Prospective study of circulating soluble CD40 Ligand concentrations and incident CVD in a nested prospective case-control study of older men and women

B.J.Jefferis*, P.H.Whincup†, P.Welsh‡, S.G.Wannamethee*, A.Rumley‡, DA.Lawlor§, S.Ebrahim¶ and G.D.O.Lowe‡

* Dept Primary Care & Population Health, UCL Medical School.
† Division of Community Health Sciences, St George's, University of London.
‡ Division of Cardiovascular and Medical Sciences, University of Glasgow.
§ MRC Centre for Causal Analyses in Translational Epidemiology, School of Social and Community Medicine, University of Bristol.
¶ Non-Communicable Disease Epidemiology Unit, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine.
Corresponding author (B Jefferis). Dept Primary Care & Population Sciences, UCL Medical School, Rowland Hill Street, London. NW3 2PF.
Tel 0207 830 2230, Fax 020 7794 1224 b.jefferis@pcps.ucl.ac.uk
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Abstract

Background: CD40L is implicated in atherosclerotic plaque formation.

Objectives: We investigate prospective associations between circulating soluble CD40L and MI or stroke in an older general population cohort, accounting for established and novel cardiovascular risk factors.

Methods: Baseline serum sCD40L was measured in incident MI (n=368) and stroke (n=304) cases and 2 controls per case, 'nested' in prospective UK studies of 4252 men and 4286 women aged 60–79 years, sampled from general practices in Britain in 1998–2000, with 7-year follow-up for fatal and non-fatal MI and stroke.

Results: Serum sCD40L was higher in smokers and negatively associated with lung function and positively associated with total cholesterol and markers of inflammation, but not with other established CVD risk factors. Geometric mean sCD40L levels did not differ between MI cases and controls (6.28 pg/mL versus 6.09 pg/mL; p=0.5) or between stroke cases and controls (5.93 pg/mL versus 5.55 pg/mL, p = 0.1). There was no strong evidence for elevated risk of MI or stroke in multivariable models comparing participants in the top to those in the bottom third of sCD40L. Age-adjusted odds ratios were 1.39(95%CI 0.98, 1.96) for MI and 1.16 (0.78, 1.73) for stroke. These attenuated to1.24 (95%CI 0.86, 1.79) and 1.18 (0.78, 1.78), respectively, after adjustment for established and novel CVD risk factors.

Conclusions: Serum sCD40L is associated with other inflammatory markers but is not itself a strong independent risk marker for either stroke or MI.

Key words

Coronary heart disease, CD40, epidemiology, myocardial infarction, prospective study, stroke

Introduction

Associations between levels of circulating inflammatory markers CRP, fibrinogen and IL-6 and raised risks of CHD onset have been demonstrated in meta-analyses, although their causal basis remains debated[1-3]. Activated T lymphocytes, vascular endothelial cells, smooth muscle cells and macrophages all play a role in mediating expression of IL-6 and other circulating inflammatory markers associated with CHD risk[4]. Furthermore cell membrane interactions between leucocytes within developing rupture-prone atheromatous plaques, mediated by receptors such as the CD40 / CD40 ligand dyad (CD40L), may play a key role in atherothrombosis. As such, CD40L–CD40 interactions between T-cells and multiple lineages of antigen presenting cells may lead to plaque rupture and triggering of acute coronary events, during which platelet expression of the CD40/CD40L dyad may be exacerbated[5].

Membrane-bound CD40L (mCD40L) can be sequestered into the circulation as soluble CD40L (sCD40L) by a poorly defined proteolytic process following T-cell activation. The biology of sCD40L is currently poorly understood. Nonetheless it has been suggested that because of the potential role of CD40L–CD40 interactions in plaque rupture, sCD40L levels will be positively associated with myocardial infarction (MI) and stroke.

