Effects of statin therapy on coronary artery plaque volume and high-risk plaque morphology in HIV-infected patients with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial

Janet Lo, Michael T Lu, Ezinne J Ihenachor, Jeffrey Wei, Sara E Looby, Kathleen V Fitch, Jinhee Oh, Chloe O Zimmerman, Janice Hwang, Suhny Abbara, Jorge Plutzky, Gregory Robbins, Ahmed Tawakol, Udo Hoffmann, Steven K Grinspoon

Summary

Background HIV-infected patients have a high risk of myocardial infarction. We aimed to assess the ability of statin treatment to reduce arterial inflammation and achieve regression of coronary atherosclerosis in this population.

Methods In a randomised, double-blind, placebo-controlled trial, 40 HIV-infected participants with subclinical coronary atherosclerosis, evidence of arterial inflammation in the aorta by fluorodeoxyglucose (FDG)-PET, and LDL-cholesterol concentration of less than 3·37 mmol/L (130 mg/dL) were randomly assigned (1:1) to 1 year of treatment with atorvastatin or placebo. Randomisation was by the Massachusetts General Hospital (MGH) Clinical Research Pharmacy with a permuted-block algorithm, stratified by sex with a fixed block size of four. Study codes were available only to the MGH Research Pharmacy and not to study investigators or participants. The prespecified primary endpoint was arterial inflammation as assessed by FDG-PET of the aorta. Additional prespecified endpoints were non-calcified and calcified plaque measures and high risk plaque features assessed with coronary CT angiography and biochemical measures. Analysis was done by intention to treat with all available data and without imputation for missing data. The trial is registered with ClinicalTrials.gov, number NCT00965185.

Findings The study was done from Nov 13, 2009, to Jan 13, 2014. 19 patients were assigned to atorvastatin and 21 to placebo. 37 (93%) of 40 participants completed the study, with equivalent discontinuation rates in both groups. Baseline characteristics were similar between groups. After 12 months, change in FDG-PET uptake of the most diseased segment of the aorta was not different between atorvastatin and placebo, but technically adequate results comparing longitudinal changes in identical regions could be assessed in only 21 patients (atorvastatin Δ –0·03, 95% CI –0·17 to 0·12, *vs* placebo Δ –0·06, –0·25 to 0·13; p=0·77). Change in plaque could be assessed in all 37 people completing the study. Atorvastatin reduced non-calcified coronary plaque volume relative to placebo: median change –19·4% (IQR –39·2 to 9·3) versus 20·4% (–7·1 to 94·4; p=0·009, n=37). The number of high-risk plaques was significantly reduced in the atorvastatin group compared with the placebo group: change in number of low attenuation plaques –0·2 (95% CI –0·6 to 0·2) versus 0·4 (0·0, 0·7; p=0·03; n=37); and change in number of positively remodelled plaques –0·2 (–0·4 to 0·1) versus 0·4 (–0·1 to 0·8; p=0·04; n=37). Direct LDL-cholesterol (–1·00 mmol/L, 95% CI –1·38 to 0·61 *vs* 0·30 mmol/L, 0·04 to 0·55, p<0·0001) and lipoprotein-associated phospholipase A_2 (–52·2 ng/mL, 95% CI –70·4 to –34·0, *vs* –13·3 ng/mL, –32·8 to 6·2; p=0·005; n=37) decreased significantly with atorvastatin relative to placebo. Statin therapy was well tolerated, with a low incidence of clinical adverse events.

Interpretation No significant effects of statin therapy on arterial inflammation of the aorta were seen as measured by FDG-PET. However, statin therapy reduced non-calcified plaque volume and high-risk coronary plaque features in HIV-infected patients. Further studies should assess whether reduction in high-risk coronary artery disease translates into effective prevention of cardiovascular events in this at-risk population.

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Program in Nutritional Metabolism (J Lo MD, E J Ihenachor BS, J Wei BA, S E Looby PhD, K V Fitch FNP, J Oh BA, C O Zimmerman BA, J Hwang MD, Prof S K Grinspoon MD), **Cardiovascular Imaging Section, Department of Radiology** (M T Lu MD, Prof S Abbara MD, Prof U Hoffmann MD), **Division of Cardiology** (A Tawakol MD), **and Division of Infectious Disease** (G Robbins MD), **Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; and Division of Cardiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA USA** (J Plutzky MD)

Correspondence to Dr Steven K Grinspoon, Massachusetts General Hospital, LON-207, 55 Fruit Street, Boston, MA USA 02114 **sgrinspoon@mgh.harvard.edu**

Introduction

Coronary artery disease is a major cause of morbidity and mortality for patients living with long-term HIV infection.[1–4] Epidemiological studies fully adjusting for numerous risk factors and investigating validated cardiovascular events show increased risk in patients with HIV compared with that in those without.[5] Efficacious cardiovascular risk reduction interventions for HIV-infected patients are urgently needed.

Several studies have shown increased prevalence of subclinical atherosclerosis, including a predominant increase in non-calcified plaque in HIV-infected patients compared with patients without HIV, controlling for traditional coronary artery disease risk factors.[6–8]

Additional studies in HIV-infected patients have shown evidence of arterial inflammation[9] and increased vulnerable plaque morphology on coronary CT angiography (CCTA).[10] Greater volumes of non-calcified plaque and vulnerable plaque morphology on CCTA are associated with future major adverse cardiac events.[11]

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (statins) reduce the numbers of cardiac events and mortality in the general population. Imaging trials in patients without HIV have also shown that intensive statin therapy can slow progression of coronary atherosclerosis and even result in disease regression.[12–14] Previous studies of statin therapy in HIV-infected patients have shown improvement in lipid concentrations and reduction in markers of inflammation, as well as improvement in endothelial function, but no studies have prospectively investigated the effects of statins on direct measurements of arterial inflammation and coronary atherosclerosis in this population of patients.[15–18] We did a randomised, double-blind, placebo-controlled trial to investigate the effects of a statin on arterial inflammation and coronary atherosclerosis in HIV-infected people without known cardiovascular disease or raised LDL cholesterol concentrations but with subclinical atherosclerosis and arterial inflammation.

We hypothesised that coronary atherosclerosis would progress at a rapid rate in this population of HIV-infected people, despite normal concentrations of baseline LDL-cholesterol, and that treatment with a statin would reduce arterial inflammation, deter the progression of coronary atherosclerosis, and reduce non-calcified plaque volume and vulnerable plaque morphology, resulting in a decrease in high-risk atherosclerotic lesions.

