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The CD14 C-260T single nucleotide polymorphism (SNP) modulates monocyte/macrophage activation in treated HIV-infected individuals

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Abstract

Background: HIV-infected individuals have an increased risk of cardiovascular disease (CVD). T-allele carriers of the CD14 C-260T single-nucleotide polymorphism (SNP) have reported increased expression of the LPS-binding receptor, CD14 and inflammation in the general population. Our aim was to explore the relationship of this SNP with monocyte/macrophage activation and inflammation and its association with sub-clinical atherosclerosis in HIV-infected individuals.

Methods: Patients with no pre-existing CVD risk factors on suppressive antiretroviral therapy were recruited from University Malaya Medical Centre, Malaysia (n = 84). The CD14 C-260T and TLR4 SNPs, Asp299Gly and Thr399Ile were genotyped and soluble(s) CD14 and sCD163 and high-sensitivity C-reactive protein, hsCRP were measured in plasma. Subclinical atherosclerosis was assessed by measuring carotid intima media thickness (cIMT). The association between CD14 C-260T SNP carriage and cIMT was assessed in a multivariable quantile regression model where a p-value of <0.05 was considered significant.

Results: We found the CD14 C-260T T-allele in 56% of the cohort and evidence of subclinical atherosclerosis in 27%. TT genotype was associated with higher sCD163 (p = 0.009) but only marginally higher sCD14 (p = 0.209) and no difference in hsCRP (p = 0.296) compared to CC/CT. In multivariable analysis, only Framingham risk score was independently associated with higher cIMT while lower sCD163 was trending towards significance. No association was found in TT-genotype carriers and cIMT measurements.

Conclusion: The CD14 C-260T SNP was associated with increased monocyte activation but not systemic inflammation or cIMT in this HIV-infected cohort with low CVD risk profile.

Keywords: HIV, Lipopolysaccharide, CD12 C-260T, Soluble CD14, Soluble CD163, Monocyte activation, C-reactive protein, Atherosclerosis, Carotid intima media thickness

Background

Persistent immune activation and inflammation have been well described in chronic HIV disease (reviewed in [1]) and have been associated with an increased risk of atherosclerosis in this population [2-6]. Many factors contribute to chronic immune activation including the translocation of microbial products including lipopolysaccharide (LPS) from damaged gut-associated lymphoid tissue sustained during early HIV disease [7,8]. Chronic elevation of LPS persists despite suppressive combination antiretroviral therapy (cART) [7,9] and may drive increased cardiovascular disease (CVD) risk in HIV-infected individuals [2,10] as has been found in HIV-negative populations [11-13].

CD14 is a co-receptor for LPS, and together with MD-2 binds to toll-like receptor 4 (TLR4) on monocytes and macrophages activating them (reviewed in [14]). There is also a soluble form for CD14 (sCD14) which is secreted by monocytes/macrophages following LPS stimulation and by liver cells as an acute-phase protein following stimulation by IL-6 [15]. Soluble CD14 plays an important role in

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LPS-mediated activation and injury of cells lacking membrane-bound CD14 including endothelial and epithelial cells [16].

A common polymorphism in the CD14 gene, C-260T has been associated with differential expression levels of CD14 on monocytes/macrophages [17-19]. The SNP (C → T) occurs in the promoter region of the CD14 gene and increases the binding affinity of specificity protein (Sp) transcription factors [20], leading to subsequent increases in CD14 production [20,21]. T vs C allele carriers have been found to have higher density of membrane-bound CD14 and circulating levels of sCD14 [18,19,22] and subsequent increased production of pro-inflammatory cytokines following LPS stimulation [19,21,23]. This in turn may influence the development and progression of atherosclerotic disease. Conversely, two non-synonymous SNPs in the TLR4 gene, Asp299Gly and Thr399Ile, have been associated with reduced cytokine responses following LPS stimulation in some studies [24,25] and the carriage of these SNPs may also modulate LPS-mediated responses.

In genotype-phenotype studies, the association between the C-260T single nucleotide polymorphism (SNP) and cardiovascular disease have been mixed with some studies reporting a positive association while others have not [18,22,26-31]. Two comprehensive meta-analyses have however suggested that this association may be more relevant in East Asians compared to Caucasians [32,33].

Given the association between LPS-mediated monocyte/macrophage activation and CVD in HIV-infected individuals [10,34,35], we hypothesised that the CD14 (C-260T) SNP would be associated with increased risk of CVD in an Asian cohort of HIV-infected individuals. To help better delineate the potential influence of this SNP from other metabolic processes known to increase CVD risk, the study was conducted in patients who reported no clinical history of CVD risk factors (IHD, diabetes, hypertension or dyslipidemia) at recruitment. Our aims were 1) to explore the relationship between the CD14 C-260T SNP on markers of monocyte/macrophage activation (including soluble(s) CD14 and sCD163) and inflammation (as measured by highly sensitive C-reactive protein (hsCRP) and 2) to assess the association between the CD14 C-260T SNP and subclinical atherosclerosis measured by carotid intima-media thickness (cIMT).