Elevated serum sCD40L levels have been observed in patients with unstable angina [6] compared to stable angina or controls, and in the circulation of patients with acute coronary syndrome (ACS) compared to controls with chest pain but no ST elevation [7]. Data from cross-sectional and prospective clinical studies suggest that elevated circulating sCD40L levels predict incident CVD events in patients with prevalent vascular disease [6-11]. One previous small study of healthy women reported an association between circulating levels sCD40L and CVD risk[12].

We therefore study the associations between sCD40L and incident MI and stroke in a cohort of men and women aged 60-79 years, drawn from across Britain, followed up for CVD events and mortality for 6-8 years; longer than previous studies. We investigate the hypothesis that higher levels of circulating sCD40L are related to raised risks of incident MI and stroke in later adult life, and whether associations vary by age, gender or presence of pre-existing CVD.

Methods

In 1998-2000, 4252 men from a single General Practice in each of 24 British towns who were already participating in a prospective study of CVD were examined at age 60-79 years (response rate 77%)[13]. In 1999-2001, a parallel study of 4286 women of the same age and in the same Practices was established, omitting two study towns (Dewsbury and Maidstone) and adding another (Bristol), response rate 60%[14]. In both studies, nurses administered questionnaires, made physical measurements (weight, height and seated blood pressure), recorded an ECG and collected fasting venous blood samples, from which serum was stored at ≤-70oC for subsequent analysis of lipids, hemostatic and inflammatory markers as described elsewhere [1;13-15]. Soluble CD40L (ng/mL) was measured in 2007 by researchers blinded to case-control status of samples, using a commercially available sandwich ELISA (R&D Systems, Oxon, UK), intra-assay variation was <5% and inter-assay variation was <10%. In two study towns, sCD40L was measured in 138 men in 1996 and again in 2000, to enable intra-individual comparisons of values over time [16]. Participants completed detailed questionnaires. Pre-existing CVD was defined from reports from General Practitioner, or self-report of MI or stroke at any questionnaire between 1978-80 and 1998-2000 in men and in the baseline questionnaire (1999/2000) in women. Data on cigarette smoking, alcohol consumption, physical activity, own longest-held occupation (or husband's occupation for non-married women, coded using the Registrar General's classification) were self-reported. Use of antiplatelet medications (BNF codes 2.9), ace inhibitors (BNF code 2.5.5.1), beta blockers (BNF code 2.4), statins (BNF code 2.12) and oral anti-diabetic medications, (BNF codes 6.1.2) was self-reported. Region of residence was recorded for women in 1998-2000 and for men at the start of their follow-up in 1978-80. Participants were followed-up for 6.25-8.5 years for mortality and cardiovascular morbidity, with a follow up loss of <2%. Fatal cases were ascertained through National Health Service Central Registers from a death certificate: ICD-9 codes 410-414 for MI and ICD-9 codes 430-438 for stroke, indicating deaths with cerebrovascular disease as the underlying cause. Non-fatal MI or stroke was diagnosed using World Health Organisation diagnostic criteria based on reports from General Practitioners, supplemented by regular reviews of General Practice records [13;17]. Participants provided written informed consent to the investigation and ethical approval was provided by all relevant local research ethics committees.

A nested case-control study was established within the cohort study, . All participants (including those with pre-existing cardiovascular disease), were eligible for selection. All

390 new MI cases occurring between examination date (1998-2000) and June 2006 in men and between examination date (1999-2001) and September 2007 in women were selected. Two controls (totalling 780) were "frequency matched" to each case on town of residence, gender and age in 5-year bands. Controls were randomly selected from among subjects who survived to the end of the study free from incident CVD. A separate nested case control study based on 324 cases of stroke and 648 controls was similarly established. The MI and stroke control populations did not overlap. Both case and control populations included prevalent MI or stroke but controls had no incident cases during follow-up.

