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High-density lipoprotein cholesterol subfraction HDL2 is associated with improved endothelial function in systemic lupus erythematosus

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ABSTRACT

Objective Patients with systemic lupus erythematosus (SLE) have increased risk of premature atherosclerosis but the exact mechanisms remains unclear. Flow-mediated dilatation (FMD) is an established non-invasive assessment of vascular endothelial function. Lipoprotein subfractions may be better predictors of FMD than conventional cholesterol measurements. We tested the hypothesis that lipoprotein subfractions are independently associated with FMD.

Methods Forty-one consecutive adult patients with SLE without known cardiovascular risk factors or disease were recruited in this cross-sectional study. Endothelial function and early atherosclerosis were assessed by brachial FMD and common carotid artery (CCA) intima-media thickness (IMT). High-density lipoprotein (HDL)/low-density lipoprotein (LDL) subfractions were measured. Machine learning models were also constructed to predict FMD and CCA IMT.

Results Median FMD was 4.48% (IQR 5.00%) while median IMT was 0.54 mm (IQR 0.12 mm). Univariate analysis showed lower LDL1 ($r=-0.313$, $p<0.05$) and higher HDL2 subfractions ($r=0.313$, $p<0.05$) were significantly associated with higher log-transformed FMD. In a multiple linear regression model, HDL2 ($\beta=0.024$, SE=0.012, $p<0.05$) remained an independent predictor of higher FMD after adjusting for age, body mass index, LDL1 and systolic blood pressure. The machine learning model included parameters such as HDL2 (positive association), prednisolone dose, LDL cholesterol and LDL1 for prediction of FMD ($r=0.433$, $p<0.01$). Age, LDL cholesterol and systolic blood pressure were independently associated with higher CCA IMT after adjusting for body mass index and HDL2.

Conclusions HDL 2, a large HDL particle, was independently associated with greater FMD and may be a biomarker of vascular health in SLE.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Patients with systemic lupus erythematosus (SLE) have increased risk of premature atherosclerosis but this comorbidity is incompletely understood and likely multifactorial in aetiology, including the interplay between high-density lipoprotein (HDL).

WHAT THIS STUDY ADDS

⇒ We show that patients with SLE without traditional cardiovascular risk factors had normal low-density lipoprotein and HDL electrophoretic profiles but HDL2 was independently associated with better endothelial function in patients with SLE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The improved understanding of HDL subfractions such as HDL2, rather than HDL cholesterol itself, may contribute to interventions to influence the future risk of atherosclerotic disease and cardiovascular outcomes in SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease with diverse clinical manifestations and systemic organ involvement that presents a challenge in treatment.[1] Outcomes in SLE have improved over the decades, with earlier diagnosis and referral, more potent microbial agents, availability of renal replacement therapy and less toxic immunosuppressive treatment.[2] However, patients with SLE have an increased risk of multiple comorbidities, which influence long-term prognosis and all-cause mortality.[3] An association with cardiovascular disease (CVD) was first noted nearly 50 years ago when Urowitz *et al* observed a bimodal mortality pattern in patients with SLE, with myocardial infarction accounting for late mortality.[4] More recently, the Systemic Lupus International Collaborating Clinics group showed that 3.6% of patients with SLE in their inception cohort had vascular events attributed to atherosclerosis.[5] In a cohort

study, it was found that women with SLE aged 35–44 years were over 50 times more likely to have a myocardial infarction than age-matched controls.[6] Another cohort study showed that the risk ratio of a cardiovascular event in women with SLE compared with those without was 2.26.[7]

The pathogenesis of accelerated atherosclerosis in SLE is incompletely understood and likely multifactorial. Mechanistic studies have identified the role of emerging non-traditional (SLE-related) risk factors such as type I interferons, autoantibodies, microparticles and neutrophil extracellular traps which induce damage to the blood vasculature, via mechanisms ranging from enhancing apoptosis of endothelial cells to reduction in quantity and function of endothelial progenitor cells, leading to impaired endothelial function in SLE.[8] These factors work in concert to predispose patients with SLE to premature atherosclerosis. When atherosclerosis manifests as clinical events such as myocardial infarction, vascular damage is already advanced, and often irremediable.[9] Thus, there is a need for biomarkers to predict endothelial damage before clinical signs appear.

