

## **Impact of Lipoprotein Apheresis on Thrombotic Parameters in Patients with Refractory Angina and Raised Lipoprotein(a): Findings from a Randomised Controlled Cross-Over Trial**

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## ABSTRACT

### Background

Raised lipoprotein(a) [Lp(a)] is a cardiovascular risk factor common in patients with refractory angina. The apolipoprotein(a) component of Lp(a) exhibits structural homology with plasminogen, and can enhance thrombosis and impair fibrinolysis.

### Objectives

To assess the effect of lipoprotein apheresis on markers of thrombosis and fibrinolysis in patients with high Lp(a).

### Methods

In a prospective, single-blind, crossover trial, 20 patients with refractory angina and raised Lp(a)>50mg/dL, were randomised to three months of weekly lipoprotein apheresis or sham. Blood taken before and after apheresis/sham was assessed using the Global Thrombosis Test, to assess time taken for *in vitro* thrombus formation (occlusion time, OT) and endogenous fibrinolysis (lysis time, LT); as well as von Willebrand Factor (vWF), fibrinogen, D-dimer, thrombin/anti-thrombin III complex (TAT), prothrombin fragments 1+2 (F1.2) and thrombin generation assays (TGA).

### Results

Lp(a) was significantly reduced by apheresis (100.2[IQR 69.6,143.0] vs. 24.8[17.2,34.0]mg/dL,  $p=0.0001$ ) but not by sham ( $p=0.0001$  between treatment arms). Apheresis prolonged OT (576±116s vs. 723±142s,  $p<0.0001$ ) reflecting reduced platelet reactivity and reduced LT (1340[1128, 1682]s vs. 847[685,1302]s,  $p=0.0006$ ) reflecting enhanced fibrinolysis, without corresponding changes with sham. Apheresis, but not sham, reduced vWF (149[89.0,164] vs. 64.2[48.5,89.8]IU/dL,  $p=0.0001$ ), and fibrinogen (3.12±0.68 vs. 2.20±0.53g/L,  $p<0.0001$ ), and increased F1.2 (158.16[128.77, 232.09] vs. 795.12[272.55,1201.00]pmol/L,  $p=0.0006$ ). There was no change in D-dimer, TAT or TGA with apheresis or sham.

### Conclusion

Lipoprotein apheresis reduces Lp(a) and improves some thrombotic and fibrinolytic parameters in patients with refractory angina. **Key words:** Lipoprotein(a), Apheresis, Thrombosis, Fibrinolysis, Angina.



## INTRODUCTION

With an ageing western population and improved survival from coronary artery disease, there is an increasing burden of patients with refractory angina,<sup>1</sup> defined as angina resistant to medical therapy, unamenable to conventional revascularization procedures.<sup>2</sup>

Raised lipoprotein(a) [Lp(a)] is common in patients with refractory angina,<sup>3</sup> and may be involved in its aetiology and symptomatology. Lp(a) is a genetically determined form of low-density lipoprotein cholesterol [LDL-C] consisting of a cholesterol-rich LDL particle with apolipoprotein B (ApoB) and an additional protein apolipoprotein(a) [Apo(a)].<sup>4</sup> Although currently available medications can achieve significant LDL-C reduction, many patients remain at residual risk of cardiovascular disease; some of which elevated Lp(a) accounts for according to several large clinical trials.<sup>5</sup> Observational prospective cohort data from 56,804 participants across Europe with up to 24 years follow-up showed that elevated Lp(a) was associated with increased risk of major coronary events and cardiovascular death, particularly amongst diabetics.<sup>6</sup> Prospective studies have demonstrated a clear relationship between Lp(a) and the incidence of coronary artery disease<sup>7</sup> and premature cardiovascular disease,<sup>8</sup> and raised Lp(a) is considered an independent cardiovascular risk factor.<sup>7,9</sup> Lp(a) may enhance coronary intimal lipoprotein deposition, and affect myocardial perfusion, microvascular function, plasma viscosity and endothelial function.<sup>10</sup>

The apolipoprotein(a) component of Lp(a) has close structural homology with plasminogen, and through competitive inhibition can inhibit plasmin formation, causing reduced fibrinolysis.<sup>10</sup> Lp(a) may also enhance coagulation by inhibiting the function of tissue factor pathway inhibitor,<sup>11</sup> and promotes thrombosis via secretion of plasminogen activator inhibitor-1.

There is currently no FDA-approved pharmacological treatment to lower Lp(a), but it can be effectively lowered with lipoprotein apheresis, an extracorporeal treatment whereby atherogenic ApoB-containing lipoproteins, including Lp(a) and LDL-C, are removed from blood or plasma.<sup>12</sup> In a prospective observational study, patients with progressive cardiovascular disease who commenced lipoprotein apheresis for elevated Lp(a) experienced a reduction in major adverse coronary events from 0.41 to 0.09 per year.<sup>13</sup> Similar results were seen in another retrospective observational study.<sup>14</sup> There remains

however, a paucity of prospective, randomised controlled trial data exploring the impact of aggressively lowering raised Lp(a) in patients with established coronary artery disease. We therefore conducted a prospective, single-blind, crossover trial in 20 patients with refractory angina and raised Lp(a) >50mg/dL, randomised to three months of weekly lipoprotein apheresis or sham. Lipoprotein apheresis led to significant improvements in myocardial perfusion, atheroma burden, exercise capacity and symptoms, with no significant change demonstrated in the sham arm.<sup>15</sup> Within the same study we also hypothesized that lowering Lp(a) in patients with refractory angina would lead to favourable changes in markers of thrombosis and fibrinolysis.