Statistical analyses

Highly skewed variables were transformed using natural logarithms and baseline (age 60-79 years) characteristics of the MI case and control populations were calculated (mean and standard deviation, median and IQR for skewed variables, or n (%) for categorical variables). Continuous variables were adjusted for gender, region of residence and age. Tertiles of sCD40L were defined in the MI control sample. Unmatched logistic regression analyses were used to examine associations between tertiles of sCD40L and MI, in the sample with complete data on outcomes and confounding variables. Regression models were adjusted for cardiovascular risk factors selected a priori: (i) gender, age and region of residence; (ii) smoking status (current, ex, never); (iii) history of diabetes, or CHD, social class (manual or non-manual), alcohol use (1-2 drinks/day or other) physical activity (more or less than 3 hours of moderate/ vigorous activity per week), systolic and diastolic blood pressure (SBP and DBP), body mass index (BMI), total and HDL cholesterol as continuous variables; (iv) CRP and IL-6. Logistic regression models of continuous sCD40L, log transformed to base 2 were used to assess the effect of a doubling of sCD40L level on MI. Differences in the sCD40L-CVD associations were tested using likelihood ratio (LR) tests for interactions by gender, age and pre-existing CVD. With the 293 MI cases and 236 stroke cases, we had 80% power to detect a relative risk of 1.68 in the top tertile of sCD40L compared to the bottom tertile, at the 5% statistical significance level, assuming 2 controls per case. As a form of sensitivity analysis, the estimates sCD40L-CVD associations were corrected for regression dilution using Rosner's method[18]; a regression dilution ratio was calculated in a subset of 138 men with sCD40L levels measured 4 years apart[16] and applied to the estimates sCD40L-CVD associations.

Results

MI

Of the 390 cases and 790 selected controls, 368 cases (270 men and 98 women aged on average 70.8 years) and 713 controls had data available on sCD40 levels. Baseline characteristics of MI cases and controls are shown in Table 1. MI cases had a higher prevalence of pre-existing CVD (MI or stroke) and diabetes than controls, were more likely to smoke cigarettes and less likely to be physically active or light drinkers (all $p<0.02$) and had higher mean SBP, total cholesterol, triglycerides, insulin, glucose, CRP, IL-6, fibrinogen, and white blood cell count, lower HDL cholesterol and forced expiratory volume (FEV_1) and similar SEP, BMI and DBP. Geometric mean serum sCD40L was similar in the MI cases and controls; 6.28 pg/mL (IQR 4.63, 8.73) compared to 6.09 pg/mL (IQR 4.36, 8.30), p(no difference)=0.5.

Stroke

sCD40L data were available for 304 of 324 stroke cases (194 men and 110 women, mean age 71.4 years) and 596 of 648 controls. Characteristics of stroke cases and controls are shown in Table 2. Cases had higher prevalence of pre-existing CVD and current smokers than controls (both $p<0.003$), but similar SEP, alcohol use, physical activity and prevalence of pre-existing diabetes. Cases had higher mean systolic and diastolic blood pressure, total cholesterol, IL-6 and white blood cell count, but lower FEV1 and similar BMI, HDL, triglyceride, insulin, glucose CRP and fibrinogen to controls. Geometric mean serum sCD40L levels was similar in the stroke cases and the controls; 5.93 pg/mL (IQR 4.18, 7.98) compared to 5.55 pg/mL (IQR 3.85, 7.71), p(no difference)=0.1.

Baseline correlates of sCD40L

Table 3 summarises the associations between sCD40L and other markers of CVD risk across thirds of the MI control population (the larger of the two control populations). sCD40L levels were higher in current than non-smokers, were inversely associated with FEV_1 but positively associated with total cholesterol in the MI control population, but not in the stroke control population (Supplementary Table 1). In the control populations, sCD40L levels were also generally weakly positively associated with CRP, white blood cell count and fibrinogen levels. There were not consistent associations between sCD40L levels and demographic or behavioural factors or established CHD risk factors such as BP or lipids, or with insulin and glucose. Similarly there were not consistent associations between sCD40L levels in the control populations with use of antiplatelet medications, statins, ACE inhibitors, beta blockers and oral anti-diabetic medications (data not presented).