Methods

Study design and participants

This study was a single-site, randomised, double-blind, placebo-controlled clinical trial. Study participants were men and women with HIV disease, no history of cardiovascular disease or cardiac symptoms, and evidence of subclinical coronary atherosclerosis, defined by presence of one or more plaques on CCTA but without clinically significant stenosis, defined as greater than 50% left main stenosis or greater than 70% stenosis in any major vessel. Additionally, participants who showed plaque on CCTA had subsequent screening with fluorodeoxyglucose (FDG)-PET to assess arterial inflammation. Patients were required to have evidence of arterial inflammation, defined as an aortic to venous target to background ratio of greater than 1·6 as measured with previously described methods[9] in addition to plaque on CCTA to be eligible for the study. Additional inclusion criteria were age 18–60 years, stable antiretroviral therapy (ART) with no changes in regimen in the preceding 6 months, and LDL cholesterol concentration between 1·81 mmol/L and 3·37 mmol/L (70–130 mg/dL). Participants did not meet guidelines in place at the time for statin therapy. Exclusion criteria were concurrent use of a statin, contraindication to statins, aspartate aminotransferase or alanine aminotransferase three-times greater than the upper limit of normal or treatment for active liver disease, renal disease, or creatinine greater than 130 μmol/L, acute infectious illness in the preceding 3 months, contraindication to β-blocker or nitroglycerin use because these drugs are given as part of the standard cardiac CT protocol, significant radiation exposure within the year before the study (eg, previous nuclear scans or radiation therapy), bodyweight greater than 136 kg because of CT scanner table limitations, allergy to iodine-containing contrast media, pregnancy, or breastfeeding. Participants were recruited via referral by infectious disease providers, newspaper and broadcast advertisements, and flyers posted in community and health centres.

Patients received either atorvastatin (starting at dose of 20 mg per day and escalating to 40 mg per day at the 3 month visit if study drug was well tolerated) or placebo. Prespecified discontinuation criteria for the study included creatine kinase greater than five times the upper limit of normal with symptoms of myalgia, myositis, or rhabdomyolysis, creatine kinase concentration greater than ten-times the upper limit of normal, and alanine aminotransferase or aspartate aminotransferase concentrations greater than three-times the upper limit of normal range. For patients in whom aspartate aminotransferase concentration was greater than two-times the upper limit of normal range at screening visit, continuation was allowed unless the concentration exceeded five-times the upper limit of normal range. If after starting the 40 mg dose, a participant developed symptoms of myalgias or an increase in creatine kinase, aspartate aminotransferase, or alanine aminotransferase concentrations above study stated limits, the dose was reduced from 40 mg back to 20 mg of atorvastatin or placebo once a day. All participants received standardised lifestyle counselling based on National Cholesterol Education Program (NCEP) guidelines at baseline, including standardised recommendations on smoking, exercise, and diet. All participants in both the atorvastatin and placebo groups received counselling on the therapeutic lifestyle changes diet by Massachusetts General Hospital (MGH) bionutritionists. Participants were encouraged to comply with ART, as prescribed by clinical carers. Participants were seen 1, 3, 6, 9, and 12 months after baseline. Primary and secondary endpoints were assessed at baseline and end of study. Safety was assessed at interim visits and final visit (1, 3, 6, 9, and 12 months) including assessment of symptoms, interim history, physical examination, aspartate aminotransferase, alanine aminotransferase, and creatine kinase. Endothelial function by reactive hyperaemia peripheral arterial tonometry was assessed at baseline and 12 months and will be reported separately.

All participants provided written informed consent and the study was approved by the institutional review board. A data and safety monitoring board consisting of an HIV specialist, a cardiologist, and a biostatistician met every 6 months for safety monitoring. All potential participants completed a brief telephone screen followed by two clinical assessments to determine eligibility for the study. Study visits were done in the Clinical Research Center at MGH.

Outcomes

The prespecified primary endpoint was arterial inflammation, as assessed by FDG-PET of the aorta. Other prespecified endpoints were coronary atherosclerotic plaque (non-calcified and calcified plaque and high risk plaque features) as assessed by coronary CT angiography, lipids, inflammatory, immunological, and biochemical measures.

Randomisation and masking

Randomisation was done by the MGH Clinical Research Pharmacy with a permuted block algorithm, stratified by sex with a fixed block size of four, with allocation (1:1) to atorvastatin or identical matching placebo. Study codes were available only to the MGH Research Pharmacy and not to study investigators or participants. Dose escalations were similarly blinded with matching increases for placebo-treated patients. Investigators analysing FDG-PET and CCTA were masked to clinical data and randomisation. Randomisation was stratified by sex, and sex effect was assessed by the Breslow Day test.

Procedures

Participants had PET imaging after an overnight fast to reduce myocardial FDG uptake. PET imaging was done 3 h after administration of 13 mCi of fluorine-18-labelled FDG (Siemens ECAT Exact HR+ PET or biograph 64 system, Knoxville, TN, USA).[19] At the time the study was initiated, the only option to assess aortic FDG-PET was via a dedicated PET scanner; a combined PET–CT scanner was not available for non-clinical use.

An experienced cardiologist who was masked to clinical data and randomisation did image analysis. Co-registration of the PET and CT images was done with anatomical landmarks including ascending aorta, left atrium, and spine. The ascending aorta was chosen for measurement. The target-to-background ratio was calculated by dividing the mean arterial standardised uptake value by the mean venous standardised uptake value.[9] The mean arterial standardised uptake value was derived from the maximum values in serial axial measurements. All images were assessed for quality before unblinding and rated for acceptability on the basis of ability to coregister CT and PET and comparability of registration between serial scans. Derivation of tissue activity was not done for images deemed to be of unacceptable quality.

ECG-gated CCTA with tube current modulation was done on a Somatom Definition Flash 128-slice dual source CT (Siemens Medical Solutions, Forchheim, Germany) according to the guidelines of the Society of Cardiac Computed Tomography[20] at enrolment and at 1 year follow-up. Before CCTA, between 0 mg and 15 mg of intravenous metoprolol was given in 5 mg doses to achieve a resting heart rate of less than 65 beats per min. 0·6 mg of sublingual nitroglycerin was given for vasodilation. The CT protocol included a non-contrast CT for calcium scoring, a timing bolus, and the CCTA. After a 20 cm³ timing bolus, intravenous contrast (iopamidol 370 g/cm³, Bracco Diagnostics, Inc, Princeton, NJ USA, 60–80 cm³ at 4·5–5·4 cm³/s based on participant size) was injected followed by a 40 cm³ normal saline flush. Acquisition parameters were tube voltage 120 kV; ECG-dependent tube current modulation with retrospective gating and with reference 320 mAs; adaptive pitch 0·2–0·4 based on heart rate; collimation 128×0·6 mm; rotation time 280 ms; and temporal resolution 75 ms. All scans were done in an inspiratory breath hold. Images were reconstructed with 5% intervals between 60% and 75% of the R-R interval and 10% intervals elsewhere in

