherosclerosis.

Aortic PWV has been shown to correlate closely with aortic atherosclerosis [26,27] and to predict fatal and non-fatal cardiovascular events, including IHD and strokes[28-30], while the presence of femoral plaques on ultrasound is a good measure of lower limb atherosclerosis [31]. Our observations showing that PMC are related to these measures of aortic and femoral atherosclerosis suggest that formation of PMC may be an indicator of the overall burden of atherosclerosis rather than atherosclerosis in a particular territory. While

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atherosclerosis is a generalized disease affecting all territories [32], it is also well recognized that the burden of atherosclerosis in an individual varies substantially [33,34], with carotid and coronary atherosclerosis tending to show closer inter-associations. Such regional differences in atherosclerosis may account for the lack of association between PMC and carotid and coronary atherosclerosis seen in our study. The aorta and femoral arteries are likely to make a much greater contribution to total atherosclerosis load than the coronary and carotid arteries, as they have a greater surface area and atherosclerosis develops earlier and more extensively at these sites [35,36]. To explore possible mechanisms linking atherosclerosis to increased PMC we performed multivariate analyses. These analyses have limitations: the sample size is relatively small and therefore may be underpowered to identify weak predictors of PMC or interactions between covariates; the use of hypertension and smoking status as categorical variables is likely to underestimate blood pressure and smoking exposures. However, current blood pressure on treatment is probably an even less satisfactory measure of lifetime blood pressure exposure and other measures of smoking exposure (e.g. pack years and cotinine) were not available to us. Despite their limitations, these exploratory analyses do suggest that inflammation, as assessed by serum CRP, could play a role in mediating the increase in PMC seen in association with atherosclerosis.

Our observation of only weak and non-significant relationships between other markers of platelet activation, PAC-1 binding and CD62P, and atherosclerosis is consistent with previous studies suggesting that PMC may be a more sensitive marker of platelet activation than PAC-1 and CD62P *in vivo* [4,5,24,37]. Platelet activation is associated with an initial increase in CD62P [38] and this results in rapid binding of platelets to monocytes, neutrophils and endothelium via an interaction with a constitutively expressed receptor, PSGL-1. This process will deplete the number of circulating unbound CD62P-positive platelets. In addition, degranulated platelets rapidly lose surface P-selectin, although they continue to circulate and function [39]. Both these mechanisms are likely to result in a tendency for platelet CD62P to underestimate the extent of platelet activation.

Although some studies have shown that platelet activation is increased in IHD [3,4,6], findings have not been consistent [40,41] and, in keeping with our observations, another study found no relation between the extent of coronary disease and platelet microparticles, although platelet microparticles were increased in patients with IHD compared with healthy controls[6]. Previous studies have also found conflicting evidence of increased platelet activation in subjects with carotid atherosclerosis [8,42,43]. Possible explanations for differences between studies include methodological differences in assessment of platelet activation status, the impact of ischemia or plaque instability on platelet activation and, on the basis of our data, variability in the overall extent of atherosclerotic disease.

It is possible that differential use of medication (e.g. statins, anti-platelet drugs other than clopidogrel) could have influenced our results. Use of aspirin and statins was predictably more common in subjects with carotid, femoral and coronary artery disease, but comparison of PMC in those receiving statin or aspirin showed no statistically significant difference in PMC (data not shown). While we cannot exclude an effect of therapy on PMC, it seems unlikely to account for our findings.

We also found that markers of platelet and leukocyte activation did not differ significantly by ethnicity and while our sample size is relatively small our observations do not suggest that there are major differences in platelet activation that are likely to account for the marked differences in risk of myocardial infarction and stroke in South Asian individuals.

In conclusion, increased circulating platelet-monocyte activation is closely related to the extent of aortic and femoral atherosclerosis rather than coronary or carotid atherosclerosis.

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The relationship between elevated CRP and increased platelet-monocyte activation suggests that inflammation in the context of generalized atherosclerosis may play an important role in this process.