Patients with SLE with coronary heart disease (CHD) are more likely to have exposure to all classic cardiovascular risk factors, including hyperlipidaemia, compared with SLE controls without CHD.[10] Low levels of high-density lipoprotein (HDL) and high levels of low-density lipoprotein (LDL) are associated with atherosclerotic complications in patients with SLE.[11] Existing studies also demonstrate significant increase in oxidation of SLE-associated lipoprotein subfractions such as HDL2 and HDL3.[12] To our best knowledge, there has been no study that shows an explicit association between lipoprotein subfractions in patients with SLE and endothelial dysfunction. In this study, we will determine whether lipoprotein subfractions are predictors of endothelial dysfunction in patients with SLE, as measured by brachial flow-mediated dilatation (FMD), a well-established surrogate biomarker of endothelial function.[13]

METHODS

Subjects

Consecutive adults who fulfilled the 1997 American College of Rheumatology revised SLE classification criteria were recruited from the outpatient clinics of National University Hospital (NUH), Singapore.[14] Demographic and relevant clinical data were abstracted from the medical records of patients with SLE for the entire duration of the study from 1 February 2017 to 29 February 2020. SLE disease activity and SLE-related disease damage were assessed using the SLE Disease Activity Index 2000 (SLEDAI-2K) for a 30-day window and the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI), respectively.[15–17] The Framingham Risk Score (FRS) and modified FRS (mFRS) for SLE were calculated for each patient.[18] Patients with SLE with known CVD, diabetes mellitus, hypertension, lipid-lowering therapy within the past month, active thyroid disease, renal impairment (estimated glomerular filtration rate <60 mL/min), proteinuria >3 g/day, current smoking history, antiphospholipid syndrome and/or on anticoagulation, antiplatelet medications, pregnancy or acute illness in the preceding 2 weeks were excluded. Community-derived healthy controls were recruited using poster advertisements in the hospital or were the nursing staff of NUH. The study was approved by National Healthcare Group Domain Specific Review Board E (reference code: 2016/01419) and carried out in accordance with the principles of the Declaration of Helsinki. All subjects gave written informed consent prior to study inclusion.

Quantification of cholesterol in lipoprotein subfractions

Serum was obtained from patients with SLE after a 12-hour fast in the morning. Processed serum was stored at −80°C until retrieval for analyses. Analyses were performed for total cholesterol, triglycerides, HDL by automated clinical chemistry analyzer (AU5800, Beckman Coulter, Florida) in NUH, a College of American Pathologists accredited laboratory. LDL was calculated using the Friedewald formula. LDL and HDL subfractions were analysed using Lipoprint LDL System (which resolves up to 12 lipoprotein subfractions: very LDL (1), mid-band (3), LDL (7) and HDL (1); Lipoprint, Quantimetrix Corporation, California) and Lipoprint HDL System (which separates HDL into 10 subfractions; Lipoprint, Quantimetrix Corporation, California), respectively. Mid-bands comprise mainly of intermediate-density lipoproteins. HDL 1–3, 4–7 and 8–10 are classified as large, intermediate and small subfractions by the Lipoprint HDL System, respectively. The relative area (%) for each lipoprotein band is determined and multiplied by the total cholesterol concentration of the sample to yield the amount of cholesterol for each band (mg/dL).

Measurement of lipocalin-2

Lipocalin-2 is a novel biomarker, which has been associated with endothelial dysfunction and carotid atherosclerosis.[19 20] The concentrations of lipocalin-2 were determined using the human Quantikine ELISA kit (R&D Systems, Minnesota).

Assessment of endothelium-dependent flow-mediated dilation

Endothelium-dependent FMD was assessed using the Prosound Alpha 10 ultrasound system (Hitachi Aloka Medical Ltd., Japan). A 10 MHz linear array probe steadied by a stereotactic clamp was used to image the brachial artery and position electronic tracking gates at the media-adventitia interface of opposing arterial walls. The eTracking application implemented on the system uses radiofrequency signals from the tracked vessel walls to determine arterial distension in real time to 0.01 mm accuracy. Reactive hyperaemia was induced by inflating a DS 66 Trigger Aneroid blood pressure (BP) device (Welch Allyn, New York) placed around the proximal forearm to a pressure of 50 mm Hg above systolic BP for

5 min, followed by rapid cuff deflation. The proprietary FMD software provides a continuous graphical display of minute vasodilatation from baseline, cuff occlusion, vasodilation and recovery and automatically calculates parameters such as vessel diameter at maximum dilatation and % FMD. Endothelial function was assessed as brachial FMD at end-diastole. All FMD studies were performed after abstention from food/exercise for 12 hours, coffee/tea for 24 hours and alcohol for 48 hours and discontinuation of vasoactive medications for at least four half-lives, if possible. Female subjects were studied 7 days after cessation of their last menstrual period to standardise/minimise the effect of sex hormones on endothelial reactivity.