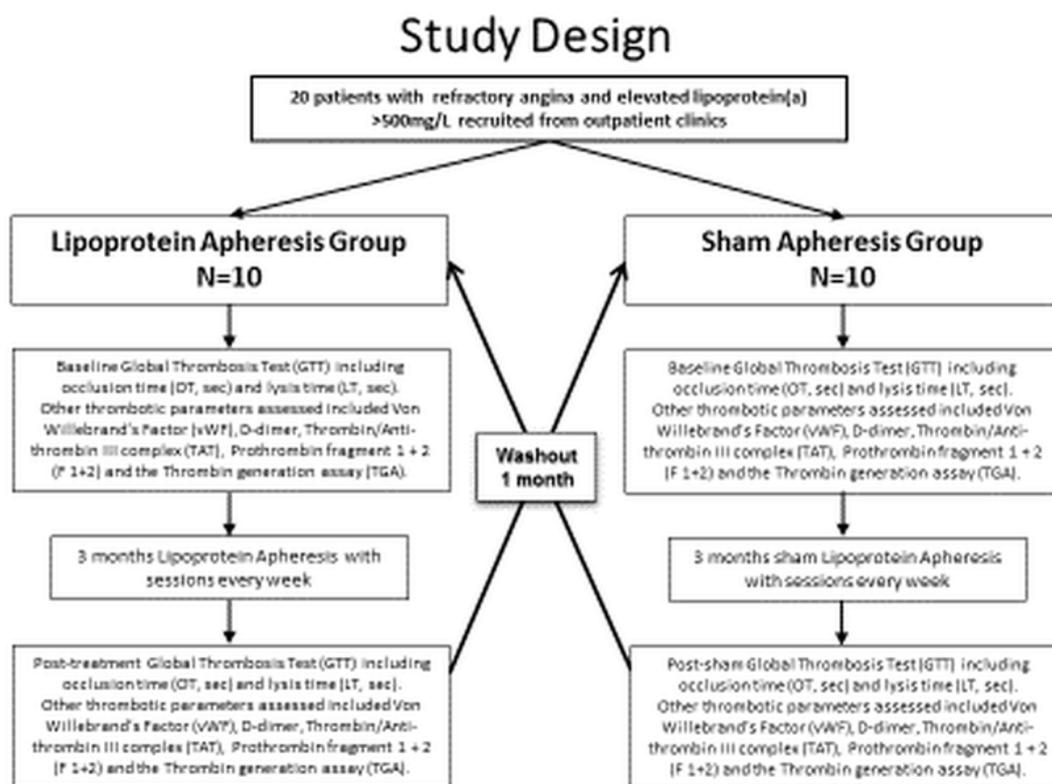
## METHODS

Ethical approval was obtained from the National Research Ethics Committee and the trial was registered with ClinicalTrials.gov (Identifier: NCT01796912). All participants gave written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. The study was conducted at the Royal Brompton and Harefield NHS Foundation Trust, between March 2013 and November 2015.

### Study design

We conducted a prospective, randomised, sham-controlled, single-blind, cross-over study of 20 patients with refractory angina and elevated Lp(a) >50mg/dL (normal range <30mg/dL) to assess the effect of lipoprotein apheresis on myocardial perfusion reserve and clinical outcomes, and in this paper, we are exploring the impact on secondary endpoints of markers of thrombosis and fibrinolysis.

Participants were randomised to an initial treatment arm (lipoprotein apheresis treatment sessions weekly for 3 months), or to an initial control group (sham apheresis sessions weekly for 3 months) (figure 1). After the first 3-month period, there was a wash-out period of 1 month with no investigations, before cross-over to the alternative arm. Baseline and post-intervention investigations were repeated before and after each three-month treatment period.



**Figure 1: Summary diagram showing trial methodology and design**

### Patient population

Patients with a diagnosis of refractory angina were recruited in out-patient clinics. Refractory angina was defined as angina resistant to medical therapy, unamenable to conventional revascularization procedures. The diagnosis of refractory angina was confirmed by at least one cardiologist, who verified the absence of revascularisation options and that the pain was of ischaemic origin, in most cases with evidence of reversible ischaemia. Inclusion criteria were: refractory angina for >3 months; two or more episodes of angina per week; evidence of significant coronary disease demonstrated by at least one of the following 3 criteria: previous myocardial infarction, bypass surgery, percutaneous coronary angioplasty (or any combination of these three criteria); optimal medical therapy for angina with at least two anti-anginal drugs; hypercholesterolaemia with elevated Lp(a) >50mg/dL and LDL-cholesterol

<4.0mmol/L, despite maximally-tolerated lipid-lowering therapy. We excluded patients who had sub-optimal peripheral veins for cannulation, other chronic systemic illness such as liver or renal failure, neoplastic disease, anaemia with haemoglobin <100 g/l, platelet count <100 x 10<sup>9</sup>/l, known bleeding diathesis, overt heart failure, unstable coronary artery disease with significant chest pain at rest, coronary revascularisation or a myocardial infarction within eight weeks, pregnancy, untreated diabetes mellitus, untreated hypertension, and those with contraindications to CMR or adenosine.

### **Randomisation and blinding**

Computer-generated block randomisation was performed using Stata software and patients randomised in a 1:1 fashion to the active or sham arms. All trial participants remained strictly blinded to treatment allocation throughout the entire protocol.

### **Lipoprotein apheresis**

Lipoprotein apheresis was performed according to clinical guidelines,<sup>16</sup> using the DX21 DHP (Direct Hemo Perfusion) Lipoprotein Apheresis machine with the Liposorber DL-75 column, Kaneka Pharma Europe; which utilises dextran sulphate to bind Apo(b)-containing lipoproteins removing them directly from whole blood. On average, each treatment lasted 2-3 hours. The control group had sham apheresis sessions with needle insertion, with tubing not connected to the machine and the apheresis machine was run to simulate active treatment. Screens and drapes were used to blind patients to treatment allocation. For safety reasons, the attending physician was not blinded to treatment allocation. With exception of the final active treatment, patients were given an intravenous bolus of 3000 IU heparin prior to treatment to prevent clotting within the instrument. We avoided administering intravenous heparin prior to the final apheresis session to avoid heparin lingering in the blood-stream after treatment, which may have affected markers of thrombosis and coagulation.

### **Blood sample collection**

Blood samples were taken at four time-points; namely pre- and post-apheresis, and pre- and post-sham. Pre-apheresis and pre-sham blood tests were taken just before commencing the first apheresis or sham

treatment session. Post-apheresis and post-sham blood tests were taken at least one hour after finishing the final treatment session via a fresh venepuncture (not via the treatment access, to avoid errors from potential haemodilution) and were conducted once we had confirmed that blood heparin levels were at 0 IU/ml, to avoid the potential confounding impact on tests of thrombosis and coagulation.

Blood samples were taken from an antecubital vein using a 21-G butterfly cannula using a 2-syringe technique, avoiding prolonged tourniquet time. The first 5 mL blood was used for routine blood tests and the next 4 mL sample used for assessment of thrombotic status (see below).

Blood samples were also assessed for lipid profile including total cholesterol, Lp(a), LDL, HDL, total cholesterol to HDL ratio, TG; ~~Apo(a) and Apo(b)~~; B-natriuretic peptide (BNP), coagulation screen, haematocrit and C-reactive protein (CRP). Lp(a) was measured using Lp(a) Ultra: a quantitative immunoturbidimetric ~~isoform-insensitive~~ assay, Randox Laboratories Ltd. London.

