PhD THESIS

TITLE:
CLINICAL OUTCOMES, PERFUSION AND VASCULAR FUNCTION IN PATIENTS WITH REFRACTORY ANGINA AND RAISED LIPOPROTEIN(a) TREATED WITH LIPOPROTEIN APHERESIS

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Declaration of Originality

I can confirm that the work presented in my thesis is original and entirely my own. Work of others, for example literature referred to in the background or discussion sections to place my work in the context of prior evidence; or contribution from collaborators has been appropriately referenced or acknowledged.

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Dedication

I would like to dedicate this work to my family, who have provided me with inspiration and support throughout; and to the committed patients who participated in my clinical trial, without whom this work would not have been possible.
ABSTRACT

Background:

Angina which is refractory to conventional medical therapy and revascularisation is challenging to manage and novel therapeutic options are needed. Raised lipoprotein(a) is common in refractory angina and is an independent cardiovascular risk factor that can be reduced by lipoprotein apheresis. To date there is no randomised controlled data assessing the clinical benefit of lipoprotein apheresis in patients with refractory angina and raised lipoprotein(a).

Methods:

We conducted a randomised controlled trial in 20 patients with refractory angina and raised lipoprotein(a), with three months of blinded weekly lipoprotein apheresis or sham, followed by crossover. The primary endpoint was change in quantitative myocardial perfusion reserve (MPR) assessed by cardiovascular magnetic resonance. Secondary endpoints included measures of atheroma burden, exercise capacity, symptoms and quality of life.

Results:

The primary endpoint MPR increased by 0.47 [95% CI, 0.31 to 0.63] from 1.45±0.36 to 1.93±0.45 following apheresis, but decreased during sham by -0.16 [95% CI, -0.33 to 0.02] from 1.63±0.43 to 1.47±0.30; yielding a net treatment increase of 0.63 [95% CI 0.37 to 0.89; p<0.001 between groups]. Median total carotid wall volume (mm³) reduced during apheresis from 2482 [IQR 1910, 2836] before apheresis to 2251 [IQR 1719, 2437] after apheresis, but increased from 2342 [IQR 1997, 2644] pre-sham to 2455 [IQR 2166, 2831] post-sham (p<0.001 between groups). The Six Minute Walk Test (6MWT) distance(m) improved by a median value of 70.5[IQR 41.5,105.5]; there was no change in the sham arm (P=0.001 between groups). Significant improvements were also demonstrated in 4 of 5 domains of the Seattle Angina Questionnaire (all p<0.02 between groups) and quality of life physical component summary by the Short Form 36 Survey (p=0.001 between groups).
Conclusions:

Lipoprotein apheresis is an effective novel treatment option for patients with refractory angina and raised lipoprotein(a) improving myocardial perfusion, atheroma burden, exercise capacity and symptoms.

Funding:

This project was supported by the NIHR Cardiovascular Biomedical Research Unit of Royal Brompton and Harefield NHS Foundation Trust and Imperial College London.
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Chapter 1: Summary and Objectives

Based on good quality epidemiological evidence, it is well established that Lipoprotein(a) [Lp(a)] is an independent cardiovascular risk factor and predictor of major adverse cardiovascular events.\(^1\) Lipoprotein apheresis is currently the most effective approved treatment available for raised Lp(a), with minimal effect conferred by conventional lipid lowering agents. A growing body of evidence suggests that aggressively lowering raised Lp(a) may improve cardiovascular and clinical outcomes, although more prospective randomised controlled research is required to objectively address this question.\(^1\)

Angina which is refractory to conventional medical therapy and revascularisation is extremely challenging to manage, and is increasing in incidence as more patients with severe coronary artery disease survive with improved revascularisation techniques, yet continue to experience troublesome angina. There is a significant unmet need to establish therapeutic options for affected patients.\(^1\)

Our goal was to determine the impact of lipoprotein apheresis on clinical parameters and symptoms of patients with refractory angina secondary to advanced coronary disease in the context of raised Lp(a).\(^1\) Determining whether we should aggressively lower Lp(a) in such patients is an important question, which could potentially provide a treatment strategy for a substantial population. In addition, our goal was to provide insights into the mechanisms via which Lp(a) increases cardiovascular risk and how lipoprotein apheresis can potentially reduce the risk.

We have therefore conducted a prospective, randomised controlled crossover study of patients with refractory angina and raised Lp(a), randomised to undergoing three months of weekly lipoprotein apheresis or sham apheresis. Patients then crossed over to the opposite study arm after a 1 month wash-out phase. We assessed myocardial perfusion, carotid atherosclerosis, endothelial vascular function, thrombogenesis, oxidised LDL and their antibodies, exercise capacity, angina and quality of life at the beginning and end of treatment, to determine the net true treatment effect on the above parameters.\(^1\) This is a
novel area of research, as to date previous studies have not assessed the role of lipoprotein apheresis in patients with refractory angina and raised Lp(a) in a prospective randomised controlled manner.

**Hypothesis and Objectives of the Randomised Controlled Trial**

**Main Hypothesis:**
That lipoprotein apheresis improves quantitative myocardial perfusion as assessed by Myocardial Perfusion Reserve (MPR) detected by stress/rest Cardiovascular Magnetic Resonance imaging (CMRI), in patients with Refractory Angina and raised Lipoprotein (a).\(^1\)

The primary objective was to determine the effect of lipoprotein apheresis on myocardial perfusion reserve, as assessed with quantitative perfusion CMR, in patients with refractory angina and raised Lp(a).\(^1\)

The secondary objectives of the project in such patients were to determine the effect of lipoprotein apheresis on:\(^1\)

1. Carotid atherosclerosis and/or plaque burden as measured by carotid CMR.
2. Functional CMR parameters: LVEF, LVM, LVEDV.
3. Endothelial vascular function as assessed with pulse amplitude tonometry.
4. The severity and frequency of angina as assessed by the Seattle Angina questionnaire.
5. Physical functioning as assessed with a Six Minute Walk test, and on the psychological well-being of patients and quality of life as assessed with a Quality of Life scale questionnaire (SF-36).
6. Lipid parameters (Lp(a), LDL-cholesterol, oxidised LDL and their antibodies, triglycerides).
7. Thrombotic risk as assessed by markers of thrombogenesis.

In addition, we have conducted genetic studies on the recruited patients with refractory angina and raised Lp(a), to see whether these patients were found to be genotype positive for the LPA locus variants (rs10455872) and/or (rs3798220) which are thought to be
associated with both an increased level of Lp(a) lipoprotein and an increased risk of coronary disease.

We have also compared lipoprotein apheresis against sham apheresis in order to try and ascertain the true effect of this treatment against any potential placebo effect. If our hypotheses are verified, lipoprotein apheresis may potentially be applied as a treatment modality in the management of patients with refractory angina and raised Lp(a). In addition, our study is assessing the impact of lipoprotein apheresis on a wide spectrum of parameters including symptoms, myocardial perfusion, atherosclerosis, endothelial vascular function, thrombogenesis and platelet function, oxidised LDL and their antibodies, exercise capacity and quality of life. Hence we will provide valuable insights into the role of Lp(a) and the mechanisms of action by which it contributes to the atherosclerotic process and how it increases the risk of ischaemic heart disease and adverse cardiovascular events.\textsuperscript{1}
Chapter 2: Lipoprotein(a)

2.1: Structure, Pathophysiology and Genetics

Lipoprotein(a) [Lp(a)] was first discovered in the 1960s by Berg.\(^2\) The exact physiological role of Lp(a) is not currently understood; however an elevated Lp(a) level (>600mg/L) has emerged as an important independent cardiovascular risk factor and predictor of adverse outcomes in atherosclerotic disease.\(^3,4\)

**Structure**

Lp(a) is an inherited, genetically determined form of LDL-cholesterol. It is a plasma lipoprotein consisting of a cholesterol-rich LDL particle, with one molecule of apolipoprotein B100 and an additional protein, apolipoprotein(a), attached via a disulphide bond.\(^5\) {See Figure 1} The presence of apo(a) increases the density of Lp(a) compared with LDL and significantly reduces its affinity for the LDL receptor.\(^5\) This may explain why raised Lp(a) levels in plasma are unaffected by statins, which work by increasing hepatic LDL receptor activity.\(^5\)

![Figure 1: The structure of lipoprotein(a)](image-url)
Pathophysiology
After Lp(a) is transferred from plasma into the arterial intima, it may be more avidly retained than LDL, as it binds to the extracellular matrix not only through apolipoprotein(a), but also via its apolipoprotein B component, thereby contributing cholesterol to the expanding atherosclerotic plaque. A recent cross-sectional cohort study performed in patients with acute coronary syndromes (ACS), showed that raised Lp(a) levels are associated with an increased atherosclerotic burden and predisposes patients to features of high risk coronary atherosclerosis. Specifically, upon optical coherence tomography (OCT), patients with higher Lp(a) levels (>300 mg/L) compared to patients with lower Lp(a) levels (<300 mg/L) exhibited a higher prevalence of lipidic plaque at the site of the culprit stenosis (67% vs. 27%; P = 0.02), a wider lipid arc (135 ± 114 vs 59 ± 111; P = 0.03) and a higher prevalence of thin cap fibroatheroma (TCFA) (38% vs. 10%; P = 0.04).

In vitro, Lp(a) binds to several extracellular matrix proteins including fibrin and defensins, a family of amino acid peptides that are released by neutrophils during inflammation and severe infection. It is likely that defensins, like lipoprotein lipase, provide a bridge between Lp(a) and the extracellular matrix. Lp(a) seems to be retained at sites of mechanical injury; and fibrin deposition appears to occur preferentially at such sites.

Lipoprotein(a) has also been shown to bind pro-inflammatory-oxidized phospholipids and is a preferential carrier of oxidized phospholipids in human plasma, which may represent one of the mechanisms by which raised Lp(a) may lead to cardiovascular risk. Furthermore, numerous previous studies measuring circulating oxidised LDL (OxLDL), have demonstrated its association with atherosclerotic disease. Matsuo et al. found MDA-LDL levels (which is a measurement of OxLDL) were associated with the presence of thin cap fibroatheromas as detected by optimal coherence tomography. This suggests that circulating MDA-LDL holds promise as a biomarker of plaque vulnerability. In a prospective study to evaluate the role of circulating MDA-LDL as a potential biomarker of CAD, a large cohort was followed up for 8 years with non-fatal MI or fatal CAD defined as endpoints. Wu et al. reported that circulating MDA-LDL was significantly related to risk of CAD in multivariate analysis prior to adjustment for other lipid markers. However, this relationship was lost after adjustment for lipid markers such HDL, LDL or TGs. Oxidative modification of LDL renders it immunogenic.
and autoantibodies to OxLDL are found in serum and recognise material in atheromatous tissue. An observational case-control cohort study showed that the titre of autoantibodies to MDA-LDL was an independent predictor of the progression of carotid atherosclerosis. In addition, in an observational case-control cohort sub-study performed in patients participating in the Helsinki Heart Study, elevated levels of antibodies against OxLDL were predictive of myocardial infarction. The effect was independent of LDL cholesterol levels, and the joint effect was additive. Lipoprotein(a) also contains lipoprotein-associated phospholipase A2 which may cleave oxidized fatty acids at the sn-2 position in oxidized phospholipids to yield short chain fatty acids and lyssolecithin.

The apolipoprotein(a) component of Lp(a) has close structural similarity with plasminogen, which endows Lp(a) with anti-fibrinolytic properties via its competitive inhibition of tissue-type plasminogen activator—mediated activation of plasminogen. Lipoprotein(a) may also enhance coagulation by inhibiting the function of tissue factor pathway inhibitor. Lp(a) also promotes the secretion of PAI-1, which may represent another potential mechanism by which Lp(a) promotes thrombosis.

Finally, small isoforms of apolipoprotein(a) have been observed to possess elevated potency in inhibiting fibrinolysis and thereby promoting thrombosis. Indeed, a recent meta-analysis demonstrated a two-fold increase in the risk of CHD and ischaemic stroke in subjects with small apolipoprotein(a) phenotypes. Furthermore, prospective findings in the Bruneck study have revealed a significant association specifically between small apolipoprotein(a) phenotypes and advanced atherosclerotic disease involving a component of plaque thrombosis. These data suggest that the determination of apolipoprotein(a) phenotype/genotype may provide clinicians with additional information by which to evaluate Lp(a)/apolipoprotein(a)-associated atherothrombotic risk.

To summarise, elevated Lp(a) is believed to promote atherosclerosis via Lp(a)-derived cholesterol entrapment in the intima, inflammatory cell recruitment and/or via the binding of pro-inflammatory-oxidised phospholipids, such as oxidised LDL. In addition, elevated Lp(a) is felt to be pro-thrombotic via the inhibition of fibrinolysis with enhancement of clot
stabilisation as well as via enhanced coagulation via the inhibition of tissue factor pathway inhibitor.21

Genetics

Lipoprotein(a) levels are co-dominantly inherited, and the LPA gene is located on chromosome 6 (6q26-27).22 The key LPA gene sequence that influences Lp(a) levels and atherogenicity is the number of kringle IV type 2 (KIV-2) repeats.22 This number largely determines the size of apo(a) and levels of Lp(a). Smaller numbers of KIV-2 repeats (ie, <22) are associated with higher levels of Lp(a) and potentially more atherogenic apo(a).22 It has been postulated that small apo(a) Lp(a) may be associated with higher Lp(a) levels because the smaller molecules are more readily synthesized in the liver and less readily degraded by cellular organelles.22

Clarke et al. used a novel gene chip containing 48,742 single-nucleotide polymorphisms (SNPs) in 2100 candidate genes to test for associations in 3145 case subjects with coronary disease and 3352 control subjects.23 Three chromosomal regions (6q26–27, 9p21, and 1p13) were strongly associated with the risk of coronary disease. The LPA gene locus on 6q26–27 encoding Lp(a) lipoprotein had the strongest association.23 They identified a common variant (rs10455872) at the LPA locus with an odds ratio for coronary disease of 1.70 (95% confidence interval [CI], 1.49 to 1.95) and another independent variant (rs3798220) with an odds ratio of 1.92 (95% CI, 1.48 to 2.49).23 Both variants were strongly associated with an increased level of Lp(a) lipoprotein, a reduced copy number in LPA (which determines the number of kringle IV–type 2 repeats), and a small Lp(a) lipoprotein size. A meta-analysis showed that with a genotype score involving both LPA SNPs, the odds ratios for coronary disease were 1.51 (95% CI, 1.38 to 1.66) for one variant and 2.57 (95% CI, 1.80 to 3.67) for two or more variants.23 The authors concluded that these SNPs explain 36% of the variation in the Lp(a) lipoprotein level. One in six people carry a variant LPA allele and thus have a risk of coronary disease that is increased by a factor of 1.5.23
2.2: Epidemiology

The plasma concentration of Lp(a) ranges from 0 to >2000 mg/L and is skewed towards lower values in European populations; 500 mg/L representing the 80th percentile based on data from non-fasting fresh serum samples from ~3000 men and 3000 women from the Copenhagen General Population Study collected from 2003 to 2004.21,24

There are significant disparities between different ethnic groups, in terms of the prevalence of raised Lp(a). Levels are lowest in non-Hispanic Caucasians [e.g. median: 120 mg/L; inter-quartile range: 50–320], Chinese [110, 40–220], and Japanese [130, 50–260], slightly higher in Hispanics [190, 80–430],25 and even higher levels in Blacks [390, 190–690].25,26

Similarly, in a study performed by Enas et al., it was demonstrated that amongst Americans of different ethnic origins, Blacks have the highest median Lp(a) levels, followed by Asian Indians. Caucasians had substantially lower median Lp(a) levels, whilst Hispanics and American Indians had the lowest levels.27 The median Lp(a) level in Blacks was approximately three times higher than that in Whites.27 Furthermore, Lp(a) confers less risk in Blacks than in Asian Indians or Whites.28 This decreased risk may be due to their less atherogenic lipid profile (slightly lower LDL-C and triglyceride levels and higher HDL-C levels compared with Whites), which may, in part, counterbalance the atherogenic potential of Lp(a).29

Asian Indians have high levels of Lp(a) second only to Blacks with more than 40% having Lp(a) levels >200 mg/L.30 The high Lp(a) levels seen in Asian Indians are in sharp contrast to levels seen in other Asian populations, which are similar to or lower than those observed in Whites.31 The adverse effects of Lp(a) in Asian Indians are significantly increased by the high prevalence of diabetes, low HDL-C levels, high TC/HDL-C ratio, high triglycerides, and hyper-homocystinaemia.32

2.3: Lipoprotein(a) as a cardiovascular risk factor

The Göttingen Risk Incidence and Prevalence Study (GRIPS) evaluated the impact of Lp(a) on the basis of a large prospective cohort study (6002 men aged 40-59.9 years at baseline with
Multivariate logistic regression models for the estimation of MI risk confirm Lp(a) as an important risk factor for IHD, ranking fifth behind LDL cholesterol, family history of MI, plasma fibrinogen and HDL cholesterol (inverse relationship). An early meta-analysis of 18 prospective studies which reported on a pooled analysis of 4000 coronary heart disease (CHD) cases, suggested that the combined relative risk of CHD for individuals in the top vs. bottom thirds of baseline Lp(a) concentrations was 1.7 (95% CI: 1.4-1.9). A more recent meta-analysis of 31 prospective studies, involving a total of 9870 CHD cases suggested that the corresponding combined risk was more modest (relative risk: 1.5; 1.3-1.8).

A very large epidemiological study on Lp(a) assessed individual records of 126 634 participants in 36 prospective studies. The association of Lp(a) with CHD was broadly continuous in shape and curvilinear, with no evidence of a threshold. The relative risk of CHD per 3.5-fold higher Lp(a) level adjusted for age and sex only was 1.16 and 1.13 (95% CI: 1.09-1.18) following further adjustment for systolic blood pressure, smoking, history of diabetes and total cholesterol. This suggests that the association is only minimally confounded by conventional risk factors. Accordingly, a recent prospective study found that the Lp(a)/CHD risk association did not depend on levels of other CVD risk factors, including LDL cholesterol levels. Similarly, amongst 18 720 participants from the European Prospective Investigation of Cancer (EPIC)-Norfolk cohort, Lp(a) levels were associated with future peripheral artery disease (PAD) and coronary artery disease (CAD) events, and the association between Lp(a) and cardiovascular disease was not modified by LDL cholesterol levels.

Previously, in a post hoc analysis of the Familial Atherosclerosis Treatment Study, it was found that lowering LDL cholesterol levels in those with high LDL and high Lp(a) levels conferred risk reduction. However, more recently Khera et al. demonstrated that in cohort of 9612 JUPITER participants with low LDL cholesterol and elevated hsCRP, Lp(a) was a significant determinant of residual risk. Furthermore, the magnitude of relative risk reduction with rosuvastatin was similar among participants with high or low Lp(a). This data
implies that despite aggressive LDL cholesterol reduction, raised Lp(a) still confers residual risk which deserves further attention. Further studies will help to directly assess the impact of specifically lowering Lp(a) concentrations for potentially reducing this residual risk.\textsuperscript{38}

In analyses adjusted for age and sex only, the association of elevated Lp(a) levels with increased risk of ischaemic stroke was less pronounced than that for CHD.\textsuperscript{34} However, the weaker association with ischaemic stroke may be due to heterogeneity of stroke aetiologies, that is, the association in atherothrombotic stroke could be diluted by weaker or no association with other stroke subtypes, such as haemorrhagic strokes.\textsuperscript{34} Assuming a log-linear association with risk, the age-and-sex-only-adjusted relative risk for ischaemic stroke was 1.11 per 3.5-fold higher than usual Lp(a) levels and was 1.10 (95% CI: 1.02–1.18) following further adjustment for traditional risk factors.\textsuperscript{34}

In patients with acute coronary syndromes, a cross-sectional coronary angiographic cohort study showed that raised Lp(a) levels are associated with an increased atherosclerotic burden and identifies a subset of patients with features of high risk coronary atherosclerosis.\textsuperscript{7}

In conclusion, elevated Lp(a) levels and presence of the relevant genotypes correlate significantly and independently with CHD risk. The association is continuous in shape without a threshold and does not depend on high levels of LDL or non-HDL cholesterol, or on the levels or presence of other cardiovascular risk factors.\textsuperscript{39} There are also significant disparities between ethnic groups in relation to the prevalence of raised Lp(a) and its conferred risk.\textsuperscript{39}

2.4: Current methods of treating Lp(a)

Most patients with raised LDL-cholesterol levels can be adequately treated with appropriate dietary measures and lipid-lowering drug therapy.\textsuperscript{40} On the other hand, the conservative therapy of elevated Lp(a), in most cases, is unsatisfactory.\textsuperscript{41} Data assessing the impact of statins on Lp(a) are limited and highly variable,\textsuperscript{21} and overall statins are ineffective at significantly lowering Lp(a). Niacin (nicotinic acid) reduces Lp(a) levels by up to 30-40% in a
dose-dependent manner and in addition exerts other potential beneficial effects by reducing LDL cholesterol, total cholesterol, triglycerides, and remnant cholesterol and by raising HDL cholesterol. However, there is a reasonably high incidence of side effects experienced with niacin, including flushing and gastro-intestinal effects. In a study assessing niacin therapy on the lipid profile of diabetic patients, 21% of the patients were unable to tolerate niacin owing to reversible side-effects, and 14% were unable to adhere to the niacin dosing regimen of three times daily. Tredaptive (a nicotinic acid based treatment also containing laropiprant) was previously felt to be modestly effective at lowering Lp(a), however the European Medicines Agency have withdrawn this drug based on findings from the HPS2-THRIVE trial showing that this drug does not reduce major adverse cardiac events and causes a higher incidence of serious non-fatal side effects.