Measurement of carotid-intima media thickness

Intima-media thickness (IMT), a measure of structural atherosclerosis, was evaluated by high-resolution B-mode ultrasonography of the common carotid artery (CCA) using the same ultrasound equipment, according to American Society of Echocardiography guidelines.[21] Both left and right CCAs were scanned in three planes and carotid IMT measured 1 cm proximal to the carotid bulb, in an area devoid of plaque. Triplicate IMT measurements were taken and averaged. For each subject, the CCA IMT was calculated to be the average of the left and right CCA IMT.

Statistical analysis and machine learning

As FMD was not normally distributed, the data were log-transformed before performing univariate and multiple linear regression. Data were assessed using Mann-Whitney U test for two comparisons and Pearson correlation coefficient. Multiple linear regression was used to identify independent predictors of FMD and CCA IMT with adjustment for potential confounders. As the number of patients with SLE was relatively small in this study, this limits the number of variables that can be included in the traditional regression models. The covariates considered in the multiple linear regression models were three prespecified variables (age, body mass index and systolic BP) that are known to affect FMD in the community and the rest of the parameters were included based on significance levels of univariate correlations.[22] This analysis was performed using SPSS, V.25.0 (IBM, New York). In addition, machine learning was attempted to enable the consideration of all the generated variables in the study with the exceptions of FRS, mFRS, small, intermediate and large HDL subfractions to avoid introducing multicollinearity into the models. Glmnet was used to fit the parameters for the prediction of FMD and CCA IMT. Parameters were filtered to ensure that at least 90% of data were present and missing data were substituted using the average value of the parameter. The glmnet implementation (glmnet package) in R V.3.6.2 was used with a 10-fold cross-validation and the model with the minimum lambda selected. The advantage of the glmnet analysis is that it attempts to prevent overfitting and attempts to select for a subset of features, which can be potentially more generalisable. Spearman Rank correlation was then used to assess the fit of the model (model predicted test outcome) to the actual FMD and CCA IMT data. A two-tailed p value <0.05 was considered statistically significant. No sample size calculation was conducted as this study is exploratory in nature.

Patient and public involvement

No patients or members of the public were involved in the design of this study.

RESULTS

Demographics and clinical characteristics

A total of 41 patients with SLE were recruited with key participant and treatment characteristics detailed in online supplemental table 1. The patients with SLE had the following characteristics: 92.7% female; median age 41.0 (IQR: 18.5) and body mass index 22.0 (IQR: 4.5). The population was multiethnic with 75.6% Chinese, 9.8% Malay and 2.4% Indian. Participants had SLE for a median duration of 72.0 (IQR: 101.5) months. Patients with SLE of 68.3%were receiving prednisolone at a median dose of 4 mg (IQR: 5). Steroid-sparing agents prescribed included hydroxychloroquine (97.6%), mycophenolate mofetil (39.0%), azathioprine (19.5%), methotrexate (9.8%) and cyclosporine A (7.3%). None of the patients with SLE patients was on lipid-lowering medications. The main clinical manifestations and laboratory findings over the course of SLE are described in online supplemental table 1. The most common lupus manifestations included arthritis (68.3%), renal disorder (36.6%) and malar rash (34.1%) at diagnosis and over time. Of note, the FRS and mFRS were 5% (IQR: 9.25) and 10% (IQR: 18.5), indicating low and moderate risks for CVD, respectively. The median SLEDAI-2K was 2 (IQR: 4) and SDI was 0 (IQR: 0) at time of study. Of 3 (7.3%) patients with SLE had current (active) lupus nephritis. The median C reactive protein (CRP) was 5 mg/L (IQR: 1).

