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Reduced endothelial progenitor cells in European and South Asian men with atherosclerosis

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Abstract

Background—Circulating endothelial progenitor cells (EPCs) play a role in the repair and regeneration of the endothelium and may represent a novel cardiovascular risk factor. South Asian subjects have an increased risk of cardiovascular disease which is not fully explained by known risk factors. This study examined associations of EPCs with atherosclerosis and possible ethnic differences in EPCs.

Materials and methods—A population sample of 58 European and South Asian adult men was enriched with the recruitment of an additional 59 European and South Asian men with known coronary disease. The coronary artery calcification score was measured by multi-slice computerized tomography (CT), carotid and femoral intima-media thickness (IMT), and femoral plaques were measured by ultrasound. The subjects were further subdivided into three categories of coronary artery disease on the basis of coronary artery calcification score and clinical history. Total EPCs and non-senescent EPCs (ns-EPCs) were quantified after 5 days cell culture and the number of late outgrowth colonies was measured over a 6-week test period. Circulating CD34+ haematopoietic precursor cells were measured by flow cytometry.

Results—Individuals with femoral plaques had reduced total and ns-EPCs. The number of ns-EPCs were reduced in individuals with the most coronary atheroma and were inversely related to the coronary calcification score and femoral IMT. These relationships persisted after multivariate adjustment for other risk factors. The numbers of late outgrowth colonies or circulating CD34+ cells were unrelated to the presence of atherosclerosis. There were no differences in the number of EPCs between European and South Asian subjects.

Conclusion—The number of EPCs are reduced in subjects with atherosclerosis independent of other risk factors. Reduction in EPC numbers may be an independent risk factor for atherosclerosis but does not explain ethnic differences in cardiovascular risk.

Keywords

Atherosclerosis; coronary calcification; endothelial progenitor cells; ethnicity; intima-media thickness

Introduction

The key role of endothelial damage in atherosclerosis and cardiovascular disease (CVD) is well recognized [1]. Although mechanisms of endothelial damage have been extensively studied, less is known regarding endothelial regeneration and repair [2]. Recent data suggest that circulating endothelial progenitor cells (EPCs) may be important in this process [3]. The majority of EPCs have limited proliferative capacity in culture [4], but can secrete a variety of angiogenic factors which may be important to angiogenesis and endothelial repair *in vivo*.

Studies in populations of largely European origin have demonstrated that reduced numbers of EPCs and/or function is associated with increased CVD risk factors [5,6] and endothelial dysfunction [6], but the relationship of EPC numbers to direct measures of atherosclerosis, such as coronary calcification or intima-media thickness (IMT), has not been examined. Moreover, ethnic differences in EPC numbers have not been investigated. This may be important in understanding any ethnic differences in CVD risk. For example, people of South Asian origin, i.e. from the Indian subcontinent, have a 1.5-fold increased risk of coronary disease compared with white Europeans [7] which cannot be fully explained by conventional risk factors [8].

The objectives of this study were to establish whether the number and viability of EPCs were proportionally related to the degree of atherosclerosis and if there were ethnic differences in these relationships.

Materials and methods

Study design and recruitment

This study was performed as a sub-study of a larger study to examine the relationship of coronary and peripheral arterial disease in a bi-ethnic population [9]. For the main study, a population sample of South Asian ($n = 43$) and European men ($n = 41$) aged ≥ 45 years was drawn from a family general practice register in north-west London (registration with a family practitioner is free and provides access to health care services; virtually 100% of the population is registered). Name recognition was used to identify migrants of South Asian origin (i.e. from India, Pakistan or Bangladesh) in the UK. However, a random population sample would provide a large number of men with little or no coronary artery disease (CAD), but few men with severe disease, so the study population sample was enriched with a similar number of men with CAD established by angiography. This allowed the study to examine relationships across a range of coronary disease (from none to severe). South Asian ($n = 41$) and European ($n = 42$) men with known CAD were recruited from cardiology clinics at St. Mary's Hospital and Ealing Hospital, London, UK. The patients came from the same communities and geographical location from which the population sample was drawn.

From the main study, 117 men (70%) of the total sample ($n = 167$) agreed to participate in a sub-study which involved an additional blood sample to measure EPCs and the data from this sub-study is reported (57 Europeans and 60 South Asians).

Men with coronary stents or atrial fibrillation were excluded, as these interfere with the assessment of coronary calcification. Individuals with unstable angina, or other severe comorbidity, which restricted full participation were also excluded from the study. None of the subjects had undergone recent surgery or had trauma which might influence EPC numbers [10].