Association of sCD40L with risk of MI

The odds ratio (OR) for MI associated with the highest compared to the lowest third of sCD40L and adjusted for age, gender and region of residence (Model 1) was 1.39 (95%CI 0.98, 1.96), with no evidence of a linear trend across the tertiles (p=0.26) (Table 3). Further adjustments for cigarette smoking (Model 2), established risk factors (Model 3) and for IL-6 and CRP (Model 4) attenuated the association somewhat. No strong evidence of association with MI was found when sCD40L was analysed as a continuous variable (Table 3). There was no evidence that sCD40L-MI associations varied by age, gender or by presence of pre-existing CVD (LR tests, all p>0.2).

Association of sCD40L with risk of stroke

There was no strong evidence of associations of sCD40L with stroke in multivariate regression models for men and women together (Table 5) and there was no evidence for interaction by age or pre-existing CVD (LR tests p=0.8 and p=0.7 respectively). However, there was some evidence that the association between sCD40L and stroke differed by gender (LR test p=0.02). For men, the OR for stroke associated with the highest compared to the lowest third of sCD40L and adjusted for age and region of residence (Model 1) was 1.57 (95%CI 0.97, 2.54), p(linear trend across tertiles)=0.04 (Supplementary data Table 2). Further adjustments for cigarette smoking (Model 2), established risk factors (Model 3) and for IL-6 and CRP (Model 4) somewhat strengthened the ORs to 1.69 (1.02, 2.80), p(trend)=0.04. A doubling of sCD40L level adjusted for age and region of residence (Model 1) was associated with an OR for stroke 1.30 (95%CI 1.01, 1.68), which was little changed by adjustments. For women, the Model 1 OR was 1.03 (95% CI 0.70, 1.54).

Extent of regression dilution

A regression dilution ratio of 0.47(95%CI 0.24, 0.71) was calculated in a subset of 138 men with sCD40L levels measured 4 years apart and applied to ORs estimated in the full sample of men and women. After correction for regression dilution, the OR among participants in the top third of sCD40L compared with the lowest third and adjusted for age, gender and region, was 2.02 (95% CI 0.96, 4.19) for MI and 1.37 (95%CI 0.59, 3.21) for stroke.

Discussion

In this population-based study of older men and women, we found that serum sCD40L levels were associated with cigarette smoking status and with other inflammatory markers (CRP, white blood cell count and fibrinogen levels), but were not consistently associated with classical cardiovascular risk factors (health behaviours, lipids and blood pressure).

Our hypothesis that higher sCD40L levels would predict onset of either MI or stroke in older women and men was not clearly supported. There was some evidence that higher levels of sCD40L were associated with increased risks of stroke in men. However, such interactions (subgroup analyses) often fail to replicate and further research is required before we could conclude that sCD40L is importantly associated with stroke in men but not women.

Associations between sCD40L and CVD risk factors

Our findings that sCD40L levels were associated with smoking[19;20] and with the inflammatory marker CRP[19;21] are in line with findings from some, but not all[22] other studies. Variations in the associations between sCD40L and inflammatory markers may be due to differences in age structure of study populations [22] as levels of inflammatory markers increase with age [23]. Our study of older adults adds to evidence accumulated from largely middle aged populations [7;8;12].

Associations between sCD40L and CVD events

Our study extends existing literature about associations between sCD40L and CVD. Firstly, it adds to the literature about generally healthy populations as it has more CVD cases and a longer follow-up period than the one previous case-control study. It also has longer follow-up than previous studies of high risk groups and is able to account for many important confounding factors. Secondly ours is the first study to examine whether the associations between sCD40L and incident CVD differ by gender, age and presence of pre-existing CVD. Thirdly it provides estimates of associations corrected for within-person variability. Prior epidemiological [12] and experimental evidence[24] gave us reason to expect associations between sCD40L and CVD events, but we found no robust evidence for this.