### **Assessment of thrombotic status**

Thrombotic status was assessed using the point-of-care automated Global Thrombosis Test (GTT, Thromboquest Ltd., UK). Venous blood obtained as above was inserted into a channel in the GTT (within 15 s of withdrawal); using the same techniques as previous studies.<sup>17</sup> This is a comprehensive test of platelet reactivity, coagulation (thrombin generation), and spontaneous (endogenous) thrombolytic activity performed on native, non-anticoagulated blood;<sup>17</sup> hence an ideal means of assessing the impact of apheresis on thrombin generation and thrombolysis. The instrument measures the time taken to create a shear-induced thrombus under physiological conditions (occlusion time [OT], in seconds); and the second phase of the test, measures the time to achieve endogenous thrombolysis of the thrombus created during the first phase (lysis time [LT], in seconds).<sup>17</sup> If lysis does not occur until 6,000 s after OT (LT cut-off time), “no lysis” is displayed and recorded.<sup>17</sup>

Venous samples at each time point were also taken to measure the following: D-dimer, von Willebrand factor (vWF) antigen, fibrinogen, thrombin/anti-thrombin III complex (TAT) and prothrombin fragments F1+2 (Enzygnost® TAT and Enzygnost® F1.2 respectively; Siemens Healthcare Diagnostics, USA) using an enzyme-linked immunosorbent assay (ELISA), and thrombin generation

tests using calibrated automated thrombography, developed by Hemker et al.<sup>18</sup> Thrombin generation is initiated by the addition of  $\text{CaCl}_2$  and a fluorogenic substrate (Z-Gly-Gly-Arg-AMC) in the presence of tissue factor (TF; 4pM) and phospholipids (4 $\mu\text{M}$  DOPS/DOPC/DOPE, 20:60:20) to trigger the reaction. The thrombin generation was quantified using the Thrombinoscope software (Synapse BV), Netherlands. To inhibit contact activation, corn trypsin inhibitor was added (65 $\mu\text{g}/\text{ml}$  plasma; Enzyme Research Laboratories, UK). All samples were run in triplicates and parameters such as lag time, peak thrombin, time-to-peak thrombin and endogenous thrombin potential were calculated from the curves generated for each sample.

### **Outcome measures**

The primary outcome measure of the main study was change in the myocardial perfusion reserve (MPR) which was assessed with magnetic resonance imaging by comparing gadolinium contrast perfusion during adenosine-induced stress versus the resting state, from baseline to three months after lipoprotein apheresis, previously published.<sup>15</sup> Here we report the change in thrombotic and fibrinolytic status, a secondary outcome measure.

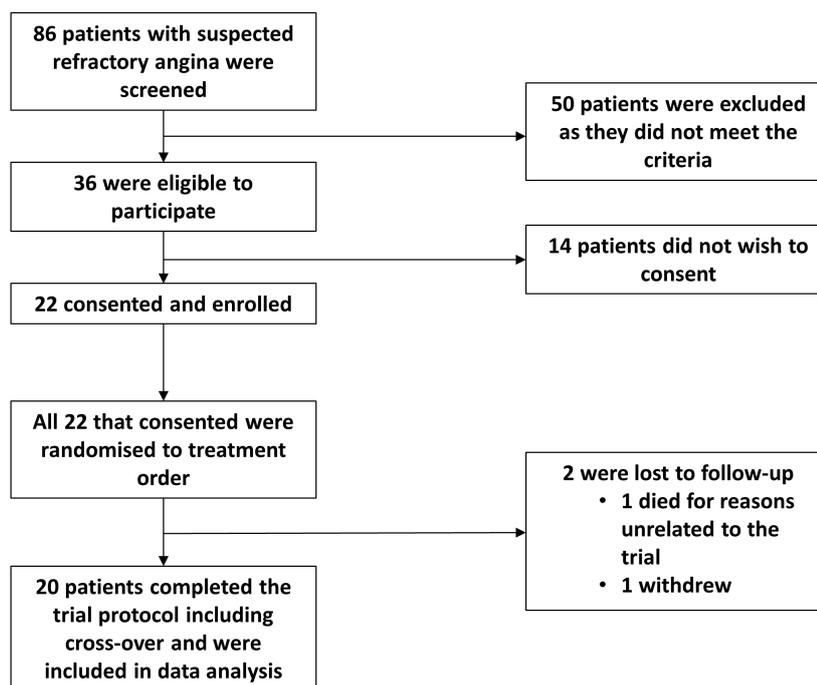
### **Statistical analysis**

A power calculation was performed based on the primary endpoint. With the cross-over design, assuming the inter-study reproducibility for MPR to have a standard deviation (SD) of 0.15 with a postulated change in MPR of 0.2 between the groups, a sample size of 20 patients was required to achieve 99% power at a p-value of 0.05.<sup>15</sup> All sample analysis was performed blinded to treatment allocation. Unblinding of data and all statistical analysis was performed after the conclusion of the trial following blinded analysis of the end points. Continuous data are presented as mean (SD) for normally distributed or median [interquartile range] for non-normally distributed data. Comparisons between groups were performed using Student's paired t-test for normally distributed data or the Mann Whitney U-test for non-normally distributed data using statistical software Stata 14.1 (Statacorp, Texas USA). Statistical significance was taken as  $p < 0.05$ .

## RESULTS

Twenty patients completed the trial protocol (figure 2). Baseline characteristics of trial patients and the order in which treatment was randomised is described in table 1. One patient was unable to tolerate the DX 21 DHP system and was switched to a double filtration HF440 lipoprotein apheresis system.

Lp(a) fell in response to apheresis from the baseline 100.2 (IQR 69.6, 143.0) to 24.8 (IQR 17.2, 34.0) mg/dL ( $p=0.0001$ ), with no significant change in Lp(a) in response to sham (94.3 [IQR 68.7, 144.1] vs. 88.6 [IQR 63.4, 146.7] mg/dL,  $p=0.79$ ); with significant difference in change in Lp(a) between treatment arms ( $p=0.0001$ ).