Lipid profile and endothelial function

Lipid profile and FMD findings are detailed in table 1. LDL cholesterol in patients with SLE was optimal at 56.0 mg/dL (IQR: 9.0) as per the Adult Treatment Panel III guidelines.[23] The LDL subfraction analysis showed that 37 out of 41 (90.2%) were phenotype A (normal LDL lipoprint profile). Although HDL cholesterol was low at 29 mg/dL (IQR: 9), the distribution (area %) of the large, intermediate and small HDL subfractions were normal at 38 (normal range (NR)≥10), 51 (NR ≥22) and 9 (NR ≤11), respectively. Of the HDL subfractions, HDL2 was the subfraction that was most highly correlated with HDL cholesterol (r=0.47, p=0.002). Triglyceride level was low at 0.87 mmol/L (IQR: 0.465). Twelve healthy controls (12 female, median age 42.5 years (IQR: 25.8)) underwent FMD and IMT assessment for comparison. FMD and IMT variables were not significantly different among the two groups although FMD trended towards being lower in

Table 1 Fasting lipoprotein subfractions and endothelial function variables in patients with SLE

Lipoprotein subfractions		Endothelial function (SLE)	
LDL-C (mg/dL)	56.0 (9.0)		
LDL1 (mg/dL)	20 (4.5)	FMD systolic (%)	4.59 (4.71)
LDL2 (mg/dL)	9 (11.5)	FMD (%)	4.48 (5.00)
LDL3 (mg/dL)	0 (2)	IMT Rt CCA (mm)	0.54 (0.13)
LDL4 (mg/dL)	0 (0)	IMT Lt CCA (mm)	0.53 (0.12)
LDL A, n (%)	37 (90.2)	IMT CCA (mm)	0.54 (0.12)
LDL B, n (%)	4 (9.8)		
VLDL (mg/dL)	15 (5)	**Endothelial function (HC)**	
HDL-C (mg/dL)	29 (9)	FMD systolic (%)	6.44 (5.61)
Area % HDL1	5 (5.5)	FMD (%)	6.51 (4.66)
Area % HDL2	14 (8.5)	IMT Rt CCA (mm)	0.51 (0.08)
Area % HDL3	17 (5.5)	IMT Lt CCA (mm)	0.50 (0.09)
Area % HDL4	15 (3.5)	IMT CCA (mm)	0.51 (0.07)
Area % HDL5	12 (2)		
Area % HDL6	19 (5.5)		
Area % HDL7	6 (2)		
Area % HDL8	5 (2)		
Area % HDL9	3 (2)		
Area % HDL10	3 (3)		
Area % large HDL	38 (16)		
Area % intermediate HDL	51 (9)		
Area % small HDL	9 (6.5)		

Data are median (IQR), n=41. Twelve healthy controls were recruited for FMD and IMT assessment for comparison.
CCA, common carotid artery; FMD, flow-mediated dilatation; HC, healthy control; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; IMT, intima-media thickness; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; Lt, left; Rt, right; SLE, systemic lupus erythematosus; VLDL, very low-density lipoprotein.

patients with SLE compared with healthy controls (4.48% vs 6.51%, p=0.059).

Factors related to endothelial function and CCA IMT in SLE

Results of univariate analyses between log-transformed FMD with demographic, clinical and lipoprotein subfraction variables are shown in table 2. Significant univariate correlations were found between log-transformed FMD with LDL1 (r=−0.313, p=0.047) and HDL2 (r=0.313, p=0.046) subfractions. In a multiple linear regression model, HDL2 (β=0.024, SE=0.012, p=0.046) remained an independent predictor of higher FMD after adjusting for age, body mass index, LDL1 and systolic BP. Using glmnet, we trained a model to predict FMD (r=0.433, p=0.005) (figure 1A). The parameters that were in the glmnet model comprised LDL cholesterol, LDL1, HDL2 (positive association) and prednisolone dose (figure 1B). In keeping with results of the univariate and multiple linear regression analyses, HDL2 was positively associated with higher FMD in the glmnet model. Significant univariate correlations were found between CCA IMT with LDL cholesterol (r=0.421, p=0.006), LDL1 (r=0.323, p=0.039), HDL9 (r=0.328, p=0.039), mFRS (r=0.722, p<0.001), small HDL (r=0.344, p=0.028), systolic BP (r=0.370, p=0.006) and total cholesterol (r=0.404, p=0.009) (table 3). Age, LDL cholesterol and systolic BP were independently associated with higher CCA IMT after adjusting for body mass index and HDL2 (table 3). Using glmnet, we trained a model to predict CCA IMT (r=0.831, p<0.0005) (figure 1C). The parameters that were in the glmnet model comprised of age, duration of SLE, LDL cholesterol, LDL1, HDL2, HDL3, HDL4, prednisolone dose and systolic BP (figure 1D). Of note, age, LDL cholesterol and systolic BP were concordantly associated with higher CCA IMT on both multivariate and machine learning-based analytics (table 3 and figure 1D).