Both the main study and the sub-study were approved by the St. Mary's Hospital local Ethics Committee and all participants gave separate informed consent to both studies.

Study investigations

In addition to baseline demographic data, sitting brachial systolic (SBP) and diastolic blood pressure (DBP) and heart rate (HR) were recorded as the mean of three readings using a validated automatic blood pressure device (Omron 705CP; Omron Healthcare UK Ltd, Milton Keynes, UK). A resting electrocardiogram (ECG) was also performed. All subjects completed a Rose Angina questionnaire [11] and an Edinburgh Claudication questionnaire [12]. Fasting blood samples were also taken for isolation of EPCs and related cells, quantification of glucose, total and high-density lipoprotein (HDL) cholesterol, triglycerides, insulin and creatinine. Insulin was measured using an ultrasensitive specific simultaneous enzyme-linked immunosorbent assay (ELISA) assay using two high affinity monoclonal antibodies in excess (Mercodia AB, Uppsala, Sweden). Assay cross-reactivity with pro-insulin was < 0.01%, and with C-peptide < 0.01%. The C-reactive protein (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). The estimated glomerular filtration rate (eGFR) was calculated according to the Modification of Diet in Renal Disease Study Group (MDRD) equation, $eGFR = 186 \times (\text{creatinine}/88.4)^{-1.154} \times (\text{age})^{-0.203}$ [13].

Assessment of atherosclerosis

Coronary calcification—All the subjects underwent coronary computerized tomography (CT) scanning using either a Philips Mx8000 4-detector multi-slice CT scanner or a Philips Mx8000 IDT 16-detector multi-slice CT scanner (Phillips Electronics UK Ltd, Guildford, UK). Identical scanning parameters were used for all subjects. Contiguous 3-mm wide sections covering the whole heart were acquired using prospective ECG gating during suspended inspiration. Scans were acquired at 120 kV, 140 mA, 250-mm field-of-view and 512×512 display matrix. Coronary artery calcification was quantified using proprietary software on a Philips MxView computer workstation and calcification was defined as an area $> 1 \text{ mm}^2$ of density > 130 Hounsfield Units (HU). The coronary artery calcification score was calculated as the sum of all lesion scores. As the distributions of coronary artery calcification scores in the group were highly-skewed, subjects were also categorized into three coronary atheroma groups on the basis of known clinical history and coronary calcification score to give an alternative measure of coronary atheroma for the purposes of analyses. The coronary atheroma category (CAC) was classified as follows:

- mild, no history of CAD and CAC score < 49 HU,
- moderate, no history of CAD and $50 < \text{CAC score} < 400$ HU, and
- severe, history of CAD or CAC score ≥ 400 HU.

A single observer made all the coronary calcification measurements. A repeatability study of 20 scans showed a mean difference $\pm$ SD of difference between cycles of 1 ± 10 HU.

Ultrasound investigations

The B-mode, colour and pulsed Doppler measurements were performed in all subjects using a HDI 5000 ultrasound machine (Philips, Bothell, WA) equipped with a linear array broadband 7-4 MHz transducer. Carotid IMT was measured in a 10-mm segment of the far wall of the right and left common carotid artery (CCA) proximal to the carotid bulb, as previously described [14]. Femoral IMT was measured in a 15-mm segment of the far wall of the right and left common femoral artery (CFA) proximal to the bifurcation [14]. The B-mode images of CCA and CFA from five consecutive cardiac cycles were saved to PC as cine-loops for subsequent off-line measurement of IMT and lumen diameter using a validated semi-

automatic program [15]. Measurements from the right and left CCA and CFA, respectively, were averaged to give estimates of far-wall IMT in the CCA and the CFA.

Atherosclerotic plaques (defined as focal thickenings of the intima-media > 1.3 mm, or a distinct area with an IMT > 50% thicker than the adjacent region) were identified in the common femoral artery using B-mode ultrasound. colour Doppler and pulsed Doppler were also used to assist in the confirmation of possible plaques. A single observer made all the measurements and the mean difference $\pm$ SD difference of repeated measures of IMT made on separate occasions was 0.012 ± 0.059 mm ($n = 15$).