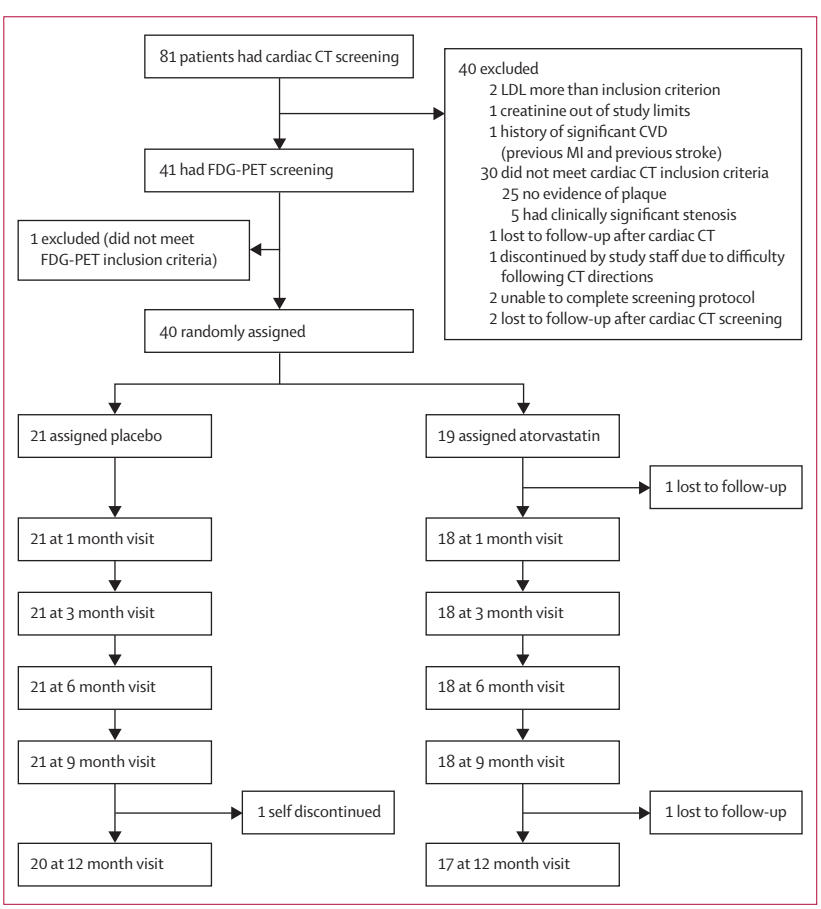

Figure 1: **Study profile**
CVD=cardiovascular disease. MI=myocardial infarction. FDG=fluorodeoxyglucose.

the cardiac cycle, a slice thickness of 0·75 mm, and an increment of 0·7 mm. The cardiac phase or phases that minimised motion artifact for each of the coronary arteries were identified and used for image analysis.

All image data at baseline and 1 year follow-up were analysed with an offline workstation (Syngo Multimodality Workplace, Siemens, Forchheim, Germany) by an experienced cardiac radiologist who was masked to clinical data and randomisation. For assessment of total and non-calcified plaque in CCTA data sets, cross sectional and multiplanar reconstructed images were assessed for the presence and qualitative composition of atherosclerotic plaque for each coronary artery segment defined by the Society of Cardiac Computed Tomography model.[20] Non-calcified plaque was defined as any discernible structure that could be assigned to the coronary artery wall with a density less than 130 HU that could be identified in at least two independent planes.[21] Luminal stenosis was graded as less than 25%, 25–50%, 50–69%, or 70% or greater according to the guidelines of the Society of Cardiac Computed Tomography.[20] Substantial progression in coronary artery stenosis was defined as an increase to greater than 50% stenosis from a baseline stenosis of less than 50% or an increase to greater than 70% stenosis in patients with greater than 50% but less than 70% stenosis in any coronary artery segment at baseline.

Semiautomated plaque volume measurements were done on a second analysis package (Aquarius iNtuition, Terarecon, Foster City, CA, USA). The software generated curved planar reformats around a centre line through the coronary artery lumen, which was edited as necessary.[22,23] The plaque length was established visually with a marker in the proximal and distal plaque limits. Plaque length was kept constant between the enrolment and follow-up CT to minimise variability.[24] The inner and outer borders of the vessel lumen and plaque were established with a semiautomatic tracer and plaque volume was calculated automatically. When the contours deviated from the vessel or plaque border, they were manually corrected. Voxels with attenuation less than 130 HU were assigned to the non-calcified volume of the plaque. This volumetric plaque measurement technique has excellent intraobserver, interobserver, and interscan reproducibility.[23,25–29]

Each coronary segment was then further assessed for high-risk plaque features defined as positive remodelling, low attenuation plaque, and spotty calcium. Positive remodelling was defined as a ratio of plaque segment outer diameter to reference segment outer diameter greater than 1·05.[30] If low attenuation was visually noted in non-calcified plaque, five region-of-interest measurements (area 1·0 mm²) were placed within the low attenuation portion. Low attenuation plaque was defined when the smallest mean attenuation number in these regions of interest was less than 40 HU.[30] This threshold was also used in our previous report establishing high prevalence of low attenuation plaque in people with HIV.[10] Spotty calcium was defined by calcified plaque with a maximum diameter of 3 mm occupying only one side of the vessel wall.[31] Our group and others have previously described excellent intraobserver and interobserver variability for these high-risk features.[32,33]

	Placebo (n=21)	Atorvastatin (n=19)
Age (years)	50·0 (5·6)	52·2 (3·8)
Male	17 (81%)	15 (79%)
Race or ethnic group		
White	13 (68%)	13 (68%)
Black	3 (16%)	3 (16%)
Asian	1 (5%)	0
Hispanic	1 (5%)	1 (5%)
More than one race	1 (5%)	2 (11%)
Framingham 10 year risk estimate (%)	5·4% (4·4)	6·9% (4·1)
Hypertension	2 (10%)	4 (21%)
Diabetes mellitus	2 (10%)	2 (11%)
Current smoker	6 (29%)	5 (26%)
Lipids		
Total cholesterol (mmol/L)	4·97 (0·70)	5·14 (0·98)
HDL cholesterol (mmol/L)	1·31 (0·39)	1·34 (0·50)
Direct LDL cholesterol (mmol/L)	3·23 (0·83)	3·20 (0·95)
Triglycerides (mmol/L)	1·28 (1·04–1·53)	1·36 (1·10–2·31)
HIV disease related parameters		
CD4 T-lymphocytes	590 (289)	522 (263)
HIV RNA viral load (copies per mL)	<48 (<48–48)	<48 (<48–<48)
Undetectable HIV RNA (<48 copies per mL)	17 (81%)	16 (84%)
Duration since HIV diagnosis (years)	15·0 (6·9)	16·8 (5·1)
Currently on antiretroviral therapy	100%	100%
Duration of antiretroviral therapy (years)	11·4 (5·8)	12·4 (3·7)
Current PI treatment	8 (38%)	11 (58%)
Duration of PI treatment (years)	4·9 (5·8)	6·8 (5·6)
Current NRTI treatment	21 (100%)	17 (89%)
Duration of NRTI treatment (years)	11·3 (6·1)	10·8 (4·4)
Current NNRTI treatment	12 (57%)	7 (37%)
Duration of NNRTI treatment (years)	4·6 (5·4)	1·9 (3·0)
Anthropometric measures		
Body-mass index (kg/m²)	25·8 (4·8)	25·6 (2·9)
Visceral adipose tissue area (cm²)	142 (74)	166 (130)
Subcutaneous adipose tissue area (cm²)	202 (120)	152 (93)
Haemodynamic measures		
Systolic blood pressure (mm Hg)	119 (16)	117 (13)
Diastolic blood pressure (mm Hg)	76 (10)	73 (8)
Metabolism		
Fasting glucose (mmol/L)	4·92 (0·38)	4·76 (0·66)
Haemoglobin A_{1c} (%)	5·5 (0·3)	5·6 (0·4)
Inflammation		
C-reactive protein (mg/L)	1·1 (0·4–2·4)	0·8 (0·3–1·9)
Interleukin 6 (ng/L)	0·8 (0·5–1·2)	0·6 (0·4–1·6)

Data are mean (SD), n (%), or median (IQR). NRTI=nucleoside or nucleotide reverse transcriptase inhibitor. NNRTI=non-nucleoside reverse transcriptase inhibitor. PI=protease inhibitor.