DISCUSSION

We recruited a group of relatively young patients with SLE without established cardiovascular risk factors or CVD to study the correlations between lipoprotein subfractions and endothelial function. There are three key findings in this study. First, patients with SLE without known hyperlipidaemia or other traditional cardiovascular risk factors had isolated low HDL cholesterol but normal LDL and

Table 2 Univariate correlation and multiple linear regression examining the association between log-transformed FMD with demographic, clinical and lipoprotein subfraction variables for patients with SLE

Univariate analysis		
Variable	**Pearson correlation coefficient**	**P value**
Age	−0.116	0.470
BMI	−0.165	0.304
SBP	−0.045	0.780
Total cholesterol	−0.208	0.191
LDL-C	−0.254	0.109
LDL1	**−0.313**	**0.047**
LDL2	0.061	0.706
LDL3	0.135	0.401
HDL-C	−0.034	0.831
Area % HDL1	0.064	0.692
Area % HDL2	**0.313**	**0.046**
Area % HDL3	0.11	0.946
Area % HDL4	−0.035	0.830
Area % HDL5	−0.207	0.193
Area % HDL6	−0.158	0.325
Area % HDL7	−0.133	0.405
Area % HDL8	−0.186	0.245
Area % HDL9	−0.063	0.696
Area % HDL10	−0.17	0.288
Area % small HDL	−0.161	0.314
Area % intermediate HDL	−0.223	0.161
Area % large HDL	0.258	0.104
HbA1c	0.274	0.389
Duration of SLE	0.138	0.391
Prednisolone dose	0.212	0.182
mFRS	−0.19	0.240
SDI	−0.098	0.542
SLEDAI-2K	−0.016	0.920
Lipocalin-2	−0.020	0.902
Multiple linear regression model		
Variable	**β**	**P value**
SBP	0.006	0.972
BMI	−0.089	0.579
Age	−0.103	0.505
LDL1	−0.279	0.068
Area % HDL2	**0.024**	**0.046**
	Adjusted R^2 0.075	

Variables with significant p values are in bold.
BMI, body mass index; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; mFRS, modified Framingham risk score; SBP, systolic blood pressure; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; SLE, Systemic Lupus Erythematosus; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000.

HDL electrophoretic profiles, indicating an ostensibly non-atherogenic phenotype. Second, analyses of lipoprotein subfractions using different approaches revealed that HDL2 was positively associated with better endothelial function in patients with SLE. Third, age, LDL cholesterol and systolic BP were predictive of early atherosclerosis, as assessed by CCA IMT.

The lipid paradox in rheumatic diseases was first described for patients with rheumatoid arthritis more than 10 years ago, whereby lower total cholesterol and LDL cholesterol were associated with increased cardiovascular risk, owing to ongoing inflammatory processes and increased cholesterol catabolism.[24 25] A lupus pattern of hyperlipidaemia has also been reported, characterised by low HDL cholesterol, high triglycerides but unchanged LDL cholesterol.[26] However, higher levels of total cholesterol and LDL cholesterol have been associated with CHD and stroke in SLE.[27] Quantitative and qualitative characteristics of lipoprotein subfractions may, therefore, be more informative than total lipoprotein levels in assessing cardiovascular risk in patients with SLE.