Studies examining the prognostic value of sCD40L levels for MI have mostly examined how sCD40L during or after an MI is related to subsequent events; some, but not all [25], report that raised sCD40L levels (eg above median or fourth quintile) are associated with raised risks of subsequent CHD or mortality [7;8]. The association between sCD40L and MI has been investigated in only one primarily healthy population (restricted to women), with only 130 cases, followed over 4 years; women with plasma sCD40L levels in the top 5% of the control distribution (>3.71 ng/mL) had elevated relative risks of non-fatal MI or fatal ACS events[12]. However associations with CVD risk were not observed when less

extreme cut-points (eg median and quartiles) were used, this fits with our findings based on tertiles. Associations might be observed in our data if more extreme comparison groups were used, although we did not see any evidence of associations across the whole sCD40L distribution when it was examined as a continuous covariate.

The literature about sCD40L and stroke is sparse. A small study of patients with TIA or stroke found that the receptor CD40 was upregulated on monocytes, compared with controls at the time of event, but less so three months later[26]. One prospective study of high risk CVD patients reported that sCD40L levels above the median (>4.76 ng/ml) were predictive of subsequent stroke and MI as a composite endpoint and of stroke separately [11]. However, in a separate similarly powered study of AF patients which used a tertile analysis, plasma sCD40L had no strong association with risk of stroke [27].

Gender differences

Ours is the first study to examine and report gender differences in associations between sCD40L and onset of CVD, so findings are exploratory and hypothesis generating. There was some weak evidence that the association between sCD40L and stroke was stronger in men than in women. Due to the limited number of cases in the gender stratified analysis, these findings must be interpreted cautiously and replicated.

Strengths and Limitations

Whilst our study is large in comparison with previous studies, being of sufficient size and statistical power to exclude an OR of 1.68 between top and bottom thirds of the sCD40L distribution, we cannot exclude a modest but potentially important association between sCD40L and CVD in healthy populations. Like other studies, we are limited by having only one measure of sCD40L, however, uniquely we were able to estimate intra-individual variability in serum sCD40L levels. Estimates suggested lower stability of sCD40L levels than several established risk factors [16]. After correction for within-person variability, the ORs were less conservative. Although there was some imbalance between cases and controls with respect to smoking and diabetes and pre-existing CVD, we were able to account for these variables by adjusting for them in regression models. The nested case-control design required that controls survived to the end of the study which could introduce some bias (towards overestimating the results), however since observed adjusted associations were modest and not significant, any bias is unlikely to be problematic. Response rates were reasonably high and we do not believe that attrition would affect our estimate of the association between sCD40L and CVD, prior work on the men's cohort suggests that non-attenders did not differ substantially from attenders[28] and we have

complete data from ONS death records and GP records on our outcome measures. Our results should therefore be generalisable to other healthy older adult populations. The validity of serum and plasma measures of sCD40L has been debated[5] as serum sCD40L may be subject to post-draw platelet degranulation but this may occur in both plasma and serum[29;30]. Although care has been taken with these serum samples, we cannot rule out the possibility of some degradation in sCD40L due to storage. To date no studies of pre-analytical variability we are aware of have investigated this issue[31-33]. However, case and control populations have been treated in the same manner, and we have no reason to believe that any degradation in samples would be different in cases compared to controls. Furthermore, circulating serum concentrations of sCD40L in our stored samples from healthy men appear broadly similar to those observed in fresh serum[31;33]. Our observation that serum sCD40L is associated with markers of inflammation and smoking validates the supposition that it is indicative of inflammatory processes.

Conclusions

In our study of primarily healthy older men and women, circulating soluble sCD40L levels did not independently predict CVD events, although they were associated with other inflammatory markers which are CVD risk factors. If other studies confirm these findings, it is unlikely that sCD40L will prove a useful diagnostic tool in predicting CHD and stroke risk in primarily healthy populations. .

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Table 1. Baseline Characteristics (age 60-79) of the MI cases and controls: mean (SD) or n(%)[*].