Table 1: **Baseline characteristics**

The presence and extent of coronary artery calcification was assessed on non-contrast enhanced images and calculated using the Agatston method.[34] Calcium mass, volume, and density were also determined with the non-contrast enhanced images.

Direct LDL was measured by homogeneous enzymatic colorimetric assay (Roche COBAS INTEGRA). Other lipid concentrations including total cholesterol, HDL, and triglycerides were measured with standard techniques. Blood was drawn after a 12 h fast.

Glucose and haemoglobin A_{1c} (HbA_{1c}) were measured with standard techniques after 12 h overnight fast. C-reactive protein (CRP) concentrations were measured with ELISA. CD4 T-cell counts were assessed by flow cytometry. HIV RNA was measured in real time with clinically available ultrasensitive reverse-transcription PCR assays. From December, 2009, to November, 2011, Ampliprep Taqman V1.5(Roche) was used to measure HIV RNA (linear range 48–10 million copies per mL of plasma). From 2012 to present, Ampliprep Taqman 48 V2 (Roche) was used (linear range 20–10 million copies per mL). Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) was measured by ELISA (PLAC Test; diaDexus, Inc, South San Francisco, CA, USA). Assessment of flow cytometry and other immune activation indices was planned. These data were collected but have not yet been fully analysed. This manuscript focuses on clinically relevant inflammatory, biochemical, and immunological assessments. A Veterans Aging Cohort Study (VACS)[35] score was calculated for each patient at baseline and end of study.

Weight and anthropometric measurements were measured in the morning, before breakfast. Abdominal visceral and abdominal subcutaneous adipose tissue area (VAT and SAT, respectively) were quantified with a cross-sectional abdominal CT scan at the level of the L4 pedicle.[36,37] DEXA measures of bone were obtained, but not included in this manuscript, which focuses on cardiovascular endpoints. 4 day food records were completed by the participants and analysed with Minnesota Nutrition Data System software.

Statistical analysis

Comparisons between the two groups (atorvastatin *vs* placebo) were done with Student *t* test for normally distributed continuous variables and Wilcoxon rank sum test if the distribution was non-normal. To assess changes within each group, a paired *t* test was done. For comparison between groups of dichotomous variables, Fisher's exact test was used with cell numbers less than 5, otherwise χ^2 test was used. Two-tailed probability values are reported,

	Baseline placebo group (n=21)	Baseline atorvastatin group (n=19)	Change in placebo group (n=20)	Change in atorvastatin group (n=17)	Between-group p value
Vascular inflammation					
Mean FDG-PET TBR of aorta	2·20 (0·37) n=12	2·08 (0·32) n=12	–0·05 (–0·28 to 0·17) n=11	0·04 (–0·08 to 0·16) n=10	0·41
Mean FDG-PET TBR of most diseased segment of aorta	2·26 (0·37) n=12	2·18 (0·33) n=12	–0·06 (–0·25, 0·13) n=11	–0·03 (–0·17 to 0·12) n=10	0·77
Plaque volume (mm^3)					
Non-calcified (<130 HU) plaque volume	66·1 (14·8 to 94·8)	33·7 (19·6 to 83·5)	6·7 (–6·5 to 29·8)	–8·2 (–18·3 to 3·5)	0·03
Change in non-calcified plaque volume	..	..	20·4% (–7·1 to 94·4)	–19·4% (–39·2 to 9·3)	0·009
Total plaque volume	81·1 (30·6 to 134·9)	55·2 (23·0 to 153·6)	12·0 (0·8 to 100·4)	–0·8 (–16·8 to 14·2)	0·02
Change in total plaque volume	..	..	18·2% (1·5 to 59·9)	–4·7% (–25·4 to 15·9)	0·01
Coronary artery calcium					
Calcium score (total Agatson score)	24·4 (0·0 to 46·9)	10·9 (0·0 to 92·6)	1·7 (0·0 to 28·0)	0·9 (0·0 to 18·5)	0·74
Calcium mass (mg)	4·1 (0·0 to 6·9)	1·9 (0·0 to 12·8)	0·2 (0·0 to 2·9)	0·7 (0·0 to 3·9)	0·48
Calcium volume (mm^3)	17·8 (0·0 to 37·3)	10·5 (0·0 to 70·4)	1·2 (0·0 to 18·5)	5·0 (0·0 to 25·8)	0·63
Calcium density (mg/mm^3)	0·2 (0·2 to 0·2)	0·2 (0·2 to 0·2)	0·0 (0·0 to 0·0)	0·0 (0·0 to 0·0)	0·51
Plaque vulnerability features*†					
Low attenuation (<40 HU) plaques	0·4 (0·6)	0·8 (1·0)	0·4 (0·0 to 0·7)	–0·2 (–0·6 to 0·2)	0·03
Positively remodelled plaques (remodelling index >1·05)	2·1 (1·8)	2·0 (2·4)	0·4 (–0·1 to 0·8)	–0·2 (-0·4 to 0·1)	0·04
Plaques with spotty calcification	2·0 (2·1)	2·4 (2·9)	–0·2 (-0·4 to 0·0)	0·0 (–0·3 to 0·3)	0·25
Patients with progression of coronary stenosis beyond clinically significant thresholds‡	..	..	3/20 (15%)	0/17 (0%)	0·23‡
Patients with any regression of plaque volume					
Progression of total plaque volume	..	..	16/20 (80·0%)	6/17 (35·3%)	0·008§
Regression of total plaque volume	..	..	4/20 (20·0%)	11/17 (64·7%)	..

Non-normally distributed data presented as median (IQR). Normally distributed data presented as mean (SD). FDG=fluorodeoxyglucose. TBR=target-to-background ratio. *Changes presented as mean (95% CI). †For ease of interpretation, some non-normally distributed data are presented at mean (SD), but p values by non-parametric comparison. ‡Progression from <50% to >50% stenosis or progression from <70% to >70% stenosis. §Fisher's exact test is used for small cell numbers.

***Table 2*: Vascular inflammation and coronary plaque measurements**

and statistical significance was assumed when p<0·05. Means and 95% CIs are reported to describe changes in continuous variables with normal distribution, otherwise, medians and IQRs are used. CRP concentrations were log transformed to reach normal distribution. All statistical analyses were done with SAS JMP (SAS Institute). The prespecified primary study endpoint was arterial inflammation because data showing effects on FDG-PET of the carotid in patients without HIV were available at the time of study initiation to provide data for sample-size estimation,[38] whereas no data on effects of statin on coronary plaque volume measured by CCTA were available at time of study initiation. The main endpoint for plaque was non-calcified plaque volume. With 40 participants and a planned dropout rate of 15%, the study was designed to detect an approximate 15% difference in arterial inflammation between groups and a one SD change in other endpoints at α=0·05, 85% power. The dropout rate was less than anticipated, at 7·5%. Intention-to-treat analysis was done with all available data. No imputation was made for missing data as attrition was extremely low and equal in groups. Sensitivity analysis was done among patients who maintained undetectable viraemia throughout the study. Sensitivity analyses were also done, controlling for baseline plaque volume. The relation between the change in LDL cholesterol concentration and plaque was assessed by Wilcoxon rank sum test comparing changes in plaque variables in participants who achieved LDL cholesterol of less than 2·59 mmol/L and those who did not, and less than 1·8 mmol/L and those who did not.