Methods

Study population
This was a sub-study of a clinic-based cohort that was established to study the prevalence of metabolic syndrome and subclinical atherosclerosis among HIV-infected individuals attending the University Malaya Medical Centre (UMMC), Malaysia. Participants in the sub-study were re-consented for genetic testing. The inclusion criteria were; receiving cART for a minimum duration of 6 months, no

evidence of symptomatic AIDS in the last 6 months, no pre-existing clinical history of CVD or other atherosclerotic disease including cerebrovascular or peripheral vascular disease, pregnancy, hypertension, diabetes mellitus, dyslipidemia and malignancies.

Subclinical atherosclerosis was assessed by measurement of cIMT with high-resolution ultrasonography (Philips IU-22, USA) at 6 sites; right and left mid common carotid arteries, carotid bifurcation and the proximal site of the internal carotid arteries. Measurements of cIMT from left and right arteries were pooled and mean values were used for each patient. Subclinical atherosclerosis was defined as a mean cIMT > 0.7 mm as previously described [36], the presence of plaque (focal echogenic structure with cIMT > 1.2 mm) or both. Additionally, screening for traditional cardiovascular risk factors including fasting blood glucose, lipid profile, anthropometric measurements, resting blood pressure and assessment of smoking status and family history for premature cardiovascular disease (CVD) were performed. Data on HIV-specific characteristics including HIV RNA, CD4 T-cell counts, antiretroviral drug history and history of co-infections were obtained from patient medical records.

The study was approved by the hospitals institutional review board (MEC 975.6).

Specimen collection, genotyping and measurement of immune activation markers
Whole blood was collected in EDTA-vacutainers. Peripheral blood mononuclear cells (PBMCs), plasma and neutrophils were isolated as previously described [37]. DNA was extracted using the Qiagen Blood and Tissue kit (Qiagen, Germany) from neutrophils and genotyped for the CD14 (rs2569190) and TLR4 (rs4986790 and rs2569190) SNPs at the Australian Genome Research Facility (AGRF) using the Sequenom MassArray platform. Markers of monocyte activation including sCD14 (R&D Systems, McKinley Place, MN) and sCD163 (Trilium Diagnostics, Bangor, ME) were measured by ELISA in plasma according to the manufacturer's instructions. The systemic inflammation marker, hsCRP was determined by immunochemiluminometric assay by the hospitals clinical diagnostic laboratory.

Statistical analysis
Categorical variables were summarized using frequency and percentage. Continuous variables were tested for skew using a Shapiro-Wilk test and summarized using mean and standard deviation (SD) or median and inter-quartile range (IQR) as appropriate. Chi-square analysis was used to test Hardy-Weinberg equilibrium (HWE). The influence of CD14 C-260T genotypes on markers of immune activation, inflammation and cardiovascular risk factors were compared using Mann Whitney U or

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chi-square test. A dominant genetic model of association was chosen as the primary model of analysis given prior reports of a higher odds of SNP association when using this model compared to a recessive model in cohorts of East Asian ethnicity [33]. Median quantile regression analysis was then used to assess the independent influence of the CD14 SNP on cIMT adjusting for traditional cardiovascular risk factors and HIV-related parameters. Using quantile regression to model median cIMT was preferred over simple regression of the mean secondary to significant skew in cIMT which was not able to be corrected using standard transformations (log-, square-root or inverse Gaussian transformations). As such the key underlying assumption for outcome variable normality required by regression models of the mean was not able to be satisfied. A quantile regression by comparison does not assume underlying normality and is thus far more robust to non-normal errors and outliers. Parameters were log transformed if they were significantly skewed or if their effect sizes (coefficients) were too small or clinically meaningless if left untransformed. Co-variates for the multivariable model were selected based on a semi-backwards/semi-frontwards/semi-*a priori* modelling approach where the *a priori* assumption was that covariates significant at a p < 0.20 level in the univariable model was considered a candidate predictor to be included in the multivariable analysis. All statistical analysis was performed using Stata version 12 (StataCorp, College Station, Texas).

Results

Cohort characteristics and distribution of CD14 (C-260T) SNP

Patients were identified from a pre-existing study assessing the prevalence of metabolic syndrome and subclinical atherosclerosis among HIV-infected individuals on cART. A total of 84 patients consented for genetic testing from the initial cohort of 126. Most patients were male with a median (IQR) age of 41 (36 -46) years (Table 1). All patients had received cART for a median (IQR) duration of 4 (1-8) years. The majority received non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens (88%) while 11% received a protease inhibitor (PI)-based regimen. Only one patient had prior exposure to the nucleoside reverse transcriptase inhibitor (NRTI), abacavir (1.1%) and three with a PI-based regimen (4%). The cohort in general had low CVD risk with the median (IQR) 10-year Framingham risk score of 5% (3–11). Twenty seven percent of the cohort had evidence of subclinical atherosclerosis defined by an increase in mean cIMT of >0.7 mm or the presence of plaque.

T-allele carriage for the CD14 (C-260T) SNP was 56% and the genotype distribution was in HWE (p = 0.718). The genotype call rate was 95.2%. We additionally genotyped the TLR4 Asp299Gly and Thr399Ile SNPs to address possible gene-gene interactions with the CD14

(C-260T) SNP but found only one patient heterozygous for the TLR4 SNPs while the remaining were wild type carriers. The TLR4 SNPs were therefore not considered in subsequent analysis.