	MI Cases (n=368)	MI Controls (n=713)	Difference (p value)
Demographic/questionnaire			
Age (years)	70.83 (5.46)	70.80 (5.43)	Matched
Male, n(%)	270 (73.4)	516 (72.4)	Matched
Northern region of residence, n(%)[†]	156 (42.4)	298 (41.8)	Matched
Non-manual occupation, n(%)[†]	155 (44.5)	325 (47.7)	0.333
Prior evidence of CVD, n(%)	111 (30.2)	119 (16.7)	<0.001
History of diabetes, n(%)	66 (17.9)	76 (10.7)	0.001
1-2 alcoholic drinks/day, n(%)	129 (38.2)	304 (46.2)	0.015
Current smoker, n(%)	77 (20.9)	94 (13.2)	0.002
Physical activity (inactive/occasional), n(%)	235 (66.4)	401 (58.8)	0.017
Physical measurements			
Body Mass Index (Kg/m^2)[‡‖]	27.06 (4.23)	27.02 (3.96)	0.891
Systolic blood pressure (mm/Hg)[‡§‖]	153.89 (27.29)	148.69 (24.03)	0.001
Diastolic blood pressure (mm/Hg)[‡§‖]	83.78 (12.09)	83.28 (11.50)	0.512
FEV_1 (L/min)[‡‖**]	2.15 (0.55)	2.23 (0.60)	0.028
Lipids / Metabolic markers			
Total cholesterol (mMol/L)[‡]	6.24 (1.23)	6.09 (1.07)	0.032
HDL cholesterol (mMol/L)[‡]	1.32 (0.34)	1.42 (0.36)	<0.001
Triglyceride (mMol/L)[‡§#]	1.80 (1.31, 2.41)	1.58 (1.13, 2.09)	<0.001
Insulin (mU/L)[‡§#]	9.52 (6.09, 13.83)	8.14 (5.56, 11.23)	<0.001
Glucose (mMol/L)[‡§#]	6.16 (5.34, 6.38)	5.82 (5.31, 6.10)	<0.001
Inflammatory and hemostatic markers			
sCD40L(pg/mL)[‡#]	5.94 (4.58, 8.72)	5.82 (4.37, 8.34)	0.533
C-Reactive Protein (mg/L)[‡#]	2.39 (1.05, 5.39)	1.77 (0.89, 3.62)	<0.001
IL-6(pg/ml)[‡§#]	2.97 (1.92, 4.07)	2.43 (1.61, 3.43)	<0.001
Fibrinogen(g/L)[‡§#]	3.46 (3.04, 3.95)	3.27 (2.84, 3.76)	<0.001

White cell count (^10^9/L)[‡§#]	7.27 (6.03, 8.75)	6.80 (5.67, 7.97)	<0.001

[*] Case-control sample is maximum available

[†] reported in 1978-1980(age 40-59 years) for men and in 1998-2000 (age 60-79 years) for women

[‡] adjusted for gender, age and region of residence (Scotland, North, Midlands and South).

[§] adjusted for time of day

[‖] adjusted for inter-observer variation

[#] analysed as natural log transformed variable, geometric mean (IQR) reported on original scale.

[**] adjusted for height squared

Table 2. Baseline Characteristics (age 60-79) of the stroke cases and controls: mean (SD) or n(%)[*].