Isolation and counting of total and senescent EPCs

Mononuclear cells were isolated from a fasting sample of 20-mL citrated peripheral venous blood by density centrifugation using histopaque-1077 at room temperature, as described previously [5]. Cells were seeded at a density of 2×10^6 cells well⁻¹ onto 13-mm cover-slips which had previously been coated overnight at 4 °C with fibronectin ($5 \mu\text{g cm}^{-2}$) in 24-well plates containing EBM-2 + EGM-2-MV-Single Quots medium with an addition of 10% foetal calf serum (FCS) and cultured at 37 °C in a humidified environment with 5% CO₂. After 2 days in culture, non-adherent cells were removed by washing with phosphate buffered saline (PBS) and fresh media was added. Subsequently, the medium was replaced daily. Total EPCs were quantified at 5 days as the number of cells which stained positively with both 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate-acetylated low-density lipo-protein (acLDL) and fluorescein isothiocyanate (FITC), labelled Ulex Europaeus Agglutinin I (ulex-lectin). Cells on cover-slips were incubated with acLDL ($2.4 \mu\text{g mL}^{-1}$) at 37 °C for 1 h and then fixed with 2% paraformaldehyde and stained with ulex-lectin ($10 \mu\text{g mL}^{-1}$) for 1 h. The number of dual-stained cells positive for both acLDL and ulex-lectin were counted per cover-slip. Total EPCs were estimated by multiplying the cells per cover-slip by the total number of isolated mononuclear cells. Overall, EPCs represented approximately 2% of the total mononuclear cells, as previously reported [16]. Senescence-associated β -galactosidase (SA- β -Gal) activity was also measured at 5 days as previously described [17]. Cells were fixed for 1 h in 0.5% glutaraldehyde and then permeabilized with 0.02% Nonidet P-40 (NP-40) per 0.1% sodium deoxycholate and stained overnight in the dark in a 1 mg mL⁻¹ solution of X-Gal substrate (5-bromo-4-chloro-3-indolyl-D-galactopyranoside) containing 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide and 2 mM magnesium chloride, together with 0.02% NP-40 at an acidic pH (6.0). The percentage of SA- β -Gal positive EPCs were counted and non-senescent EPCs (ns-EPCs) were calculated by subtracting SA- β -Gal-positive EPCs from total EPCs.

Quantification of late-outgrowth EPC colony forming ability

Late outgrowth colonies probably reflect bone-marrow derived endothelial precursors [18]. A late outgrowth EPC colony was defined as a colony rosette of > 100 polygonal cells appearing after 7 days of culture; colonies appearing before this time were excluded as they may have been derived from mature circulating endothelial cells [10]. The number of colonies were counted for each subject over a 6-week period after initial culture and the average number of colonies per cover-slip had been calculated.

Quantification of CD34+ haematopoietic progenitor cells

The CD34+ haematopoietic progenitor cells present in peripheral venous blood were measured in whole blood anti-coagulated with EDTA by flow cytometry after lysis of erythrocytes with ammonium chloride as previously described [19]. All samples were stored at 4 °C and processed within 12 h. Assays were performed in accordance with manufacturer's instructions using a commercial flow cytometry kit (BD Biosciences, San Jose, CA) with a FAC-Scan flow cytometer (Becton Dickinson, San Jose, CA) following

the guidelines for flow cytometric enumeration of CD34+ haematopoietic progenitor cells [20]. In brief, the technique employs a CD45 FITC/CD34 PE two-colour assay used in combination with TruCOUNT tubes containing fluorescently labelled beads. Counts were performed using the gating strategy as previously described [20]. Absolute counts of CD34+ were determined by relating CD34+ cellular events detected by flow cytometry to the number of fluorescent bead events present in the TruCOUNT tubes as described in the manufacturer's instructions.

Statistical analysis

Data were presented as means $\pm$ SD, or median (interquartile range), for skewed data. The skewed data were log or square-root transformed as appropriate before statistical analysis. The analyses were performed using Pearson's or Spearman's correlation, analysis of covariance (ANCOVA), logistic regression or a χ^2 -test as appropriate. Statistical analysis was performed using Stata 8.2 and Stata 9.2 (Stata-Corp LP, College Station, TX) and $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of subjects are shown in Table 1. South Asian men were younger, but tended to have a longer duration of coronary disease. They also had lower body surface area (BSA) and body mass index (BMI), reduced femoral IMT, increased prevalence of diabetes, increased glycosylated haemoglobin (HbA1c) and were less likely to be current smokers. An increased coronary atheroma category was associated with older-age, smoking, higher fasting glucose, increased creatinine and decreased eGFR, increased carotid and femoral IMT, increased presence of femoral plaques, diabetes and increased use of aspirin and cholesterol-lowering therapy (Table 2).