Association between CD14 C-260T SNP and markers of monocyte activation and systemic inflammation

We assessed the influence of CD14 C-260T SNP on markers of monocyte activation and systemic inflammation. There was no significant difference in the concentration of sCD14 between CC/CT vs TT genotype carriers (p = 0.266) but TT genotype carriers had significantly higher sCD163 levels compared to CC/CT carriers (p = 0.008) (Table 2). The associations did not change when controlling for the effects of smoking, current CD4 T-cell counts, age and gender, factors previously shown to strongly influence monocyte/macrophage activation levels [38-40]. There was no significant difference in the median concentration of hsCRP in the two groups, however hsCRP was significantly correlated with levels of sCD14 (p = 0.030) and sCD163 (p = 0.022) (Figure 1). These associations remained unchanged when analysis was done using a recessive model (CC vs CT/TT, data not shown).

Association between CD14 C-260T SNP and increased cIMT

We next assessed if the CD14 C-260T SNP was independently associated with increased cIMT using a multivariate regression model adjusting for both HIV-specific clinical parameters and traditional cardiovascular risk factors. In univariable analysis, we found only increasing age and Framingham score were significantly associated with increased cIMT, while log current CD4 T-cell counts and log sCD163 levels were candidate predictors based on p-value thresholds set *a priori*, p < 0.20. In the multivariable model however, only higher Framingham risk score was independently associated with higher cIMT while there was a trend that lower log sCD163 levels was associated with increased cIMT (Table 3).

Discussion

This is the first study to assess the potential modifying effect of the CD14 C-260T SNP on monocyte activation and inflammation in HIV-infected individuals. In this clinic-based cohort of HIV-infected patients on cART who had a no clinical history of CVD, diabetes, hypertension and dyslipidemia at recruitment, 27% had evidence of sub-clinical atherosclerosis defined by increased cIMT >0.7 mm. The CD14 C-260T T-allele was the major allele carried in 56% of the cohort and consistent with the reported distribution among Asians in Hapmap. In a multivariable analysis, the CD14 C-260T SNP was however not associated with sub-clinical atherosclerosis as measured by cIMT in this treated HIV cohort.

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Table 1 Cohort characteristics according to the CD14 C-260T SNP distribution

Patient characteristics	CD14 C-260T genotype[#]			Total cohort (N = 84)*
	CC (n = 15)*	CT (n = 41)*	TT (n = 24)*	
Age, years	38 (32–48)	41 (37–45)	41 (36–48)	41 (36–46)
Gender, n (%) male	13 (86.7%)	33 (80.5%)	17 (70.8%)	67 (79.8%)
Ethnicity, n (%)				
- Malay	7 (46.7%)	7 (17.1%)	3 (12.5%)	19 (22.6%)
- Chinese	7 (46.7%)	28 (68.3%)	19 (79.2%)	56 (66.7%)
- Indian	1 (6.7%)	6 (14.6%)	2 (8.3%)	9 (10.7%)
Baseline CD4 T-cell count, cells/µl	30 (8–253)	106 (13–223)	60 (22–197)	63 (16–228)
Current CD4 T-cell count, cells/µl	300 (155–450)	386 (286–536)	468 (292–631)	387 (271–547)
Current CD4:CD8 ratio	0.36 (0.17–0.55)	0.54 (0.33–0.73)	0.59 (0.28–0.79)	0.51 (0.28–0.74)
Baseline HIV RNA, copies/ml	163693 (59611–559681)	137600 (50158–316679)	105659 (33069–404288)	136423 (52214–398660)
Duration on ARV, years	2 (1–6)	5 (3–10)	7 (2–10)	4 (1–8)
Current ARV regimen, n (%)				
- NNRTI-based	13 (86.7%)	36 (87.8%)	21 (87.5%)	74 (88.1%)
- PI-based	2 (13.3%)	5 (12.2%)	3 (12.5%)	10 (11.9%)
Previous abacavir use, n (%)		1 (2.4%)		1 (1.1%)
Previous PI use, n (%)		3 (7.3%)		3 (3.6%)
Positive hepatitis C antibody, n (%)	0 (0.0%)	0 (0.0%)	1 (4.2%)	2 (2.4%)
Positive hepatitis B surface antigen, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Smoking, n (%)				
- Never	9 (60.0%)	24 (58.5%)	12 (50.0%)	46 (54.8%)
- Former	2 (13.3%)	4 (9.8%)	4 (16.7%)	10 (11.9%)
- Current	4 (26.7%)	13 (31.7%)	8 (33.3%)	28 (33.3%)
Framingham risk score, %	5.85 (1.88–11.45)	5.05 (2.93–8.63)	4.75 (2.23–14.80)	5.00 (2.75–11.05)
Body mass index, kg/m^2	22.3 (19.8–25.1)	22.6 (21.4–25.4)	24.1 (20.8–26.2)	23.0 (21.2–25.5)
Family history CVD, n (%)	1 (6.7%)	7 (17.1%)	0 (0.0%)	8 (9.5%)
Diabetes, n (%)	0 (0.0%)	1 (2.4%)	2 (8.3%)	4 (4.8%)
Hypertension, n (%)	9 (60.0%)	14 (34.1%)	8 (33.3%)	32 (38.1%)
Fasting glucose, mmol/L	4.8 (4.5–5.5)	5.2 (4.9–5.5)	5.3 (4.7–5.8)	5.1 (4.7–5.6)
Total cholesterol, mmol/L	5.4 (4.2–5.8)	5.2 (4.7–6.2)	5.2 (4.8–5.6)	5.2 (4.6–5.8)
LDL, mmol/L	2.8 (2.5–3.6)	3.1 (2.5–3.9)	3.1 (2.6–3.5)	3.0 (2.5–3.6)
HDL, mmol/L	1.2 (0.9–1.5)	1.2 (1.0–1.5)	1.1 (0.9–1.3)	1.2 (0.9–1.4)
Triglycerides, mmol/L	2.0 (1.4–2.8)	1.8 (1.5–2.3)	2.1 (1.5–3.3)	1.9 (1.4–2.5)
Increased cIMT, (>0.7 mm), n (%)	3 (20.0%)	13 (31.7%)	6 (25.0%)	23 (27.4%)
TLR4 frequency, n (%)				
- TLR4 Asp299Gly (c.896A > G) (rs4986790)		1 (2.4%)		1 (1.1%)
- TLR4 Thr399Ile (c.1196C > T) (rs2569190)		1 (2.4%)		1 (1.1%)