**Figure 2: Consort diagram**

**Table 1: Patient baseline characteristics**

Variable	Apheresis/Sham	Sham/Apheresis	All subjects
n	9	11	20
Age years	59.1 (10.4)	62.4 (9.0)	60.9 (9.5)
Male	9 (100)	10 (91)	19 (95)
Ethnicity: White	4 (44.4)	3 (27.3)	7 (35.0)
Asian	5 (55.6)	8 (72.7)	13 (65.0)
Body-mass index (kg/m <sup>2</sup> )	27.3 (1.9)	27.5 (4.1)	27.4 (3.2)
Diabetes	2 (22.2)	1 (9.1)	3 (15.0)
Hypertension	4 (44.4)	8 (72.7)	12 (60.0)
Smoking: Never	3 (37.3)	7 (63.6)	10 (50.0)
Ex	4 (44.4)	2 (18.2)	6 (30.0)
Current	2 (22.2)	2 (18.2)	4 (20.0)
Family history of CAD	7 (77.8)	9 (81.8)	16 (80.0)
Prior coronary artery bypass graft surgery	6 (66.7)	6 (54.6)	12 (60.0)
Prior percutaneous coronary intervention	7 (77.8)	9 (81.8)	16 (80.0)
Prior myocardial infarction	8 (88.9)	9 (81.8)	17 (85.0)
Anti-anginal drugs			
Oral nitrate	7 (77.8)	7 (63.6)	14 (70.0)
Beta blocker	7 (77.8)	11 (100)	18 (90.0)
Calcium channel blocker	3 (33.3)	5 (45.5)	8 (40.0)
Ivabradine	2 (22.2)	2 (18.2)	4 (20.0)
Ranolazine	1 (11.1)	0	1 (5.0)
Statin	9 (100.0)	11 (100.0)	20 (100.0)
Anti-platelet agents*	9 (100.0)	11 (100.0)	20 (100.0)
Oral anticoagulants**	1 (11.1)	3 (27.3)	4 (20)
Systolic blood pressure (mmHg)	125.6 (8.5)	125.5 (9.1)	125.5 (8.6)
Diastolic blood pressure (mmHg)	72.2 (9.4)	71.4 (2.3)	71.8 (6.3)
Baseline Lp(a) (mg/dL)	112.0 (77.1, 166.0)	108.0 (90.2, 152)	110.0 (77.1, 159)
Total cholesterol (mmol/L)	3.46 (0.82)	4.25 (0.74)	3.90 (0.86)
LDL cholesterol (mmol/L)	1.85 (0.74)	2.41 (0.64)	2.16 (0.73)
HDL cholesterol (mmol/L)	1.11 (0.31)	1.11 (0.28)	1.11 (0.28)
TG cholesterol (mmol/L)	1.22 (0.54)	1.22 (0.45)	1.22 (0.48)
Haemoglobin (g/L)	135.1 (16.9)	134.9 (12.4)	135.0 (14.2)
Platelet count x 10 <sup>9</sup> /L	294.11 (36.92)	194.45 (28.11)	198.80 (31.85)

Data are mean (SD), n (%), median (interquartile range). LDL=low-density lipoprotein, HDL=high-density lipoprotein, TG=Triglyceride. CAD=coronary artery disease. \* Antiplatelet agents comprised of aspirin +/-clopidogrel. \*\* anticoagulants comprised of warfarin or apixaban.

**Thrombotic status**

Apheresis treatment resulted in significant increase in OT (pre-apheresis  $576s \pm 116$  vs. post-apheresis  $723s \pm 142$ ,  $p < 0.0001$ ). There was no change in OT with sham ( $585s \pm 142$  vs.  $573s \pm 113$ ,  $p = 0.61$ ) (table 2). The change in OT ( $\Delta$ ) between the apheresis and sham treatment arms was significant ( $\Delta$  OT  $147$  (95% CI 97.9, 196) vs.  $-12.7$  (95% CI  $-63.1$ , 37.8),  $p = 0.0002$  between treatment arms). Apheresis treatment resulted in significant shortening of LT ( $1340s$  [IQR 1128, 1682] vs.  $847s$  [685, 1302],  $p = 0.0006$ ), with no change in LT in response to sham ( $1098$  [IQR 983, 1573] vs.  $1248$  [IQR 987, 1592],  $p = 0.36$ ). The difference in change in LT ( $\Delta$ ) between the apheresis and sham treatment arms was significant ( $\Delta$  LT  $-355$  [IQR  $-738$ ,  $-88.5$ ] vs.  $\Delta$  LT  $36.0$  [IQR  $-56.0$ , 204],  $p = 0.005$  between treatment arms) (table 2).

**Table 2: Change in blood markers in response to apheresis and sham, compared to baseline, shown as mean (standard deviation) or median [inter quartile range].**

Variable	Apheresis	Sham	P (between groups)
GTT Occlusion time (s)	147.0 (25.0)	-12.7 (25.7)	<0.001
GTT Lysis time (s)	-355 [-738, -88.5]	36.0 [-56.0, 204]	0.005
APTT (s)	16.2 (2.1)	-0.69 (0.54)	< 0.001
PT (s)	1.45 [0.80, 2.35]	-0.1 [-0.6, 0.65]	0.003
INR	0.1 [0.1, 0.2]	0 [-0.1, 0.1]	0.004
Hb (g/L)	-8.8 (1.9)	1.0 (1.6)	0.001
Platelets (10 <sup>9</sup> /L)	-49.7 (8.3)	-2.0 (5.5)	<0.001
PCV	-0.025 (0.006)	0.0045 (0.005)	0.002
vWF (IU/dL)	-60.5 (6.9)	9.24 (10.6)	< 0.001
D dimer (ng/mL)	3 [-40.5, 56]	14 [-26, 32]	0.88
TAT (µg/L)	0.32 [0.03, 1.54]	0.02 [-0.26, 0.70]	0.13
F1.2 (pmol/L)	670 [141, 951]	0.96 [-75.8, 62.2]	0.001
TGA LAG time (min)	0.05 [-0.44, 0.83]	-0.17 [1.34, 0.34]	0.13
TGA ETP (nM/min)	-4.42 (55.9)	-59.3 (53.9)	0.48
TGA peak (nM)	-7.40 (15.4)	-3.19 (13.4)	0.82
TGA tt peak (min)	-0.28 [-0.89, 0.89]	-0.28 [-1.95, 0.78]	0.30
Lp(a) Ultra latex assay (mg/dL)	-68.0 [-110.2, -45.3]	-5.5 [-48.9, 51.5]	<0.001
Total cholesterol (mmol/L)	-1.7 [-2.2, -1.5]	0.1 [-0.25, 0.30]	<0.001
LDL cholesterol (mmol/L)	-1.55 [-1.90, -1.17]	-0.03[-0.04, 0.07]	<0.001
HDL cholesterol (mmol/L)	-0.12 (0.05)	-0.002 (0.04)	0.006
TC:HDL ratio	-1.635 [-1.75, -1.24]	0.025 [-0.28, 0.35]	<0.001
Triglycerides (mmol/L)	-0.28 (0.11)	0.18 (0.10)	0.007
Apolipoprotein (a) (g/L)	-0.09 (0.04)	-0.01 (0.03)	0.074
Apolipoprotein (b) (g/L)	-0.41 (0.03)	-0.04 (0.04)	<0.001
Fibrinogen (g/L)	-0.93 (0.11)	-0.15 (0.08)	< 0.001
BNP (ng/L)	3.9 (8.1)	-3.7 (10.1)	0.57
CRP (mg/L)	-0.5 [-3.5, 0]	0 [-1.0, 0.5]	0.19

GTT= Global Thrombosis Test, APTT= Activated partial thromboplastin time, PT= Prothrombin time, INR= International Normalized Ratio, Hb= Haemoglobin, PCV= Packed cell volume, vWF= Von Willebrand factor, TAT= Thrombin/anti-thrombin III, F1.2 = Prothrombin fragments F1+2, TGA= Thrombin generation assay, Lp(a)= Lipoprotein(a), LDL= Low density lipoprotein, HDL= High density lipoprotein, BNP= Brain natriuretic peptide, CRP= C-reactive protein.