**Lipoprotein Apheresis:**

Lipoprotein apheresis is a selective lipid-lowering extracorporeal treatment by which excess atherogenic ApoB100-containing lipoproteins, including Lp(a) and LDL cholesterol, are removed from blood or plasma. Currently it remains the most effective available means of lowering Lp(a) levels. Stefanutti et al. compared the efficacy of lipoprotein apheresis with standard lipid-lowering therapy such as statins in patients with raised levels of Lp(a) and angiographically documented coronary artery disease. They found that the lipoprotein apheresis group averaged an Lp(a) reduction of 57.8 ± 9.5% (p<0.001) compared to the group treated with standard lipid-lowering therapy in whom Lp(a) increased in a year by 14.7% ± 36.5% (p=0.66). Lipoprotein apheresis may improve myocardial perfusion and attenuate the progression of coronary artery disease. It has also been demonstrated to improve various haemo-rheological parameters including plasma viscosity, native blood viscosity, red cell aggregation, and red cell deformability in hyper-cholesterolaemic patients. However, it is not fully determined whether similar effects exist in patients with elevated Lp(a) as observational and single studies suggest.

Lipoprotein apheresis can be carried out using several methods. The most commonly used are dextran sulphate cellulose adsorption (DSA), heparin-induced extracorporeal LDL-cholesterol precipitation (HELP), immunoadsorption, double filtration plasmapheresis.
(DFPP) and direct adsorption of lipoproteins (DALI).\textsuperscript{51} In the DSA, HELP, immunoadsorption and DFPP systems, plasma is separated from red blood cells prior to removal of LDL-cholesterol and Lp(a), whereas in DALI and direct haemoperfusion (DHP), these lipoproteins are removed directly from whole blood.\textsuperscript{51}

The DX21 lipoprotein apheresis machine is a DHP system manufactured by Kaneka Pharma which utilises Liposorber\textsuperscript{®} D columns which contain dextran sulphate to covalently bind Apo-B containing lipoproteins to remove them directly from whole blood.\textsuperscript{1} (See Figure 2)
There is no significant difference with respect to the clinical outcome or in regards to total cholesterol, LDL-, HDL-cholesterol or triglyceride concentrations between the different systems, however the immune-adsorption method is reported to be the most effective in reducing Lp(a) levels.\textsuperscript{52}
Lp(a)-specific apheresis has been developed, although it is not yet widely available currently in clinical practice. It involves separating the plasma and then passing it through an immunoadsorption column with sheep polyclonal mono-specific antibodies against human apolipoprotein(a), and has been demonstrated to reduce Lp(a) levels by up to 88%. Other plasma compounds, including LDL and plasminogen, are reported to remain practically unchanged.

Potential future treatments:

The proprotein convertase subtilisin kexin type 9 (PCSK9) monoclonal antibody, Evolocumab (AMG 145) offers a potential future pharmacological approach to Lp(a) reduction. Recently the effect of evolocumab (AMG 145) on Lp(a) was assessed from a pooled analysis of data from 1,359 patients involved in 4 phase II trials. Evolocumab treatment for 12 weeks resulted in significant (p < 0.001) mean (95% confidence interval) dose-related reductions in Lp(a) compared to control: 29.5% (23.3% to 35.7%) and 24.5% (20.4% to 28.7%) with 140 mg and 420 mg, dosed every 2 and 4 weeks, respectively. This data lends further support to studying the impact of PCSK9 inhibition on Lp(a) in a phase III clinical outcomes trial. In addition, it would be useful to assess the impact of monoclonal antibodies to PCSK9 in patients with exclusively raised Lp(a), as the majority of patients assessed in these phase II studies had concomitant raised levels of LDL cholesterol, given that the impact on LDL cholesterol was primarily being assessed.

Another potential pharmacological treatment that holds promise for the future are antisense oligonucleotides (ASO) directed to apolipoprotein (a) [Apo(a)], thereby reducing apo(a) and Lp(a) levels. ISIS-APO(a)Rx, is a second-generation antisense drug designed to reduce the synthesis of apolipoprotein(a) (apo[a]) in the liver. To date, animal studies have shown that this may provide an effective approach to lower elevated Lp(a) levels. More recently, a randomised, double-blind, placebo-controlled, phase 1 study was conducted in 47 healthy volunteers with were randomly assigned to receive ISIS-APO(a)Rx as a single-dose or multi-dose of ascending concentrations or placebo. Whereas single doses of ISIS-APO(a)Rx (50-400 mg) did not decrease Lp(a) concentrations at day 30, six doses of ISIS-APO(a)Rx (100-300 mg) resulted in dose-dependent, mean percentage decreases in plasma...
Lp(a) concentration of 39·6% from baseline in the 100 mg group (p=0·005), 59·0% in the 200 mg group (p=0·001), and 77·8% in the 300 mg group (p=0·001). Similar reductions were observed in the amount of oxidized phospholipids associated with apolipoprotein B-100 and apolipoprotein(a). Mild injection site reactions were the most common adverse events. However, further human trial data is necessary to confirm the safety and efficacy of this treatment and in particular, phase II and III trials will have to be completed in patients with raised Lp(a) before it can be established for widespread use.
Chapter 3: Refractory Angina

3.1: Definition

Cardiovascular disease remains the leading cause of death in the developed world. The majority of patients with angina, resulting from coronary heart disease (CHD) are successfully treated with conventional medical therapy and revascularisation techniques such as coronary artery bypass graft (CABG) surgery or percutaneous coronary interventions (PCI). However, there is a subset of patients who have severe disabling angina from coronary artery disease which is refractory to conventional therapy, for whom management is particularly challenging. Refractory angina, as defined by Mannheimer and colleagues in 2002, is 'a chronic condition characterised by the presence of angina pectoris caused by coronary insufficiency in the context of reversible myocardial ischaemia, in the presence of coronary artery disease which cannot be controlled by a combination of medical therapy, angioplasty and coronary bypass surgery. Chronic is defined as a duration of more than 3 months'.

3.2: Epidemiology

There are no accurate figures on the occurrence or frequency of refractory angina, though there is universal agreement that its prevalence is increasing. The European Society of Cardiology estimates that 15% of patients who experience angina can be characterized as having refractory angina. Estimates based on rejection rates for further intervention among angina patients in Europe suggest that between 30,000 to 50,000 patients per year develop the condition. Most of these patients are relatively young and have a moderately impaired left ventricular ejection fraction. A Swedish study performed in 1998 which involved an inventory of patients referred for coronary angiography to the national cardiothoracic centre showed that 5–15% of patients referred for coronary angiography probably have refractory angina. An estimated 300,000 to 900,000 patients in the United States have refractory angina, with between 25,000 and 75,000 new cases diagnosed each year. It is however acknowledged that there is a need for systematic registration and
recording of cases of refractory angina to assess the burden of this disease, in order to obtain more accurate figures for prevalence and incidence data.\textsuperscript{59}

There is variable data regarding the long-term survival of patients with refractory angina. Based on observations from a prospective clinical database which included detailed baseline and yearly follow-up information regarding 1200 patient with refractory angina; by Kaplan-Meier analysis, mortality was 3.9% (95% CI 2.8-5.0) at 1 year, 17.5% (95% CI 15.2-19.9) at 5 years and 28.4% (95% CI 24.9-32.0) at 9 years.\textsuperscript{63} Cause of death was determined for 213 (88.4% of deaths) of which 153 (71.8%) were of cardiovascular cause and 60 (28.2%) were non-cardiovascular deaths.\textsuperscript{63} Amongst individuals who died of cardiovascular causes, 45 (29.4%) died of progressive CHF/ischaemic cardiomyopathy, 33 (21.6%) died suddenly, 36 (23.5%) died of MI and 39 (25.4%) were not classifiable (ie. natural causes).\textsuperscript{63} Peri-procedural death occurred in 20 (9.3%), including 13 (6.1%) following cardiac procedures (11 peri-CABG, 2 peri-PCI). The multivariate predictors of mortality in patients with refractory angina are similar to those in patients with other cardiovascular conditions: baseline age, diabetes mellitus, angina class, chronic kidney disease, left ventricular (LV) dysfunction and congestive heart failure (CHF).\textsuperscript{63} Besides age, angina class (3 and 4) and LV dysfunction/CHF were found to be the strongest predictors of mortality and therefore patients with these characteristics deserve special attention for additional alternative treatment strategies.\textsuperscript{63}

3.3 Existing treatments

The epidemiological and economic burden of refractory angina is significant and the management of affected patients is challenging. There remains a relatively limited number of effective treatment options, exposing a significant unmet clinical need to develop further therapeutic options.\textsuperscript{55} Sufficient prospective evidence supporting the efficacy of existing treatments also remains to be established.\textsuperscript{55}

Aside from conventional anti-anginal agents such as beta-blockers, calcium channel blockers and nitrates, newer pharmacological agents include ranolazine, which works by altering the trans-cellular late sodium current; and ivabradine, an If\textsuperscript{-} channel inhibitor.\textsuperscript{64} Current non-pharmacologic options for patients with refractory angina include neurostimulation
(transcutaneous electrical nerve stimulation and spinal cord stimulation), enhanced external counterpulsation (EECP) therapy, laser revascularization, gene therapy, and newer procedures such as extracorporeal shockwave myocardial revascularization.\textsuperscript{64}

**New pharmacological treatments:**

**Ranolazine** is a piperazine derivative anti-anginal and anti-ischaemic agent thought to work via alteration of the intracellular sodium level, which in turn through sodium-dependent calcium channels, prevents calcium overload that causes cardiac ischaemia.\textsuperscript{64,65} It has been shown to decrease angina episodes and improve exercise tolerance in individuals with CAD on maximal doses of amlodipine, atenolol or diltiazem.\textsuperscript{64} Unlike traditional anti-anginal medications such as nitrates and beta-blockers, ranolazine does not significantly alter either the blood pressure or heart rate, and is therefore particularly useful in individuals with angina refractory to maximal tolerated doses of these medications.\textsuperscript{64} The U.S. Food and Drug Administration (FDA) approved ranolazine in 2002; which is indicated for the treatment of chronic angina, in combination with amlodipine, beta-blockers or nitrates, in patients who do not adequately respond to other anti-anginal drugs.\textsuperscript{64} In order to assess the safety, tolerability and efficacy of ranolazine specifically in refractory angina, an observational registry study was conducted, which monitored 153 patients with refractory angina treated with ranolazine.\textsuperscript{66} Patients were followed up at 1, 6 and 12 months to obtain angina class and ranolazine use. For patients completing 1 year follow-up, 92 (65.3\%) remained on ranolazine. Reasons for discontinuation were side effects (n=21), revascularization (n=7), cost (n=5), ineffective (n=8), cost and ineffective (n=4), death (n=2), unknown (n=2).\textsuperscript{66} The proportion of patients with ≥2 class improvement in angina was higher for those who had remained on ranolazine compared to those who had discontinued use (51.8\% vs. 23.1\%; p=0.003).\textsuperscript{66}

**Ivabradine** selectively and specifically inhibits I(f), a primary sino-atrial node pacemaker current,\textsuperscript{67} thereby reducing heart rate at rest and during exercise. Borer et al. investigated the safety and efficacy of ivabradine in terms of relieving angina and underlying ischemia.\textsuperscript{67} In a double-blind, placebo controlled trial of 360 patients with chronic stable angina, 10 mg of ivabradine twice daily led to a 12\% increase in the time to onset of 1-mm ST-segment
depression, and a 9.5% increase in exercise tolerance. Ivabradine use also resulted in a 77% decrease in the frequency of angina (p < 0.001). The most common side effect reported was visual disturbance, which occurred in 14.8% of patients. The results of the BEAUTIFUL study were presented in 2008 at the European Society of Cardiology, which investigated the value of ivabradine in addition to optimal medical therapy. This randomized, double-blind, placebo-controlled, parallel-group trial involved 10,917 patients with coronary artery disease and left ventricular ejection fraction < 40%. Patients received ivabradine 5 mg, with the intention of up-titrating to 7.5 mg twice daily (n = 5,479) or placebo (n = 5,438) on top of conventional recommended medication. The majority of patients were concurrently receiving beta-blockade (87%). Although the primary composite endpoint (cardiovascular death, hospitalization for acute MI, or hospitalization for new onset or worsening heart failure) was not reached for the whole group, it was found to be beneficial in a prespecified subgroup of patients with HR ≥ 70 bpm. More recently, a randomised, double-blind, placebo-controlled trial of ivabradine, added to standard therapy, in 19,102 patients who had stable coronary artery disease, without clinical heart failure, and a heart rate of more than 70 beats per minute has been performed. There was no significant difference between the ivabradine group and the placebo group in the incidence of the primary endpoint of a composite of death from cardiovascular causes or nonfatal myocardial infarction (6.8% and 6.4%, respectively; hazard ratio, 1.08; 95% CI, 0.96 to 1.20; P = 0.20). It should however be noted that the two aforementioned large scale randomised controlled trials were not specifically assessing patients with refractory angina and indeed further randomised controlled data assessing the use of ivabradine in refractory angina will be required, in order to establish a clear mandate for the role of ivabradine.

Non-pharmacological treatments:

Enhanced external counterpulsation therapy (EECP):
EECP is considered a safe, beneficial, low-cost, non-invasive treatment for refractory angina with or without left ventricular dysfunction/heart failure and is recommended as a potential therapeutic option in the European Society of Cardiology (ESC) guidelines in the management of refractory angina as well as the American Heart Association (AHA) guidelines. EECP therapy consists of electrocardiogram-gated rapid, sequential compression of the lower extremities during diastole, followed by simultaneous decompression during
systole. These manoeuvres produce haemodynamic effects similar to those of an intra-aortic balloon pump (IABP); but unlike IABP, EECP therapy also increases venous return. A full course of therapy typically consists of 35 sessions of one hour per day. EECP was evaluated in a randomized, placebo-controlled multi-centre trial to determine its safety and efficacy. 139 patients with chronic stable angina, documented CAD, and a positive exercise treadmill test were randomly assigned to receive EECP (35 hours of active counterpulsation) or inactive EECP over a 4- to 7-week period. The authors concluded that EECP decreased angina frequency (\(P<0.05\)) and improved time to exercise-induced ischaemia (\(P=0.01\)). Two multi-centre registry studies involving 978 patients from 43 centres, and 2289 patients from more than 100 centres evaluated the safety and effectiveness of EECP in treating chronic stable angina. These studies found the treatment to be generally well tolerated and efficacious; with improvement of angina symptoms in approximately 75% to 80% of patients. However, although the ESC and AHA guidelines recommend that EECP should be considered for patients with refractory angina; they also both state that additional clinical trial data are necessary before EECP can be recommended definitively.

Neurostimulatory techniques:

- Transcutaneous electrical nerve stimulation

Transcutaneous electrical neural stimulation (TENS) involves applying a low voltage electrical current through pads placed on the skin in the area of pain. The technique is thought to work primarily via the ‘gate control’ theory of pain. Stimulation of large-diameter afferent fibres inhibits input from small diameter fibres in the substantia gelatinosa of the spinal cord. The activation of an endogenous opioid pathway or an increased endorphin concentration in blood and cerebrospinal fluid may also be involved. In a small study of patients with pacing-induced angina, TENS demonstrated an increased tolerance to pacing, improved lactate metabolism, and less-pronounced ST-segment depression. Although no data on the long-term efficacy has yet been reported, the benefits of TENS are that it is a passive, non-invasive, non-addictive modality, which is not thought to have potentially harmful side effects.
- Spinal cord stimulation (SCS)

SCS blocks pain by stimulating the dorsal columns, which inhibits transmission through the pain-conducting spinothalamic tract.\textsuperscript{76,77} A meta-analysis of seven randomised trials, including 270 patients with refractory angina, demonstrated that SCS improved outcomes (specifically exercise capacity, health-related quality of life (QoL) and a trend in ischaemic burden) when compared with 'no-stimulation'.\textsuperscript{78} Few adverse events were reported. These included infection (1%) and lead migration/fracture (7.8%). Thus, SCS is a reasonable therapeutic option in patients with refractory angina that has the potential to ameliorate symptoms and improve QoL. However, the ESC and AHA guidelines both acknowledge that evidence regarding reduction in both ischaemia burden and mortality is currently lacking.

**Transmyocardial laser revascularisation (TMR) and Percutaneous laser revascularisation (PLR):**

A systematic review of TMR and PLR was undertaken by the National Institute of Clinical Excellence (NICE) in the UK.\textsuperscript{79} The evaluation of TMR included 10 randomized, controlled clinical trials, involving a total of 1359 patients. It was demonstrated that while there was an improvement in the more subjective outcome measures (including exercise tolerance testing, angina score, and QoL) this was counter-balanced by a higher risk of post-operative mortality and morbidity (including MI, heart failure, thrombo-embolic events, pericarditis, acute mitral insufficiency, and neurological events). In the same way, the evaluation of PLR included five randomized trials.\textsuperscript{79} As a conclusion, overall mortality was not increased. However, morbidity (MI, ventricular perforation and tamponade, cerebrovascular events, and vascular complications) was also increased by PLR. Thus current evidence on both TMR and PLR for refractory angina pectoris shows no efficacy and may pose unacceptable procedural-related risks. On these grounds, NICE recommends that these procedures should not be used.\textsuperscript{79}

**Extracorporeal shockwave myocardial revascularization:**

This newer therapy, which is still under review, uses low-intensity shockwaves that are delivered to myocardial ischaemic tissue.\textsuperscript{64} Shockwaves, created by a special generator, are focused using a shockwave applicator device and the treatment is guided by
echocardiography. The shockwaves are delivered in synchronization with the patient’s R-wave to avoid arrhythmias. Prior to treatment, stress SPECT imaging is performed to identify the ischaemic areas. Following that, the shockwaves are focused to the identified ischaemic area, guided by ultra-sound. Several treatments are required for optimal results. A small study (involving 9 patients with refractory angina) has reported an improvement in symptoms and in functional class score and led to reduced nitroglycerine use. However, more substantial prospective evidence is necessary before establishing a potential recommendation.

**Coronary sinus reducing device:**
Recently, coronary-sinus reducing devices have been developed which are balloon expandable, stainless steel, hourglass-shaped devices which create a focal narrowing and increase pressure in the coronary sinus, thus redistributing blood into ischaemic myocardium. Investigators randomly assigned 104 patients with Canadian Cardiovascular Society (CCS) class III or IV angina and myocardial ischaemia, who were not candidates for revascularization, to implantation of the device (treatment group) or to a sham procedure (control group). A total of 35% of the patients in the treatment group (18 of 52 patients), as compared with 15% of those in the control group (8 of 52), had an improvement of at least two CCS angina classes at 6 months (P = 0.02), which was the primary end point. In addition, quality of life as assessed with the use of the Seattle Angina Questionnaire was significantly improved in the treatment group, as compared with the control group (17.6 vs. 7.6 points; P = 0.03). There were no significant between-group differences in improvement in exercise time assessed with the use of a symptom-limited stress test or in the mean change in the wall-motion index as assessed by means of dobutamine echocardiography. However, the authors commented that the study was statistically under-powered to detect an improvement in ischaemia via stress testing or wall-motion index. Larger phase three randomised studies will be necessary to confirm these promising findings and establish whether coronary sinus reducing devices have an established role in refractory angina.
The need for further treatment options in Refractory Angina:

Although as discussed, a few treatment options exist for refractory angina, further evidence to support widespread use of these treatments is required. In addition, there is a pressing need to identify further treatments and therapeutic targets.¹

3.4 Treatment of raised Lp(a) in Refractory Angina: A Case History

In our centre, we have experience of treating a patient with refractory angina and raised lipoprotein(a) with lipoprotein-apheresis and demonstrated that aggressive reduction of Lp(a) successfully ameliorated the progression of coronary stenosis and provided effective and durable relief of angina symptoms.² At the age of 42, this gentleman presented with unstable angina. He had no previous cardiac history, had a balanced diet and had never smoked. Coronary angiography revealed diffuse multi-vessel coronary artery disease. Subsequently, in the context of ongoing angina he was treated with quadruple coronary artery bypass grafts and a total of 12 stents over a three year period. He averaged a new coronary stent every four to six weeks. During this period, there was no evidence of myocardial infarction, but there was severe and dynamic progression of the native coronary disease and venous grafts. Despite these multiple and extensive coronary interventions and despite being treated with optimal medical therapy throughout, he continued to experience ongoing angina which was adversely affecting his quality of life. At this point, his Lp(a) was screened and was found to be significantly elevated at 1200mg/L (normal range: 0-300). The remainder of his fasting lipid profile revealed normal total cholesterol (TC) (3.2mmol/L, LDL-C (1.5mmol/L) and plasma triglycerides (TG) (1.4mmol/L). As lipoprotein apheresis is the most effective means of reducing Lp(a) levels, the patient was started on a fortnightly regimen of lipoprotein apheresis using the Kaneka whole blood system (DX21). Lipoprotein apheresis drastically reduced the plasma levels of LDL-C, TC, TG and most importantly Lp(a). Figure 3 demonstrates his Lp(a) levels pre- and post- treatment. Since the institution of regular apheresis, the patient has shown improvement in his functional status, with significant improvement of his angina chest pain and quality of life. The patient is now active and able to walk unrestricted and is engaged in fulltime employment. Lipoprotein apheresis has also slowed the rate of progression of his coronary disease, with a reduction in the rate
of revascularisation procedures since the institution of lipoprotein apheresis. During the five year period the patient has been undergoing lipoprotein apheresis, in terms of revascularisation, the patient has had four prophylactic stents to an in-stent stenosis in the distal right coronary artery; in contrast to the multiple frequent interventions he was requiring prior to starting apheresis.