Relationship between EPCs and atherosclerosis

Total EPCs were lower in the highest CAC [severe = 5.4, (1.6-10.4); moderate = 6.4 (3.6-15.3); mild = 6.0 (1.4-10.1)] cells $\times 10^5$, but this was not statistically significant ($P = 0.13$). There was also a weak inverse relationship between coronary artery calcification and total EPCs ($r = -0.1$, $P = 0.4$). Total EPCs were significantly lower in those with femoral plaques (Fig. 1). There was a significant inverse relationship between total EPCs and femoral IMT ($r = -0.24$, $P = 0.02$) and this relationship was not affected by statistical adjustment for possible confounders, namely age, ethnicity, BMI, systolic BP, glucose, total cholesterol, HDL, triglycerides, insulin, eGFR, CRP, smoking, statin and aspirin. Total EPCs did not correlate significantly with carotid IMT ($r = 0.04$, $P = 0.6$).

The ns-EPCs were significantly reduced in the group with the highest CAC (Fig. 2). There was also a significant inverse relationship between ns-EPCs and coronary artery calcification score ($r = -0.23$, $P = 0.02$). The ns-EPCs were also significantly reduced in subjects with femoral plaques (Fig. 1) and the number of ns-EPCs were inversely related to femoral IMT ($r = -0.25$, $P = 0.013$). These relationships between ns-EPCs and measures of atherosclerosis were not significantly affected by adjustment for age, ethnicity, BMI, SBP, glucose, total cholesterol, HDL, triglycerides, insulin, eGFR, CRP, smoking, statin or aspirin use. The number of ns-EPCs was not significantly related to carotid IMT ($r = 0.05$, $P = 0.6$).

There was no statistically significant relationship of the number of colonies or CD34+ cell count with the coronary calcification group ($P = 0.9$ and $P = 0.3$, respectively), coronary calcification score ($P = 0.6$ and $P = 0.13$, respectively), femoral IMT ($P = 0.3$ and $P = 0.7$, respectively) or presence of femoral artery plaque ($P = 0.1$ and $P = 0.2$, respectively). However, CD34+ cell count was strongly related to eGFR ($r = 0.34$, $P = 0.007$) and inversely related to creatinine ($r = -0.29$, $P = 0.02$).

There was no significant difference in total or ns-EPCs between ethnic groups (Table 3). This lack of difference remained after statistical adjustment for age, CAC, femoral IMT, diabetes, BSA or BMI, or all these possible confounders combined. There was also no ethnic difference in the number of late outgrowth colonies or CD34+ cell counts (Table 3).

Discussion

There is increasing evidence that circulating EPCs contribute to endothelial repair, neovascularization and CVD [5,6,21] and that circulating EPCs may have therapeutic potential in CVD [3]. However, the relationship of EPCs with direct measures of atherosclerosis has not been studied. This study used coronary artery calcification and both carotid and femoral IMT as measures of atherosclerosis. Coronary artery calcification has been shown to predict coronary atherosclerosis in both European [22] and South Asian people [23] and correlates with total coronary atheroma assessed by histology [24]. Similarly, measurement of IMT is widely used as a measure of atherosclerosis in the carotid and femoral arteries [25]. Using these techniques, it was shown that EPCs measured after 5 days of cell culture were reduced in subjects with coronary and femoral atherosclerosis and patients with more advanced coronary disease had significantly fewer ns-EPCs. Other measures, such as the number of late outgrowth colonies or circulating CD34+ counts, were not related to different levels of disease, although CD34+ levels were reduced in individuals with decreased eGFR, as previously reported [26]. While these data indicate that the number of EPCs was reduced in advanced atherosclerosis they also suggest that different measures of EPCs are not synonymous. This observation has important implications for which aspects of EPCs number and function should be measured in future studies.

Previous studies of associations between the number of EPCs and conventional cardiovascular risk factors in populations of European origin are inconsistent. In one study of male volunteers, the number of EPC colonies was inversely related to a number of cardiovascular risk factors, such as cholesterol, diabetes and hypertension, but not smoking, and was also inversely related to endothelial function [6]. However, in another study of patients with known coronary disease, no such associations between number of EPCs or CD34+ cells with individual CVD risk factors were observed, except for smoking, which accounted for the association with overall cardiovascular risk [5].

In terms of documented atheromatous disease, again there are conflicting findings. In one study no difference was found in circulating EPCs and the number of bone marrow CD34+/CD133+ cells between healthy controls and patients with coronary disease [27], while a reduced number of circulating CD34+ cells and CD34+/CD133+ cells was observed in a further study [28].