### Tests of coagulation and thrombogenesis

APTT increased following apheresis from  $33.3s \pm 4.6$  to  $49.5s \pm 11.1$  ( $p=0.0001$ ), but not following sham ( $p=0.096$ ). PT increased in response to apheresis from  $12.1s [11.4, 13.3]$  to  $13.0s [12.7, 14.2]$  ( $p=0.0006$ ), but not in response to sham ( $11.9 [11.2, 13.0]$  vs.  $12.2 [11.4, 13.0]$ ,  $p=0.96$ ). There was a significant change between the apheresis and sham arms ( $p=0.0034$  between treatment arms). There was a very small rise in INR in response to apheresis ( $1.0 [1.0, 1.15]$  vs.  $1.1 [1.1, 1.2]$ ,  $p=0.002$ ); but no change in response to sham ( $1.0 [1.0, 1.1]$  vs  $1.0 [1.0, 1.1]$ ,  $p=0.6$ ).

Apheresis was associated with a reduction in vWF (IU/dL) ( $149 [89.0, 164]$  vs.  $64.2 [48.5, 89.8]$ ,  $p=0.0001$ ), with no significant change with sham ( $95.8 [86.2, 160]$  vs.  $111 [99.5, 157]$ ,  $p=0.31$ ). There was a significant difference between the change in vWF in the treatment arm and sham arm ( $\Delta -60.8 [-75.1, -47.1]$  during apheresis, vs.  $\Delta 5.02 [IQR-6.4, 30.6]$  during sham,  $p<0.0001$  between treatment arms). There was no significant net change in D-dimer or TAT in response to either apheresis or sham. Apheresis was associated with a significant rise in F1.2 (pmol/L) ( $158.16 [128.77, 232.09]$  vs.  $795.12 [272.55, 1201.00]$ ,  $p=0.0006$ ), which was not observed with sham ( $207.11 [128.72, 255.52]$  vs.  $218.75 [152.31, 267.53]$ ,  $p=0.97$ ). There was a significant difference in the change (delta  $\Delta$ ) in F1.2 between the two study arms ( $\Delta 458 [286, 892]$  during apheresis, vs.  $\Delta -0.51 [-66.9, 54.2]$  during sham;  $p=0.0008$  between treatment arms). In response to apheresis, there was a reduction in fibrinogen from  $3.12 \pm 0.68$  to  $2.20 \pm 0.53$ ,  $p<0.0001$ , but this was not seen with sham.

There was no significant change in any of the 4 parameters of the thrombin generation assay (TGA) namely lag time (TGA LAG time), endogenous thrombin potential (TGA ETP), peak thrombin concentration (TGA Peak), time-to-peak thrombin (TGA tt Peak) in response to either apheresis or sham.

## DISCUSSION

This is the first randomised controlled trial to examine the effect of lipoprotein apheresis on markers of thrombotic status in patients with raised Lp(a). Our data demonstrates that apheresis achieves a marked reduction in Lp(a) that is also associated with favourable changes in thrombotic status. In particular, we

observed a reduction in platelet reactivity (demonstrated by increase in occlusion time) and improvement in endogenous fibrinolysis (demonstrated by reduction in lysis time), accompanied by reduction in vWF and fibrinogen. VWF is a marker of endothelial dysfunction and reduction in vWF levels may therefore represent improvement in endothelial function. Previous studies have shown that lipoprotein apheresis results in induction of vasodilation and improved blood flow through stimulation of expression of endothelium-derived nitric oxide.<sup>19</sup> Lp(a) has been shown to affect myocardial perfusion, microvascular function, plasma viscosity and endothelial function.<sup>10</sup> Vascular resistance,<sup>20</sup> a major determinant of microvascular perfusion, as well as blood viscosity,<sup>21</sup> have been shown to be significantly reduced following lipoprotein apheresis. In keeping with this, quantitative myocardial perfusion reserve (MPR) assessed with cardiovascular magnetic resonance imaging, the primary end point of our trial; increased by 0.47 [0.31, 0.63] from  $1.45 \pm 0.36$  to  $1.93 \pm 0.45$  with apheresis, but was unchanged during sham with a change of  $-0.16$  [ $-0.33, 0.02$ ] from  $1.63 \pm 0.43$  to  $1.47 \pm 0.30$  ( $0.47$  vs.  $-0.16$ ;  $p < 0.001$  between groups).<sup>15</sup>

The likelihood of coronary thrombosis is determined partly by the balance between pro-thrombotic drivers, such as enhanced platelet reactivity, on the one hand, and the effectiveness of the endogenous fibrinolytic system to spontaneously dissolve forming thrombi, on the other.<sup>22</sup> Enhanced platelet reactivity, in particular as assessed using platelet function testing in patients taking P2Y<sub>12</sub> inhibitors<sup>23</sup> and more recently, impaired endogenous fibrinolysis<sup>22</sup> have been demonstrated as biomarkers that predict an increased risk of future cardiovascular thrombotic events in patients with coronary artery disease. Whilst potent antiplatelet agents can be given to reduce enhanced platelet reactivity, little is known about therapeutic interventions to favourably modulate endogenous fibrinolysis. In patients with ACS, impaired endogenous fibrinolysis is associated with reduced spontaneous reperfusion in patients with ST-elevation myocardial infarction<sup>24</sup> and a high rate of recurrent cardiovascular thrombotic events in patients with non-ST elevation ACS.<sup>17</sup> Although the baseline fibrinolytic status in our population was similar to that previously reported in the normal population or low risk patients,<sup>17</sup> we demonstrated a statistically significant reduction (improvement) in lysis time in response to apheresis. Although the

sample size was small; the fact that patients acted as self-controls and given similar results were not seen in the sham arm, lends confidence to our findings.

Since the apolipoprotein(a) component of Lp(a) has close structural homology with plasminogen, thereby inhibiting plasmin formation,<sup>10</sup> and given Lp(a) promotes the secretion of plasminogen activator inhibitor-1; raised levels of Lp(a) are known to impair fibrinolysis and may promote a pro-thrombotic state. ~~We therefore postulate that~~ Therefore, the association we observed of apheresis-related Lp(a) lowering with some favourable improvements in thrombotic and fibrinolytic parameters ~~fibrinolysis, is likely to be causal~~ interesting and may warrant further investigation in future with more specific Lp(a) lowering therapies.