This case highlights several noteworthy lessons regarding the management of refractory angina in the context of raised lipoprotein(a). Firstly, the important role of Lp(a) as a risk factor for refractory angina and the progression of coronary artery disease. Lp(a) measurement is often a missed feature of the biochemical profile of these challenging patients. Secondly, it shows that lipoprotein apheresis is a powerful tool in normalizing Lp(a), which can impact on the rate of progression of dynamic coronary artery disease and lead to an improvement in angina symptoms. Further studies at the clinical and the mechanistic level are needed to validate and understand this phenomenon.
Chapter 4: Impact of treating Lipoprotein(a): the existing evidence

4.1 Observational Cohort Studies

Although it is now well established that Lp(a) is an important independent cardiovascular risk factor and predictor of adverse cardiovascular events, more research is required to demonstrate that vigorously treating it can objectively improve clinical outcomes. The body of evidence supporting this notion is growing slowly, but is still limited.\(^{39}\)

Jaeger et al. conducted a retrospective longitudinal cohort study in Germany to assess whether combined lipoprotein apheresis and lipid-lowering medication can reduce extremely high levels of Lp(a) and thus prevent major adverse coronary events (MACE) more efficaciously than lipid-lowering medication alone.\(^{83}\) Eligible patients had coronary artery disease and Lp(a) levels $\geq 2.14 \, \mu\text{mol/l}$ or $>600 \text{mg/L}$ (95th percentile). All patients received lipid-lowering medications alone until maximally tolerated doses were no longer effective, followed by combined lipoprotein apheresis and lipid-lowering medication. The rates of the primary outcome, MACE, were recorded for both periods. A total of 120 patients were included. The mean duration of lipid-lowering therapy alone was $5.6 \pm 5.8$ years, and that of apheresis was $5.0 \pm 3.6$ years. Median Lp(a) concentration was reduced from $4.00 \, \mu\text{mol/l}$ to $1.07 \, \mu\text{mol/l}$ or $1120 \text{mg/L}$ to $300 \text{mg/L}$ with apheresis treatment ($P < 0.0001$); the corresponding mean annual MACE rate per patient was $1.056$ versus $0.144$ ($P < 0.0001$).\(^{83}\) The authors concluded that lowering of Lp(a) levels by apheresis is efficacious and safe and they recommend apheresis for patients in whom maximally tolerated doses of medication alone have failed to control coronary artery disease-associated events.\(^{83}\)

Similarly and more recently, Leebman et al. performed a prospective observational multicentre study in Germany which 170 patients were investigated who commenced lipoprotein apheresis because of Lp(a)-hyperlipoproteinemia and progressive cardiovascular disease.\(^{84}\) Specifically it was a prospective observational study comparing the incidence rates of cardiovascular events in patients with raised Lp(a) and progressive CVD retrospectively before and prospectively after commencing chronic lipoprotein apheresis with a pre-
specified uniform observation period. At the time of completed enrollment, this group of 170 patients represented ≈60% of all German patients receiving chronic lipoprotein apheresis owing to elevated Lp(a). Before lipoprotein apheresis, mean low-density lipoprotein cholesterol and Lp(a) were 2.56±1.04 mmol/L and Lp(a) 1049±45.7 mg/L respectively. At the time of the first apheresis session, 156 (91.8%) patients had CAD, 77 (45.3%) had concomitant or sole cerebrovascular disease, and 65 (38.2%) had concomitant or sole peripheral atherosclerosis. The incidence rates of cardiovascular events 2 years before (y-2 and y-1) and prospectively 2 years during LA treatment (y+1, y+2) were compared. Mean annual rates for major adverse coronary events declined from 0.41 for 2 years before LA to 0.09 for 2 years during LA (P<0.0001). From their data, the authors extrapolated that in total, 142 MACE before the lipoprotein apheresis period versus 31 MACE during the lipoprotein apheresis period could be translated into a number needed to treat of 3 to prevent 1 MACE per patient per year.

Although both studies are observational cohort studies, they do suggest a treatment effect and provide rationale for a well-designed randomised controlled trial. It should also be noted that both of these studies included patients with established coronary artery disease and were not specifically assessing patients with refractory angina.

### 4.2 Existing prospective trial data

There is a paucity of prospective randomised controlled trial data which aims to examine the impact of aggressively treating raised Lp(a) in the context of established coronary heart disease. Infact, to the best of our knowledge there are just two studies with a randomised controlled design which attempt to explore this question. Again, as for the aforementioned observational studies, these two randomised studies were assessing patients with coronary artery disease and were not assessing patients with refractory angina.

Bohl et al. explored the effects of a single lipoprotein apheresis session on myocardial perfusion in patients with elevated Lp(a) and coronary artery disease using cardiac magnetic resonance imaging. Twenty patients with Lp(a) >600 mg/L and coronary artery disease were randomized into a control or a treatment group. Both groups underwent cardiac
magnetic resonance imaging with assessment of left ventricular function, perfusion and viability, and the treatment group underwent lipoprotein apheresis immediately afterwards. Repeat magnetic resonance imaging was performed at 24 h for both groups and at 96 h for just the treatment group. The trans-myocardial perfusion gradient (i.e. endo-epi ratio [EER]) was determined and a comprehensive parameter of resting and adenosine-induced stress perfusion was derived (EER-S/R). The EER-S/R at 24 h was lowered by therapy (ΔEER-S/R 5%; p<0.03), whereas this effect disappeared at 96 h. The ejection fraction (EF) was slightly improved at 24 h (67.07 ±6.28% vs. 64.89 ±6.39%; ΔEF 2.2%, p<0.05) and returned to baseline at 96 h. In the control group no corresponding changes were detected. They concluded that cardiac magnetic resonance imaging detects subtle treatment-related changes in regional myocardial perfusion in patients with elevated Lp(a) and coronary artery disease undergoing lipoprotein apheresis. The study explored the immediate impact of a single treatment, hence the impact on perfusion of sustained regular treatments over a longer period has been unknown prior to our research.

Safarova et al. assessed the impact of specific Lp(a) apheresis versus statin therapy on coronary atherosclerosis regression in stable CHD patients with high Lp(a) levels. A total of 30 subjects with CHD verified by angiography, Lp(a) >500 mg/L, and low density lipoprotein cholesterol (LDL-C) ≤2.5 mmol/L on chronic statin treatment were prospectively evaluated for 18 months. Patients were allocated to receive specific weekly Lp(a) apheresis (n=15), or atorvastatin only (n=15). Blinded quantitative coronary angiography analyses of percent diameter stenosis and minimal lumen diameter (MLD) were performed at baseline and after the 18-month treatment period. Median percent diameter stenosis was reduced by -2.0 (95% confidence interval [CI], -5.0-0.0) with apheresis (p <0.01 in comparison with baseline), and increased by 3.5 (0.0-6.9) with atorvastatin (p <0.001 between the groups). The effect on MLD was more favourable with apheresis than with atorvastatin: 0.20 ±0.39 mm, as compared with 0.01 ±0.34 mm, p =0.04. This suggests that specific Lp(a) apheresis may produce coronary atherosclerosis regression in stable CHD patients with high Lp(a). As part of the same study, in the apheresis group, changes in carotid intima–media thickness (IMT) at 9 and 18 months from baseline were −0.03 ± 0.09 mm (p = 0.05) and −0.07 ± 0.15 mm (p = 0.01), respectively. In the atorvastatin group no significant changes in lipid and lipoprotein parameters as well as in IMT occurred over the 18-month period. Two years
after study termination carotid IMT increased by an average of 0.02 ± 0.08 mm in apheresis group and by 0.06 ± 0.10 mm in the control group (p = 0.033). The authors concluded that isolated extracorporeal Lp(a) elimination over an 18 months period produced regression of carotid IMT in stable CHD patients with high Lp(a) levels and that this effect was maintained for two years after the end of study. The limitation of this study is that it included stable asymptomatic patients and did not objectively explore the impact of treatment on angina symptoms and quality of life and functional clinical parameters such as myocardial perfusion, endothelial function, exercise capacity, rheological parameters and thrombotic risk. Our study will explore the impact of treatment on all of these additional parameters and will include a clinically more relevant group of patients with symptomatic accelerated coronary disease causing refractory angina, whom potentially stand to benefit more from therapy.

The following table summarises the key studies performed to date that explore the impact of apheresis in patients with established coronary disease and raised Lp(a) (See Table 1).

Table 1: Summary table of key studies assessing the impact of apheresis in patients with CAD and raised Lp(a).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Trial design</th>
<th>Sample size</th>
<th>Primary endpoints/findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaeger et al.</td>
<td>Retrospective longitudinal</td>
<td>120</td>
<td>Mean annual MACE rate per patient was 1.056 before LA versus 0.144 during LA (P &lt;0.0001).</td>
</tr>
<tr>
<td></td>
<td>observational cohort study</td>
<td></td>
<td></td>
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<tr>
<td>Leebman et al.</td>
<td>Prospective longitudinal</td>
<td>170</td>
<td>Mean annual MACE rate per patient declined from 0.41 for 2 years before LA to 0.09 for 2 years during LA (P&lt;0.0001).</td>
</tr>
<tr>
<td></td>
<td>observational cohort study</td>
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<td>Bohl et al.</td>
<td>Randomised controlled study (no</td>
<td>20</td>
<td>The CMR trans-myocardial perfusion gradient (i.e. endo-epi ratio [EER]-S/R) at 24 h following a single LA session was lowered by therapy (∆EER-S/R 5%; p&lt;0.03).</td>
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<td></td>
<td>cross-over)</td>
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<td>Safarova et al.</td>
<td>Randomised controlled study (no</td>
<td>30</td>
<td>Quantitative coronary angiographic median percent diameter stenosis was reduced by -2.0 (95% CI, -5.0-0.0) with LA, and increased by 3.5 (95% CI, 0.0-6.9) with atorvastatin (p &lt;0.001 between groups).</td>
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<td>cross-over)</td>
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4.3 Current guidelines on Lipoprotein(a) reduction

The European Atherosclerosis Society Consensus Panel recommended that Lp(a) levels should be reduced below 500 mg/L, in extreme cases, by lipoprotein apheresis. The HEART UK guidelines recommend lipoprotein apheresis for patients with Lp(a) levels >600mg/L with progressive coronary heart disease and LDL levels >3.2 mmol/L despite treatment with maximally tolerated combined drug therapy. However, objective prospective evidence to support these recommendations is currently limited. Adoption of these guidelines in clinical practice may potentially improve if good quality prospective evidence is established to validate them.

4.4 Addressing the gap in the evidence base

Although it is now well established that raised Lp(a) is an important independent cardiovascular risk factor and predictor of adverse cardiovascular events, more research is required to demonstrate that vigorously treating it can objectively improve clinical outcomes. This important question lies at the heart of what our study hopes to achieve. The body of evidence supporting this notion is still limited.

The European Atherosclerosis Society Consensus Panel stated that further international effort is required to assess the athero-thrombotic risk due to Lp(a) and unravelling the mechanisms by which Lp(a) contributes to cardiovascular disease. Good quality lab-based research and well-designed prospective randomised controlled intervention trials with reduction of plasma Lp(a) are urgently needed to assess the clinical benefit of treating raised Lp(a) and determining the role of Lp(a) treatment in the primary and secondary prevention of coronary disease and its sequelae. Without robust evidence in this field, we will not be certain whether Lp(a) should be a therapeutic target and whether vast amounts of NHS funding should be devoted to treating it. In addition, as our case example highlights, further research is needed to explore raised Lp(a) as a risk factor for refractory angina or accelerated coronary artery disease and to determine the clinical and symptomatic benefit of aggressively lowering Lp(a) in such individuals. In addition, even if it is proven that there is clinical benefit from treating raised Lp(a) in individuals with refractory angina, mechanistic
studies that define the way in which lowering Lp(a) improves cardiovascular health are still needed.

Although the existing evidence, albeit limited, is suggestive of a beneficial effect of lipoprotein apheresis in progressive coronary disease in the context of raised Lp(a), as yet a randomised controlled trial has not been conducted specifically to evaluate the potential role of lipoprotein apheresis in patients with refractory angina and raised Lp(a).

We have therefore conducted a randomised sham controlled cross-over trial of lipoprotein apheresis in patients with refractory angina and high Lp(a) levels. We specifically chose to include sham control treatment, to exclude the possibility that there may be a significant placebo effect, particularly on symptoms, from regular apheresis sessions involving frequent contact with healthcare professionals. The cross-over design was specifically chosen to improve statistical power, thereby reducing the required sample size and easing the challenges of recruitment. A cross-over design also mitigates potential randomisation issues that would otherwise arise in a limited sample size, since all of the same patients undergo both treatment and sham apheresis hence can effectively act as their own controls. On a pragmatic level, the cross-over design also helps clinicians to make considered decisions about implementing lipoprotein apheresis for the trial subjects in the long term as the net effect of treatment for each individual receiving active treatment versus sham would be known.

4.5 Rationale for our chosen endpoints

We chose fully quantitative CMR perfusion as our primary end point, given that it has the capability to quantify absolute myocardial blood flow with high spatial resolution, and thereby provides a meaningful and physiological clinical marker for patients with coronary disease. In addition, we felt that a fully quantitative approach would provide data which is more amenable to interpretation than a semi-quantitative approach.
Furthermore, we were encouraged that a previous trial had detected an improvement in the trans-myocardial perfusion gradient using semi-quantitative methods following a single lipoprotein apheresis session in patients with elevated Lp(a) and coronary artery disease using CMR.\textsuperscript{41} We therefore had rationale to explore the possibility that a more sustained regimen of regular apheresis in patients with refractory angina and raised Lp(a) may lead to an improvement in myocardial perfusion, which may potentially be depicted more clearly using fully quantitative methods.

Previously, as part of the LDL-Apheresis Atherosclerosis Regression Study (LAARS) it was demonstrated that in patients with severe hypercholesterolemia with significantly raised LDL cholesterol and extensive coronary artery disease, LDL apheresis led to some improvement in regional myocardial perfusion, assessed by digital subtraction angiography with video-densitometric calculation of hyperaemic mean transit time (HMTT) of contrast medium.\textsuperscript{88} 42 patients were established on treatment with simvastatin and were randomised to either receive LDL apheresis every 2 weeks for 2 years or simvastatin alone. In the LDL apheresis group, regional HMTT decreased over 2 years from $3.35 \pm 1.18$ (mean $\pm$ SD) to $2.87 \pm 0.82$ s (-14%, $p = 0.001$), whereas no change in the medication group was observed.\textsuperscript{88} This study specifically involved patients with raised LDL cholesterol rather than raised Lp(a) and included patients with extensive coronary artery disease, who were not required to have refractory angina. However, given that sustained apheresis led to improvement in regional myocardial perfusion in patients with raised LDL cholesterol, we hypothesised that myocardial perfusion assessed using fully quantitative CMR methods may feasibly improve in patients with raised Lp(a) and refractory angina associated with extensive coronary artery disease.

In addition, we considered that it would be worthwhile to quantitatively determine the impact of apheresis on atherosclerosis in patients with refractory angina and raised Lp(a) using a non-invasive method. Assessment of carotid atheroma using accurate quantitative CMR techniques offers the ideal means of evaluating this, whilst avoiding radiation and invasive cardiac catheterisation procedures; and to the best of our knowledge has not previously been applied to prospectively assess the impact of apheresis in patients with raised Lp(a) in the context of a randomised controlled trial. Furthermore, CMR imaging of
the carotids can provide considerably more quantitative information than carotid intima-media thickness (IMT) measured with ultra-sound; which was the imaging modality previously applied in a trial assessing Lp(a) apheresis in stable CHD patients. Grimm et al. carried out a cross-sectional, single centre observational study using CMR carotid imaging at a single time point to assess the composition of atherosclerotic carotid plaques in 16 patients undergoing chronic lipoprotein apheresis compared to 32 patients in a control group matched according to the degree of carotid stenosis, who had recently suffered an ischaemic stroke. Patients on chronic lipoprotein apheresis had smaller maximum wall areas (49.7 vs. 59.6mm², p<0.05), showed lower prevalence of lipid cores (28.1% vs. 56.3%, p<0.05) and the lipid content was smaller than in the control group (5.0 vs. 11.6%, p<0.05); despite the fact that the apheresis group had a higher prevalence of hypercholesterolemia and of established coronary heart disease. Only 3 out of the 16 patients receiving apheresis in this study had isolated raised Lp(a) with progressive vascular disease, with the remaining 13 being treated for severe hypercholesterolemia refractory to medical therapy; therefore although the study is suggestive of a beneficial effect of apheresis in general in hyper-cholesterolaemic patients, it cannot necessarily be extrapolated to patients with isolated raised Lp(a) without raised LDL cholesterol. The authors acknowledged that the cross-sectional design of the study allowed by definition only a snapshot of plaque burden and composition at a single time point, and therefore that definitive conclusions could not be drawn on the evolution of plaque composition over time. They suggested that prospective longitudinal studies were needed to better assess the atheromatous process over time during chronic lipoprotein apheresis.

We also thought that it would be useful to determine the impact of apheresis on endothelial function in patients with raised Lp(a) and refractory angina. Several previous studies have shown that statin therapy can lead to improvement in endothelium-mediated vasomotion within relatively short periods of time. A more recent study found that lipid-lowering drugs such as statins and bile sequestrant resins, reverse coronary endothelial dysfunction assessed with the quantitative angiographic response to acetylcholine (Ach), but that the effect is heterogeneous, with most coronary segments showing improvement, whilst other segments showed declination of dilation. They also found that improvement in vasomotion correlated most significantly with markers of plasma-oxidized low density
lipoprotein. Therefore, it would appear to be plausible that lipoprotein apheresis may also improve endothelial function. One study found that coronary endothelial function similarly assessed with the quantitative angiographic response to Ach improved after a single apheresis session in 15 patients with familial hypercholesterolemia. In contrast, a more recent study showed that a single apheresis session did not improve vascular endothelial function (VEF) responses measured brachial artery flow-mediated dilation (FMD), oxidative stress, or nitric oxide homeostasis in 5 patients treated chronically for hypercholesterolaemia. With these conflicting findings, the impact of lipoprotein apheresis on endothelial function remains inconclusive and certainly this question has not been addressed for patients with isolated raised Lp(a) and refractory angina, as the previous studies involved patients with familial hypercholesterolaemia rather than patients with specifically raised Lp(a).

We chose to examine the impact of apheresis on exercise capacity in our trial cohort, given that the LDL-Apheresis Atherosclerosis Regression Study (LAARS) demonstrated that in patients with significantly raised LDL and severe coronary atherosclerosis, LDL apheresis led to a significant improvement in exercise variables versus statin therapy alone. On bicycle exercise tests, the time to 0.1 mV ST-segment depression increased significantly by 39% and the maximum level of ST depression decreased significantly by 0.07 mV in the apheresis group versus no changes in the statin group. Therefore, given that a positive impact on exercise capacity was demonstrated with apheresis in patients with raised LDL, we hypothesised that a similar impact on exercise capacity may occur in patients with raised Lp(a) with refractory angina. In fact, although the LAARS trial included patients with severe coronary atherosclerosis, the trial subjects were not required to be symptomatic with angina, whereas all of the patients in our trial were symptomatic with refractory angina. For this reason, assessing the impact of apheresis on exercise in our trial subjects becomes even more relevant; given that the baseline exercise capacity in our trial cohort is poor, therefore potentially representing greater scope for treatment related improvement.
METHODS

Chapter 5:

5.1 Methods of a randomised controlled cross-over trial assessing the impact of lipoprotein apheresis in patients with refractory angina and raised Lp(a)

Methods:

The study was a prospective, randomised controlled blinded pilot study with a cross-over design involving patients with refractory angina and elevated Lp(a). I previously published a detailed account of the methodology of the trial.\(^1\) All patients provided written informed consent. Eligible patients were identified from cardiology outpatient clinics and cardiac catheterisation lists of the Royal Brompton and Harefield NHS Foundation Trust, London, United Kingdom, who satisfied the following criteria.\(^1\)

**Inclusion criteria:**

- Patients diagnosed with refractory angina for more than three months.
- Two or more episodes of angina per week.
- Previous history of myocardial infarction, CABG, PCI or any combination of the above.
- Prescribed optimal medical therapy with at least two anti-anginal agents.
- Hypercholesterolaemia with an elevated Lp(a) > 500mg/L and an LDL-cholesterol less than 4.0mmol/L, despite optimal lipid lowering drug therapy.