The institutional review board approved the protocol anticipated screening of up to 100 patients to enrol 40 into the trial. A plan to enrol patients without plaque into a non-randomised longitudinal natural history study was not done because of constraints on time and expense. The trial is registered with ClinicalTrials.gov, number NCT00965185.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

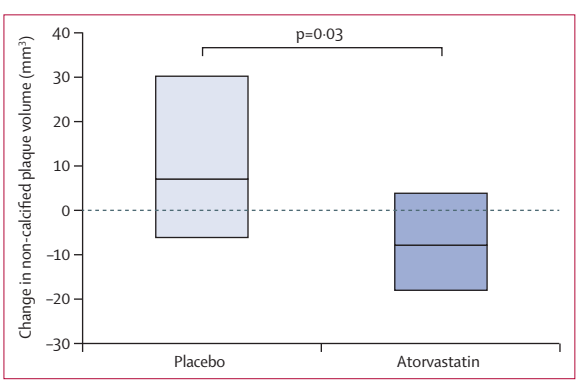

Figure 2: **Comparison of the 1 year change in non-calcified plaque volume in study participants**

Median and IQR.

See Online for appendix

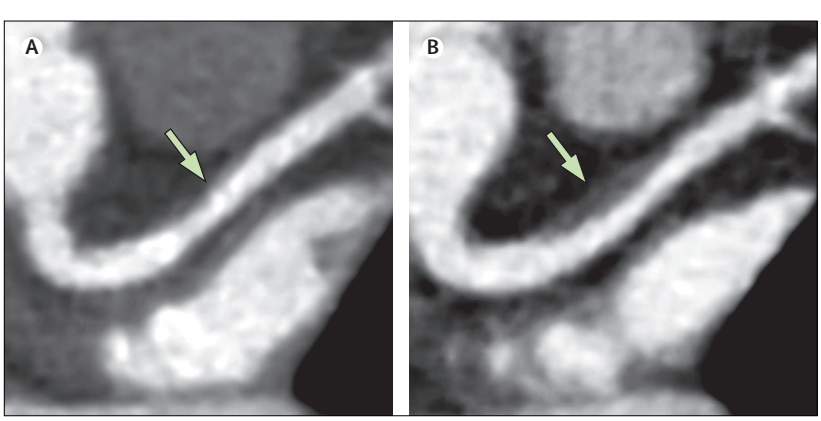

Figure 3: **Increasing non-calcified plaque in proximal left anterior descending (LAD) coronary artery in patient on placebo**

CT angiography of LAD coronary artery (arrow) at baseline (A) and 12 month follow-up (B) in a 58-year-old woman with HIV. Plaque volume increased from 11 to 124 mm³ (arrow). High risk morphology features including positive remodelling and low attenuation lipid core developed on follow-up.

Results

The study ran from Nov 13, 2009, to Jan 13, 2014. We screened 81 HIV-infected patients; screening was sequential such that the presence of plaque was first established by CCTA (figure 1). The study took several years to complete because treatment duration was 1 year and recruitment was at a single site. 40 participants were randomly assigned to receive either atorvastatin (n=19) or placebo (n=21). Age, sex, race and ethnicity, body-mass index, and Framingham 10 year risk estimate were similar between both groups at baseline (table 1). Prevalence of hypertension, diabetes mellitus, and current smoking were similar between groups as were blood pressure, fasting glucose, HbA_{1c}, total cholesterol, HDL, LDL, and CRP measurements. HIV disease variables, including duration and type of ART, were also similar in the two groups. Participants were all on ART and most patients had undetectable viraemia with similar immunological and virological indices between groups (table 1). Four patients in the placebo group and three in the atorvastatin group had detectable viraemia but the maximum viral load at baseline was 355 copies per mL and all but three had viral loads less than 100 copies per mL. Patients were all on ART for a mean of 11–12 years without differences between the groups. All patients had started ART more than 2 years before the study and all were on a stable regime for at least 6 months before study initiation. Baseline VACS scores were not different between the atorvastatin and placebo groups (appendix p 1). Viral-load information was given to referring physicians responsible for ongoing clinical care of patients. At baseline, no significant differences were noted between groups in arterial inflammation, plaque volume, or other atherosclerosis variables (table 2).

Discontinuation rates were similar between groups (figure 1). Adherence was determined by pill count and was similar between groups at each visit (appendix p 2).

After manual coregistration of PET and CT scans, change from baseline to 12 months was assessed. Although manual coregistration was adequate for assessment of baseline data for screening, identical anatomical regions were difficult to assess with manual coregistration on serial scans. Of the available data pairs, 21 were of acceptable image quality to permit assessment of change over time in identical regions in serial scans. In this group, no statistically significant differences between the atorvastatin and placebo groups were detected (table 2).

Atorvastatin decreased non-calcified coronary plaque volume as compared with placebo over 12 months (figure 2, table 2; p=0·03). On a percentage basis, patients receiving atorvastatin had a decrease in plaque volume compared with an increase in non-calcified plaque volume in the placebo group (table 2; p=0·009). Additionally, atorvastatin reduced total coronary plaque volume compared with placebo (p=0·02); patients taking atorvastatin had a decrease in total plaque volume, which increased over 12 months in those taking placebo (p=0·01).

The number of segments with low attenuation plaques (<40 HU) and number of positively remodelled plaques (remodelling index >1·05) were significantly reduced by atorvastatin compared with placebo (p=0·03 and 0·04, respectively, table 2). Change in number of plaques with spotty calcifications was not significantly different between the two groups (table 2).

11 (64·7%) of 17 patients in the atorvastatin group had regression of coronary atherosclerosis (based on any reduction in plaque volume) compared with four (20%) of 20 in the placebo group (p=0·008). Conversely, 16 patients (80%) in the placebo group had progression of coronary atherosclerosis (based on any increase in plaque volume) compared with six patients (35·3%) patients in the atorvastatin group (table 2). No obvious effect of sex on plaque regression or progression was seen by Breslow Day test (p=0·17). Figure 3 shows an example of progression of coronary plaque in the proximal left anterior descending coronary artery of an HIV-infected participant randomly assigned to receive placebo.