	Stroke Cases (n=304)	Stroke Controls (n=596)	Difference (p value)
Demographic/questionnaire			
Age (years)	71.36 (5.32)	71.39 (5.26)	Matched
Male, n(%)	194 (63.8)	377 (63.3)	Matched
Northern region, n(%)[†]	111 (36.5)	227 (38.1)	Matched
Non-manual occupation, n(%)[†]	125 (46.5)	263 (47.2)	0.632
Prior evidence of CVD, n(%)	86 (28.3)	90 (15.1)	<0.001
History of diabetes, n(%)	43 (14.1)	73 (12.3)	0.422
1-2 alcoholic drinks/day, n(%)	107 (38.8)	213 (38.4)	0.913
Current smoker, n(%)	53 (17.4)	58 (9.8)	0.002
Physical activity (inactive/occasional), n(%)	199 (71.7)	383 (66.8)	0.213
Physical measurements			
Body Mass Index (Kg/m^2)[‡‖]	27.06 (4.33)	27.06 (4.09)	0.828
Systolic blood pressure (mm/Hg)[‡§‖]	154.32 (24.96)	149.37 (24.42)	0.005
Diastolic blood pressure (mm/Hg)[‡§‖]	84.98 (12.61)	82.45 (11.29)	0.002
FEV_1 (L/min)[‡‖**]	2.09 (0.57)	2.23 (0.53)	<0.001
Lipids / Metabolic markers			
Total cholesterol (mMol/L)[‡]	6.13 (1.21)	6.32 (1.17)	0.023
HDL cholesterol (mMol/L)[‡]	1.43 (0.40)	1.43 (0.36)	0.924
Triglyceride (mMol/L)[‡§#]	1.64 (1.11, 2.32)	1.69 (1.23, 2.29)	0.468
Insulin (mU/L) [‡§#]	8.53 (5.61, 13.16)	7.96 (5.22, 11.60)	0.121
Glucose (mMol/L)[‡§#]	6.05 (5.30, 6.17)	5.88 (5.29, 6.10)	0.105
Inflammatory and hemostatic markers			
sCD40L (pg/ml)[‡#]	5.61 (4.25, 7.93)	5.28 (3.88, 7.69.)	0.106
C-Reactive Protein (mg/L)[‡#]	2.12 (1.04, 4.35)	1.92 (0.91, 4.10)	0.173
IL-6(pg/ml)[‡§#]	2.83 (1.87, 4.14)	2.56 (1.65, 3.55)	0.021
Fibrinogen(g/L)[‡§#]	3.35 (2.96, 3.85)	3.28 (2.88, 3.70)	0.092

White cell count (^10^9/L)[‡§#]	7.22 (6.14, 8.60)	6.90 (5.78, 8.19)	0.011

[*] case control sample is maximum available

[†] reported in 1978-1980(age 40-59 years) for men and in 1998-2000 (age 60-79 years) for women

[‡] adjusted for gender, age and region of residence (Scotland, North, Midlands and South).

[§] adjusted for time of day

[‖] adjusted for inter-observer variation

[#] analysed as natural log transformed variable, geometric mean (IQR) reported on original scale.

[**] adjusted for height squared

Table 3. Association between sCD40L (tertiles) and cardiovascular risk factors in the MI control sample (n=713)

	Low (0.41-4.88) N= 238	Medium (4.89-7.46) N= 238	High (7.46-20.37) n= 237	Trend p-value*
Demographic/questionnaire				
Age (years)	70.79	70.88	70.74	0.929
Male, n(%)	161 (67.7)	178 (74.8)	177 (74.7)	0.087
Northern region, n(%)†	87 (36.6)	109 (45.8)	102 (43.0)	0.152
Non-manual occupation, n(%)†	109 (48.2)	123 (53.7)	93 (41.2)	0.132
Evidence of CVD, n(%)	46 (19.3)	35 (14.7)	38 (16.0)	0.336
History of diabetes, n(%)	30 (12.6)	24 (10.1)	22 (9.3)	0.242
1-2 alcoholic drinks/day, n(%)	105 (51.8)	123 (55.4)	118 (54.1	0.631
Current smoker, n(%)	16 (6.7)	29 (12.2)	49 (20.7)	<0.001
Physical activity (inactive/occasional), n(%)	134 (58.3)	134 (60.1)	133 (58.1)	0.969
Physical measurements				
Body Mass Index (Kg/m^2)‡‖	27.07	27.00	26.99	0.759
Systolic blood pressure (mm/Hg)‡§‖	148.48	148.97	148.62	0.957
Diastolic blood pressure (mm/Hg)‡§‖	83.42	83.05	83.38	0.965
FEV_1 (L/min)‡‖**	2.25	2.30	2.13	0.030
Lipids / Metabolic markers				
Total cholesterol (mMol/L)‡	5.91	6.14	6.21	0.002
HDL cholesterol (mMol/L)‡	1.41	1.42	1.43	0.571
Triglyceride (mMol/L)‡§#	1.54	1.60	1.61	0.313
Insulin (mU/L)‡§#	8.54	7.97	7.92	0.165
Glucose (mMol/L)‡§#	5.87	5.79	5.81	0.575
Inflammatory and hemostatic markers				
C-Reactive Protein (mg/L)‡#	1.53	1.81	2.00	0.008
IL-6(pg/ml)‡§#	2.37	2.39	2.51	0.306
Fibrinogen(g/L)‡§#	3.15	3.22	3.44	<0.001
White cell count ($\wedge 10^9$/L)‡§#	6.37	6.81	7.26	<0.001