The European League Against Rheumatism recommendations for cardiovascular risk management in SLE stated that hyperlipidaemia management should follow that of the general population.[27] However, no specific laboratory method of measuring total cholesterol, LDL cholesterol or HDL cholesterol was endorsed in the recommendations. Different assays, such as density gradient ultracentrifugation, nuclear magnetic resonance, non-denaturing gradient gel electrophoresis and the lipoprint system, a linear, polyacrylamide gel electrophoresis system, have been used to resolve LDL and HDL subfractions with respect to particle size.[28–30] A considerable body of experimental and epidemiological reports has shown that small-sized LDL and HDL particles are crucial players of atherogenesis compared with larger particles, although the nomenclature may differ slightly based on the assay used. European League Against Rheumatism also outlined several recommendations to reduce cardiovascular morbidity of patients with SLE through interventions targeted against traditional risk factors.[27] Lipid management was recommended to follow the general population.[27] Aggressive BP control to a target of less than 130/80 mm Hg is recommended in SLE, like that of non-SLE patients with diabetes mellitus or chronic kidney disease.[27] These recommendations are particularly relevant given our findings of LDL cholesterol and systolic BP being independent predictors of higher IMT in patients with SLE.

Despite the substantial body of evidence of an inverse relation between HDL cholesterol and cardiovascular event risk, interventions to raise HDL cholesterol as the only therapeutic target have not uniformly demonstrated benefit.[31] This may be because HDLs are a class of structurally and functionally heterogeneous particles. HDL can be classified on the basis of density, resulting in the large buoyant HDL2 and the small-dense HDL3, which can be further subfractionated into five distinct