Since patients with refractory angina and high Lp(a) are at increased risk of recurrent cardiovascular events, identifying patients at high risk with targeted modulation to improve fibrinolytic status with lipoprotein apheresis, may be a novel method to reduce future thrombotic events. Our results do not prove causation and the results may be attributable to confounders including the effects of the plasma exchange circuit or a spurious result. However, our findings are supported by observational data in 1435 patients followed for 4 years, showing that lipoprotein apheresis lowered the incidence of cardiovascular events in patients with high LDL-C and/or high Lp (a) level, and progressive CVD.<sup>25</sup> In patients with raised Lp(a) levels, who continued to experience a high rate of major adverse coronary events despite effective LDL-C-lowering treatment, lipoprotein apheresis lowered Lp(a) by 73% and the rate of major adverse coronary by 86% over 5-year follow-up.<sup>14</sup>

The causality of the relationship between Lp(a) and fibrinolytic status could be explored further by exploring the effect of pharmacological lowering of Lp(a) with agents such as cholesteryl ester transfer protein (CETP) inhibitors, fibrates or statins<sup>26</sup> or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Although agents<sup>19</sup> such as niacin, PCSK9 inhibitors,<sup>27,28,29</sup> the CETP inhibitor anacetrapib,<sup>26</sup> and mipomersen (not approved in Europe) can lower Lp(a) by 25-30%, the benefit of specifically lowering Lp(a) using such drugs on cardiovascular risk reduction is unknown.<sup>30</sup> In contrast, lipoprotein apheresis lowered Lp(a) by some 68% after 3 months, which is an order of magnitude greater than that achieved with pharmacological approaches and thus may have greater benefits. Anti-sense oligonucleotides directed against apolipoprotein(a) [Apo(a)], thereby reducing Apo(a) and Lp(a) levels

hold promise as an effective pharmacological means of lowering Lp(a), although are not yet established in clinical use pending phase II trials. ISIS-APO(a)Rx, a second-generation antisense drug designed to reduce the synthesis of Apo(a) achieved an average decrease in plasma Lp(a) concentration from 39.6% at baseline to 77.8% using variable dosing regimens.<sup>31</sup>

Plasma fibrinogen may have an impact on atherosclerotic plaque growth, in addition to luminal or mural thrombosis.<sup>32</sup> Therefore, the significant reduction in fibrinogen that we observed with lipoprotein apheresis, which has also been documented previously,<sup>33</sup> may have a beneficial effect on atherosclerotic plaque progression as well an anti-thrombotic effect.

Renal dialysis circuits have many features in common with apheresis circuits. A single session of haemodialysis through a synthetic polysulfone membrane has been shown to induce increases in platelet, endothelial, and coagulation activation with increase in TAT ( $p < 0.001$ ), D-dimer ( $p < 0.001$ ), vWF ( $p < 0.001$ ) and reduction in thrombin receptor activating peptide (TRAP)-induced platelet aggregation.<sup>34</sup> Our results showing reduction in vWF, and reduction in platelet reactivity, are supported by earlier observations of reduced platelet aggregation in response to dialysis.<sup>34</sup> We observed no change in D-dimer and previously the effect of dialysis on D-dimer levels has been controversial, with some studies suggesting no change and other studies suggesting an increase post-dialysis.<sup>34</sup> Prothrombin fragments 1+2 (F1.2) reflect *in vivo* thrombin generation and increased significantly during apheresis. This could represent contact activation from the extra-corporeal circuit which may have led to acutely increased thrombin generation, although this was not seen in the thrombin generation assay. This could be due the fact that TAT complexes compared to F1.2 have much shorter half-lives (15 minutes<sup>35</sup> and 90 minutes respectively<sup>36</sup>) and post-apheresis and post-sham blood tests were taken at least one hour after finishing the final apheresis. Therefore, changes in F1.2 can still be detected due to contact activation whilst TAT can be normal. The confounding effects of the plasma exchange circuit could be removed by examining the isolated impact of a specific Lp(a)-lowering treatment such as the novel anti-sense oligonucleotides on thrombosis and fibrinolysis.

### Study Limitations

Limitations of our study include the small sample size and the lack of a true control arm. The sham set up did not replicate the effects of an extra-corporeal circulation, therefore the observed effects may reflect the effects of the extracorporeal circulation. Blood samples were taken 1 hour after completing apheresis, when heparin levels were undetectable. Although citrate was used as the regional extracorporeal anticoagulant, with Acid Citrate Dextrose A (ACD-A) solution run at 2.0-2.5% of blood flow rate, it is highly unlikely that this represents a confounding factor since citrate has a short systemic half-life of approximately 5 minutes. There was a small but statistically significant drop in both haemoglobin and platelet count following apheresis, that has previously been observed using the same dextran-sulphate column we used.<sup>37</sup> This raises the possibility that the reduction in platelet reactivity seen here may reflect a reduction in platelet count or haemodilutional effect. This is unlikely, since the platelet count although reduced, was still very much within the normal range. Coagulation factors themselves may be removed from the apheresis column, and previous studies have shown that plasma exchange transfusion results in marked changes in haemostatic parameters, with changes in platelet count and significant reduction in all coagulation factors and antithrombin III which took up to 24 hours to normalise.<sup>38</sup> Although statistically significant, the magnitude of change in INR was negligible following apheresis in our study. The change in APTT and PT may reflect some removal of coagulation factors, but the magnitude of change is small in relation to the magnitude of change in lysis time. Another limitation is the heterogenous nature of the patient population, including use of antiplatelet and anticoagulant medications in some, although the cross-over design ensured that patients acted as self-controls, making comparisons valid. How long the effects of lipoprotein apheresis on thrombotic markers persist, and whether there is a sustained benefit or whether a rebound effect occurs, remains unknown. The study design which compared samples taken before the first apheresis or sham procedure versus samples taken following 3 months after the last apheresis or sham procedure, does not allow one to distinguish long-term changes in haemostatic variables from changes induced by a single procedure. Therefore, the improvements in these variables do not accurately measure improvements averaged over time. Finally, as previously stated, we cannot prove causation, but simply report an association between

reduction of Lp(a) with lipoprotein apheresis and an improvement in some thrombotic and fibrinolytic parameters ~~status~~.

### **Conclusion**

In patients with refractory angina and raised Lp(a), lipoprotein apheresis is associated with significant improvements in thrombotic and fibrinolytic parameters. Further studies are needed to assess whether this favourable effect on thrombotic status can translate into a reduction in cardiovascular risk.