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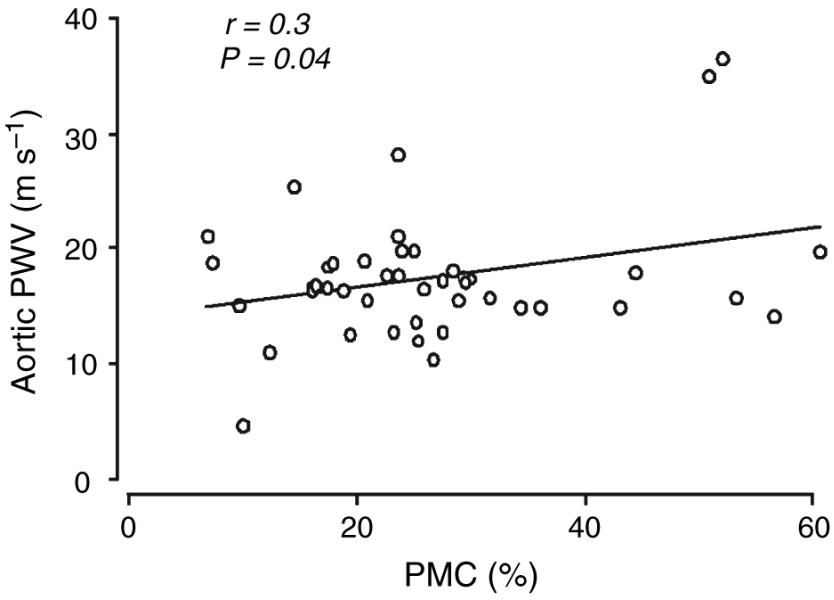

Fig. 1.
Relationship between platelet-monocyte complexes and carotid-femoral pulse wave velocity (aortic PWV).

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Table 1

Subject characteristics

Parameter	All subjects (*n* = 54)	Europeans (*n* = 30)	South Asians (*n* = 24)	*P*-value
Age, years	64.3 ± 7.7	65.3 ± 7.2	63.0 ± 8.1	0.3
BMI, kg m^{-2}	27.2 ± 3.8	28.7 ± 3.9	25.3 ± 2.6	< 0.001
Systolic blood pressure, mmHg	140.3 ± 15.1	142.6 ± 15.6	137.4 ± 14.1	0.2
Diastolic blood pressure, mmHg	83.2 ± 8.4	84.3 ± 8.1	81.9 ± 8.7	0.3
Fasting glucose, mmol L^{-1}	5.9 ± 1.4	5.9 ± 1.2	5.8 ± 1.7	0.8
HbA1c, %	6.0 ± 0.9	5.8 ± 0.6	6.3 ± 1.1	0.048
Total cholesterol, mmol L^{-1}	4.3 ± 1.0	4.2 ± 1.0	4.4 ± 1.1	0.7
HDL cholesterol, mmol L^{-1}	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	0.9
Fasting triglyceride, mmol L^{-1}	1.2 (0.9–1.7)	1.2 (0.9–1.8)	1.2 (1.0–1.7)	0.7
CRP, mmol L^{-1}	1.9 (0.9–3.76)	2.1 (1.1–4.8)	1.3 (0.9–3.0)	0.08
Coronary calcification, Hounsfield Units	214 (12–768)	241 (35–697)	134 (12–768)	0.3
Aortic PWV, m s^{-1}	17.5 ± 5.3	16.8 ± 5.5	18.3 ± 5.1	0.3
Femoral plaque, *n* (%)	28 (52)	18 (60)	10 (42)	0.2
Carotid plaque, *n* (%)	13 (24)	9 (30)	4 (17)	0.3
Ischemic heart disease, *n* (%)	27 (50)	14 (47)	13 (54)	0.6
Diabetes mellitus, *n* (%)	9 (17)	3 (10)	6 (26)	0.1
Hypertension, *n* (%)	20 (37)	8 (27)	12 (50)	0.08
Smokers, *n* (%)	26 (55)	19 (68)	7 (37)	0.04
Subjects on statins, *n* (%)	28 (52)	16 (53)	12 (50)	0.8
Subjects on aspirin, *n* (%)	30 (56)	18 (60)	12 (50)	0.5

BMI, body mass index; HDL, high density lipoprotein; CRP, C-reactive protein; PWV, pulse wave velocity.

Data are means ± SD, medians (interquartile range) or frequency (%). *P*-value is derived from a Student's *t*-test comparing the two ethnic groups.

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Table 2

Relationship of platelet leukocyte measures to aortic pulse wave velocity

	β coefficient (SE)	*P*-value
PMC	0.92 (0.39)	0.02
PAC-1	0.40 (0.40)	0.3
PAC-1 + ADP	0.26 (3.5)	0.9
CD62P	−0.01 (0.03)	0.8
CD62P + ADP	0.56 (0.31)	0.08
CD61	0.20 (0.14)	0.14
L-selectin	−2.3 (2.5)	0.4

PMC, platelet-monocyte complexes; PAC-1, PAC-1 binding.

Data are regression (β) coefficients and SE of age-adjusted data.