*Data shown are median (IQR) unless otherwise stated; [#]4 patients did not have CD14 C-260T genotype data; ARV: antiretroviral therapy, NNRTI: non-nucleoside reverse transcriptase inhibitors, PI: protease inhibitors; CVD: cardiovascular disease; LDL: low-density lipoprotein; HDL: high-density lipoprotein, cIMT: carotid intima media thickness, TLR4: toll-like receptor 4.

We found that sCD163 was significantly higher in TT homozygous vs CC/CT carriers. Soluble CD163 is a hemoglobin-heptoglobin scavenging receptor that is shed from monocyte/macrophages following pro-inflammatory stimulation including LPS [41,42] and therefore considered a marker of monocyte activation. Functionally however, sCD163 has been described to have a role in attenuating inflammatory processes (reviewed in [43]). In HIV-infected individuals, sCD163 levels positively correlated with monocyte and T-cell activation and inversely with CD163

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Table 2 Comparison of markers of immune activation, inflammation and cardiovascular risk factors among the CD14 C-260T CC/CT vs TT carriers

	CC/CT (n = 56)*	TT (n = 24)*	p	p$^\Delta$
Immune activation and inflammatory markers				
Log sCD14	6.20 (6.11–6.28)	6.24 (6.15–6.30)	0.266§	0.188
Log sCD163	2.94 (2.84–3.07)	3.03 (2.97 3.20)	0.008§	0.013
Log hsCRP	-0.77 (-0.32 - -1.08)	-0.53 (-0.26 - -0.95)	0.296§	0.232
Cardiovascular risk factors				
Body mass index, kg/m^2	22.4 (20.9–25.2)	23.9 (20.6–26.0)	0.482§	
Fasting sugar, mmol/L	5.0 (4.6–5.5)	5.1 (4.6–5.8)	0.298§	
Total cholesterol, mmol/L	5.2 (4.6–6.0)	5.1 (4.8–5.6)	0.858§	
Triglyceride, mmol/L	1.8 (1.4–2.3)	2.0 (1.5–2.8)	0.239§	
HDL, mmol/L	1.24 (0.99–1.49)	1.12 (0.90–1.31)	0.087§	
LDL, mmol/L	2.9 (2.5–3.8)	3.1 (2.6–3.5)	0.978§	
Age, years	41 (36–46)	41 (36–48)	0.961§	
Male gender, %	82.1%	70.8%	0.257#	
Current smoker, %	30.4%	33.3%	0.792#	
Framingham risk score	5.05 (2.80–9.08)	4.75 (2.23–14.80)	0.925§	

*Data shown are median (IQR) unless otherwise stated; §p-value for Mann-Whitney U; #p-value for Chi-square test; $^\Delta$p-value adjusted for smoking status, current CD4 T-cell counts, age and gender.

expression on CD14 + CD16+ monocytes [44]. To date, we have not found any prior studies that have assessed the association between the CD14 C-260T SNP and sCD163 in the general population. Our findings that sCD163 are increased with the CD14 C-260T TT genotype implies that carriers of this genotype have increased monocyte activation.

Prior studies in HIV-infected individuals on cART found increased monocyte activation was an independent predictor of CVD [10,34,35,45-48] and mortality [49]. A recent study found that monocyte activation and not T-cell activation was strongly associated with markers of systemic inflammation and coagulation (IL-6, hsCRP and D-dimers) which has been shown to predict many serious non-AIDS events (SNAEs) and mortality in HIV-infected individuals [50]. Therefore in HIV-infected individuals, TT genotype carriers may be at a higher risk of SNAEs due to increased monocyte activation. Larger

prospective studies assessing the association of this SNP with more detailed characterisation of monocyte activation markers and clinical end-points will be needed to confirm this.