## HIGHLIGHTS

- We performed a prospective, single-blind, crossover trial in 20 patients with refractory angina and raised Lp(a) > 50 mg/dL, randomised to three months of weekly lipoprotein apheresis or sham.
- Apheresis prolonged Occlusion time (seconds) ( $576 \pm 116$ s vs.  $723 \pm 142$ s,  $p < 0.0001$ ) reflecting reduced platelet reactivity; and reduced Lysis time (seconds) ( $1340 [1128, 1682]$ s vs.  $847 [685, 1302]$ s,  $p = 0.0006$ ) reflecting enhanced fibrinolysis, without corresponding changes with sham.
- Apheresis, but not sham, reduced von Willebrand Factor ( $149 [89.0, 164]$  vs.  $64.2 [48.5, 89.8]$  IU/dL,  $p = 0.0001$ ), and fibrinogen ( $3.12 \pm 0.68$  vs.  $2.20 \pm 0.53$  g/L,  $p < 0.0001$ ), and increased prothrombin fragments 1+2 ( $158.16 [128.77, 232.09]$  vs.  $795.12 [272.55, 1201.00]$  pmol/L,  $p = 0.0006$ ).
- There was no change in D-dimer, thrombin/anti-thrombin III complex or thrombin generation assays with apheresis or sham.
- Lipoprotein apheresis reduces Lp(a) and improves thrombotic and fibrinolytic profile in patients with refractory angina.

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**Contributorship**

TK, DG, DA and JA contributed to the conception and design of the evaluation, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content. SR and JD contributed to the acquisition and analysis of the data. WB contributed to the statistical analysis of the data. AP, MB and DJP contributed to the conception and design of the evaluation and revising the article critically for important intellectual content. All authors critically reviewed the manuscript and approved the final version for submission.



## REFERENCES

- <sup>1</sup> Mannheimer C, Camici P, Chester MR, Collins A, DeJongste M, Eliasson T, Follath F, Hellemans I, Herlitz J, Lüscher T, Pasic M, Thelle D. The problem of chronic refractory angina; report from the ESC joint study group on the treatment of refractory angina. *Eur Heart J* 2002; 23: 355–370.
- <sup>2</sup> Henry TD, Satran D, Hodges JS, Johnson RK, Poulouse AK, Campbell AR, Garberich RF, Bart BA, Olson RE, Boisjolie CR, Harvey KL, Arndt TL, Traverse JH. Long-term Survival in Patients With Refractory Angina. *Eur Heart J* 2013; 34: 2683-8.
- <sup>3</sup> Khan TZ, Rhodes S, Pottle A, Banya W, Smith R, Kabir T, Ilsley C, Pennell DJ, Barbir M. High prevalence of raised lipoprotein(a) in patients with refractory angina. *Glob Cardiol Sci Pract* 2015; 2, 28.
- <sup>4</sup> Berg K. A new serum type system in man: the Lp system. *Acta Pathol Microbiol Scand* 1963; 59:369-82.
- <sup>5</sup> Kelly E, Hemphill L. Lipoprotein(a): A Lipoprotein Whose Time Has Come. *Curr Treat Options Cardiovasc Med* 2017 Jul;19(7):48.
- <sup>6</sup> Waldeyer C, Makarova N, Zeller T, Schnabel RB, Brunner FJ, Jørgensen T, Linneberg A, Niiranen T, Salomaa V, Jousilahti P, Yarnell J, Ferrario MM, Veronesi G, Brambilla P, Signorini SG, Iacoviello L, Costanzo S, Giampaoli S, Palmieri L, Meisinger C, Thorand B, Kee F, Koenig W, Ojeda F, Kontto J, Landmesser U, Kuulasmaa K, Blankenberg S. Lipoprotein(a) and the risk of cardiovascular disease in the European population: results from the BiomarCaRE consortium. *Eur Heart J* 2017; 38(32):2490-2498.
- <sup>7</sup> Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. *Circulation* 2000; 102: 1082-5.
- <sup>8</sup> Emerging Risk Factors Collaboration, Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke and non-vascular mortality. *JAMA* 2009;302:412–423.
- <sup>9</sup> Cremer P, Nagel D, Labrot B, Mann H, Mucche R, Elster H, Seidel D. Lipoprotein(a) as predictor of myocardial infarction in comparison to fibrinogen, LDL cholesterol and other risk factors: results from the prospective Gottingen Risk Incidence and Prevalence Study (GRIPS). *Eur J Clin Invest* 1994; 24: 444-53.

- <sup>10</sup> Thompson GR, Seed M. Lipoprotein(a): the underestimated cardiovascular risk factor. *Heart* 2014;100(7):534-5.
- <sup>11</sup> Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, Ginsberg H, Amarencu P, Catapano A, Descamps OS, Fisher E, Kovanen PT, Kuivenhoven JA, Lesnik P, Masana L, Reiner Z, Taskinen MR, Tokgözoğlu L, Tybjærg-Hansen A; European Atherosclerosis Society Consensus Panel. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur Heart J* 2010; 31:2844–53.
- <sup>12</sup> Borberg H. Comparison of different Lp(a) elimination techniques: A retrospective evaluation. *Trans Apheresis Sci* 2009; 41:61-65.
- <sup>13</sup> Leebmann J, Roseler E, Julius U, Heigl F, Spitthoever R, Heutling D, Breitenberger P, Maerz W, Lehmacher W, Heibges A, Klingel R for the Pro(a)LiFe Study Group. Lipoprotein apheresis in patients with maximally tolerated lipid-lowering therapy, lipoprotein(a)-hyperlipoproteinemia, and progressive cardiovascular disease. Prospective observational multicenter study. *Circulation* 2013; 128: 2567-76.
- <sup>14</sup> Jaeger BR, Richter Y, Nagel D, Heigl F, Vogt A, Roeseler E, Parhofer K, Ramlow W, Koch M, Utermann G, Labarrere CA, Seidel D; Group of Clinical Investigators. Longitudinal cohort study on the effectiveness of apheresis treatment to reduce high lipoprotein(a) levels and prevent major adverse coronary events. *Nat Clin Pract Cardiovasc Med* 2009; 6: 229-39.
- <sup>15</sup> Khan TZ, Hsu LY, Arai AE, Rhodes S, Pottle A, Wage R, Banya W, Gatehouse PD, Giri S, Collins P, Pennell DJ, Barbir M. Apheresis as novel treatment for refractory angina with raised lipoprotein(a): a randomized controlled cross-over trial. *Eur Heart J*. 2017;38(20):1561-1569.
- <sup>16</sup> Thompson GR, HEART-UK LDL Apheresis Working Group. Recommendations for the use of LDL apheresis. *Atherosclerosis* 2008; 198: 247-255.
- <sup>17</sup> Saraf S, Christopoulos C, Salha I, Stott D, Gorog D. Impaired Endogenous Thrombolysis in Acute Coronary Syndrome Patients Predicts Cardiovascular Death and Nonfatal Myocardial Infarction. *JACC* 2010; 55:2107–2115.
- <sup>18</sup> Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenvoort R, Lecompte T, Beguin S. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb*. 2003;33(1):4–15.