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Table 3

Platelet and leukocyte measures in subjects with and without femoral and carotid plaques

Femoral (%)	**Without plaque (*n* = 26)**	**With plaque (*n* = 28)**	***P*-value**
PMC	21.8 (18.1–25.5)	30.0 (23.7–36.3)	0.04
PAC-1	14.4 (8.7–20.1)	18.4 (13.1–23.7)	0.3
PAC-1 + ADP	82.0 (75.7–88.3)	84.3 (78.0–90.6)	0.6
CD62P	1.7 (1.3–2.1)	2.0 (1.6–2.4)	0.4
CD62P + ADP	81.4 (77.1–85.7)	82.9 (78.6–87.2)	0.8
L-selectin (mean fluorescence intensity)	201 (169.6–232.4)	195 (165.6–224.4)	0.8
Carotid (%)	**Without plaque (*n* = 41)**	**With plaque (*n* = 13)**	
PMC	27.1 (22.6–31.6)	22.2 (14.6–29.8)	0.3
PAC-1	14.3 (10.0–18.6)	23.1 (15.5–30.7)	0.06
PAC-1 + ADP	82.1 (77.0–87.2)	86.3 (77.7–94.9)	0.4
CD62P	2.0 (1.6–2.4)	1.5 (0.9–2.1)	0.2
CD62P + ADP	76.2 (70.1–82.3)	82.2 (72.0–92.4)	0.3
L-selectin (mean fluorescence intensity)	210.6 (187.3–233.9)	153.5 (112.5–194.5)	0.02

PMC, platelet-monocyte complexes; PAC-1, PAC-1 binding.

Data are means (95% CI) of age-adjusted data. *P*-values were calculated by ANCOVA.

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Table 4

Platelet and leukocyte measures by coronary artery calcification category

	Lowest CAC category (17)	Middle CAC category (15)	Highest CAC category (22)	*P*-value
PMC	26.8 (20.1–33.5)	25.9 (22.0–29.8)	25.0 (18.9–31.1)	0.7
PAC-1	15.2 (8.7–21.7)	16.4 (12.5–20.3)	17.7 (11.8–23.6)	0.6
PAC-1 + ADP	86.6 (79.2–94.0)	83.5 (79.2–87.8)	80.5 (74.0–87.0)	0.3
CD62P	1.7 (1.1–2.3)	1.8 (1.4–2.2)	2.0 (1.6–2.4)	0.4
CD62P + ADP	85.5 (80.2–90.8)	82.6 (79.7–85.5)	79.7 (75.4–84.0)	0.14
CD62L	181 (146–216)	197 (175–219)	212 (181–243)	0.2

PMC, platelet-monocyte complexes; PAC-1, PAC-1 binding.

Data are means (95% CI) of age-adjusted data. *P*-values were calculated by ANCOVA.

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Table 5

Platelet and leukocyte measures in European and South Asian men

	European (30)	South Asian (24)	*P*-value
PMC	24.2 (18.9–29.5)	27.7 (22.0–33.4)	0.4
PMC + ADP	34.7 (27.4–42.0)	38.2 (30.4–46.0)	0.5
PAC-1	16.7 (11.4–22.0)	16.2 (10.3–22.1)	0.9
PAC-1 + ADP	84.4 (78.5–90.3)	81.6 (75.1–88.1)	0.5
CD62P	1.8 (1.4–2.2)	2.0 (1.6–2.4)	0.5
CD62 + ADP	80.5 (76.2–84.8)	83.9 (79.6–88.2)	0.3
CD62L	181 (152–210)	218 (187–249)	0.09

PMC, platelet-monocyte complexes; PAC-1, PAC-1 binding.

Data are means ± SEM of age adjusted data. *P*-values were calculated by ANCOVA.

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Table 6

Independent predictors of platelet-monocyte complexes (PMC)

Variable	Std. Beta	β coefficient (SE)	*P*-value
C-reactive protein (CRP)	0.54	0.72 (0.17)	< 0.001
Aortic pulse wave velocity (PWV)	0.43	0.96 (0.28)	0.002
Hypertension	0.39	10.3 (3.7)	0.009
Smoking	0.30	7.9 (3.3)	0.023
Constant		54.0 (14.5)	0.001

Adjusted $r^2 = 0.51$; $P < 0.001$.

Age, serum total cholesterol, high density lipoprotein cholesterol, triglycerides, creatinine, fasting glucose, HbA1c, body mass index, CRP, presence of femoral or carotid plaques, coronary artery calcification category, carotid and femoral intima media thickness, aortic PWV, diabetes, hypertension and smoking status were entered into a forward stepwise regression model. The significance level for retention in the model was set at $P = 0.05$. Data shown are the standardized beta coefficients (Std. beta), the regression coefficient (β coefficient) with SE and the relevant *P*-value.