In this study, we found no significant difference in sCD14 levels in homozygous TT vs CC/CT carriers with and without adjusting for current CD4 T-cell counts, smoking status, age and gender. The lack of statistical significance could potentially be due to the small sample numbers in our study or due to the influence of other SNPs not measured in this study that could have additional modulating effects on sCD14 levels. A recent genome wide association study (GWAS) in 5000 older individuals (>65 years) identified 164 SNPs which were associated with sCD14 levels and responsible for approximately 33% of the phenotypic variance [13]. Some studies of the CD14 C-260T SNP in the general population have found T vs C allele carriers had increased
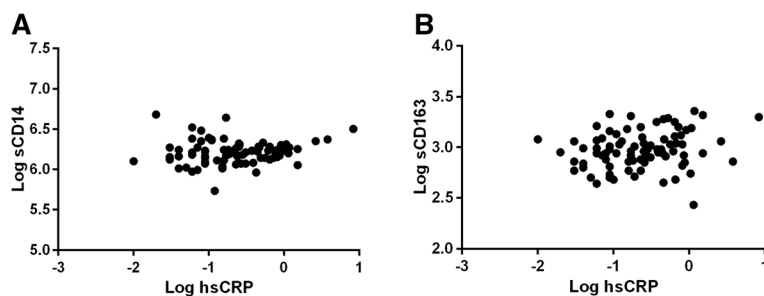

Figure 1 Correlation between systemic inflammation and monocyte activation markers. Correlation between levels of Log hsCRP and markers of monocyte activation, **(A)** Log sCD14 (p = 0.030, Spearman correlation) and **(B)** Log sCD163 (p = 0.022) measured in the cohort (n = 84).

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Table 3 Risk factors associated with carotid intima media thickness (cIMT) using univariable and multivariable models

Predictors	Co-efficient (95% confidence interval)	p-value
Univariable model		
Age, years	0.007 (0.004, 0.011)	<0.001*
Body mass index, kg/m^2	0.006 (-0.005, 0.017)	0.273
Total cholesterol, mmol/L	0.015 (-0.021, 0.051)	0.415
Triglyceride, mmol/L	0.003 (-0.029, 0.029)	0.985
HDL, mmol/L	-0.001 (-0.105, 0.103)	0.984
LDL, mmol/L	0.019 (-0.022, 0.060)	0.369
Fasting glucose, mmol/L	-0.017 (-0.053, 0.020)	0.366
Framingham score, %	0.011 (0.006, 0.016)	<0.001
Baseline CD4 T-cell counts, per 100 cells/μl	-0.011 (-0.044, 0.022)	0.527
Log baseline HIV RNA	-0.006 (-0.028, 0.016)	0.585
Log current CD4 T-cell counts	0.043 (-0.016, 0.104)	0.151
Log sCD14	0.020 (-0.083, 0.123)	0.700
Log sCD163	-0.054 (-0.135, 0.027)	0.190
Log hsCRP	0.0005 (-0.025, 0.026)	0.970
CD14 C-260T genotype; CC/CT vs TT	-0.013 (-0.099, 0.071)	0.758
Multivariable model		
Framingham score, %	0.009 (0.004, 0.014)	<0.001
Log current CD4 T-cell counts	0.038 (-0.033, 0.108)	0.289
Log sCD163	-0.084 (-0.182, 0.013)	0.087

*Age was omitted from the multivariable model given that it is one of the components used to derive the Framingham risk score.

membrane-bound CD14 and sCD14 [18,19,22] though not all studies have found this association [30,51]. *In vitro* studies have also found the T vs C allele was associated with increased inducible sCD14, TNFα and IL-6 production following LPS-stimulation of whole blood [19,21,23] but we did not measure these parameters in this study.

We did not find a significant difference in the median levels of hsCRP in TT vs CC/CT carriers. Increases in hs-CRP have been shown to correlate with the extent of vascular disease measured by the presence of vascular dysfunction in multiple vascular beds (carotid, coronary and brachial artery) [34]. Indeed, most studies that have found an association between hsCRP and the CD14 C-260T SNP [23,52,53] have so far been in populations that have already developed cardiovascular disease and unlike our study participants who had subclinical disease. This difference in cohort characteristic may have precluded our ability to show significant differences by genotype though hsCRP was significantly correlated with both sCD14 and sCD163 levels.

Only increasing age and Framingham risk score were significantly associated with cIMT in univariable analysis while log current CD4 T-cell counts and log sCD163 levels were candidate predictors. In the multivariable model however only increased Framingham risk score was significantly associated with increased cIMT while log sCD163 and log current CD4 T-cell count were not significantly associated with cIMT levels. Framingham risk score is a composite index of multiple established risk factors which are individually associated with CVD risks and therefore its association with cIMT in HIV-infected individuals is expected as previously found [54]. Prior studies in HIV-infected individuals have found sCD163 levels to be associated with non-calcified plaques [45,47], increased progression of cIMT [10] and coronary artery calcification [35], though not all studies have reported an association with CVD as we have found [55]. The exact role of sCD163 as a driver of atherosclerosis progression is unclear. Though sCD163 is a marker of increased monocyte activation, it has also been described to have an anti-inflammatory role by increasing the production of IL10 and inhibiting T-cell activation [56,57].

The CD14 C-260T SNP was not significantly associated with cIMT in this cohort. Prior studies have reported both significant [17-19,22] and non-significant associations [29,38,58] between the C-206T SNP and cIMT. Our failure to demonstrate a significant association if one truly existed could have been affected by the small sample size (power = 33%) and the low CVD risks in this cohort. Indeed, all prior studies in East Asians that have reported a significant association between the CD14 C-260T SNP and CVD were in populations of high CVD risks [33].