**Exclusion criteria:**

- Patients with poor calibre veins for cannulation.
- Patients with any other chronic systemic illness such as liver or renal failure, neoplastic disease, overt cardiac failure, unstable coronary artery disease, coronary revascularisation or a myocardial infarction within the previous eight weeks.
- Pregnancy, untreated diabetes mellitus, untreated arterial hypertension, and those with general contraindications to undergoing CMR or contraindications to adenosine.
With-drawl criteria:

- Patient withdraws consent.
- Poor venous access resulting in inability to cannulate.
- Inability to tolerate the lipoprotein apheresis treatment.

A total of 20 patients with refractory angina and elevated Lp(a) levels were included in the study. Half of the patients were randomly assigned to either a treatment arm who received lipoprotein apheresis treatment sessions every week for a total period of three months (12 sessions in total), or to a control group who received placebo ‘sham’ apheresis treatment sessions also every week for a three month period. All enrolled patients attended a baseline study visit which is detailed below. Subsequently, all participants had either 12 sessions of lipoprotein apheresis or ‘sham’ apheresis depending on randomisation. At the end of the three-month period, both groups had a repeat of the tests and assessments performed at baseline for comparison.

After a wash-out period of at least 1 month, the patients who started with the treatment arm crossed over to three months of weekly ‘sham’ apheresis; and the patients who started with ‘sham’ apheresis subsequently had lipoprotein apheresis. Baseline and post-intervention investigations were again conducted before and after the second three-month treatment period. Due to the complex nature of the trial protocol, involving multiple hospital visits, a detailed trial schedule was provided for each participant.

Baseline visit: Study introduction, followed by informed consent and randomisation. Physiological measurements including resting ECG, blood pressure and heart rate, weight, BMI, waist and hip circumference. A total of 30ml of venous blood was withdrawn from the patient’s antecubital fossa vein to measure a full fasting lipid profile including total cholesterol, Lp(a), LDL cholesterol, HDL cholesterol, total cholesterol to HDL ratio, TG; Apolipoprotein(A) (Apo(A)) and Apolipoprotein(B) (Apo(B)), fasting glucose, urea and electrolytes, liver function tests, thyroid function tests, B-natriuretic peptide (BNP), coagulation screen, ferritin, haematocrit, bone profile and C-Reactive protein (CRP). At baseline all recruited patients also had genetic testing for the LPA locus variants (rs10455872) and (rs3798220). In view of Lp(a)’s pro-thrombotic characteristics; to specifically assess the impact of lipoprotein apheresis on thrombotic risk, we also checked a
thrombogenic parameters such as tissue factor pathway inhibitor (TFPI), D-dimer, thrombin/antithrombin, thrombin generation assay and VWF ELISAs on plasma. We also conducted a Global-Thrombosis-Thrombolysis test (GTT), which is a validated bedside test performed on the patient’s native blood which assesses endogenous thrombotic and thrombolytic status and has been used successfully to identify patients at risk of future cardiac events following acute coronary syndromes. We felt that a marker of spontaneous thrombolysis would be an important parameter to assess given that increasing evidence has emerged to support the assumption that AMI is a failure of timely spontaneous thrombolysis. We also measured plasma levels of oxidised LDL which are believed to be increased in the presence of elevated Lp(a), which is the preferential carrier of oxidised phospholipids. We predicted that levels of oxidised LDL would be reduced by apheresis.

All patients had CMR at baseline assessing functional MR parameters including left ventricular volumes and left ventricular ejection fraction (LVEF), first-pass quantitative perfusion at stress and rest and late gadolinium enhancement (LGE), as well as quantitative assessment of carotid atherosclerosis burden. All patients had testing of endothelial vascular function using pulse amplitude tonometry (PAT), an assessment of exercise capacity using a Six Minute Walk test and an objective assessment of their anginal symptoms using a validated angina scoring system (the Seattle Angina Scoring Questionnaire). Quality of life assessments at baseline were assessed with a Quality of Life scale questionnaire (SF-36). Participants were also given a symptom-monitoring diary to be filled in during the course of the study.

Post intervention visit: All baseline investigations as described above were repeated.
**Aims and Hypotheses:**

The **primary aim** was to determine the effect of lipoprotein apheresis on myocardial perfusion reserve, as assessed with quantitative perfusion CMR, in patients with refractory angina and raised Lp(a).

The **secondary aims** of the project in such patients were to determine the effect of lipoprotein apheresis on:

1. Carotid atherosclerosis and/or plaque burden as measured by carotid CMR.
2. Functional CMR parameters: LVEF, LVM, LVEDV.
3. Endothelial vascular function as assessed with pulse amplitude tonometry.
4. The severity and frequency of angina as assessed by the Seattle Angina questionnaire.
5. Physical functioning as assessed with a Six Minute Walk test, and on the psychological well-being of patients and quality of life as assessed with a Quality of Life scale questionnaire (SF-36).
7. Thrombotic risk as assessed by markers of thrombogenesis.

**Main Hypothesis:**
That lipoprotein apheresis improves quantitative myocardial perfusion as assessed by Myocardial Perfusion Reserve (MPR) detected by stress/rest Cardiovascular Magnetic Resonance imaging (CMRI), in patients with Refractory Angina and raised Lipoprotein (a).

**Secondary Hypotheses:**
That in patients with refractory angina and raised Lp(a), lipoprotein apheresis leads to:

1. Reduction in carotid atherosclerosis and/or plaque burden as measured by carotid CMR.
2. Improvement in endothelial vascular function as assessed with pulse amplitude tonometry.
3. Reduction in the severity and frequency of angina as assessed by the Seattle Angina questionnaire.
4. Improvement in physical functioning as assessed with a Six Minute Walk test, and on the psychological well-being of patients and quality of life as assessed with a Quality of Life scale questionnaire (SF-36).
5. Reduction in lipid parameters (LDL-cholesterol, oxidised LDL and their antibodies, Lp(a), triglycerides).
6. Improvement in thrombotic risk as assessed by markers of thrombogenesis.

In addition, genetic studies were conducted on the recruited patients with refractory angina and raised Lp(a) to confirm whether these patients were found to be genotype positive for the LPA locus variants (rs10455872) and (rs3798220), which are felt to be associated with both an increased level of Lp(a) lipoprotein and an increased risk of coronary disease.²³
Consent:
A specific trial recruitment clinic was set up at Harefield Hospital that ran alongside the NHS clinic for one of the clinical supervisors of the study. This enabled the supervisor to review each patient that ultimately participated in the study to confirm suitability and feasibility of undergoing the treatment and trial related investigations. The trial was widely publicised at scientific and multi-disciplinary meetings at both Royal Brompton and Harefield Hospital to raise the awareness of interventional cardiologists working at both sites, to attract referrals for the trial. Clinic lists were also screened for potentially suitable patients who were believed to have a diagnosis of refractory angina. All patients screened had the trial explained to them in detail so that they understood the scientific purpose of the study as well as what the trial protocol would involve. A full history and clinical examination was conducted to assess whether they fulfilled the entry criteria. Bloods samples were also taken to check for Lp(a) levels as well as a full lipid profile and renal and liver function tests. In addition, all screened individuals were taken up to the Apheresis department to obtain a first-hand overview of what the treatment would involve and were given patient information sheets to read in their own time covering the trial protocol and the purpose of the study. We were very open about the potential risks that apheresis may involve as we wanted to ensure that patients were as well informed as possible upon entering the trial. We also encouraged patients to contact us with any further queries they may have following the initial screening clinic. Apheresis nurses also examined the veins of all patients screened to confirm whether felt that venous access would be adequate for extra-corporeal treatment. Patients were generally given at least 2 weeks to consider whether they wished to participate to allow them sufficient time to reach a decision and were encouraged to discuss these decisions with their relatives or friends if necessary. Consent was only sought after the patient had confirmed via telephone discussion that they definitely wished to proceed with the trial. A specific clinic appointment was then arranged devoted to the signing of the trial consent forms and to answer any outstanding queries that the patients had regarding the trial. A logistical time frame in which the patient would be able to complete the trial protocol was then agreed with the patient, to enable them to plan ahead. The right of the participants to refuse to participate without giving reasons was respected. Ethically it was approved that the clinician would remain free to give alternative treatment to that specified in the protocol at any stage if he/she felt it was in the participant’s best
interests, but the reasons for doing so would be recorded. In such cases, the participants remained within the study for the purposes of follow-up and data analysis. All participants were free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

Recruitment:
A total of 86 patients with suspected refractory angina were invited to be screened for the trial. Out of these 86 patients, 78 were considered to actually have refractory angina. Of the 86 patients screened, 52 (60.5%) had Lp(a) >500mg/L. After screening 75 patients with refractory angina, we reported on the fact that 60% of the patients had raised Lp(a) levels of >500mg/L. Ultimately, 36 out of the 86 screened met the eligibility criteria; of which 22 patients consented to participate in the trial. One patient withdrew during the study and 1 patient died from causes unrelated to the trial. As planned, 20 patients completed the trial protocol including cross-over (See Figure 5: Consort diagram).

Figure 5: Consort Diagram
Lipoprotein apheresis:

Lipoprotein apheresis was performed on the treatment group. The control group received placebo effect ‘sham’ apheresis sessions. The sessions for both groups were carried out in the Apheresis Unit in Harefield Hospital, Middlesex which is the largest Apheresis unit in the UK. The DX21 DHP (Direct Hemo Perfusion) Lipoprotein Apheresis machine manufactured by Kaneka Pharma with the Liposorber DL-75 column, was used to provide treatments. The DX21 DHP utilises dextran sulphate to covalently bind Apo-B containing lipoproteins to remove them directly from whole blood. The DX21 machine fulfils safety standards (International Standard IEC 60601) and has been shown to be efficient and safe in a multicentre trial.99

Patients received 12 weekly lipoprotein apheresis treatments delivered over a three-month treatment period. On average, two blood/plasma volumes were treated on each patient each treatment day. The exact volumes to be treated were decided on an individual basis dependent on starting cholesterol levels and response to treatment. Treatment length varied between two and four hours depending on body size and the flow rate of the machine. Patients in the control group had a masked sham apheresis session, each lasting two hours, on a similar schedule - one session weekly over a three-month period (12 sessions in total).

All patients had two 17-gauge dialysis cannulae inserted into peripheral veins in the antecubital fossa. If possible they were placed in different veins in the same arm to allow some freedom of movement during treatment. Prior to starting each session, all patients were shrouded from the neck down with their arms covered, to prevent them from determining their randomisation group. A partition was positioned in front the apheresis machine so the patient could not view it (See Figure 6). The machine was activated regardless of the treatment arm so that the patient heard the background noise of the powered machine.
Figure 6: Blinding of treatment allocation throughout trial with use of drapes and partitions

For those in the treatment arm of the study, the inserted cannulae were connected by means of plastic tubing to the apheresis machine, which incorporated the standard treatment filtration column containing dextran sulphate (See Figure 7). Dextran sulphate covalently binds lipoproteins from the patient’s blood as it passes through the machine.
Those in the control arm of the study were not connected to the tubing circuit of the apheresis machine. They had the sham apheresis procedure as described, complete with frequent machine alarms and checking of intravenous tube positions.

For the treatment arm, the initial flow of the machine was set at 30ml/min and was gradually increased to 65-80ml/min with individual patient variations. Anticoagulation was achieved with the use of Acid Citrate Dextrose A (ACD-A) solution run at 2.0-2.5% of blood flow rate. Most patients also required an initial bolus of 2000-5000IU Heparin prior to treatment to prevent clotting within the machine.

Lipoprotein apheresis was performed on the treatment group. The control group received placebo ‘sham’ apheresis sessions where the lipoprotein absorber column was omitted from the apparatus and replaced by a “dummy” column {See Figure 8}. The sham treatment was set up with the dummy column and treatment was conducted by connecting the tubing to saline bags rather than connecting the tubing to the patient’s venous access cannulae.
Patients were blinded to their treatment allocation and screens and surgical drapes were used to cover both the venous access and the apheresis machines so that patients were not able to determine their treatment allocation (See Figure 6).

**STUDY OUTCOME MEASURES:**

The study **primary outcome measure or endpoint** was a change in the Myocardial Perfusion Reserve (MPR) from baseline to the value after three months of lipoprotein apheresis treatment.

The secondary outcome measures were the changes in the following parameters after three months of lipoprotein apheresis:

- Carotid atherosclerosis and/or plaque burden as measured by CMR
- LV functional parameters as measured by CMR
- Endothelial vascular function
- Markers of thrombogenesis
• Lipid parameters (LDL-cholesterol, oxidised LDL and their antibodies, Lp(a), triglycerides)
• Symptoms of angina as assessed with the Seattle Angina Scoring Questionnaire
• Quality of life as assessed by SF-36 Questionnaire
• Exercise capacity as assessed by the Six Minute Walk test

We also determined the genotypic presence of LPA locus variants (rs10455872) and (rs3798220) in our study patients with raised Lp(a) and refractory angina.

5.2 CMR methods in assessment of quantitative perfusion

All CMR scans were performed at Royal Brompton Hospital CMR Unit using a 3T MR scanner (Siemens Skyra, Erlangen, Germany). A 3T system was specifically chosen over 1.5T due to its increased signal-to-noise ratio (SNR) and contrast enhancement and superior spatial resolution and image quality. A diagrammatic summary of the protocol for all of the CMR imaging performed for each scan is shown in Figure 9 below.
Figure 9: Summary of CMR scanning protocol for carotid, functional, perfusion and viability imaging.
Acquisition Protocol

For CMR assessment of quantitative perfusion we used a Siemens perfusion (WIP 810) sequence including arterial-input-function imaging (on software version VD13) written by Dr Shivraman Giri, Siemens Chicago.

Typical acquisition parameters are described below. These were occasionally adapted specifically for a patient (for examples, larger field of view [FOV] required against phase-encode wrap-around, or the modification of the gap between slices along the ventricle). Any necessary changes were kept constant for all four perfusion appointments in that patient for the purpose of consistency and reproducibility.

Shimming to second-order including reference-frequency adjustment was optimised over a 15cm cube centred on the mid left-ventricle. Typically 18 receiver coil elements of an anterior chest phased-array and 12 elements of a flat spine-coil array posterior to the heart were enabled.

Immediately after each R-wave trigger, an arterial input function (AIF) image and three 8mm short-axis slices were acquired in each cardiac cycle in basal to apical order at typically 12mm gaps, in a total acquisition time of 520ms per cardiac cycle.

The AIF image was a spoiled gradient-echo sequence at 8° flip angle with centre-out phase-encode ordering such that central raw-data was acquired following composite saturation with low T1 sensitivity aiming to avoid compression of the peak blood response to contrast agent. The delay from the third composite saturation RF to first RF excitation for central raw data was 5.0ms, but T1 recovery time was more likely to be towards 10ms over the central raw data as a whole (see limitations below). The AIF image was acquired with a 10mm slice thickness at the same FOV and location as the mid-LV myocardial slice, but at coarser image resolution for a short duration¹⁰¹ (FOV oversampled x 2, 128 FE samples in 256 μs, 48 phase-encodings with no parallel imaging, frequency-encode 340mm/64 = 5.3mm, phase-encode 275mm/48 = 5.7mm, TE 0.4ms, TR 1.2ms; total AIF slice duration 77ms, of which 58ms was image data acquisition).
Each 8mm myocardial slice included its own non-selective composite saturation 100ms before each central raw-data acquisition, and chemical-shift based fat suppression, requiring a total 151ms per slice of which image data acquisition lasted for 41x2.5ms = 102ms. Acquired resolution: frequency-encode 340mm/192samples = 1.8mm (nominally, due to an asymmetric echo) by phase-encode 340mmx80.2%=275mm at a coarser resolution of 2.25mm interpolated to 1.8mm during reconstruction. Each slice used balanced steady-state free-precession (bSSFP) (TE 1.1ms, TR 2.5ms, ADC sampling 1240Hz/1.8mm) with rate 3 temporal parallel imaging (T-GRAPPA) and linearly-ordered acquisition of 41 phase-encoded raw-data lines per slice per cycle.

Motion-correction aligned the in-plane components of respiratory motion between perfusion frames acquired in gentle free breathing\textsuperscript{102} but no further motion filtering across frames or through-plane slice tracking was used.

Before each perfusion study, typically 10-cycles of non-contrast test images of myocardium and AIF were reviewed for correct slice locations and if necessary adjustments against artefacts (see limitations below).

Proton density images were nominally acquired using 8° flip angle applied to the bSSFP in the first three cardiac cycles (see limitations below).

Typically 70 frames were acquired at each of the 3 slices (basal, mid and apical). The mid LV was positioned in the z=0 slice through the iso-centre.

First-level specific absorption rate (SAR) was enabled for the perfusion acquisition where the predicted SAR limit typically reduced the acquired flip-angle to 30-40° which was highly patient-dependent. As stress was performed first, the likely further SAR reduction due to the incidental elevated heart-rate was copied to the rest scan so that identical settings were used for both perfusion runs.
Limitations

The T1-weighting of the AIF image should be carefully optimised to suit the range of peak blood concentrations likely at the half-dose and injection parameters used. A longer T1 recovery dispensing with the center-out ky coverage (and its associated phase-encode artefacts) would be advisable in further work. For the short TE of the AIF image, it has been shown that T2* loss of peak AIF signal at the 0.05mmol/kg dose used in this work would not be excessive, even at 3T main field; but at full dose a multi-echo based correction of T2* signal-attenuation may be required.

In practice, bSSFP off-resonance artefacts occasionally required correction of the shimming, adjustment of center-frequency and in-plane rotation after scout images to move such artefacts off the LV myocardium.

Spoiled-gradient-echo imaging at the required image resolution within a similar time per image acquisition did not generate sufficient SNR of myocardial peak-enhancement at 0.05mmol/kg. At 0.1mmol/kg this was more acceptable but immediately generated concerns about not only nonlinear myocardial response but also multi-compartmental T1 within peak-perfused myocardium. In keeping with general experience on this topic, a lower dose protocol was adopted requiring bSSFP imaging for sufficient SNR. The half-dose bSSFP protocol was also adopted by Drs Peter Kellman and Andrew Arai at NIH Bethesda who supplied an example protocol which formed the basis for the protocol used in this study. An important problem for quantification of myocardial blood flow (MBF) is the possibility of signal saturation. Some studies demonstrate the use of gadolinium contrast dose up to 0.04-0.05mmol/kg for the quantification of myocardial perfusion with a linear relation between signal change and gadolinium concentration. Some investigators have found that gadolinium contrast doses greater than 0.05mmol/kg results in signal saturation of the AIF. However, CMR experts have observed that signal saturation can occur at a gadovist dose of 0.04mmol/kg given that it has a high T1 relaxivity; therefore this could potentially have represented a limitation in our perfusion quantification, given that gadovist was the contrast agent used in this study. However, immediately after acquisition of each perfusion study, ROIs were drawn on the myocardial and AIF series and images were sent to our collaborators at NIH (who had supplied us the perfusion protocol and advised the
0.05mmol/kg gadovist dose) for external quality control validation. In all studies used in analysis it was immediately confirmed by the team at NIH that signal saturation had fortunately not occurred. However, for future work, in order to avoid the potential issue of signal saturation with 0.05mmol/kg; a lower dose may be advisable.

Particularly at 3T, accurate saturation of magnetisation over the range of resonance frequencies and B1 non-uniformity over the volume of interest cannot be assumed. Optimisation of the composite-saturation performance was also evaluated using scout images, and if found insufficiently dark over the LV myocardium a trial-and-error iterative process was employed adjusting the transmitter RF voltage of the composite saturation RF pulses. Further work is in progress by Dr Kellman NIH regarding optimisation of saturation RF pulses at 3T. We hope to transfer that work locally to the Royal Brompton Hospital Skyra scanner for further improved perfusion work, where there will also be an MSc student during 2016 working on ensuring optimal saturation and inversion performance on the Skyra system.

Clinical aspects of the CMR perfusion scans

Prior to each perfusion scan a 12 lead ECG was performed to ensure that there were no contra-indications to adenosine administration. Patients were also instructed to avoid any dietary intake of caffeine for at least 24 hours prior to each stress perfusion scan to avoid the potential impact of caffeine on the bio-availability of adenosine. In addition, a caffeine assay was performed on a blood sample taken from each patient prior to each of the four scans to exclude the presence of caffeine. Prior to the trial starting, in order to define the thresholds for the presence or absence of caffeine, the assay used was tested on a normal subject who had been caffeine-free for 24 hours. A second sample was also taken after caffeine consumption.