- <sup>19</sup> Stefannuti C, Julius U, Watts G, Harada-Shiba M, Cossu M, Schettler VJ, De Silvestro G, Soran H, Roeters Van Lennep J, Pisciotta L, Klor HU, Widhalm K, Moriarty P and the MIGHTY MEDIC Multinational Society . Toward an international consensus—Integrating lipoprotein apheresis and new lipid-lowering drugs. *Journal of Clinical Lipidology* 2017; 11: 858–871.
- <sup>20</sup> Moriarty PM, Hemphill L. Lipoprotein apheresis. *Cardiol Clin.* 2015; 33(2):197–208.
- <sup>21</sup> Moriarty PM, Gibson CA, Kensey KR, Hogenauer W. Effect of low-density lipoprotein cholesterol apheresis on blood viscosity. *Am J Cardiol.* 2004; 93(8):1044–1046.
- <sup>22</sup> Okafor ON, Gorog DA. Endogenous Fibrinolysis: An Important Mediator of Thrombus Formation and Cardiovascular Risk. *J Am Coll Cardiol* 2015;65:1683-1699.
- <sup>23</sup> Tantry US, Bonello L, Aradi D, Price MJ, Jeong Y-H, Angiolillo DJ, Stone GW, Curzen N, Geisler T, Ten Berg J, Kirtane A, Siller-Matula J, Mahla E, Becker RC, Bhatt DL, Waksman R, Rao SV, Alexopoulos D, Marcucci R, Reny JL, Trenk D, Sibbing D, Gurbel PA. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. *J Am Coll Cardiol* 2013;62(24):2261-73.
- <sup>24</sup> Christopoulos C, Farag M, Sullivan K, Wellsted D, Gorog DA. Impaired Thrombolytic Status Predicts Adverse Cardiac Events in Patients Undergoing Primary Percutaneous Coronary Intervention. *Thrombosis and Haemostasis* 2016 Dec 22. doi: 10.1160/TH16-09-0712.
- <sup>25</sup> Schettler VJ, Neumann CL, Peter C, Zimmermann T, Julius U, Roeseler E, Heigl F, Grutzmacher P, Blume H, Vogt A; Scientific Board of GLAR for the German Apheresis Working Group. The German Lipoprotein Apheresis Registry (GLAR) - almost 5 years on. *Clin Res Cardiol Suppl.* 2017;12(1):44-49.
- <sup>26</sup> Sahebkar A, Simental-Mendia L, Watts GF, Serban MC, Banach M. Comparison of the effects of fibrates versus statins on plasma lipoprotein(a) concentrations: a systematic review and meta-analysis of head-to-head randomized controlled trials. *BMC Med* 2017;15(1):22.
- <sup>27</sup> Raal FJ, Honarpour N, Blom DJ, Hovingh GK, Xu F, Scott R, Wasserman SM, Stein EA; TESLA Investigators. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015;385(9965):341-50.

- <sup>28</sup> Raal FJ, Hovingh GK, Blom D, Santos RD, Harada-Shiba M, Bruckert E, Couture P, Soran H, Watts GF, Kurtz C, Honarpour N, Tang L, Kasichayanula S, Wasserman SM, Stein EA. Long-term treatment with evolocumab added to conventional drug therapy, with or without apheresis, in patients with homozygous familial hypercholesterolaemia: an interim subset analysis of the open-label TAUSSIG study. *Lancet Diabetes Endocrinol* 2017; 5(4): 280-90.
- <sup>29</sup> Robinson JG, Farnier M, Kremph M, Bergeron J, Luc G, Averna M, Stroes ES, Langslet G, Raal FJ, El Shahawy M, Koren MJ, Lepor NE, Lorenzato C, Pordy R, Chaudhari U, Kastelein JP, for the ODYSSEY LONG TERM Investigators. Efficacy and Safety of Alirocumab in Reducing Lipids and Cardiovascular Events. *N Engl J Med* 2015;372:1489-99.
- <sup>30</sup> Stein EA; Raal F. Future Directions to Establish Lipoprotein(a) as a Treatment for Atherosclerotic Cardiovascular Disease. *Cardiovasc Drugs Ther.* 2016; 30(1):101-8.
- <sup>31</sup> Tsimikas S, Viney NJ, Hughes SG, Singleton W, Graham MJ, Baker BF, Burkey JL, Yang Q, Marcovina SM, Geary RS, Crooke RM, Witztum JL. Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled phase 1 study. *Lancet* 2015; 386(10002): 1472 – 1483.
- <sup>32</sup> Smith EB. Fibrin deposition and fibrin degradation products in atherosclerotic plaques. *Thromb Res.* 1994;75(3):329-35.
- <sup>33</sup> Hovland A, Lappegard KT, Mollnes TE. LDL Apheresis and Inflammation – Implications for Atherosclerosis. *Scandinavian J of Immunology* 2012; 76: 229–236.
- <sup>34</sup> Milburn JA, Cassar K, Ford I, Fluck N, Brittenden J. Prothrombotic changes in platelet, endothelial and coagulation function following hemodialysis. *Int J Artif Organs* 2011; 34(3): 280 – 287.
- <sup>35</sup> Pelzer H, Schwarz A, Heimbürger N. Determination of human thrombin-antithrombin III complex in plasma with an enzyme-linked immunosorbent assay. *Thromb Haemost* 1988; 59:101-6.
- <sup>36</sup> Greenberg CS, Hursting MJ, Macik BG, Ortel TL, Kane WH, Moore BM. Evaluation of preanalytical variables associated with measurement of prothrombin fragment 1.2. *Clin Chem* 1994; 40: 1962-9.
- <sup>37</sup> Yuasa Y, Osaki T, Makino H, Iwamoto N, Kishimoto I, Usami M, Minamino N, Harada-Shiba M. Proteomic Analysis of Proteins Eliminated by Low-Density Lipoprotein Apheresis. *Ther Apher Dial* 2014; 18: 93–102.

<sup>38</sup> Flaum MA, Cuneo RA, Appelbaum FR, Deisseroth AB, Engel WK, Gralnick HR. The hemostatic imbalance of plasma-exchange transfusion. *Blood* 1979; 54:694-702.