There were a number of important limitations in our study. First, we only genotyped the CD14 C260T SNP in this study while numerous other SNPs have recently been described to additionally modulate sCD14 levels [13]. In contrast to other SNPs associated with CVD, the CD14 C-260T SNP has been associated with multiple other inflammatory diseases [59-62] consistent with involvement in important non-redundant biological pathways. Second, many of our findings were probably confounded by the small sample numbers in our cohort. The recruitment in our study was dependent on patients re-consenting for genetic testing from an established clinic-based cohort of patients reporting no clinical history of CVD risk factors. We only had 33% power to show a significant association of cIMT with this SNP if one existed (at the 5% significance level, presuming a ratio of 1:1) taking into account the average increased attributable risk to the T-allele in non-HIV infected East Asians [33] and the baseline rate of events (defined as increased cIMT >0.7 mm) in C-allele carriers in our cohort.

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Conclusion

This is the first study to assess the relationship between the CD14 C-260T SNP and monocyte activation, inflammation and cIMT in HIV-infected individuals. We found that the CD14 C-260T SNP was associated with increased monocyte activation but not systemic inflammation or cIMT in this HIV-infected cohort with low CVD risk profile. Given that drivers of chronic immune activation persist despite cART and that increased monocyte activation has been associated with morbidity and mortality in HIV-infected patients on cART, the potential influence of this SNP in chronic HIV disease warrants further investigation.

Abbreviations

HIV: Human immunodeficiency virus; CVD: Cardiovascular disease; SNP: Single nucleotide polymorphism; UMMC: University Malaya Medical Centre; hsCRP: Highly sensitive C-reactive protein; sCD14: Soluble CD14; sCD163: Soluble CD163; TLR4: Toll-like receptor; cIMT: Carotid intima media thickness; cART: Combination antiretroviral therapy; LPS: Lipopolysaccharide; IQR: Inter-quartile range; NNRTI: Non-nucleoside reverse transcriptase inhibitor; NRTI: Nucleoside reverse transcriptase inhibitor; PI: Protease inhibitors; SNAE: Serious non-AIDS events; AIDS: Acquired immunodeficiency syndrome.

Competing interests

The authors declared no competing interests.

Authors' contribution

RR, SP and MYY conceived the study and participated in its design. YYK, RN and NA coordinated recruitment, processed samples and performed the immunoassays. TS, RR and MYY analysed and interpreted the data. RR, SL and AK wrote the manuscript. All authors read and approved the final manuscript.

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References

1. Lederman MM, Funderburg NT, Sekaly RP, Klatt NR, Hunt PW. Residual immune dysregulation syndrome in treated HIV infection. Adv Immunol. 2013;119:51–83.
2. Duprez DA, Kuller LH, Tracy R, Otvos J, Cooper DA, Hoy J, et al. Lipoprotein particle subclasses, cardiovascular disease and HIV infection. Atherosclerosis. 2009;207:524–9.
3. Tincati C, Bellistri GM, Casana M, Merlini E, Comi L, Bai F, et al. CD8+ hyperactivation and senescence correlate with early carotid intima-media thickness in HIV+ patients with no cardiovascular disease. J Acquir Immune Defic Syndr. 2009;51:642–4.
4. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. J Acquir Immune Defic Syndr. 2009;51:268–73.
5. Funderburg NT, Mayne E, Sieg SF, Asaad R, Jiang W, Kalinowska M, et al. Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. Blood. 2010;115:161–7.
6. Steele AK, Lee EJ, Vestal B, Hecht D, Dong Z, Rapaport E, et al. Contribution of intestinal barrier damage, microbial translocation and HIV-1 infection status to an inflammaging signature. PLoS One. 2014;9:e97171.
7. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12:1365–71.
8. Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, et al. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. J Infect Dis. 2009;199:1177–85.
9. Rajasuriar R, Booth D, Solomon A, Chua K, Spelman T, Gouillou M, et al. Biological Determinants of Immune Reconstitution in HIV-Infected Patients Receiving Antiretroviral Therapy: The Role of Interleukin 7 and Interleukin 7 Receptor α and Microbial Translocation. J Infect Dis. 2010;202:1254–64.
10. Kelesidis T, Kendall MA, Yang OO, Hodis HN, Currier JS. Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection. J Infect Dis. 2012;206:1558–67.
11. Dauphinee SM, Karsan A. Lipopolysaccharide signaling in endothelial cells. Lab Invest. 2006;86:9–22.
12. Wiedermann CJ, Kiechl S, Dunzendorfer S, Schratzberger P, Egger G, Oberhollenzer F, et al. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. J Am Coll Cardiol. 1999;34:1975–81.
13. Reiner AP, Lange EM, Jenny NS, Chaves PH, Ellis J, Li J, et al. Soluble CD14: genomewide association analysis and relationship to cardiovascular risk and mortality in older adults. Arterioscler Thromb Vasc Biol. 2013;33:158–64.
14. Triantafilou M, Triantafilou K. Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. Trends Immunol. 2002;23:301–4.
15. Bas S, Gauthier BR, Spenato U, Stingelin S, Gabay C. CD14 is an acute-phase protein. J Immunol. 2004;172:4470–9.
16. Pugin J, Schurer-Maly CC, Leturcq D, Moriarty A, Ulevitch RJ, Tobias PS. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc Natl Acad Sci U S A. 1993;90:2744–8.
17. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5′ flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. Am J Respir Cell Mol Biol. 1999;20:976–83.
18. Hubacek JA, Rothe G, Pit'ha J, Skodova Z, Stanek V, Poledne R, et al. C (−260)−>T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. Circulation. 1999;99:3218–20.
19. Eng HL, Wang CH, Chen CH, Chou MH, Cheng CT, Lin TM. A CD14 promoter polymorphism is associated with CD14 expression and Chlamydia-stimulated TNF alpha production. Genes Immun. 2004;5:426–30.
20. LeVan TD, Bloom JW, Bailey TJ, Karp CL, Halonen M, Martinez FD, et al. A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. J Immunol. 2001;167:5838–44.
21. Gu W, Dong H, Jiang DP, Zhou J, Du DY, Gao JM, et al. Functional significance of CD14 promoter polymorphisms and their clinical relevance in a Chinese Han population. Crit Care Med. 2008;36:2274–80.
22. Koenig W, Khuseyinova N, Hoffmann MM, Marz W, Frohlich M, Hoffmeister A, et al. CD14 C(−260)−>T polymorphism, plasma levels of the soluble endotoxin receptor CD14, their association with chronic infections and risk of stable coronary artery disease. J Am Coll Cardiol. 2002;40:34–42.
23. Rizzello V, Liuzzo G, Trabetti E, Di Giannuario G, Brugaletta S, Santamaria M, et al. Role of the CD14 C(−260) T promoter polymorphism in determining the first clinical manifestation of coronary artery disease. J Cardiovasc Med (Hagerstown). 2010;11:20–5.
24. Marsik C, Jilma B, Joukhadar C, Mannhalter C, Wagner O, Endler G. The Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms influence the late