Due to the concerns with full dose gadolinium (0.1mmol/kg) highlighted above, namely potential nonlinear myocardial response, a half-dose gadolinium protocol was adopted. For both stress and rest perfusion studies, Gadolinium (Gadovist; Schering, Berlin, Germany) contrast at a dose of 0.05mmol/kg of body weight was injected at 3.5ml/s via an 18-gauge
cannula sited in the right ACF, followed by a 25ml normal saline flush at 7ml/s. The site of
the cannula for gadolinium contrast was kept consistent for each patient across all four
scans during the trial for consistency.

For the stress perfusion adenosine was infused at 140mcg/kg/min generally for a minimum
of 3 minutes via a 20 gauge cannula in the left arm, via a consistent site for each patient
across all four scans. Heart rate was monitored continuously and blood pressure was
measured and recorded immediately preceding and 3 minutes following the initiation of
adenosine infusion. Symptomatic evidence of a stress response (eg. development of chest
pain or breathlessness) as well as the peak heart rate achieved during stress was also
recorded for each stress scan. Stress perfusion image acquisition was undertaken when we
were satisfied that a stress response based on heart rate response and symptomatic
response had been achieved. Wherever possible, we ensured that adenosine was infused
for the same time frame across all four scans to achieve as much consistency as possible.
After completing the stress perfusion acquisition, a “top-up” Gadovist contrast dose of
0.05mmol/kg at 3.5ml/s followed by a 25ml at 7ml/s normal saline flush was administered
to allow subsequent late gadolinium enhancement imaging (see below).

Rest imaging was performed at least 20 minutes after the stress study with exactly the same
Gadovist contrast dose of 0.05mmol/kg injected at 3.5ml/s via an 18-gauge cannula sited in
the right ACF, followed by a 25ml normal saline flush at 7ml/s. All acquisition parameters
were kept identical to the stress scan. As per the stress scan, heart rate and blood pressure
were also recorded at the time of the rest perfusion acquisition.

For consistency and to optimise reproducibility across all four scans in our trial protocol, we
recorded the acquisition parameters such as: Distance factor (%), Field of view (FoV) Read
(mm) and FoV Phase (%) and kept these identical across all 4 scans.

Function and viability CMR:

Functional imaging was also performed at 3T using steady-state, free precession breath-
hold cines, echo time (TE)/repetition time (TR) (1.2/2.6 ms, flip angle 50°) in long-axis planes
and sequential 7 mm short-axis slices (3 mm gap) from the atrioventricular ring to the apex. Advanced cardiac shimming and frequency scouts were used to eliminate problems with artefacts. Finally, late gadolinium enhancement (LGE) images were acquired 10 min after the intravenous “top-up” gadolinium administered following the stress imaging study described previously, in identical short-axis planes using an inversion-recovery gradient echo sequence using a protocol used in previous research performed locally. Inversion times were adjusted to null normal myocardium, and LGE images were acquired with additional phase-swapping to exclude artefact. Although the extent of LGE was not objectively quantified; a visual assessment of the presence/absence of LGE and the affected coronary territories was made to allow overall comparison for any gross changes across the scans.

All analysis of functional and viability parameters was performed used validated software that is in widespread use (CMR tools, Cardiovascular Imaging Solutions Ltd, London, UK).

**Image analysis for perfusion quantification**

All three short-axis slices of perfusion images covering the base, mid-LV and the apex were analyzed. The process of quantitative pixel-wise perfusion image analysis and myocardial blood flow (MBF) maps has been described and validated previously. The method of MBF quantification was derived from the central volume principle based on indicator-dilution experiments. Perfusion quantification was performed in collaboration with Dr Andrew Arai and his team at NIH Bethesda, USA using their custom in-house developed software suite written in Interactive Data Language (Exelis Visual Information Solutions, Boulder, Colorado, USA) for CMR image analysis. In brief, endocardial and epicardial borders of the left ventricular (LV) myocardium were first manually traced on the perfusion image series to outline myocardial regions of interest (ROI). An additional ROI was drawn in the blood pool of the low resolution dual-sequence image series to extract the arterial input function (AIF). Further image processing steps were performed on the perfusion image series to correct motion artifacts and signal intensity bias. A non-rigid image registration was used to remove cardiac and respiration motion. A surface coil intensity correction was used to compensate surface coil-related field inhomogeneity in the images, which was then followed by
baseline intensity correction. Finally, pixel-wise myocardial time-signal intensity curves were extracted and quantified using a model-constrained deconvolution to estimate myocardial blood flow on a pixel-by-pixel basis at the three myocardial levels at stress and rest.

After MBF maps were obtained, further analysis was performed to depict the regions of ischaemic myocardium. A remote region with relatively preserved perfusion was selected in the myocardial ROI from the MBF pixel maps. A 25% threshold lower than the average MBF of the remote region was used to provide an indicator of the area of ischaemic myocardium.

Calculation of average myocardial blood flows and Myocardial perfusion reserve (MPR):

The quantitative perfusion software estimates myocardial blood flow (ml/min/g) at the three myocardial levels (basal, mid, apical) at stress and rest. The total average myocardial flow was calculated as the average values of these blood flows for both stress and rest for each scan.

Myocardial perfusion reserve (MPR) was calculated as a ratio of average total myocardial flow at stress (ml/min/g) against average total myocardial flow at rest (ml/min/g).

5.3 CMR methods in assessment of carotid atherosclerosis

Hardware for Imaging

Scanner:

All of the scans were performed using a 3.0 Tesla Siemens CMR scanner.

Coils:

For all scans purpose-built bilateral four channel phased-array carotid surface coils were used (Machnet BV, Eelde, the Netherlands), which were successfully utilised in previous local work.\textsuperscript{119} In addition, specially designed neck cushions and head straps were used for immobilisation. Subjects were optimally positioned so that their neck was at the iso-centre of the magnet.\textsuperscript{120} All subjects were given ‘in-the-ear’ ear plugs to protect their hearing during imaging.
**Imaging Protocol**

A locally developed and validated carotid CMR protocol described below was used, which has been applied to previous studies involving carotid CMR assessment.\(^{119,120,121}\) Firstly, gradient echo bright blood localisers were rapidly acquired in all 3 orthogonal planes to locate the patient’s head and neck within the scanner. A stack of time-of-flight (TOF) images was then acquired perpendicular to the long axis of the carotid arteries, as identified on the localisers. A saturation band was then placed cranial to the imaging volume so that only blood flowing in the caudo-cranial direction would be of high signal. The TOF images were used to identify the carotid bifurcation on each side. Then a low resolution T1-weighted fast spin echo (FSE) image was acquired through the bifurcation on each side. The bifurcation was defined as a plane running through three points: one in the common carotid artery, one in the internal carotid artery and one in the external artery. A second image was then acquired perpendicular to this in the common carotid artery. These images were next used to rapidly assess for the presence of any carotid plaque, to determine the location and orientation of the carotid artery for further imaging, and to verify optimal coil position. This was defined as when the carotid bifurcation was in the middle of the area of high signal underlying the coil. If the coil was too high or too low, it was repositioned, and imaging was repeated to optimise image quality. Using these localising images, high-resolution cross-sectional images were subsequently acquired with a standard 2-dimensional fast spin-echo (FSE) sequence \(^{119}\) at 2mm intervals for 20mm above the bifurcation and 20mm below the bifurcation of the common carotid arteries (CCA) on both sides, such that a total of 40mm of carotid vessel was imaged bilaterally.

**Carotid Imaging Analysis**

Dedicated software to analyse carotid artery images was locally developed by Dr Varghese at the Royal Brompton Hospital CMR Unit in collaboration with Dr Merrifield of the Visual Information Processing Group, at the Department of Computing, Imperial College. This software has been validated for this application.\(^{122}\) The software is called Atheroma Tools, and it functions as a plug-in of CMRtools (Cardiovascular Imaging Solutions, London, UK), a commercial software solution for the analysis of CMR images.\(^{122}\)
• Assessment of carotid wall volumes:
Atheroma Tools was used to derive carotid artery volumes.\textsuperscript{122} For each slice, the operator contoured the internal and external carotid arterial surfaces. The software then measured the luminal and adventitial areas for each slice and, using the slice thickness and number of slices, converted these into luminal and adventitial volumes.\textsuperscript{122} Subtracting the luminal volume from the adventitial volume derived the total carotid arterial wall volume (TWV) (mm$^3$), which is a marker for total carotid plaque volume.\textsuperscript{120} Total carotid wall volume (mm$^3$), measured separately on the LEFT and RIGHT, then added to give a combined total carotid wall volume was the main variable measured in this clinical trial, to provide a quantitative and direct indicator of atheroma burden. Given that luminal volume can be affected by multiple variables, such as the haemodynamic status of the patient, it was felt that this parameter may be less meaningful in our study; especially as it does not directly measure atheroma volume and hence would be less likely to change in the time frame of treatment periods in this study. The accuracy of the CMR vessel wall sequence described above for measuring carotid lumen, adventitial, and wall volumes has been found to be good when validated against ex vivo specimens, with minor overestimation.\textsuperscript{121} The protocol used has also been shown to have good inter-study reproducibility,\textsuperscript{120} making it suitable for longitudinal assessments of carotid atheroma progression or response to therapy.

• Assessment of carotid distensibility:
Using TSE localiser images, a single gradient echo cine image was acquired perpendicular to the common carotid artery on each side to evaluate arterial distensibility. A standard prospectively ECG-gated balanced steady state free precession (SSFP) sequence was used, with sequence parameters FOV 105mm x 52mm, flip angle 60, bandwidth 977 Hz/pixel, giving a voxel size of 0.66 x 0.82 x 6mm. The cine image was then manually contoured at the internal border of the common carotid artery at end diastole and end systole for each side. Atheroma tools\textsuperscript{122} was then used to derive the cross-sectional areas (mm$^2$) and carotid distensibility was calculated as the percentage change in area using the following equation:

\[
\text{Strain (\%)} = \frac{\text{CCA cross-section area (systole)} - \text{CCA cross-section area (diastole)}}{\text{CCA cross-section area (diastole)}}
\]
As a limitation, it is acknowledged that in fact the above equation actually represents strain rather than distensibility which should be derived by correcting for pulse pressure, which has not been done in our study.

5.4 Assessment of Endothelial function
Measurement of endothelial vascular function was done using pulse amplitude tonometry (PAT), (EndoPAT device, Itamar Medical, Caesarea, Israel). This method was chosen given that it is non-invasive and can conveniently be performed within 15-20 minutes. We did not opt to use CMR methods of measuring endothelial function such as flow mediated dilatation (FMD) of the brachial artery, given that this would have made the CMR protocol (which already included a full cardiac study, involving stress perfusion, as well as carotid imaging) even lengthier. Endothelial function measurements were carried out by testing endothelial vasomotor function after reactive hyperaemia by PAT (RH-PAT) as measured in the fingertips via plethysmographic probes.\textsuperscript{123}[See Figure 10] The EndoPat device is a fingertip plethysmograph capable of measuring volume changes in the finger with each arterial pulse.\textsuperscript{123} Volume changes in the fingertip are recorded digitally as pulse amplitude and can be tracked over time. The 3 phases of digital pulse amplitude tonometry (PAT) include baseline, occlusion and dilatation or hyperaemia.\textsuperscript{123} [See Figure 11]
Figure 10: The EndoPAT plethysmographic probe

Figure 11: Pulse amplitude recordings from the baseline, occlusion and dilatation or hyperaemia phases of the EndoPAT test
The procedure was conducted according to the manufacturer’s instructions, for which the protocol has previously been published and is described as follows:\textsuperscript{123} A PAT probe was positioned on one finger of each hand which was inflated to either 10mmHg below diastolic pressure or 70 mmHg, whichever was lower. Recordings were taken simultaneously from both fingers throughout the study. Baseline measurements were taken for a minimum of 5 minutes, then a blood pressure cuff was inflated on the non-dominant arm to supra-systolic pressure for 5 minutes to achieve the occlusion phase. After 5 minutes, the cuff was deflated and the post-occlusion hyperaemic or dilatation response was measured. Pulse amplitude recordings were digitised and analysed by an automated, proprietary algorithm. During the occlusion period, signals continued in the control finger whilst signals were absent from the test finger. After cuff release, pulse amplitude should increase in subjects with normal endothelial function. Endothelial function measurements were carried out using the patient’s non-dominant arm as the test arm before and after each three-month treatment period.

RHI stands for Reactive Hyperemia Index (RHI). This is the final concluded result of the EndoPAT\textsuperscript{™} and gives an indication of the endothelial vasodilator function.\textsuperscript{123} The RHI is the post-to-pre occlusion PAT signal ratio in the occluded arm, relative to the same ratio in the control arm, and corrected for baseline vascular tone of the occluded arm\textsuperscript{123} where:

- Normal: RHI > 1.67
- Abnormal: RHI ≤ 1.67

The LnRHI is a natural log transformation of the same index, where:

- Normal: LnRHI > 0.51
- Abnormal: LnRHI ≤ 0.51

This transformation is a monotonic transformation; therefore it does not change the dichotomous diagnosis (normal/abnormal) for any individual test. LnRHI provides a better double sided distribution that is closer to a normal distribution.\textsuperscript{123}

5.5 Assessment of lipid parameters, oxidised LDL and thrombogenesis

At the four data collection time points in the trial ie. pre- and post-apheresis and pre-and post-sham, blood tests were taken for: a full lipid profile including total cholesterol, Lp(a),
LDL cholesterol, HDL cholesterol, total cholesterol to HDL ratio, TG; Apolipoprotein(A) (Apo(A)) and Apolipoprotein(B) (Apo(B)), fasting glucose, urea and electrolytes, liver function tests, thyroid function tests, B-natriuretic peptide (BNP), coagulation screen, ferritin, haematocrit, bone profile and C-Reactive protein (CRP). All blood samples were analysed by the Royal Brompton and Harefield Hospital Trust’s laboratory using standard methods. 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 apheresis or sham treatment session via a fresh venepuncture (ie. not via the treatment access to avoid errors from potential haemodilution) and were only conducted once we had confirmed that blood heparin levels were at 0.001IU/ml. The presence of heparin was excluded specifically to avoid the confounding impact this may have otherwise had on the thrombogenic tests. Generally, we attempted to avoid administering an intra-venous heparin bolus prior to the final apheresis session to avoid the chances of heparin lingering in the blood-stream after completing the treatment.

Lp(a) measurement:
At the start of the trial the standard assay for Lp(a) that was being used for clinical purposes at Royal Brompton and Harefield NHS Foundation trust was the nephelometric IMMAGE assay; which measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction. Fourteen months after the study had commenced, the standard assay used for clinical purposes was changed to (Lp(a) Ultra: a quantitative immunoturbidimetric assay, on the grounds that it is regarded as isoform-insensitive. According to the manufacturers, the principle of the Lp(a) Ultra method is that when an antigen-antibody reaction occurs between Lp(a) in a sample and anti-Lp(a) antibody which has been adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change, with the magnitude of change being proportional to the quantity of Lp(a) in the sample.

To ensure consistency of analysis for the purposes of the trial all samples were analysed with the initial IMMAGE assay throughout the trial. In addition, samples had been frozen and stored at -80C, hence we retrospectively measured the Lp(a) Ultra in all relevant
samples up until the date of introduction of the newer assay, and prospectively applied the Lp(a) Ultra assay for all samples after its introduction. In other words, Lp(a) levels for the 4 important time points pre-apheresis, post-apheresis, pre-sham and post-sham were measured using both assays across the entire study. Therefore, we were able to report on the results for the entire study using both assays. Given that all of the patients were recruited prior to the introduction of the Lp(a) Ultra assay, selection of patients using the Lp(a) >500mg/L inclusion requirement was based on the Lp(a) IMMAGE assay.

Assessment of oxidised LDL and Anti-oxidised LDL antibodies:
Samples were collected for oxidised LDL (OxLDL) and Anti-oxidised LDL antibodies prior to starting the first apheresis or sham session as well as after the final apheresis or sham session, at the time as the sample collection for the full lipid profile. Analysis of these tests was performed in the Department of Vascular Sciences, Hammersmith Hospital laboratories in collaboration with Dr Ramzi Khamis and Professor Dorian Haskard and their team. It was surmised that given that Lp(a) is known to be potent carrier of oxidised phospholipids;\textsuperscript{10} it may be worth exploring the changes that occur in oxidised LDL and Anti-oxidised LDL antibodies before and after a period of treatment with apheresis, particularly as to the best of our knowledge experiments have not thus far been conducted to investigate this in patients with raised Lp(a), in the absence of elevated LDL cholesterol. We hypothesised that exploring the changes in oxidised LDL prior to starting and after treatment with apheresis may shed more light on the pathophysiology of Lp(a) and its link with oxidised LDL; as well as the impact of apheresis on both.

Measuring Circulating OxLDL:
The development of assays to measure circulating OxLDL levels has been enabled by the development of monoclonal antibodies binding oxidation-specific epitopes (e.g. EO6 binding PC, 4E6 binding MDA).\textsuperscript{124} Holvoet was the first to demonstrate using the specific murine monoclonal antibody 4E6 in a competition ELISA, that patients with CAD had significantly elevated plasma levels of MDA-LDL.\textsuperscript{125}
Professor Haskard’s group isolated LO1, the first spontaneously arising IgG anti-OxLDL monoclonal antibody using hybridoma generated from the splenocytes of 1-year-old female LDLr−/− mice. LO1 reacts with MDA-LDL but minimally with native LDL. They have implemented LO1 as the capture antibody in developing a new sandwich ELISA assay. Specific methods used by their group to perform the analysis of oxidised LDL and anti-oxidised LDL antibodies in our trial patients are described in detail below:

Plasma samples were all stored at -80°C and thawed to room temperature before use in ELISA. All samples were anonymised and assigned digital codes and personnel conducting assays were fully blinded to patient treatment allocation and order and unaware of the statistical analysis plan.

Generation of MDA-LDL:
MDA-LDL was prepared as described previously by Palinski et al. (1990). Native LDL (Calbiochem, Cat No. 437644, conc. 5.00 mg/mL) was incubated for 3 hours at 37°C with 0.5M MDA solution prepared through the acid hydrolysis of MDA-bis-dimethylacetal (Sigma Aldrich, Cat No. 10,838-3) at a ratio of 100µL MDA solution per milligram of ApoB followed by neutralization to pH 7.4. Following modification, the MDA-LDL conjugate was then eluted with PBS through a PD-10 column and 0.01% EDTA added to prevent further oxidation. The optical properties of the MDA adduct prevented the measurement of MDA-LDL concentration by spectrophotometry. Thus, an ELISA assay was carried out to accurately determine the ApoB protein concentration of MDA-LDL, comparing it to a standard curve of human native LDL of known concentration (5.00 mg/mL provided by manufacturers) by probing ApoB. The generation of the MDA-LDL epitope on the LDL was confirmed using 15µg/mL LO1 and biotinylated polyclonal goat anti-mouse IgG antibody (Southern Biotech, Cat No. 1030-08) (concentration 1:5000) followed by HRP-conjugated streptavidin (R&D Systems, Minneapolis, MN Cat No. 890803.01) (concentration 1:200) for 20 minutes. The generated MDA-LDL was stored at 4°C.

Enzyme-linked Immunosorbent Assays:
A general ELISA protocol was adopted for all ELISAs carried out. 96-well (NUNC Scientific, Waltham, MA) Maxisorp plates were used. They were coated overnight at room
temperature with capture antibody made to different concentrations with Phosphate Buffered Solution (PBS)(Gibco, Life Technologies Cat No. 20012-09). Plates were washed (3 cycles of 400µL buffer per wash) and non-adherent material removed by Thermo Scientific Mk4 plate washing machine with wash buffer (0.05% Tween in PBS) after each of the incubation steps. Plates were blocked with 300µL/well of 2% filtered Bovine Serum Albumin (BSA) (Sigma Aldrich, Poole, UK Cat No. A3803) for 1 hour following the overnight incubation. Reagent diluent (PBS with 0.5% BSA and 0.05% tween) was used to produce the required concentrations of reagents. The concentrations of the consecutive layers of the ELISA assay (i.e. plasma samples, capture and detection antibodies) were determined from assay development experiments. Antibody and plasma layers were both incubated for 1 hour whereas streptavidin-HRP was incubated for 20 minutes only. The IgG and IgM anti-MDA-LDL antibody assays were used as described previously by Khamis et al.\textsuperscript{128} in the ASCOT anti-OxLDL antibodies substudy. Neat 3,3’, 5,5’-tetramethylbenzidine (TMB) (Sigma-Aldrich, Poole, UK Product No. T0440) 100µL/well was added to produce a reaction which was stopped by sulphuric acid (0.5M H\textsubscript{2}SO\textsubscript{4} Acros Organics Code: 124240010) 100µL/well. An equal number of wells remained coated with 2% BSA to act as a negative control and to allow for correction for non-specific binding. Control plasma from a healthy volunteer was used as the positive control. The plates were covered with airtight adherent plate-sealing films (Sigma Aldrich, Poole, UK Cat No. Z369659) during all incubation steps up to the addition of TMB to prevent contamination or oxidation of reagents including LDL. The optical density of each well was measured at 450nm wavelength using a synergy HT multi-mode microplate reader (Southern Biotech, Birmingham, AL). On testing patient plasma samples, reference plasma from a healthy volunteer was used to correct against in a 2-in-1 dilution series. In addition values were corrected to background measured using 2% BSA as a negative control to plasma.