Three participants in the placebo group developed clinically significant increases in the extent of stenosis (two participants in the placebo group had stenosis that progressed to beyond 70% at 12 months and one participant in the placebo group developed >50% stenosis at 12 months). Although of clinical relevance,

	Baseline placebo group (n=21)	Baseline atorvastatin group (n=19)	Change in placebo group (n=20)	Change in atorvastatin group (n=17)	Between-group p value
Lipids					
Total cholesterol (mmol/L)	4·97 (0·70)	5·14 (0·98)	0·12 (–0·12 to 0·37)	–1·23 (–1·53 to –0·93)	<0·0001
HDL cholesterol (mmol/L)	1·31 (0·39)	1·34 (0·50)	–0·04 (–0·17 to 0·09)	0·02 (–0·11 to 0·16)	0·48
Direct LDL-cholesterol (mmol/L)	3·23 (0·83)	3·20 (0·95)	0·30 (0·04 to 0·55)	–1·00 (–1·38 to 0·61)	<0·0001
Triglycerides (mmol/L)	1·28 (1·04 to 1·53)	1·36 (1·10 to 2·31)	0·08 (–0·46 to 0·38)	–0·10 (–0·46 to 0·44)	0·64
HIV disease-related variables					
CD4 T-lymphocytes (cells per μL)	590 (289) n=20	522 (263)	4 (–72 to 80) n=19	17 (–68 to 101)	0·81
HIV RNA viral load (copies per mL)	<48 (<48, 48)	<48 (<48 to <48)	0	0	0·40
Anthropometric variables					
Body-mass index (kg/m²)	26 (5)	26 (3)	–0·1 (–0·9 to 0·8)	–0·4 (–1·0 to 0·3)	0·60
Visceral adipose tissue area (cm²)	142 (74)	166 (130)	8 (–8 to 24)	–5 (–26 to 17)	0·31
Subcutaneous adipose tissue area (cm²)	202 (120)	152 (93)	2 (–20 to 24)	6 (–8 to 19)	0·76
Haemodynamic variables					
Systolic blood pressure (mm Hg)	119 (16)	117 (13)	3 (–1 to 7)	7 (3 to 11)	0·15
Diastolic blood pressure (mm Hg)	76 (10)	73 (8)	3 (1 to 6)	6 (3 to 8)	0·14
Metabolic variables					
Fasting glucose (mmol/L)	4·92 (0·38)	4·76 (0·66)	1·00 (–0·37 to 2·36)	0·10 (–0·22 to 0·41)	0·21
Haemoglobin A_{1c}	5·5% (0·3) n=19	5·6% (0·4) n=17	0·3% (–0·5 to 1·0)	–0·1% (–0·2 to 0·1)	0·36
Inflammatory variables					
Log CRP (mg/L)	0·1 (0·5)	–0·1 (0·6)	0·1 (–0·2 to 0·3)	–0·3 (–0·6 to 0·.0)	0·06
Interleukin 6 (ng/L)	0·8 (0·5 to 1·2)	0·6 (0·4 to 1·6)	0·1 (–0·2 to 0·4)	0·2 (–0·8 to 0·3)	0·77
Marker of vascular inflammation					
Lp-PLA_2 (ng/mL)	272·5 (73·7)	285·6 (75·0)	–13·3 (–32·8 to 6·2)	–52·2 (–70·4 to –34·0)	0·005

Non-normally distributed data are presented as median (IQR). Normally distributed data are presented as mean (SD). For change columns, normally distributed data are presented as mean (95% CI). CRP=C-reactive protein. Lp-PLA_2=lipoprotein-associated phospholipase A_2.

Table 3: **Metabolic, inflammatory, and body composition variables**

the difference between groups was not statistically significant. None of these patients developed symptoms of coronary artery disease. By contrast, no participants in the atorvastatin group developed clinically significant increases in extent of stenosis (table 2).

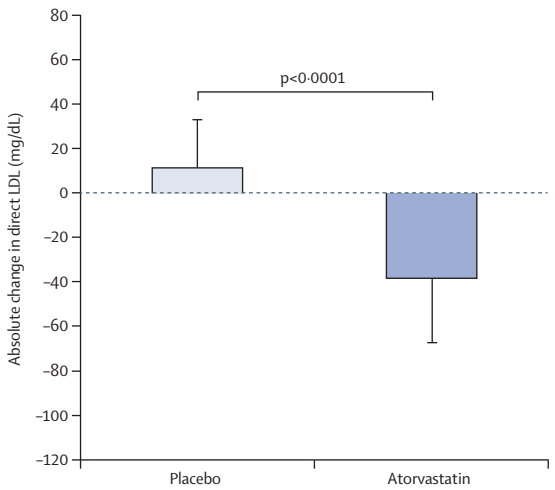

Figure 4: **Comparison of the 1 year change in direct LDL in study patients**
Mean (box) and SD (bar).

	Placebo	Atorvastatin
Any serious adverse event	1	2
Any adverse event leading to study-drug discontinuation	0	0
Any adverse event leading to study-drug dose decrease*	1	1
Any adverse event leading to non-escalation of dose at 3 months†	2	0
Loose stools	1	3
Nausea	2	1
Muscle aches or cramps	5	6
LFT abnormalities (≥three-times upper limit of normal)	2	3
Creatine kinase elevation (≥five-times upper limit of normal)	0	0
Elevated fasting blood glucose (>126 mg/dL)‡	2	0
Rash	1	0
Abdominal pain	1	0
Allergic reaction (to study medication)	0	0

Data are number of patients. In the atorvastatin group, initial presentation of myalgias occurred at 15 days, 1, 2, 3, 5, and 9 months. In the placebo group, initial presentation of myalgias occurred at 16 days, 1 (two patients), 2, and 3 months. LFT abnormalities occurred at 3 and 6 months (two patients) in the atorvastatin group. LFT abnormalities occurred at 3 and 6 months in the placebo group. Serious adverse events included hepatocellular carcinoma (one patient in placebo group); detection of large cyst at routine ultrasound, for which the patient had partial hysterectomy (one in atorvastatin group); and treated in an emergency department for gastrointestinal virus-induced dehydration (one in atorvastatin group). LFT=liver function test. *Alanine aminotransferase elevation (placebo group); myalgia (atorvastatin group). †Myalgia led to non-escalation until resolved at 9 month visit; myalgia temporarily led to non-escalation until 6 month visit. ‡Fasting glucose checked at 12 month visit.

Table 4: **Adverse events**

In a supplementary analysis, the effect of atorvastatin on plaque segments was analysed. Number of segments with plaque and number of segments with non-calcified plaque both decreased in the group assigned to atorvastatin compared with those assigned placebo, but segments with calcified plaque or mixed plaque did not change between the groups (appendix p 3).

No significant differences were noted in coronary artery calcium score, calcium mass, calcium volume or calcium density at baseline or the change over time between the two groups (table 2).

Total cholesterol and direct LDL significantly decreased with atorvastatin compared with placebo: total cholesterol change –1·23 mmol/L (95% CI –1·53 to –0·93) versus 0·12 mmol/L (–0·12 to 0·37; p<0·0001); direct LDL change –1·00 mmol/L (–1·38 to 0·61) versus 0·30 mmol/L (0·04 to 0·55; p<0·0001; table 3; figure 4). There was no statistically significant relation between change in LDL and plaque (appendix p 5). No statistically significant differences in changes in fasting glucose or HbA_{1c} were detected between the two groups (table 3).

Lp-PLA_2 significantly decreased with atorvastatin compared with placebo: –52·2 ng/mL (95% CI –70·4 to –34·0) versus –13·3 ng/mL (–32.8 to 6·2; p=0·005). Log CRP tended to decrease in the atorvastatin group compared with that in the placebo group (p=0·06; table 3).