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inflammatory response in human endotoxemia. Clin Chem. 2005;51:2178–80.

25. Montes AH, Asensi V, Alvarez V, Valle E, Ocana MG, Meana A, et al. The Toll-like receptor 4 (Asp299Gly) polymorphism is a risk factor for Gram-negative and haematogenous osteomyelitis. Clin Exp Immunol. 2006;143:404–13.

26. Unkelbach K, Gardemann A, Kostrzewa M, Philipp M, Tillmanns H, Haberbosch W. A new promoter polymorphism in the gene of lipopolysaccharide receptor CD14 is associated with expired myocardial infarction in patients with low atherosclerotic risk profile. Arterioscler Thromb Vasc Biol. 1999;19:932–8.

27. Arroyo-Espliguero R, El-Sharnouby K, Vazquez-Rey E, Kalidas K, Jeffery S, Kaski JC. CD14 C(−260) T promoter polymorphism and prevalence of acute coronary syndromes. Int J Cardiol. 2005;98:307–12.

28. Hermann M, Fischer D, Hoffmann MM, Gasser T, Quitzau K, Meinertz T, et al. CRP and CD14 polymorphisms correlate with coronary plaque volume in patients with coronary artery disease–IVUS substudy of the ENCORE trials. Atherosclerosis. 2012;220:172–6.

29. Koch W, Kastrati A, Mehilli J, von Beckerath N, Schomig A. CD14 gene -159C/T polymorphism is not associated with coronary artery disease and myocardial infarction. Am Heart J. 2002;143:971–6.

30. Haberbosch W, Unkelbach K, Schuster D, Gardemann A, Tillmanns H, Holschermann H. CD14 promoter polymorphism (− 159C–> t) is not associated with myocardial infarction or coronary artery disease in patients with assumed high genetic risk. Thorac Cardiovasc Surg. 2009;57:386–90.

31. Elghannam H, Tavackoli S, Ferlic L, Gotto Jr AM, Ballantyne CM, Marian AJ. A prospective study of genetic markers of susceptibility to infection and inflammation, and the severity, progression, and regression of coronary atherosclerosis and its response to therapy. J Mol Med (Berl). 2000;78:562–8.

32. Zhang HF, Zhong BL, Zhu WL, Xie SL, Qiu LX, Zhu LG, et al. CD14 C-260T gene polymorphism and ischemic heart disease susceptibility: a HuGE review and meta-analysis. Genet Med. 2009;11:403–8.

33. Pu H, Yin J, Wu Y, Zhang D, Wang Y, Zhou R, et al. The association between CD14 gene C-260T polymorphism and coronary heart disease risk: a meta-analysis. Mol Biol Rep. 2013;40:4001–8.

34. Longenecker CT, Jiang Y, Orringer CE, Gilkeson RC, Debanne S, Funderburg NT, et al. Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection. AIDS. 2014;28:969–77.

35. Baker JV, Hullsiek KH, Singh A, Wilson E, Henry K, Lichtenstein K, et al. Immunologic predictors of coronary artery calcium progression in a contemporary HIV cohort. AIDS. 2014;28:831–40.

36. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr. 2008;21:93–111. quiz 189–190.

37. Solomon A, Cameron PU, Bailey M, Dunne AL, Crowe SM, Hoy JF, et al. Immunological and virological failure after antiretroviral therapy is associated with enhanced peripheral and thymic pathogenicity. J Infect Dis. 2003;187:1915–23.

38. Amar J, Ruidavets JB, Bal dit Sollier C, Bongard V, Boccalon H, Chamontin B, et al. CD14 C(−260) T gene polymorphism, circulating soluble CD14 levels and arteriosclerosis. J Hypertens. 2004;22:1523–8.

39. Armah KA, McGinnis K, Baker J, Gibert C, Butt AA, Bryant KJ, et al. HIV status, burden of comorbid disease, and biomarkers of inflammation, altered coagulation, and monocyte activation. Clin Infectious Dis Off Publ Infectious Dis Soc Am. 2012;55:126–36.