Aside from any differences in populations studied, the assessment of risk factors and disease outcomes, small sample sizes and confounding by age and other risk factors, there are a number of possible reasons which could account for the above discrepancies. Peripheral blood CD34+ cells do not bear simple correspondence to endothelial precursors and comparisons with acLDL/ulex-lectin positive EPCs are not likely to give identical results [29]. Similarly, cells which form colonies account for only a small fraction of acLDL/ulex-lectin positive EPCs identified at 5 days of culture [4]. This has led to the suggestion that acLDL/ulex-lectin-positive EPCs should be termed circulating angiogenic cells rather than EPCs [4], as their role may be to secrete angiogenic factors rather than proliferate within the vascular wall [4,30]. A further complication being that, even for cells which form colonies, there is evidence that different hierarchies of EPCs exist, with some cells giving increases to small colonies (< 50 cells) and only a minority producing larger colonies [31]. In this study, a colony was defined as a cluster of > 100 cells and smaller clusters of cells were not

counted as colonies. This could account for differences between these observations and some other studies [6], but the intrinsic diversity of lineages within colony forming cells suggests that colony counting may have limitations as an approach to assess the proliferative capacity of EPCs. Myocardial ischaemia and systemic inflammation may also influence findings, as both have been associated with increased EPCs [19,32]. However, the study did not observe any relationship between a widely measured marker of inflammation, CRP and total EPCs nor ns-EPCs.

There were no differences in total or ns-EPCs, late outgrowth colony numbers or circulating CD34+ cells between European and South Asian subjects. There was no difference between the two ethnic groups even after statistical adjustment for differences in age, CAC and femoral IMT. This suggested that a difference in the number of EPCs was not the explanation for the markedly increased risk of coronary disease in South Asian people.

This study had a number of limitations. The methodology to measure EPCs is widely used, but, in addition to the limitations discussed above, it was not possible to completely exclude a contribution from circulating endothelial cells shed from the vascular wall to EPCs counts [33]. Any shed endothelial cells may be more prone to senescence and this could account, in part, for the somewhat weaker relationships between total EPCs number and atherosclerosis. The study sample was selected to ensure an adequate representation of the range of coronary atherosclerosis in two ethnic groups, but was not representative of the general population. Many of the subjects in this study were also receiving drugs (e.g. anti-hypertensive agents, statins or aspirin) which could affect the number of EPCs. While associations remained significant after statistical adjustment for these therapies, we cannot exclude some residual confounding. However, as statins have been reported to increase EPCs, it would appear unlikely that the increased use of statins in individuals with atherosclerosis could account for the reduction in ns-EPCs with increased atherosclerosis; rather, it may have resulted in an under-estimate of differences. In terms of possible ethnic differences in EPCs, as use of these agents did not differ between the two groups, we suggest this was not a major confounder.

In conclusion, this study has shown that acLDL/ulex-lectin-positive EPCs are reduced in individuals with coronary and lower limb atherosclerosis and that this is independent of other risk factors. The EPCs did not differ between Europeans and South Asian subjects and it is doubtful that differences in EPCs explain ethnic differences in CVD risk.

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Figure 1. Total (white) and non-senescent (grey) endothelial progenitor cells (EPCs) in subjects with (+) and without (-) femoral plaque. P -values calculated after square-root transformation of EPC values and age adjustment by ANCOVA.

Figure 2.

Non-senescent endothelial progenitor cells (ns-EPCs) by coronary atheroma category. ** $P=0.007$ by post-hoc test of groups 1 + 2 vs. group 3 following ANCOVA, $P=0.02$.