Changes in CD4 count and HIV RNA did not differ between the atorvastatin and placebo groups after 1 year (table 3). Change in VACS score was not different between groups including individual components such as haemoglobin and fibrosis-4 index (appendix p 1).

No statistically significant differences in changes in BMI, visceral adipose tissue, or subcutaneous adipose tissue areas were detected between the two treatment groups (table 3). Food records were returned from only a subset of patients and showed intake of total calories, fat, protein, or carbohydrates did not differ between the groups at baseline and the changes over the course of the study did not differ significantly between the groups (appendix p 4).

Adverse events were distributed fairly evenly between the atorvastatin and placebo treatment groups. Myalgias and liver-function-test abnormalities occurred in both treatment and placebo groups at similar rates without any differences in the timing of adverse events (table 4). No adverse event led to discontinuation from the study (table 4). One participant had myalgias and was reduced to 10 mg, which was tolerated for the duration of the trial. After unblinding at the conclusion of the study, this participant had received atorvastatin. A second participant had a dose reduction from 40 mg back to 20 mg because of a rise in alanin aminotransferase. Unblinding after the conclusion of the study revealed this participant received placebo.

In participants who maintained undetectable viral loads throughout the study, similar results in plaque and

other variables were seen (appendix p 5). Sensitivity analyses controlling for baseline non-calcified or total plaque volume using ANCOVA, in respective analyses, showed similar results, with significant effects of atorvastatin to reduce non-calcified (atorvastatin effect estimate –16·2 mm³, 95% CI –31·3 to –1·0; p=0·04, and total plaque volume –23·0 mm³, 95% CI –44·6 to –1·4; p=0·04]).

Discussion

In this randomised, double-blind, placebo-controlled study of HIV-infected patients with subclinical atherosclerosis, atorvastatin did not seem to reduce arterial inflammation in the aorta, but treatment deterred overall coronary plaque progression and induced coronary plaque regression in HIV-infected patients, largely through effects on non-calcified plaque volume. Atorvastatin reduced coronary non-calcified plaque volume by 19·4% in 1 year in the patients in our study, compared with an increase of 20·4% in the placebo group. Atorvastatin reduced high-risk plaque features such as low attenuation and positive remodelling, which have been linked to acute coronary events.[39] These changes occurred in the context of a net change in LDL cholesterol concentration of 1·30 mmol/L (1·00 mmol/L reduction in atorvastatin group and 0·30 mmol/L increase in placebo group) in a group in whom baseline LDL cholesterol was not raised by design (eg, ≤3·37 mmol/L or 130 mg/dL). The study population, on ART, without a clinical history of cardiovascular disease, but with subclinical plaque and arterial inflammation is representative of patients with HIV at risk for coronary artery disease. The VACS score in our population was comparable to that seen in other HIV populations including those described in the ART Cohort Collaboration.[35]

One objective of this study was to assess the effects of statins on specific inflammatory endpoints. Previous studies of statin therapy measured biomarkers of inflammation, immune activation,[16,17,40] and endothelial function[15] in HIV-infected patients but have not assessed direct measures of arterial inflammation. A significant reduction of CRP was not seen in a recent study of rosuvastatin in HIV-infected patients.[16] We recorded a small reduction in log CRP concentration with atorvastatin at 40 mg/day, the effect was not significant. We did record a significant reduction in Lp-PLA_2 by 18·3%, consistent with findings from another study.[17] Lp-PLA_2 is an inflammatory enzyme secreted by monocytes, macrophages, and other inflammatory cells that hydrolyses phospholipids on lipoproteins including oxidised LDL particles within the arterial intima, and leads to recruitment of monocytes to the intima.[41,42] Concentrations of Lp-PLA_2 and its activity are predictive of cardiovascular events in many large epidemiological cohorts;[43] however, darapladib, a selective oral inhibitor of Lp-PLA_2, did not reduce cardiovascular events in a study of non HIV-infected patients.[44]

We assessed FDG uptake in the wall of the aorta as a key endpoint to try to define potential effects of statins on direct indices of arterial inflammation in this cohort in whom arterial inflammation is increased.[9] At the time the study was initiated, however, we had no option of using a combined PET/CT scanner at our institution. The use of a dedicated PET scanner (which lacks integrated CT imaging) adversely affected the ability to coregister the molecular and structural imaging data, and limited the ability to follow regions of arterial inflammation over time. Thus, we were able to obtain interpretable data in only a subset of patients, which limits our ability to obtain meaningful data on the change in FDG uptake within this study. Further studies with advanced molecular imaging techniques might be useful to assess atherosclerotic inflammation to complement studies on plaque morphology.

Using CCTA, we previously found a high prevalence of subclinical coronary atherosclerosis even in HIV-infected patients with low Framingham risk estimates and that the increased plaque type in HIV-infected patients is non-calcified plaque rather than calcified plaque.[6] Non-calcified plaque is lipid-laden, has higher macrophage content, and is more vulnerable to rupture. Thus, a decrease in non-calcified plaque in HIV patients is meaningful, suggesting that statin therapy reduces the lipid-rich necrotic core of these plaques.[39] We had also previously shown that vulnerable plaque features were common in HIV-infected patients and were more prevalent than in matched controls.[10] Now, we show that statin therapy can reduce the number of plaques with these vulnerability features in patients with HIV as well as the volume of non-calcified plaque. This study is relevant to the many patients with HIV and subclinical atherosclerotic disease—more than half of the asymptomatic patients screened in our study. We did not see a statistically significant relation of change in LDL to plaque, suggesting other mechanisms might also be operative to achieve these results. Additional studies might further determine the relation of LDL lowering to plaque reduction in this population. Results were similar in terms of plaque endpoints in analyses restricted to patients who maintained viraemic control.

The rapid progression and nature of coronary plaque in patients randomly assigned to placebo provide evidence regarding the natural history of atherosclerosis in HIV. Not only did plaque volume increase, but three of 20 patients in the placebo group had progression of clinically significant coronary stenosis after 1 year follow-up. By contrast, none of the statin-treated group progressed to clinically significant stenosis. Evidence for increased atherosclerotic disease progression in HIV-infected patients suggests the need for more aggressive treatment with statins as in other high-risk populations.

Our study is the first to investigate the effects of a statin on coronary atherosclerosis in patients with HIV (panel). In the non-HIV-infected population, statins at

Panel: Research in Context

Systematic Review

We did a systematic review of all clinical trials of the effect of statins on cardiovascular endpoints in patients with HIV disease in the last 10 years using PubMed, using the search terms "statin" and "HIV". Additionally, we searched for clinical trials assessing effects of statins on plaque volume by imaging with either intravascular ultrasound or coronary CT angiography (CCTA) in patients with HIV. We searched for studies of these variables in the general population to provide a reference for our results. Searches were last done on Nov 17, 2014. Although a few randomised trials in HIV-infected patients have assessed the effects of statins on lipids, inflammatory markers, HIV disease variables, and safety, no randomised trial has assessed the effects of a statin on direct measures of arterial inflammation with fluorodeoxyglucose-PET or coronary atherosclerosis, either by intravascular ultrasound or CCTA, in HIV-infected patients. In the general population, randomised trials investigating effects of statins with intravascular ultrasound have been done but randomised studies have not assessed the effects of statins with CCTA. With respect to events, results of meta-analyses of data from more than 170 000 individuals in 27 randomised trials in the general population have shown the benefit of statins in reducing major vascular events,[45] but no such data exist for the HIV population.