* sample (n=713) MI controls with SCD40L value. Trend test adjusted for age, gender, region of residence

† reported in 1978-1980(age 40-59 years) for men and in 1998-2000 (age 60-79 years) for women

‡ adjusted for gender, age and region of residence (Scotland, North, Midlands and South).

§ adjusted for time of day

|| adjusted for inter-observer variation

geometric mean, p value from linear regression with ln(variable)

** adjusted for height squared

Table 4. Odds Ratio (95% CI) of MI in men and women with sCD40L values in the highest compared to lowest tertile (n=902[*])

SCD40L Tertiles	MI cases	MI controls	OR (95% CI) Model 1	Model 2	Model 3	Model 4
Range (pg/mL)						
7.46-20.37	113	198	1.39 (0.98, 1.96)	1.30 (0.91, 1.84)	1.26 (0.88, 1.81)	1.24 (0.86, 1.79)
4.89-7.46	105	198	1.29 (0.91, 1.83)	1.26 (0.89, 1.79)	1.25 (0.87, 1.80)	1.26 (0.88, 1.82)
0.41-4.88	84	204	1	1	1	1
Total	302	600	p=0.263	p=0.531	p=0.674	p=0.813
			OR (95%CI)			
Doubling of SCD40L[†]	302	600	1.11 (0.93, 1.33)	1.06 (0.88, 1.27)	1.04 (0.86, 1.26)	1.02 (0.85, 1.24)

[*] complete case analysis sample. Tertiles based on control group. P value for test for trend over tertiles

[†] OR of MI per 1 $\log_2$ increase in $\log_2$(SCD40L) ie doubling of SCD40L

Model 1= age, gender and region

Model 2= model 1 + smoking

Model 3= model 2+ SEP, alcohol, physical activity, history of diabetes, history of CVD, BMI, SBP, DBP, TC, HDL

Model 4 =model 2 + IL-6 + CRP

Table 5. Odds Ratio (95% CI) of stroke in men and women with SCD40L values in the highest compared to lowest tertile (n=734)[*]

SCD40L Tertiles	Stroke cases	Stroke controls	OR (95 % CI) Model 1	Model 2	Model 3	Model 4
Range (pg/mL)						
6.98-28.61	78	161	1.16 (0.78, 1.73)	1.18 (0.80, 1.76)	1.20 (0.79, 1.80)	1.18 (0.78, 1.78)
4.48-6.97	90	169	1.29 (0.88, 1.88)	1.29 (0.88, 1.89)	1.27 (0.85, 1.87)	1.25 (0.84, 1.85)
0.32-4.47	69	167	1	1	1	1
Total	237	497	p=0.132	p=0.118	p=0.121	p=0.139
			OR (95% CI)			
Doubling of SCD40L[†]	237	497	1.17 (0.95, 1.44)	1.18 (0.96, 1.45)	1.18 (0.96, 1.47)	1.18 (0.95, 1.46)

[*] complete case analysis sample. Tertiles based on control group. P value for test for trend over tertiles

[†] OR of MI per 1 $\log_2$ increase in $\log_2$(SCD40L) ie doubling of SCD40L

Model 1= age, gender and region

Model 2= model 1+ smoking

Model 3= model 2+ SEP, alcohol, physical activity, history of diabetes, history of CVD, BMI, SBP, DBP, TC, HDL

Model 4 =model 3 + IL-6 + CRP