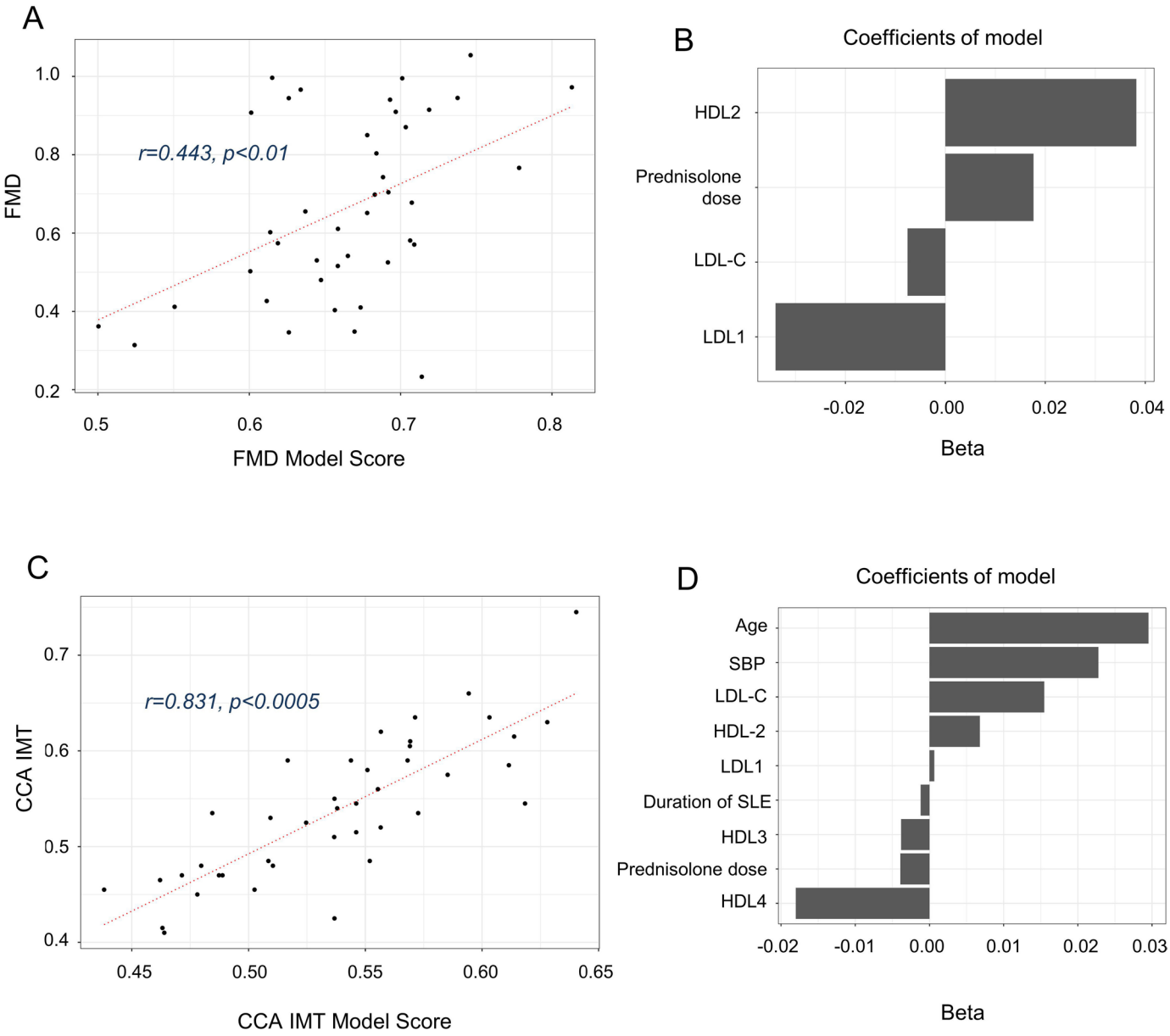

Figure 1 Using glmnet to predict FMD and CCA IMT in patients with SLE. (A) FMD model score versus actual FMD. (B) Using glmnet, a model comprising of four parameters was trained to predict FMD. (C) CCA IMT model score vs actual CCA IMT. (D) Using glmnet, a model comprising of 9 parameters was trained to predict CCA IMT. CCA, common carotid artery; FMD, flow-mediated dilatation; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; SLE, systemic lupus erythematosus.

subpopulations (HDL2b, HDL2a, HDL3a, HDL3b and HDL3c) based on size.[32] HDL subclasses can be classified as small HDL, intermediate HDL and large HDL,[33] with small HDL particles being associated with adverse cardiometabolic risk profile.[32] A study by Didichenko *et al* demonstrated the divergence in specific HDL functions according to particle size with larger particles showing stronger antioxidant function in inactivating lipid peroxidases in oxidised LDL.[34 35] In general, HDL2, HDL2b and HDL2a, as determined by ultracentrifugation, non-denaturing gradient gel electrophoresis or the lipoprint system, are considered large HDL particles.

Several studies have investigated lipoprotein subfractions in SLE, but the impact of such changes on the development of subclinical atherosclerotic plaque and CHD remains to be established.[36] The first study determined the LDL subfractions in 53 patients with SLE and 53 age-match and gender-match controls using disc polyacrylamide gel electrophoresis.[37] Patients with SLE were found to have higher LDL scores and this was associated with high levels of oxidative stress and elevated CRP.[37] Two further studies adopted the method of nuclear magnetic resonance spectroscopy to quantify LDL and HDL subfractions in SLE.[26 38] In the study by Chung *et al* with 105 patients with SLE and 77 healthy controls, the levels of sdLDL did not differ significantly and were not associated with coronary calcification measured using electron beam CT.[38] However, concentration of sdLDL in patients

Table 3 Univariate correlation and multiple linear regression examining the association between CCA IMT with demographic, clinical and lipoprotein subfraction variables for patients with SLE

Univariate analysis		
Variable	**Pearson correlation coefficient**	**P value**
Age	0.261	0.059
BMI	0.175	0.210
SBP	**0.370**	**0.006**
Total cholesterol	**0.404**	**0.009**
LDL-C	**0.421**	**0.006**
LDL1	**0.323**	**0.039**
LDL2	0.157	0.338
LDL3	−0.067	0.812
HDL-C	0.183	0.252
Area % HDL1	−0.018	0.913
Area % HDL2	0.017	0.915
Area % HDL3	−0.277	0.079
Area % HDL4	−0.259	0.101
Area % HDL5	−0.220	0.167
Area % HDL6	0.072	0.653
Area % HDL7	0.090	0.575
Area % HDL8	0.251	0.113
Area % HDL9	**0.328**	**0.039**
Area % HDL10	0.325	0.080
Area % small HDL	**0.344**	**0.028**
Area % intermediate HDL	−0.119	0.459
Area % large HDL	−0.108	0.501
HbA1c	0.479	0.115
Duration of SLE	0.014	0.931
Prednisolone dose	−0.235	0.139
mFRS	**0.722**	**<0.001**
SDI	0.124	0.441
SLEDAI-2K	−0.074	0.644
Lipocalin-2	−0.163	0.309
Multiple linear regression model		
Variable	**β**	**P value**
BMI	0.017	0.899
Area % HDL2	0.104	0.396
LDL-C	**0.260**	**0.045**
SBP	**0.283**	**0.035**
Age	**0.465**	**<0.001**
	Adjusted R^2 0.455	