Interpretation

Our findings provide the first evidence of the effects of statin therapy on coronary atherosclerois in a randomised double-blind trial of HIV-infected patients with subclinical coronary disease using CCTA to quantify and characterise coronary plaque. Our study is also the first randomised double-blind trial to show reduction in coronary plaque using CCTA. The results of our study, showing a reduction in plaque volume and plaques with vulnerability features by atorvastatin in HIV-infected patients, extend data from studies in the general population suggesting overall reduction in plaque burden and especially reduction of lipid-rich vulnerable plaque by statin therapy.

high doses can induce regression of atheroma volume measured by intravascular ultrasound[13,14] in patients with known coronary disease. In the current study, we investigated rates of plaque regression over 1 year in HIV-infected patients with subclinical plaque using CCTA and showed a 1 year plaque regression rate of 64·7% with atorvastatin up to 40 mg a day. Previous studies with CCTA to assess treatment effects of statins in the general population were not randomised trials, but also shed light on the potential effects of statins on atherosclerotic lesions. In a retrospective observational study[46] in non-HIV patients, statin therapy reduced the progression of low attenuation plaque and non-calcified plaque measured by CCTA. In another non-randomised study of patients without HIV, fluvastatin reduced low attenuation plaque volume on serial cardiac CTA assessment, similar to what we found in HIV-infected patients.[47] Therefore non-randomised studies in non-HIV infected patients suggest a potential effect of statins on non-calcified plaque. The current study is the first randomised placebo-controlled trial to show an effect of statins on non-calcified plaque and plaque regression in any population by CCTA.

Because of the concern for statin tolerability in patients with HIV, particularly given the high proportion who use protease inhibitors, we used a dose-escalation algorithm to increase the dose from 20 mg to 40 mg after 3 months in which tolerability was shown in case of potential ART effects on statin metabolism. Newer statins, such as pitavastatin, do not seem to interact with ART, but were not available at the time of study initiation. Participants in the current study were aware of the potential risks of statin therapy including myositis and other effects in the informed consent document. Using this dose escalation algorithm, we showed that atorvastatin seemed generally safe and well tolerated. 40 mg of atorvastatin lowered LDL cholesterol concentration by 1·00 mmol/L over 1 year in patients with HIV with LDL cholesterol less than 3·37 mmol/L (130 mg/dL). Other studies in people without HIV have shown an adverse effect of statins, including rosuvastatin and atorvastatin, on glucose metabolism.[48,49] Moreover, rosuvastatin worsened glucose homoeostasis in the recent SATURN trial in HIV-infected patients[50] whereas pitavastatin did not affect glucose measures in patients with HIV.[51] By contrast, we did not detect any adverse changes in glucose or HbA_{1c} in the current study but further larger studies assessing effects of statins on glucose measures in the HIV population are needed. Myopathies and mylagias were seen in similar numbers in the placebo and atorvastatin groups and no participants had creatine kinase concentrations greater than five-times the upper limit of normal. No specific pattern of timing of appearance of adverse events related to study drug initiation or dose escalation was seen.

We used state of the art techniques to observe changes in coronary plaque, including sensitive volumetric measurements and morphological assessments. With CCTA, we were able to show in a randomised, placebo-controlled trial, significant and clinically relevant changes in coronary plaque, with significant overall regression and reduction in non-calcified plaque as well as improvement in vulnerable plaque morphology. This study therefore shows for the first time that statins successfully target several specific pathophysiological features that might uniquely contribute to high cardiovascular disease rates in HIV. Larger and longer trials are needed to show effects on events related to these changes in plaque, but this was not the purpose of the current study.

Our study had very low attrition and comparable baseline characteristics in the groups, including immune function. Given the limitation we faced in assessing identical regions of aortic arterial inflammation in serial scans due to manual co-registration, the study did not have adequate power to assess changes in arterial inflammation because data were available for only a subset of participants. The patients in our study were similar in disease characteristics to those on ART in developed countries, most of whom do not have clinical cardiovascular disease. In line with our earlier studies[6] and those of Post and colleagues,[7] a high proportion of participants had subclinical disease on CT angiographic

screening, but referral bias might have led to a higher percentage than in the general population. VACS scores in these participants were comparable to those seen in other cohorts of patients with HIV on ART.[35] Although all patients were on ART, a few had a low level of detectable viraemia, but this did not differ between groups and did not affect the results as shown in sensitivity analyses. Our study does not prove that statins will prevent coronary heart disease, and further, larger, longer-term studies, investigating hard endpoints, will be needed to determine if the reduction in plaque and improvement in high-risk plaque morphology resulting from statins will translate into a protective effect on cardiovascular disease in this population.

In conclusion, our findings show that statin therapy can reduce coronary plaque in patients with HIV with subclinical atherosclerotic disease. The results show that statin therapy can reduce both the volume of lipid-laden non-calcified plaque and high-risk morphology. We showed that statins reduced markers of generalised vascular inflammation, but we were not able to show an effect on arterial inflammation using FDG-PET. The results of this study on coronary plaque are likely to be relevant to the many HIV-infected patients who have subclinical atherosclerosis but do not have raised LDL cholesterol concentrations. The responses seen to statin therapy in this study argue for further study of early statin intervention strategies to prevent cardiovascular events in this population of HIV-infected patients.

Contributors

JL was involved in reference search, preparation of figures, study design, data collection, data analysis, data interpretation, and writing; MTL was involved in the reference search, figure preparation, data collection, data analysis, data interpretation, and writing; EJI was involved in figure preparation, data collection, and data analysis; JW was involved in figure preparation, data collection, and data analysis; SEL was involved in data collection and data analysis; KVF was involved in data collection and data analysis; JO was involved in data analysis; COZ was involved in data analysis, figures, and writing; JH was involved in data collection; SA was involved in study design and data collection; JP was involved in study design; AT was involved in study design, data analysis, data interpretation, and writing; GR was involved in data collection; UH was involved in data analysis, data interpretation, and writing; SKG was involved in the reference search, figure preparation, study design, data collection, data analysis, data interpretation, and writing. All authors were involved in critical revision of the manuscript.

Declaration of interests

SKG has consulted with Navidea, AstraZeneca, NovoNordisk, and Theratechnologies, and received grant support from Gilead, Amgen, KOWA Pharmaceuticals, and Theratechnologies, unrelated to this manuscript. GR has received grant support from Gilead Sciences unrelated to this manuscript. UH received grant support from Siemens Healthcare, American College of Radiology Imaging Network, and HeartFlow Inc, unrelated to this manuscript. AT has received grant support from Genentech/Roche, Bristol-Myers Squibb, Takeda, GlaxoSmithKline, and VBL and personal fees from Novartis, Genentech/Roche, Bristol-Myers Squibb, Cerenis, Takeda, Actelion, GlaxoSmithKline, and Amgen unrelated to this manuscript. All other authors declare no competing interests.

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