**Antibody level measurement by ELISA**

IgG and IgM anti-MDA-LDL antibody levels were estimated by ELISA against solid phase MDA-LDL. Briefly, MDA-LDL was prepared as described earlier.\textsuperscript{127} The extent of modification was determined by the relative electrophoretic mobility of the LDL using pre-cast 0.6% agarose, 1.0% barbital buffer gels (Beckman Coulter, Fullerton, Ca) and with a carbonyl assay.\textsuperscript{126} IgG and IgM antibody binding to solid phase antigens was identified by mouse anti-
human IgG (Cambridge Bioscience, Cambridge, UK) or biotinylated mouse anti-human IgM (Cambridge Bioscience) followed by horse radish peroxidase (HRP) conjugated rabbit anti-mouse Ig (Dako, Cambridgeshire, UK) or HRP-conjugated streptavidin (R&D Systems, Minneapolis, MN). Antibody binding was detected with 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma Aldrich, UK), and the reaction was stopped with 0.5M H2SO4. The optical density was then measured with a Synergy HT microplate reader (Biotek, USA) at wavelength 450nm. The plates were read at an optical density of 450nm (OD450), after which the background was subtracted. All serum anti-MDA-LDL sample values were corrected to a reference serum with a standard curve used on all plates and results expressed in Units (U). Total IgG and IgM antibody levels were measured using capture ELISA. Briefly, either goat anti-human IgG or mouse anti-human IgM (Southern Biotech, Birmingham AL) was used to capture either IgG or IgM respectively. Detection antibodies used were either biotinylated goat F(ab')² anti-human IgG or biotinylated mouse anti-human IgM (both Southern Biotech). Detection was as above with HRP-conjugated streptavidin at an optical density of 450nm. Once background was subtracted, results were expressed in Units (U). Cut off and tertile as well as interquartile values in mg/ml for total IgG and IgM were interpolated via standard curve fit using standard ELISA methodology in PRISM 6 (GraphPad, La Jolla, Ca). The ELISA assay range for total IgG and IgM was wide enough to span from well below to well above the expected normal reference ranges.

Ultimately, for the 4 relevant time points results were processed for: Oxidised LDL (MDA-LDL), Anti-IgG-MDA-LDL, Total IgG, and Anti-IgM-MDA-LDL, Total IgM.

**Global Thrombosis Test**

Given that Lp(a) is felt to be pro-thrombotic via the inhibition of fibrinolysis with enhancement of clot stabilisation as well as via enhanced coagulation, we chose to assess the impact of treating raised Lp(a) with apheresis on numerous markers of thrombosis, to assess whether treatment can potentially help to reverse this risk. The global thrombosis test (GTT) (Montrose Diagnostics, London, United Kingdom), is a comprehensive test of platelet reactivity, coagulation (thrombin generation), and spontaneous (endogenous) thrombolytic activity, and hence we felt that it would be an ideal means of assessing the impact of apheresis on thrombin generation and thrombolysis. The GTT is a point-of-care
assay that employs native (non-anticoagulated) blood. The instrument measures the time taken to create a shear-induced thrombus under physiological conditions and in the second phase of the test, measures the time to achieve endogenous thrombolysis of the thrombus created during the first phase of the test. The instrument measures the time (d) between 2 consecutive blood drops. This time interval increases gradually as flow slows down and at an arbitrary point (d ≥15 s, before reaching complete occlusion), the end point of the measurement is displayed (occlusion time [OT], in seconds). Restart of blood flow after occlusion is due to spontaneous thrombolysis (lysis time [LT], in seconds). If lysis does not occur until 6,000 s after OT (LT cut-off time), “no lysis” is displayed and recorded.

**Assessment of thrombotic and thrombolytic status.**

Blood samples were taken from an antecubital vein using a 21-G butterfly cannula using a 2-syringe technique according to the manufacturer’s instructions. The first 5 ml blood was used for routine blood tests and the next 4-ml sample was aspirated into a standard polypropylene syringe, which was directly, and immediately inserted into the fitting in the GTT instrument (within 15 s of withdrawal), that was positioned next to the subject to minimize sampling delay; using the same techniques as other research studies that have implemented use of the GTT. The measurement is automated and starts as soon as blood is introduced. Prior to the test as mentioned previously, care was taken to exclude the presence of heparin specifically with a blood sample to avoid the confounding impact this may have otherwise had on the GTT and the other thrombogenic tests conducted.

**Additional Markers of Thrombogenesis:**

In addition to the GTT, we also collaborated with Professor Michael Laffan, Professor of Haemostasis & Thrombosis, Imperial College London, who advised us on other haematological markers of thrombogenesis which would be useful to evaluate the impact of treating raised Lp(a) with apheresis which consisted of:

D-Dimer:

D-dimer was measured by the routine clinical methods on the ACL TOP 500 CTS Analyser (Werfen UK, Warrington, Cheshire UK). D-Dimer is contained in the soluble derivatives formed upon plasmin degradation of Factor XIIIa cross-linked fibrin (XDP). D-Dimer is
becoming a widespread tool for diagnosing thrombosis and is found to be raised in clinical conditions such as deep vein thrombosis (DVT), pulmonary embolism (PE) and disseminated intravascular coagulation (DIC).\textsuperscript{131} D-dimer value can be elevated in a number of other normal physiologic, as well as pathologic states.\textsuperscript{132}

Von Willebrand factor (vWF):
VWF was measured by the routine clinical methods on the ACL TOP 500 CTS Analyser (Werfen UK, Warrington, Cheshire UK). It is a blood glycoprotein involved in hemostasis and it is important in platelet adhesion to wound sites.\textsuperscript{133}

Thrombin generation test:
Hemker et al. developed the conceptual methodology of the calibrated, automated thrombin generation test.\textsuperscript{134} The aim was to develop a simple method for quantifying continuous and dynamic properties of thrombin generation,\textsuperscript{135} in clotting plasma. Thrombin generation is initiated by the addition of a combined calcium/fluorogenic substrate (Z-Gly-Gly-Arg-AMC) in the presence of tissue factor (TF)/phospholipid trigger. The fluorescent signal generated is transmitted to and processed by a computer with specific software (Thrombinoscope TM)) to assess /measure thrombin generation. The fluorescent signal from the sample is then compared to that of the calibrator by the Thrombinoscope software. A thrombin generation curve is subsequently generated which has a waveform, from which certain parameters can be calculated (lag time, thrombin generation velocity, peak thrombin concentration, time-to-peak thrombin, and endogenous thrombin potential).\textsuperscript{136} The thrombin generation test was conducted using this method by Professor Laffan’s team at Hammersmith Hospital.

Thrombin/Anti-thrombin III complex (TAT):
TAT is an enzyme-linked immunosorbent assay (ELISA) which was developed for the determination of thrombin-antithrombin III complex (TAT) in human plasma,\textsuperscript{137} and is used as an aid in the diagnosis and monitoring of thrombosis. In this trial TAT was measured using the Enzygnost ® TAT assay, Siemens Healthcare Diagnostics.
F 1+2
The conversion of pro-thrombin to active thrombin accompanied by fragment formation is a key component of the coagulation cascade. Prothrombin fragment 1 + 2 (F1 + 2) is a useful parameter for diagnosis of thrombosis or hyper-coagulatory states. A previous study showed that F1 + 2, soluble fibrin, D-dimer, and thrombin-antithrombin complex have similar diagnostic ability in detecting thrombosis. In addition, a decrease of F 1+2 can be seen in patients undergoing oral anti-coagulant therapy or heparin. In this trial F 1+2 was measured using the Enzygnost® F 1+2 (monoclonal) assay, Siemens Healthcare Diagnostics.

TFPI
Inhibition of the function of tissue factor pathway inhibitor (TFPI) is thought to be one of the mechanisms by which Lp(a) may enhance coagulation. Tissue factor pathway inhibitor (TFPI) is a major regulator of tissue factor-mediated coagulation. A study demonstrated that Lp(a) binds and inactivates TFPI, and that the apo(a) portion of Lp(a) is most likely involved in TFPI binding. We therefore predicted that lowering Lp(a) may raise the level of circulating TFPI, thereby improving coagulation risk. In this trial, TFPI was measured using the IMUBIND® TFPI ELISA, which is an enzyme-linked sandwich immunoassay for the measurement of TFPI in human plasma.

5.6 Assessment of exercise capacity, symptoms and quality of life
The Six Minute Walk test was chosen as the assessment method of exercise capacity due to its feasibility, practicality and simplicity and also given that the test has been widely used for measuring the response to therapeutic interventions for pulmonary and cardiac disease. The exercise treadmill test was avoided, given that a significant proportion of patients with refractory angina are unable to perform them due to the limitations of their condition.

Description: The 6MWT is a practical simple test that requires a 100-ft hallway but no exercise equipment or advanced training for technicians. This test measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes (the 6MWD). The self-paced 6MWT assesses the submaximal level of functional capacity. Most patients do not achieve maximal exercise capacity during the 6MWT; instead, they choose
their own intensity of exercise and are allowed to stop and rest during the test, which we felt would be ideal for patients with refractory angina. However, because most activities of daily living are performed at submaximal levels of exertion, the 6MWD is a good reflection of the functional exercise capacity for daily physical activities. \cite{142}

We followed the guidelines issued by the American Thoracic Society (ATS) \cite{142} as follows to ensure that we performed the 6MWT with accurate methods in a reproducible manner.

**Technical aspects:** The 6MWT was performed indoors, along a long, flat, straight, enclosed corridor in Harefield Hospital, with a hard non-slippery surface that was seldom travelled. The length of the corridor was marked every 3 m. The turnaround points were marked with orange traffic cones, placed 18m apart. \cite{142}

**Patient preparation:** \cite{142}
1. Comfortable clothing was worn.
2. Appropriate walking shoes were worn.
3. Patients used their usual walking aids during the test (cane, walker, etc.) if necessary.
4. The patient’s usual medical regimen was continued.
5. A light meal was acceptable before tests.
6. Patients had not exercised vigorously within 2 hours of beginning the test.

**Measurements:** \cite{142}
1. Repeat testing was performed at the same time of day to minimize intra-day variability.
2. A “warm-up” period before the test was not performed.
3. Prior to the test we checked for contraindications, measured pulse and blood pressure, and made sure that clothing and shoes were appropriate and completed the first portion of the record sheet.
4. Pulse oximetry was performed, with measurement of the heart rate and oxygen saturation (SpO2) just prior to and immediately following the test. \cite{142}
5. We asked the patients to rate their baseline dyspnoea and overall fatigue using the Modified Borg Scale (which quantifies breathless or fatigue from a scale of 0 corresponding to “Nothing at all” to 10 corresponding to “Very, very severe (maximal)” and repeated this immediately after the test.
6. A timer was set to 6 minutes.
7. We instructed the patients as follows in accordance with the ATS guidelines:
   “The object of this test is to walk as far as possible for 6 minutes, but don’t run or jog. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will be exerting yourself. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able. You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. You must stop at the end of 6 minutes.”
8. We recorded the total distance (m) walked in 6 minutes. In addition, we recorded whether the walk test had induced chest pain. We also recorded the Modified Borg score for the level of perceived dyspnoea and fatigue on a scale from 0-10, at baseline and immediately after the test.

Assessment of their angina symptoms was assessed using a validated angina scoring system (the Seattle Angina Scoring Questionnaire), a 19-item self-administered questionnaire measuring five dimensions of coronary artery disease: physical limitation, anginal stability, anginal frequency, treatment satisfaction and quality of life, with higher scores in each domain depicting improved clinical status. This questionnaire was selected because sensitive to clinical change and is regarded as a valuable measure of outcome in cardiovascular research. In addition, it has previously been applied to other studies of interventions in patients with refractory angina. The SAQ was administered at Harefield Hospital at the four data collection time points in the trial ie. pre- and post-apheresis and pre-and post-sham, with patients remaining completely blinded to treatment allocation and order throughout.

Quality of life (QOL) was assessed with a validated QOL scale questionnaire (SF-36v2) as patients with refractory angina are often shown to have a relatively poor QOL, and we wanted to explore the potential impact of lipoprotein apheresis on quality of life. This particular questionnaire was utilised because it has been demonstrated to be a useful QOL scale for the differentiated clinical forms of ischaemic heart disease, with the additional capability of reflecting the level of anxiety in these patients and it has specifically been
applied previously in the assessment of refractory angina. The Optum™ SF-36v2® Health Survey asks 36 questions to measure functional health and well-being from the patient's point of view. The SF-36v2® programme provides scores for each of eight health domains and calculates psychometrically-based physical component summary (PCS) and mental component summary (MCS) scores. The SF-36 was administered at Harefield Hospital at the four data collection time points in the trial ie. pre- and post-apheresis and pre-and post-sham, with patients remaining completely blinded to treatment allocation and order throughout.

5.7 Statistics and Power calculations

Power Calculations:
The primary endpoint was a change in Myocardial Perfusion Reserve (MPR). A power calculation was performed by the trial statistician prior to commencing the study. With the cross-over design, assuming the inter-study reproducibility for MPR to have a standard deviation (SD) of 0.15 to detect a change in MPR of 0.2 between the groups, following treatment versus control, a sample size of 20 patients was required to achieve 99% power at a p-value of 0.05. The cross-over design helped to improve statistical power and eliminated potential randomisation issues, since all the same patients had treatment and sham apheresis, hence effectively acted as their own controls.

Statistical Analysis:
Statistical analysis was performed by the trial statistician Dr Winston Banya, Imperial College London according to the statistical analysis plan that was defined prior to commencement of the trial. Categorical data are presented as numbers and percentages in brackets. Groups were compared using a chi squared or Fishers Exact Test. Quantitative analysis of continuous data are presented as mean (SD) or median (interquartile range) according to whether the data is normally distributed or not. The groups were compared using the Paired Student’s t-test for normally distributed data or the Mann Whitney U-test for data that was not normally distributed. Analysis of variance was used to assess the clinical factors associated with improvement in the primary and secondary outcomes between the two groups.
5.8 Ethical approvals and study oversight
We obtained approval from the National Research Ethics Committee (London, Fulham) to conduct this clinical trial [REC reference: 11/LO/1976]. We also obtained site-specific Royal Brompton and Harefield NHS Foundation Trust R&D approval before accepting participants into the study. The local R&D department conducted audits to ensure quality control during the trial. The study adhered to the Guidelines for Good Clinical Practice and was conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions. Prior to patient recruitment, the trial was also registered with ClinicalTrials.gov, Identifier: NCT01796912.

5.9 Adverse event reporting
All adverse events were recorded in the patient’s research files and were defined in the trial protocol as follows:

**Adverse Event (AE):** any untoward medical occurrence in a patient or clinical study subject. This was further subdivided into **expected adverse events** that can potentially occur during sustained apheresis therapy and **unexpected adverse events**, which included any event that was not typically expected during apheresis therapy. Expected adverse events included bruising or swelling of the cannulation site, minor bleed from the cannula site, lightheadedness, transient hypotension during apheresis treatments, vasovagal episodes and mild chest pain during treatment.

**Serious Adverse Event (SAE):** any untoward and unexpected medical occurrence or effect that:
- **Results in death**
- **Is life-threatening** – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- **Requires hospitalisation or prolongation of existing inpatients’ hospitalisation**
- **Results in persistent or significant disability or incapacity**
Reporting procedures

All adverse events were reported according to the trial protocol as follows. Depending on the nature of the event the following reporting procedures were followed. Any questions concerning adverse event reporting were directed to the Chief Investigator in the first instance.

Non serious AEs

All such events, whether expected or not, were recorded and a summary logbook of all of these events was sent to the National Research Ethics Committee and the trial sponsor.

Serious AEs

An SAE form was to be completed and faxed or emailed to the Chief Investigator within 24 hours.

All SAEs were to be reported to the National Research Ethics Committee and the trial sponsor where in the opinion of the Chief Investigator, the event was:

- ‘related’, ie resulted from the administration of any of the research procedures; and

- ‘unexpected’, ie an event that was not listed in the protocol as an expected occurrence.
RESULTS

Chapter 6: Patient demographics, protocol compliance and safety reporting

6.1 Study Population

As planned, 20 patients completed the trial protocol including cross-over. Baseline characteristics of all trial patients and the order in which treatment was randomised is described in Table 2.

Table 2: Randomisation order and baseline characteristics: number ±SD (%).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apheresis/Sham</th>
<th>Sham/Apheresis</th>
<th>All subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.1 ± 10.4</td>
<td>62.4 ± 9.0</td>
<td>60.9 ± 9.5</td>
</tr>
<tr>
<td>Gender (Male)</td>
<td>9 (100)</td>
<td>10 (91)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>Ethnicity: White</td>
<td>4 (44.4)</td>
<td>3 (27.3)</td>
<td>7 (35.0)</td>
</tr>
<tr>
<td>Asian</td>
<td>5 (55.6)</td>
<td>8 (72.7)</td>
<td>13 (65.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ± 1.9</td>
<td>27.5 ± 4.1</td>
<td>27.4 ± 3.2</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>125.6 ± 8.5</td>
<td>125.5 ± 9.1</td>
<td>125.5 ± 8.6</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>72.2 ± 9.4</td>
<td>71.4 ± 2.3</td>
<td>71.8 ± 6.3</td>
</tr>
<tr>
<td>Lp(a) (mg/L)</td>
<td>1120 (771, 1660)</td>
<td>1080 (902, 1520)</td>
<td>1100 (771, 1590)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>3.46 ± 0.82</td>
<td>4.25 ± 0.74</td>
<td>3.90 ± 0.86</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>1.85 ± 0.74</td>
<td>2.41 ± 0.64</td>
<td>2.16 ± 0.73</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (22.2)</td>
<td>1 (9.1)</td>
<td>3 (15.0)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4 (44.4)</td>
<td>8 (72.7)</td>
<td>12 (60.0)</td>
</tr>
<tr>
<td>Smoker No</td>
<td>3 (37.3)</td>
<td>7 (63.6)</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>Current</td>
<td>4 (44.4)</td>
<td>2 (18.2)</td>
<td>6 (30.0)</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>7 (77.8)</td>
<td>9 (81.8)</td>
<td>16 (80.0)</td>
</tr>
<tr>
<td>Anti-anginal drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral Nitrates</td>
<td>7 (77.8)</td>
<td>7 (63.6)</td>
<td>14 (70.0)</td>
</tr>
<tr>
<td>Beta Blockers</td>
<td>7 (77.8)</td>
<td>11 (100)</td>
<td>18 (90.0)</td>
</tr>
<tr>
<td>Ca Channel Blockers</td>
<td>3 (33.3)</td>
<td>5 (45.5)</td>
<td>8 (40.0)</td>
</tr>
<tr>
<td>Ivabradine</td>
<td>2 (22.2)</td>
<td>2 (18.2)</td>
<td>4 (20.0)</td>
</tr>
<tr>
<td>Ranolazine</td>
<td>1 (11.1)</td>
<td>0</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>Statin</td>
<td>9 (100.0)</td>
<td>11 (100.0)</td>
<td>20 (100.0)</td>
</tr>
<tr>
<td>Prior CABG</td>
<td>6 (66.7)</td>
<td>6 (54.6)</td>
<td>12 (60.0)</td>
</tr>
<tr>
<td>Prior PCI</td>
<td>7 (77.8)</td>
<td>9 (81.8)</td>
<td>16 (80.0)</td>
</tr>
<tr>
<td>Prior MI</td>
<td>8 (88.9)</td>
<td>9 (81.8)</td>
<td>17 (85.0)</td>
</tr>
</tbody>
</table>

In terms of baseline coronary status, 12 out of the 20 (60%) patients had prior bypass graft surgery and of those, 3 of 12 (25%) had undergone redo surgery. According to angiographic studies that had been performed for clinical purposes prior to recruitment, 9 of 12 (75%) with prior bypass surgery had at least 1 occluded graft, none had occluded all grafts ie. all 12 had at least 1 patent graft remaining. Coronary stents had been inserted for 16 out of the total 20 (80%) patients. Amongst those with prior stents the average number of stents
performed was 4. Of the 16 with prior stents, 11 (69%) had evidence of occlusion of at least one stent at the time of recruitment.