40. Martin GE, Gouillou M, Hearps A, Angelovich TA ACC, Lynch F, Cheng WJ, et al. Age-associated changes in monocyte and innate immune activation markers occur more rapidly in HIV infected women. Plos One. 2013;8(1):e55279.

41. Weaver LK, Pioli PA, Wardwell K, Vogel SN, Guyre PM. Up-regulation of human monocyte CD163 upon activation of cell-surface Toll-like receptors. J Leukoc Biol. 2007;81:663–71.

42. Hintz KA, Rassias AJ, Wardwell K, Moss ML, Morganelli PM, Pioli PA, et al. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. J Leukoc Biol. 2002;72:711–7.

43. Moestrup SK, Moller HJ. CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. Ann Med. 2004;36:347–54.

44. Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, et al. Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. J Infect Dis. 2011;204:154–63.

45. Fitch KV, Srinivasa S, Abbara S, Burdo TH, Williams KC, Eneh P, et al. Noncalcified coronary atherosclerotic plaque and immune activation in HIV-infected women. J Infect Dis. 2013;208:1737–46.

46. Subramanian S, Tawakol A, Burdo TH, Abbara S, Wei J, Vijayakumar J, et al. Arterial inflammation in patients with HIV. JAMA. 2012;308:379–86.

47. Burdo TH, Lo J, Abbara S, Wei J, DeLelys ME, Preffer F, et al. Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. J Infectious Dis. 2011;204:1227–36.

48. Funderburg NT, Zidar DA, Shive C, Lioi A, Mudd J, Musselwhite LW, et al. Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome. Blood. 2012;120:4599–608.

49. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, et al. Plasma Levels of Soluble CD14 Independently Predict Mortality in HIV Infection. J Infect Dis. 2011;203:780–90.

50. Wilson EM, Singh A, Hullsiek KH, Gibson D, Henry WK, Lichtenstein K, et al. Monocyte activation phenotypes are associated with biomarkers of inflammation and coagulation in chronic HIV infection. J Infect Dis. 2014;210:1396–406.

51. Heesen M, Blomeke B, Schluter B, Heussen N, Rossaint R, Kunz D. Lack of association between the −260 C–> T promoter polymorphism of the endotoxin receptor CD14 gene and the CD14 density of unstimulated human monocytes and soluble CD14 plasma levels. Intensive Care Med. 2001;27:1770–5.

52. Bernardo E, Angiolillo DJ, Ramirez C, Cavallari U, Trabetti E, Sabate M, et al. Influence of the CD14 C260T promoter polymorphism on C-reactive protein levels in patients with coronary artery disease. Am J Cardiol. 2006;98:1182–4.

53. Morange PE, Saut N, Alessi MC, Frere C, Hawe E, Yudkin JS, et al. Interaction between the C-260T polymorphism of the CD14 gene and the plasma IL-6 concentration on the risk of myocardial infarction: the HIFMECH study. Atherosclerosis. 2005;179:317–23.

54. Westhorpe S, Maisa A, Spelman T, Hoy J, Dewar EK S, Hearps A, et al. Associations between surface markers on blood monocytes and carotid atherosclerosis in HIV-positive individuals. Immunol Cell Biol. 2014;92(2):133–8.

55. Knudsen A, Moller HJ, Katzenstein TL, Gerstoft J, Obel N, Kronborg G, et al. Soluble CD163 does not predict first-time myocardial infarction in patients infected with human immunodeficiency virus: a nested case–control study. BMC Infect Dis. 2013;13:230.

56. Hogger P, Sorg C. Soluble CD163 inhibits phorbol ester-induced lymphocyte proliferation. Biochem Biophys Res Commun. 2001;288:841–3.

57. Frings W, Dreier J, Sorg C. Only the soluble form of the scavenger receptor CD163 acts inhibitory on phorbol ester-activated T-lymphocytes, whereas membrane-bound protein has no effect. FEBS Lett. 2002;526:93–6.

58. Morange PE, Tiret L, Saut N, Luc G, Arveiler D, Ferrieres J, et al. TLR4/Asp299Gly, CD14/C-260T, plasma levels of the soluble receptor CD14 and the risk of coronary heart disease: The PRIME Study. Eur J Hum Genet. 2004;12:1041–9.

59. Wang Z, Hu J, Fan R, Zhou J, Zhong J. Association between CD14 gene C-260T polymorphism and inflammatory bowel disease: a meta-analysis. PLoS One. 2012;7:e45144.

60. Jarvelainen HA, Orpana A, Perola M, Savolainen VT, Karhunen PJ, Lindros KO. Promoter polymorphism of the CD14 endotoxin receptor gene as a risk factor for alcoholic liver disease. Hepatology. 2001;33:1148–53.

61. Wang F, Tahara T, Arisawa T, Shibata T, Nakamura M, Fujita H, et al. Genetic polymorphisms of CD14 and Toll-like receptor-2 (TLR2) in patients with ulcerative colitis. J Gastroenterol Hepatol. 2007;22:925–9.

62. Zambelli-Weiner A, Ehrlich E, Stockton ML, Grant AV, Zhang S, Levett PN, et al. Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. J Allergy Clin Immunol. 2005;115:1203–9.