Variables with significant p values are in bold.
BMI, body mass index; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; mFRS, modified Framingham risk score; SBP, systolic blood pressure; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; SLE, Systemic Lupus Erythematosus; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000.

with SLE was associated with impaired insulin sensitivity, higher body mass index and higher levels of inflammation (CRP and erythrocyte sedimentation rate).[38] In Hua *et al*'s study, 26 SLE women with history of CVD, 26 age-matched SLE women without CVD and 26 age-matched healthy controls were analysed for sdLDL.[26] Surprisingly, sdLDL was more common in healthy controls than patients with SLE without CVD and tended to be more common in patients with SLE with CVD compared with those without CVD.[26] However, there was an association between sdLDL and presence of plaques and IMT in patients with SLE with CVD.[26] Another finding from this study is that small HDL, which is often assumed to be less atheroprotective than larger HDL particles, were less common among patients with SLE with or without CVD.[26] One study measured sdLDL in 50 patients with SLE and 50 age-matched and gender-matched healthy controls using gradient gel electrophoresis.[39] The LDL particle size was smaller in patients with SLE than healthy controls and the prevalence of the atherogenic phenotype was higher in patients with SLE.[39] Two further studies focusing on HDL subfractions demonstrated an atherogenic phenotype with increased levels of HDL3 and reduced HDL2 in patients with SLE compared with healthy controls.[40 41] A recent study by Purmalek *et al* found that large HDL particles have a negative association with non-calcified plaque burden while the opposite was observed for smaller HDL particles.[42] Finally, Chan *et al* recently reported that electronegative L5 LDL was associated with increased CCA IMT.[43] However, none of the above studies assessed the relationship between lipoprotein subfractions and FMD.[26 37–41 43]

HDL 2 was not independently associated with lower IMT in our study. Endothelial dysfunction appears to be an early event in atherosclerosis, preceding atherosclerotic changes in the vascular wall.[44] Our findings may indicate that the atheroprotective properties of HDL2 could be overshadowed by factors, which promote atheroma formation, including age, LDL cholesterol and systolic BP. Alternatively, endothelial dysfunction and hyperplasia of the arterial intima and media may be distinct stages, involving different pathogenic pathways in the atherosclerotic process, as suggested by the absence of correlation between FMD and IMT in various cohorts, including patients with rheumatoid arthritis without clinically evident CVD.[45–47]

The current study has several limitations. First, its cross-sectional nature provides no information on the longitudinal effects of lipoprotein profile on endothelial function in patients with SLE. Second, the relatively small sample size may have limited the statistical significance of our findings. While HDL2 was a statistically significant predictor of greater FMD, the magnitude of this association was not large. In addition, our machine-learning model requires further prospective validation to predict FMD and CCA IMT in other SLE cohorts of larger sample sizes. Third, our generally young and healthy SLE study population

may have relatively 'favourable' lipoprotein and FMD/CCA IMT profiles, and it is unclear if our findings are replicable in older cohorts with more advanced disease and comorbidities. Fourth, the 10 subfractions obtained by the Lipoprint HDL system have not been correlated to the HDL subfractions reported in the literature.[48] However, studies regarding the effect of age, gender, total cholesterol and triglycerides on the distribution of HDL subfractions are in agreement with results obtained using other methodologies.[49] Last, an earlier meta-analysis by Mak *et al* has shown a lower FMD in patients with SLE free of CVD compared with healthy controls while our study reported demonstrated a tendency for impaired endothelial function in the lupus patients.[9] The lack of statistical significance could result from our relatively small sample size and because we have further restricted our study population to CVD-free patients with SLE naïve of cardiovascular risk factors.

In conclusion, we have shown that HDL2, a large, buoyant HDL subfraction, is a predictor of higher FMD even after adjustment for other risk factors. Larger, more buoyant HDL particles, which are more effective in reverse cholesterol transport, may be useful biomarkers for assessing endothelial function in SLE.[50] Although promising, the clinical application of HDL subfractions is currently hampered by lack of access in routine clinical laboratories and standardisation. As such, longitudinal studies and readily available clinical laboratory assays are required to investigate if HDL subfractions such as HDL2, rather than HDL cholesterol itself, influence the future risk of atherosclerotic CVD and cardiovascular outcomes in SLE.

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