Angiographic details and when this was last performed prior to patient recruitment was recorded at baseline for each patient (See Table 3). In addition, baseline CMR features such as the presence or absence of infarction as determined by LGE, whether there was a visible perfusion defect upon stress and LVEF(%) were also recorded (See Table 4). Data regarding total carotid wall volume and endothelial function determined by the EndoPAT test were also documented (See Table 5). Finally, baseline data for the 6MWT distance(m), SAQ angina frequency score and SF-36 questionnaires has also been presented (See Table 6).

In terms of optimisation of anti-anginal medications, the average dose used by the study participants of each class of medication has been calculated as a percentage of the maximum possible dose (See Table 7).
Table 3: Angiography procedure details and date last performed prior to patient recruitment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date of last angiogram</th>
<th>Details of last angiogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11/06/2007</td>
<td>Patent LIMA to LAD, Occluded VG to OM and VG to RCA. Occluded native LAD, patent native Cx, heavily diseased native RCA.</td>
</tr>
<tr>
<td>2</td>
<td>05/12/2012</td>
<td>Patent LIMA graft and a patent Cx graft. Occluded VG to RCA and subtotal occlusion of proximal to mid RCA.</td>
</tr>
<tr>
<td>3</td>
<td>08/02/2013</td>
<td>PCI x2 to LMS to LAD and to LIMA to LAD, patent VG to PDA and Ix.</td>
</tr>
<tr>
<td>4</td>
<td>19/09/2012</td>
<td>Heavy native disease. VG to the LAD occluded at the insertion point. VG to OM proximally occluded, VG to Cx patent, LIMA to LAD patent but has insertional disease. No revascularisation or PCI targets.</td>
</tr>
<tr>
<td>5</td>
<td>09/03/2006</td>
<td>VG to OM1 occluded, Patent LIMA and SVG to RCA. Graft to D1 small but patent.</td>
</tr>
<tr>
<td>6</td>
<td>17/04/2008</td>
<td>Significant disease at insertion of LIMA to LAD, VG to RCA patent, significant native 3VD.</td>
</tr>
<tr>
<td>7</td>
<td>10/08/2013</td>
<td>Occlusion of previous RCA stent. Several failed attempts were made to unblock this occlusion. Mild LAD disease.</td>
</tr>
<tr>
<td>8</td>
<td>01/05/2011</td>
<td>Occluded RCA and LAD-hence PCI to LAD/RCA.</td>
</tr>
<tr>
<td>9</td>
<td>15/10/2007</td>
<td>Moderate proximal and mid vessel stenosis in the LAD. D1 had ostial and moderate mid vessel stenoses. Unobstructed Cx. The RCA had some irregularity although the mid RCA stent was widely patent.</td>
</tr>
<tr>
<td>10</td>
<td>30/08/2009</td>
<td>Severe 3VD following NSTEMI hence underwent Triple CABG- LIMA-LAD, VG-OM, VG-PDA.</td>
</tr>
<tr>
<td>11</td>
<td>10/10/2013</td>
<td>Patent stent in Cx. Severe disease in proximal Cx extending back to ostium. Severe disease in proximal and distal RCA. Decision to treat Cx and proximal RCA disease.</td>
</tr>
<tr>
<td>13</td>
<td>17/02/2014</td>
<td>The LIMA to LAD and VG to Ix are patent. The VG to D1 is thrombosed within the stent.</td>
</tr>
<tr>
<td>14</td>
<td>22/03/2013</td>
<td>Patent LIMA to LAD with insertional stenosis, VG to D1 occluded with in-stent restenosis. No other grafts seen. Pressure wire to LAD 0.79 across level of LIMA insertion, therefore proceeded to PCI of LAD</td>
</tr>
<tr>
<td>15</td>
<td>01/06/2012</td>
<td>Patent LMS. LAD occluded. Cx - in stent restenosis distally. RCA occluded. LIMA to LAD graft patent. VG to RCA patent.</td>
</tr>
<tr>
<td>16</td>
<td>01/08/2011</td>
<td>Rotablation and PCI to the D1 and LAD. RCA had moderate disease.</td>
</tr>
<tr>
<td>17</td>
<td>04/05/2014</td>
<td>Early vein graft occlusion, but patent LIMA to LAD. Had 2 PCI procedures: PCI to OM and PLA branch of RCA.</td>
</tr>
<tr>
<td>18</td>
<td>23/04/2014</td>
<td>Moderate disease in the LAD, and 60 – 70% in stent restenosis in the proximal Cx. The RCA was occluded proximally and filled retrogradely from collateral from the LAD. Complex PCI performed to CTO of RCA.</td>
</tr>
<tr>
<td>19</td>
<td>13/02/2014</td>
<td>Patent LIMA to LAD supplying a diseased LAD. Occluded VG. LAD and RCA both occluded proximally, with severe proximal Cx stenosis.</td>
</tr>
<tr>
<td>20</td>
<td>20/07/2010</td>
<td>Complex PCI x3 to a chronic total occlusion of RCA. Further residual Cx disease which was not intervened upon.</td>
</tr>
</tbody>
</table>

Abbreviations: LIMA= Left internal mammary artery graft, VG= Vein graft, LMS=Left main stem, LAD=Left anterior descending artery, D1= first diagonal branch of LAD, Cx=Circumflex artery, OM= Obtuse marginal branch of Cx, Ix=Intermediate artery, RCA= Right coronary artery, PCI=Percutaneous coronary intervention, CTO=Chronic total occlusion.
Table 4: Baseline CMR- presence of LGE, visible perfusion defect(s) upon stress & LVEF (%)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Presence of infarction/LGE</th>
<th>Presence of visible perfusion defect(s) upon stress</th>
<th>LVEF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>Yes</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>74</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>67</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>Yes</td>
<td>44</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>Yes</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>Yes</td>
<td>54</td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>Yes</td>
<td>57</td>
</tr>
<tr>
<td>9</td>
<td>Yes</td>
<td>Yes</td>
<td>51</td>
</tr>
<tr>
<td>10</td>
<td>Yes</td>
<td>Yes</td>
<td>53</td>
</tr>
<tr>
<td>11</td>
<td>Yes</td>
<td>Yes</td>
<td>32</td>
</tr>
<tr>
<td>12</td>
<td>Yes</td>
<td>Yes</td>
<td>54</td>
</tr>
<tr>
<td>13</td>
<td>Yes</td>
<td>Yes</td>
<td>56</td>
</tr>
<tr>
<td>14</td>
<td>Yes</td>
<td>Yes</td>
<td>54</td>
</tr>
<tr>
<td>15</td>
<td>Yes</td>
<td>Yes</td>
<td>71</td>
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<tr>
<td>16</td>
<td>Yes</td>
<td>Yes</td>
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<td>17</td>
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<td>18</td>
<td>Yes</td>
<td>Yes</td>
<td>73</td>
</tr>
<tr>
<td>19</td>
<td>Yes</td>
<td>Yes</td>
<td>76</td>
</tr>
<tr>
<td>20</td>
<td>Yes</td>
<td>Yes</td>
<td>66</td>
</tr>
</tbody>
</table>
Table 5: Baseline Left and Right combined total carotid wall volume (TCWV) (mm$^3$), left and right distensibility and Endothelial function (LnRHI)

<table>
<thead>
<tr>
<th>Patient</th>
<th>L+R TCWV (mm$^3$)</th>
<th>L carotid distensibility(%)</th>
<th>R carotid distensibility(%)</th>
<th>LnRHI (Normal &gt;0.51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4467.88</td>
<td>10.10</td>
<td>13.11</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
<td>3073.93</td>
<td>20.12</td>
<td>17.69</td>
<td>0.62</td>
</tr>
<tr>
<td>3</td>
<td>1770.89</td>
<td>28.22</td>
<td>15.50</td>
<td>0.71</td>
</tr>
<tr>
<td>4</td>
<td>2590.84</td>
<td>28.60</td>
<td>23.01</td>
<td>0.56</td>
</tr>
<tr>
<td>5</td>
<td>2365.76</td>
<td>18.60</td>
<td>19.68</td>
<td>0.66</td>
</tr>
<tr>
<td>6</td>
<td>2351.34</td>
<td>11.42</td>
<td>16.11</td>
<td>0.40</td>
</tr>
<tr>
<td>7</td>
<td>3284.81</td>
<td>21.52</td>
<td>20.25</td>
<td>1.07</td>
</tr>
<tr>
<td>8</td>
<td>1802.33</td>
<td>18.64</td>
<td>22.89</td>
<td>0.75</td>
</tr>
<tr>
<td>9</td>
<td>2498.14</td>
<td>28.26</td>
<td>20.88</td>
<td>0.67</td>
</tr>
<tr>
<td>10</td>
<td>1972.09</td>
<td>10.57</td>
<td>12.53</td>
<td>1.37</td>
</tr>
<tr>
<td>11</td>
<td>2757.99</td>
<td>12.86</td>
<td>14.24</td>
<td>0.59</td>
</tr>
<tr>
<td>12</td>
<td>2628.59</td>
<td>14.99</td>
<td>18.89</td>
<td>0.80</td>
</tr>
<tr>
<td>13</td>
<td>2732.07</td>
<td>12.19</td>
<td>28.69</td>
<td>0.80</td>
</tr>
<tr>
<td>14</td>
<td>2333.07</td>
<td>14.11</td>
<td>9.43</td>
<td>0.55</td>
</tr>
<tr>
<td>15</td>
<td>2310.16</td>
<td>21.80</td>
<td>31.53</td>
<td>1.32</td>
</tr>
<tr>
<td>16</td>
<td>1873.80</td>
<td>6.15</td>
<td>10.30</td>
<td>1.12</td>
</tr>
<tr>
<td>17</td>
<td>4072.67</td>
<td>20.85</td>
<td>12.86</td>
<td>0.85</td>
</tr>
<tr>
<td>18</td>
<td>2029.49</td>
<td>27.07</td>
<td>24.40</td>
<td>0.76</td>
</tr>
<tr>
<td>19</td>
<td>2003.72</td>
<td>18.36</td>
<td>15.97</td>
<td>0.91</td>
</tr>
<tr>
<td>20</td>
<td>2458.70</td>
<td>9.69</td>
<td>16.61</td>
<td>0.74</td>
</tr>
</tbody>
</table>
Table 6: Baseline 6MWT distance (m), SAQ angina frequency (AF) score and SF-36 physical component (PC) and mental component (MC) questionnaire scores

<table>
<thead>
<tr>
<th>Patient</th>
<th>6MWT (m)</th>
<th>SAQ AF score</th>
<th>SF-36 PC score</th>
<th>SF-36 MC score</th>
</tr>
</thead>
<tbody>
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<td>20</td>
<td>409</td>
<td>50</td>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 7: Average dose used by the study participants of each class of anti-anginal medication calculated as a percentage of the maximum possible dose

<table>
<thead>
<tr>
<th>Medication Class</th>
<th>Average dose (% of maximum possible dose)</th>
</tr>
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<tbody>
<tr>
<td>Oral Nitrates</td>
<td>55%</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>42%</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>60%</td>
</tr>
<tr>
<td>Ivabradine</td>
<td>65%</td>
</tr>
<tr>
<td>Ranolazine</td>
<td>50%</td>
</tr>
</tbody>
</table>
6.2 Compliance to trial

Considering the demanding nature of the trial protocol which involved 24 treatment sessions (12 active sessions, 12 sham sessions) and an additional 8 hospital visits for the pre- and post-treatment investigations, a high level of compliance and commitment was demonstrated by the participants.

In terms of compliance to the pre- and post-treatment investigations, 100% attendance was achieved; hence there was no missing data for the primary and secondary end points due to non-attendance. Compliance to the treatment sessions was also high, with 98.33% compliance or attendance overall for the active sessions and 95.83% attendance for the sham sessions. Reasons for non-attendance were recorded in all cases in the patient’s research files.

General feedback that we subsequently received from patients was that they were committed to comply with the trial related treatments and investigations because they felt they were well informed about what the trial would involve at the beginning and throughout. In addition, receiving a trial schedule enabled them to plan attendance for the trial appointments. Furthermore, given that refractory angina is such a debilitating condition with limited treatment options, they were willing to participate in research involving the investigation of a new potential therapeutic option. Many of the patients also chose to participate as they felt that the research may help their children and future generations, given that raised Lp(a) can be genetically inherited. Patients also expressed approval of the cross-over design of the study as it ensured that all participants ultimately received the active treatment, as opposed to a simple RCT design without cross-over in which they would have had a 50:50 chance of receiving apheresis.

6.3 Deviations from trial protocol on clinical grounds

One of the patients experienced tingling sensations affecting his face and ears during the return phase of treatment at the end of the third active treatment, which resolved upon stopping the treatment. He did not experience any symptoms indicative of anaphylaxis such as laryngoedema. These symptoms did not occur during the subsequent three treatments, but did recur in the same manner at the end of the seventh treatment, again with no
evidence of a full blown anaphylactic reaction. Upon discussion with the senior clinical supervisor of the trial, given that these symptoms represented a possibility that the patient might be having an allergic response to either a component of the DX21 DHP apheresis system or the Acid Citrate Dextrose A (ACD-A) solution required to prevent clotting of the treatment column; in his best interests it was decided that he should be switched to a Double filtration lipoprotein apheresis system. He tolerated the alternative system very well with no further complications during the remaining treatments.

Another of the patients was incidentally noted to have an apical thrombus in his left ventricle upon performing his initial pre-treatment CMR and was also found to have evidence of atrial fibrillation on the ECG that was taken on the same day prior to the CMR. He had been randomised to begin with apheresis, followed by sham. He was promptly commenced on oral anti-coagulation to treat the thrombus and to protect him from further thrombo-embolic events that may have arisen from the atrial fibrillation. Upon discussion with the principle investigator and the supervisors of the trial it was decided that in the patient’s best interests, his initial randomisation order should be changed such that he started with sham; in view of the uncertainty over the safety of commencing an extra-corporeal treatment in a patient with an apical thrombus acutely commencing warfarin. He subsequently completed the trial protocol successfully with no procedure related complications. In the three subsequent CMR scans that followed in the protocol it was noted that his thrombus was improving and reducing in size. Analysis of his results was performed according to his actual treatment order, to allow appropriate interpretation of his results.

These deviations from the trial protocol were clearly covered from an ethics perspective in our trial protocol which stated that “after the participant has entered the study the clinician will remain free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant’s best interest, but the reasons for doing so will be recorded. In these cases, the participants will remain within the study for the purposes of follow-up and data analysis.”
There was no breach of the blinding of any of the patients during the trial at any stage. In general, the patients understood the purpose and importance of remaining blinded to treatment allocation and did not make attempts to determine their treatment order.

6.4 Adverse events during the trial

No adverse events occurred during the pre- and post-treatment stress perfusion CMR scans or EndoPAT tests. Only a single adverse event occurred during one of the 6MWTs, in which one patient complained of a “tight hamstring” and terminated the test early. This did not lead to any serious injury and the test was rescheduled and completed subsequently.

The apheresis treatments themselves were generally well tolerated and no patient from the trial had to be withdrawn on the grounds of intolerance of the procedure and nor was anyone withdrawn due to the inability to obtain adequate venous access to conduct treatment. Data from the World Apheresis Association (WAA) registry has reported side effects in 5–10% of treatments.149 Hence, as expected, there were a few minor side effects related to the procedure which were recorded as adverse events as per the trial protocol. Minor adverse events occurred in 16 out of 236 treatments ie. 6.78% of treatments. These consisted of significant bruising or swelling of the cannulation site in 2 treatments, minor bleed from the cannula site in 1 treatment, light-headedness in 4 treatments, hypotension in 3 treatments, vasovagal episodes in 3 treatments, mild chest pain during 1 treatment and mild allergic symptoms in 2 treatments [See Table 8]. With the exception of the mild allergic symptoms occurring in 2 treatments, the remaining adverse events were deemed to be “expected”, which was also confirmed when reviewed by the trial sponsor and the National Research Ethics Committee, to whom all adverse events were promptly reported in writing.
Table 8: Frequency of adverse events during active apheresis sessions

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Frequency (%) of all 236 active apheresis sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant bruising or swelling</td>
<td>0.85</td>
</tr>
<tr>
<td>Minor bleed from the cannula site</td>
<td>0.42</td>
</tr>
<tr>
<td>Light-headedness</td>
<td>1.69</td>
</tr>
<tr>
<td>Hypotension</td>
<td>1.27</td>
</tr>
<tr>
<td>Vasovagal episodes</td>
<td>1.27</td>
</tr>
<tr>
<td>Mild chest pain</td>
<td>0.42</td>
</tr>
<tr>
<td>Mild allergic symptoms</td>
<td>0.85</td>
</tr>
</tbody>
</table>

For two of the patients, during their treatment periods (sham in one case and active treatment for the other) after completing a few treatments, due to worsening of angina they had elective PCI performed. As per the trial protocol, which stated that coronary revascularisation or a myocardial infarction should not have occurred within the preceding eight weeks, participation in the trial was paused whilst the patients recovered post-intervention. In both cases the patients were restarted on the trial after 3 months had elapsed following their procedures (i.e. >8 weeks), with all baseline investigations repeated upon return, to eliminate the possibility that the PCI procedures may have had a confounding impact on their results. In addition, we also ensured that they still fulfilled the entry criteria for the trial upon re-entry. After repeating the pre-treatment tests, they proceeded to have 12 sessions of either active therapy or sham depending on their randomisation. In other words, the few sessions of treatment that had occurred prior to their PCI procedures and the preceding set of investigations were disregarded for the purposes of data analysis, such that there was no breach of the recruitment criteria that patients should not have had PCI or revascularisation within the preceding eight weeks prior to starting the trial.
Chapter 7: The impact of lipoprotein apheresis on perfusion, carotid atherosclerosis and endothelial function in patients with refractory angina and raised Lp(a)

7.1: Introduction
We chose fully quantitative CMR perfusion as our primary end point, given that it has the capability to quantify absolute myocardial blood flow and provides a meaningful and physiological clinical marker for patients with coronary disease. In addition, we felt that a fully quantitative approach would be preferable to a semi-quantitative approach.

For the reasons previously discussed in detail in the introductory chapters, we also chose fully quantitative CMR assessment of total carotid wall volume, as well assessment of endothelial function as secondary endpoints, in order to try and gain some insight into the mechanistic effects of apheresis in patients with refractory angina and raised Lp(a).

7.2: Methods
Technical aspects of the methods used when performing the CMR perfusion and carotid scans as well as the EndoPAT test have already been described in detail in Chapter 5.

In practical terms, we were cautious to ensure that we were as consistent as possible when performing the CMR scans for the perfusion and carotid scans as well the EndoPAT test. We therefore ensured that these tests were always scheduled on Monday mornings. In all cases, for the post-apheresis or post-sham assessments the scan was conducted approximately 72 hours after completing the final treatment session performed on the preceding Friday. We felt that this level of consistency was important for two reasons. Firstly, we wanted to strictly ensure that there was no variability whatsoever in the time between the final treatment and the perfusion scan to exclude the impact that any variability may have had. Secondly, we wanted to exclude any impact that diurnal variation may have had on perfusion. However, since the planning stages of the trial it has more recently been shown that no significant diurnal variation in perfusion occurs.\textsuperscript{150}
We also performed caffeine testing directly prior to each CMR scan to exclude the presence of caffeine, in order to avoid the potential impact of caffeine on the bio-availability of adenosine, which could then alter the stress perfusion results. For all 80 CMR scans that were used in data analysis the caffeine assay was negative (i.e. below the detectability threshold) for the presence of caffeine, hence we can be certain that we were able to exclude this potential confounder.

The EndoPAT test was consistently performed in the same treatment room, using the same device at approximately the same time, following the CMR scan and prior to the patient eating lunch, given that it is recognised that there can be diurnal variability in endothelial function,\textsuperscript{151} as well as potential impairment of endothelial function transiently following high fat meals.\textsuperscript{152,153,154,155} However, we cannot account for the fact that there would have been some variability in the actual room temperature, especially as the protocol spanned across different seasons; thereby introducing a potential confounder.