Table 1

Baseline characteristics of the study subjects

Variable	All subjects (n = 117)	Europeans (n = 57)	South Asians (n = 60)	P-value
Age (years)	62.6 ± 9.1	65.5 ± 8.3	59.9 ± 9.0	<0.001
SBP (mmHg)	138 ± 17	141 ± 17	135 ± 15	0.06
DBP (mmHg)	82 ± 9	82 ± 9	82 ± 8.7	0.9
Heart rate (b min ⁻¹)	64 ± 10	62 ± 10	65 ± 10	0.16
Fasting glucose (mmol L ⁻¹)	6.0 ± 1.7	5.7 ± 1.2	6.2 ± 2.0	0.13
HbA1c (%)	6.2 ± 1.0	5.8 ± 0.6	6.6 ± 1.2	<0.001
Total cholesterol (mmol L ⁻¹)	4.4 ± 1.0	4.4 ± 0.9	4.4 ± 1.0	0.9
HDL (mmol L ⁻¹)	1.11 (0.95-1.28)	1.11 (0.98-1.28)	1.11 (0.89-1.32)	0.4
Triglycerides (mmol L ⁻¹)	1.24 (0.92-1.8)	1.15 (0.90-1.81)	1.27 (0.95-1.80)	0.4
Creatinine (mmol L ⁻¹)	103 ± 22	104 ± 21	101 ± 22	0.4
eGFR (mL min ⁻¹ 1.73 m ²)	70.8 ± 14.8	68.6 ± 14.2	72.9 ± 15.0	0.12
Insulin (pmol L ⁻¹)	46.7 (30.1-79.9)	44.7 (29.1-68.1)	52.3 (34.6-88.3)	0.10
C-reactive protein (mg L ⁻¹)	1.63 (0.81-3.78)	1.95 (0.83-5.3)	1.28 (0.77-2.95)	0.049
Body surface area (m ²)	1.92 ± 0.19	2.00 ± 0.18	1.86 ± 0.16	<0.001
Body mass index (kg m ⁻²)	27.3 ± 3.7	28.2 ± 4.0	26.4 ± 3.17	0.01
Coronary calcification score (HU)	126 (11-556)	149 (13-601)	118 (8-554)	0.3
Carotid IMT (mm)	0.90 ± 0.25	0.91 ± 0.22	0.89 ± 0.27	0.7
Femoral IMT (mm)	1.96 ± 1.16	2.25 ± 1.11	1.68 ± 1.14	0.007
Femoral plaque (n) [%]	57 [49]	33 [58]	24 [41]	0.06
Diabetes (n) [%]	24 [21]	6 [11]	18 [30]	0.03
Hypertension (n) [%]	59 [50]	31 [54]	28 [47]	0.5
Coronary disease (n) [%]	58 [50]	25 [44]	25 [42]	0.97
Duration of coronary disease (years)	0.82 (0.11-9.86)	0.74 (0.00-5.44)	3.25 (0.43-11.4)	0.3
Cardiovascular disease (n) [%]	76 [65]	39 [68]	37 [62]	0.4
Current smoker (n) [%]	17 [15]	12 [21]	5 [8]	0.009
Statin use (n) [%]	68 [58]	32 [58]	36 [60]	0.7
Aspirin use (n) [%]	63 [54]	30 [53]	33 [55]	0.8

Continuous data are means ± SD, or median (interquartile range) for skewed data. Categorical data are frequency (percentages). Continuous data were compared using an unpaired Student's *t*-test after log transformation of skewed data and categorical data were compared by χ^2 -test.

DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; IMT, intima media thickness; SBP, systolic blood pressure.

Table 2

Significant correlates of increased coronary atheroma category

Continuous variable	r	P-value
Age (years)	0.4642	<0.001
Fasting glucose (mmol L ⁻¹)	0.2215	0.02
Creatinine (mmol L ⁻¹)	0.1860	0.04
eGFR (mL min ⁻¹ 1.73 m ²)	-0.2116	0.02
Carotid IMT (mm)	0.2478	0.007
Femoral IMT (mm)	0.553	<0.001
Categorical variable	χ^2	P-value
Femoral plaque	24.3	<0.001
Diabetes	14.4	0.006
Current smoker	14.0	0.007
Aspirin	23.7	<0.001
Statins	11.0	0.004

Data are correlation coefficients for continuous variables or Chi² for categorical variables.

eGFR, estimated glomerular filtration rate; IMT, intima media thickness.

Table 3

Endothelial progenitor cells and related cells in European and South Asian subjects

	Europeans (<i>n</i> = 57)	South Asians (<i>n</i> = 60)	<i>P</i> -value
EPCs ($\times 10^5$ cells)	5.1 (2.4-11.6)	6.39 (1.84-11.37)	0.8
Non-senescent EPCs ($\times 10^5$ cells)	1.07 (0.28-4.36)	1.82 (0.29-5.41)	0.3
CD34 count (cells μL^{-1})	3.62 ± 2.24	3.51 ± 2.63	0.6
Average colonies per cover-slip	4.8 ± 8.9	7.0 ± 12.7	0.4
Total cells isolated ($\times 10^6$ cells)	27.9 ± 8.4	27.9 ± 10.0	0.5

Data are means $\pm$ SD or median (interquartile range). Data were compared by an unpaired Student's *t*-test.

CD34, haematopoietic precursor cells; EPCs, endothelial progenitor cells.