7.3: Results
Table 9: Change in quantitative perfusion, carotid atheroma and endothelial function endpoints during apheresis and sham shown as mean (lower 95% CI, upper 95% CI) or median [lower quartile, upper quartile].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apheresis</th>
<th>Sham:</th>
<th>P (between groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Outcome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPR</td>
<td>0.47 (0.31, 0.63)</td>
<td>-0.16 (-0.33, 0.02)</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Secondary Outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress myocardial perfusion mL/min/g</td>
<td>0.44 [0.18, 0.67]</td>
<td>-0.07 [-0.14, 0.09]</td>
<td>0.0004</td>
</tr>
<tr>
<td>Rest myocardial perfusion mL/min/g</td>
<td>0.002 (-0.09, 0.10)</td>
<td>0.06 (-0.05, 0.17)</td>
<td>0.42</td>
</tr>
<tr>
<td>Percentage (%) myocardium hypoperfused at stress under 25th centile</td>
<td>-25.04 (-31.04, -19.04)</td>
<td>7.53 (0.73, 14.32)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Percentage (%) myocardium hypoperfused at rest under 25th centile</td>
<td>-16.03 (-24.46, -7.60)</td>
<td>6.97 (2.31, 11.63)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LVEF %</td>
<td>1.50 (-0.78, 3.76)</td>
<td>-0.7 (-3.52, 3.92)</td>
<td>0.66</td>
</tr>
<tr>
<td>Total carotid wall volume (left &amp; right) mm(^3)</td>
<td>-335 [-423, -247]</td>
<td>127.35 [72.2, 183]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total carotid wall volume (left) mm(^3)</td>
<td>-200 (-268, -133)</td>
<td>93.40 (40.0, 147)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total carotid wall volume (right) mm(^3)</td>
<td>-135 (-187, -81.8)</td>
<td>34.0 (-19.3, 87.2)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Left carotid distensibility %</td>
<td>4.9 (0.6, 9.2)</td>
<td>-0.8 (-4.4, 2.8)</td>
<td>0.035</td>
</tr>
<tr>
<td>Right carotid distensibility %</td>
<td>7.1 (3.3, 10.9)</td>
<td>-0.8 (-3.8, 2.1)</td>
<td>0.007</td>
</tr>
<tr>
<td>EndoPat LnRHI</td>
<td>-0.05 (-0.08, 0.19)</td>
<td>-0.03 (-0.18, 0.11)</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Primary endpoint

Myocardial perfusion reserve (MPR) is calculated as a ratio of average total myocardial flow at stress (ml/min/g) against average total myocardial flow at rest (ml/min/g). Assessing stress and rest perfusion separately, average total myocardial flow at stress (ml/min/g) increased by 0.44 [95% CI, 0.18 to 0.67] from 1.40 ± 0.39 prior to apheresis to 1.85 ± 0.50 following apheresis and did not change during sham from 1.45 ± 0.38 to 1.44 ± 0.36 (P<0.001 between groups) {See Table 9}. In contrast, average total myocardial flow at rest (ml/min/g) did not change significantly during apheresis from 0.97 ± 0.18 to 0.97 ± 0.22, or during sham from 0.93 ± 0.29 to 0.99 ± 0.21 (P=0.42 between groups) {See Table 9}. These results suggest that the improvement in MPR was primarily driven by improvements in stress perfusion, with insignificant change in rest perfusion. {See Figure 12}

MPR assessed by cardiovascular magnetic resonance (CMR) increased by 0.47 [95% CI, 0.31 to 0.63] from 1.45 ± 0.36 prior to apheresis to 1.93 ± 0.45 following apheresis and decreased slightly during sham by -0.16 [95% CI, -0.33 to 0.02] from 1.63 ± 0.43 to 1.47 ± 0.30 (P<0.001 between groups) {See Table 9}, giving a net treatment effect of an average increase in MPR by 0.63 [95% CI, 0.37 to 0.89]. A similar result was obtained from the linear mixed model analysis which gave the net treatment effect as an average increase in MPR by 0.55 [95% CI 0.36 to 0.75, p < 0.001 between groups]. Individual data for MPR are also presented below pre-apheresis, post-apheresis, pre-sham and post-sham such that individual response to treatment may be observed {See Table 10}.

Correlational analysis was performed to look for a correlation at the individual level between baseline Lp(a) level and baseline MPR (r= 0.25, p=0.28); demonstrating no statistically significant relationship. In addition, there was no significant individual correlation between apheresis related change in MPR and change in Lp(a) (r= 0.10, p=0.69).
Figure 12: A) Quantitative CMR perfusion pixel maps pre and post apheresis and pre and post sham. The colour scale shows perfusion from 0-4mL/min/g as low (black-green), medium (mauve- pink) and high (orange-white), therefore brighter colours represent greater perfusion. In this single patient example, there is clear improvement in stress perfusion after apheresis compared with baseline, but no change is seen during sham treatment. B) Group data are shown from myocardial perfusion at rest (left), perfusion with stress (middle) and the myocardial perfusion reserve (right). There are no changes in rest perfusion with apheresis or sham, but stress perfusion increases significantly with apheresis compared with sham. The myocardial perfusion reserve increases with apheresis because of the improved stress perfusion.
Table 10: Individual data for MPR pre-apheresis, post-apheresis, pre-sham and post-sham

<table>
<thead>
<tr>
<th>Patient</th>
<th>MPR Pre-Apheresis</th>
<th>MPR Post-Apheresis</th>
<th>MPR Pre-Sham</th>
<th>MPR Post-Sham</th>
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</thead>
<tbody>
<tr>
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<td>1.68</td>
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<td>1.49</td>
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<td>1.84</td>
<td>2.4</td>
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<td>2.03</td>
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<tr>
<td>6</td>
<td>0.75</td>
<td>1.33</td>
<td>1.7</td>
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<td>1.89</td>
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<td>1.56</td>
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<td>1.5</td>
<td>2.28</td>
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<td>1.87</td>
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<td>20</td>
<td>1.09</td>
<td>1.16</td>
<td>0.81</td>
<td>1.09</td>
</tr>
</tbody>
</table>

To evaluate the pattern and relative distribution of myocardial perfusion even further, a 25% threshold lower than the average myocardial blood flow of the remote region with relatively preserved perfusion, was used to provide an indicator of the area of ischaemic myocardium, both at stress and rest. Percentage (%) myocardium hypo-perfused at stress under 25th centile of remote myocardium reduced significantly during apheresis by -25.04 (95% CI -31.04, -19.04) from 50.84 ± 14.79 to 25.80 ± 10.52; and conversely increased by a lesser degree by 7.53 (95% CI 0.73, 14.32) from 47.97 ± 18.72 to 55.50 ± 14.35 during sham (P < 0.001 between groups) (See Table 9). To a lesser degree, a similar pattern in the relative distribution of myocardial blood flow also occurred at rest. Percentage (%) myocardium
hypo-perfused at rest under 25th centile of remote myocardium reduced during apheresis by \(-16.03\) (95\% CI \(-24.46, -7.60\)) from \(39.47 \pm 17.71\) to \(25.43 \pm 11.38\); and conversely increased by a lesser degree by \(6.97\) (95\% CI \(2.31, 11.63\)) from \(33.57 \pm 13.91\) to \(40.54 \pm 14.89\) during sham (\(P < 0.001\) between groups) {See Table 9}.

Although quantitative comparisons of the extent of LGE burden was not performed; a visual comparison was made of the presence/absence of LGE and the affected coronary territories. In all 20 subjects, there was no significant visually discernible change in the presence of LGE in subsequent scans compared to the baseline scan in this short study.

Median total carotid wall volume [LEFT + RIGHT] (cubic mm) reduced during apheresis by \(-334.85\) [95\% CI \(-423.08, -246.62\)] from \(2482.47\) [IQR 1910.00, 2835.71] before apheresis to \(2251.39\) [IQR 1719.15, 2437.36] after apheresis. During sham, median total carotid wall volume [LEFT + RIGHT] (cubic mm) increased by \(127.35\) [95\% CI 72.20, 182.51] from \(2342.20\) [IQR 1997.28, 2644.27] pre-sham to \(2454.73\) [IQR 2165.87, 2831.14] post-sham (\(P<0.001\) between groups) {See Table 9}. The same treatment related reduction in carotid wall volume was also observed when the data was analysed separately for the left and right side. Total carotid wall volume on the left (cubic mm) reduced during apheresis by a mean of \(-200.28\) [95\% CI, \(-267.66, -132.89\)] from a median of \(1207.28\) [IQR 1013.55, 1511.78] before apheresis to a median of \(1078.40\) [IQR 890.89, 1254.02] after apheresis (\(P<0.001\)). Similarly, total carotid wall volume on the right (cubic mm) reduced during apheresis by a mean of \(-134.57\) [95\% CI, \(-187.39, -81.76\)] from a median of \(1168.56\) [IQR906.27, 1421.69] before apheresis to a median of \(1021.98\) [IQR 837.19, 1237.42] after apheresis (\(P<0.001\)). During sham, median total carotid wall volume on the left (cubic mm) went from \(1187.30\) [IQR 1028.88, 1340.13] pre-sham to \(1314.22\) [IQR 1126.38, 1486.13] post-sham, with a mean change of \(93.40\) (95\% CI, 40.0, 147) (\(P<0.001\)). On the right side, median total carotid wall volume (cubic mm) went from \(1101.49\) [IQR 922.78, 1277.0] pre-sham to \(1157.78\) [IQR 982.34, 1288.32] post-sham, with a mean change of \(34.0\) (95\% CI, \(-19.3, 87.2\)) (\(P<0.001\)) {See Table 9}. Figure 13 shows an example of carotid plaque regression observed during apheresis, demonstrated with CMR cross-sectional carotid imaging before and after 3 months of apheresis in the same patient in the same location of the left common carotid artery.
Figure 13: Cross-sectional CMR carotid imaging with overlying internal and external carotid wall contours depicting plaque regression in the same patient before and after 3 months of apheresis.

Individual data for total carotid wall volume [Combined LEFT + RIGHT] (cubic mm) are also presented pre-apheresis, post-apheresis, pre-sham and post-sham such that individual response to treatment may be observed (See Table 11).
Table 11: Combined [LEFT + RIGHT] total carotid wall volume (TCWV) (mm$^3$) are presented pre-apheresis, post-apheresis, pre-sham and post-sham

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<th>Patient</th>
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<th>Combined TCWV (mm$^3$) Post-Apheresis</th>
<th>Combined TCWV (mm$^3$) Pre-Sham</th>
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Along with the improvement in carotid atheroma burden, mean left carotid distensibility (%) increased by 4.9 [95% CI, 0.6 to 9.2] during apheresis from 17.21 [IQR 12.53, 21.22] to 22.89 [IQR 14.04, 27.61] and did not change during sham with (%) change -0.8 [95% CI, -4.4 to 2.8] from 16.26 [IQR 11.45, 20.96] to 15.22 [IQR 13.19, 18.62] (P=0.035 between groups). Similarly, mean right carotid distensibility (%) increased by 7.1 [95% CI, 3.3 to 10.9] during apheresis from 17.74 ± 4.36 to 24.89 ± 8.07 and did not change during sham with mean (%)
change of -0.8 [95% CI, -3.8 to 2.1] from 17.90 ± 5.56 to 17.06 ± 5.84 (P=0.007 between groups) {See Table 9}.

CMR functional parameters including systolic and diastolic LV volumes as well as LV ejection fraction (LVEF) were measured using validated software (CMR tools, Cardiovascular Imaging Solutions Ltd, London, UK). LVEF (%) did not change significantly during apheresis with median change of 1.50 [IQR -0.78, 3.76] from 57.85 ± 12.28 to 59.35 ± 10.67. Similarly, LVEF (%) did not change significantly during sham with median change of 0.7 [IQR -3.52, 3.92] from 59.00 ± 12.42 to 59.70 ± 12.31 (P=0.66 between groups) {See Table 9}.

Endothelial function, assessed peripherally via the finger-tips using the EndoPAT device did not change significantly during apheresis or sham. Median LnRHI went from 0.72 [IQR 0.49, 0.84] to 0.78 [IQR 0.49, 0.95] during apheresis with a median change of 0.05 [IQR -0.08, 0.19]; and from 0.76 [IQR 0.63, 0.90] to 0.66 [IQR 0.53, 0.90] during sham with a median change of -0.03 [IQR -0.18, 0.11] (P=0.14 between groups) {See Table 9}.

### 7.4: Discussion

These results demonstrate a substantial statistically significant impact on fully quantitative myocardial perfusion as determined by MPR (our primary end point) with apheresis, fulfilling our primary hypothesis. This improvement in MPR appears to be primarily driven by a statistically significant increase in stress perfusion during apheresis, with insignificant change in rest perfusion. In comparison, during the sham phase, there was no statistically significant change in either stress or rest perfusion.

We were also able to examine relative myocardial blood flow by defining the area of myocardium perfused below a 25% threshold lower than the average myocardial blood flow of the relatively well perfused remote region. This was used to provide an indicator of the area of ischaemic myocardium. However, it is acknowledged that this method has limitations in that it is a relative measurement and is dependent on the average myocardial blood flow of the relatively well perfused remote region; and can therefore only provide a surrogate marker for the extent of ischaemia. Several studies suggest that segmental MPR
with a threshold of <1.5 can provide a reasonable indicator of coronary lesions of functional significance confirmed with FFR.\textsuperscript{156,157,158} Some studies suggest that navigator-gated 3-dimensional BOLD imaging at 3.0-T can reliably detect stress-induced myocardial ischaemia and may be considered a valid alternative to first-pass perfusion studies.\textsuperscript{159,160}

Interestingly we found that during apheresis, the area of relatively hypo-perfused myocardium decreased substantially in stress conditions and also to a lesser degree in rest conditions, with statistical significance. In addition to detecting absolute global improvements in myocardial blood flow, this relative blood flow analysis sheds more light on the mechanism of improvement in myocardial blood flow and distribution and suggests that apheresis may lead to the reduction of ischaemic burden. Conversely, during sham, the area of relatively hypo-perfused myocardium increased slightly during sham in stress conditions and also increased slightly in rest conditions. This may help to explain the progressive nature of angina symptoms and illustrates the chronic accumulation of ischaemic burden in patients with refractory angina and raised Lp(a), predisposing them to the recurrent MACE that they suffer from when left untreated. The few other studies that have explored the impact of apheresis in patients with coronary disease with raised Lp(a) have not objectively assessed the impact of treatment on ischaemia as an end-point, using either CMR or any other imaging modalities. Bohl et al. assessed CMR derived trans-myocardial perfusion gradient (i.e. endo-epi ratio [EER]) as their primary end point and demonstrated subtle improvement after a single apheresis treatment in patients with raised Lp(a) and proven coronary disease.\textsuperscript{41} And although Safarova et al. assessed the impact of Lp(a)-specific apheresis on quantitative coronary angiography analyses of percent diameter stenosis and minimal lumen diameter (MLD), in patients with stable CHD and raised Lp(a),\textsuperscript{85} the impact on ischaemia was not assessed.

Although numerous other studies in different settings have used quantitative MPR as end points; to the best of our knowledge this is the first randomised controlled clinical trial in humans which has used fully quantitative CMR techniques to assess perfusion as its primary end-point. This supports the potential application of quantitative CMR as a meaningful research tool in further clinical trials assessing the impact of interventions in patients with cardiac disease.
The test-retest reproducibility of MPR in our patient cohort was not formally assessed and therefore has to be acknowledged as a limitation when interpreting these results. It must also be acknowledged that for the power calculations the supposition that the inter-study reproducibility for MPR has a standard deviation (SD) of 0.15 using our local protocols was based on an estimate from the primary supervisor of my PhD, who is an expert in CMR; rather than being supported by reproducibility data in the literature. In hindsight, performing formal test-retest reproducibility of MPR in our patient cohort using our local methods prior to starting the trial would have produced a more reliable power calculation. We have provided some indication of test-retest variability by presenting the pre-sham and post-sham MPR values for each individual, although we acknowledge that this does not truly represent intra-subject variability as the scans are separated by 12 weeks.

It is also acknowledged as a limitation, that we did not quantitatively compare the extent of LGE burden before and after treatments. However, qualitative visual assessments did not reveal any major changes in the extent and distribution of LGE/viability; which is perhaps not surprising given the short duration of the trial protocol. In addition, none of the trial subjects presented clinically with an overt myocardial infarction or a troponin positive event whilst participating in the trial.

Statistical analysis did not reveal a significant correlation between the baseline Lp(a) and baseline MPR, which was perhaps contrary to expectation. We would have expected MPR to be lower in individuals with higher Lp(a) levels. It may be that the sample was insufficient to detect such a relationship if it exists, and that multiple co-existing determinants of perfusion such as coronary anatomy and extent of previous revascularisation, extent of non-viable or infarcted myocardium, presence of diabetes etc. were confounding factors. Another potential explanation is that the Lp(a) range within this study was a relatively small window of high levels: median Lp(a) (mg/L) = 1100 (IQR 771, 1590), at which this relationship may be less clear or non-linear. Perhaps if we had performed the correlation including data from individuals with low as well as high Lp(a), we may have seen a significant correlation. In addition there was no correlation between the reduction or change in Lp(a) and the change in MPR with apheresis. A plausible explanation for this is that aside from Lp(a), LDL
cholesterol as well as numerous factors including fibrinogen, coagulation factors, thrombogenic factors, complement factors, which all contribute to plasma viscosity are also removed by the apheresis column; hence we are unlikely to find an independent correlation between Lp(a) reduction and improvement in MPR.

The results also clearly demonstrate statistically significant regression in quantitative total carotid wall volumes when analysed separately on the left and right as well as the combined total carotid wall volume on both sides; fulfilling our secondary hypothesis that apheresis may lead to improvements in carotid atheroma burden. In comparison, during the sham phase there is to a lesser degree a concomitant increase in both combined total carotid wall volume, as well as an increase on both sides analysed separately. This may reflect the progressive nature of the atherosclerotic process and plaque development in patients with raised Lp(a) who are left untreated, and help to explain why such patients may be at risk of accelerated progression of coronary disease and recurrent MACE. Again, to the best of our knowledge, this is the first randomised controlled clinical trial assessing the impact of apheresis compared to a control group that has utilised fully quantitative CMR techniques to objectively measure the treatment effect on carotid atherosclerotic burden. Previously, Safarova et al. used carotid intima-media thickness (IMT) rather than quantitative CMR to evaluate the impact of apheresis in patients with raised Lp(a) and stable CHD patients. In this study, IMT of the right and left CCA in the anterior plane were measured at a distance of 1 cm proximal to the carotid bifurcation, using ultrasound duplex scanning. The primary efficacy end-point for was the absolute change from baseline of the mean posterior wall IMT of the left and right CCA. Carotid IMT provides a 1-dimensional index at a fixed location (1 cm proximal to the carotid bifurcation) and therefore provides limited information about the quantitative plaque burden. In contrast the techniques we have used in our trial are able to fully quantify 3-dimensional total carotid wall volume, thereby providing a more informative, complete and quantitative evaluation of the impact of apheresis on atheroma burden. In retrospect, opting for a non-invasive marker of the atherosclerotic process rather than invasive angiographic assessment helped in regards to patient recruitment and compliance. In their feedback, several patients reported that would not have been willing to participate in a trial involving invasive cardiac catheterisation investigations and would have found this prohibitive, particularly as their underlying
condition already predisposes them to multiple invasive investigations and revascularisation procedures which they are generally keen to limit to essential procedures. On the other hand, quantitative coronary angiographic assessments of coronary plaque burden would have shed light on the impact of apheresis on coronary atherosclerosis; which has not been addressed by this study.

As for MPR assessment, the test-retest reproducibility of quantitative total carotid wall volume measurement in our patient cohort was not formally assessed, and therefore has to be regarded as a limitation when interpreting the results.

It is interesting to observe that the improvement in perfusion is accompanied with an improvement in carotid atheroma. Physiologically this implies that that regression of atherosclerosis may be a contributing factor towards improved perfusion, which may then translate to improvement in angina symptoms and exercise capacity which the trial also demonstrated and which is discussed in Chapter 9. However, in order to examine this relationship more directly at the individual level, I performed a correlation between individual improvement in MPR during apheresis (ie. the difference between post-apheresis and pre-apheresis MPR) with individual reduction in LEFT + RIGHT combined total carotid wall volume during apheresis (ie. the difference between post-apheresis and pre-apheresis total carotid wall volume). Although this correlation was a negative relationship as expected $r=-0.268$, it did not reach statistical significance ($P=0.25$). It may be that our sample size is insufficient to detect a clear linear relationship between apheresis related changes perfusion and carotid wall volume, if it exists; especially in the presence of multiple concurrent heterogenous variables within this small cohort that may potentially confound this relationship. In chapter 9, after presentation of the exercise capacity results I have also performed a correlation between individual improvement in MPR versus improvement in exercise distance.

We did not detect any improvement in LV functional parameters such as LVEF. However, we were not in reality expecting that an extra-corporeal treatment should lead to a significant change in the LVEF; hence this result does not surprise us. In addition, the LVEF was measured in resting conditions and we did not in fact detect an improvement in rest
perfusion during apheresis. In hindsight, it would have been interesting to measure LVEF in stress conditions, to see if there was any improvement in LV function and contractility to correlate with the improvement we have detected in treatment related stress perfusion. In this trial, we did not specifically measure the impact of treatment on diastolic function, which could potentially have been explored with existing CMR techniques such as feature tracking; which may potentially be regarded as a limitation.

Endothelial function, assessed peripherally via the finger-tips using the EndoPAT device did not change significantly during either apheresis or sham.

Literature regarding the validity of EndoPAT as a reliable indicator of endothelial function is divided, making interpretation of the test contentious.

An experiment using the EndoPAT device showed that the RHI score reflected the bioavailability of nitric oxide. Another study showed that digital reactive hyperaemia, as measured by EndoPAT, is attenuated in patients with coronary endothelial dysfunction measured compared with individuals with normal coronary endothelial function. In this study the presence of endothelial function was determined invasively via coronary angiography where the threshold for normal coronary endothelial function was defined as an increase in coronary blood flow (CBF) of >50% in response to the maximum dose of acetylcholine. Another study showed that EndoPAT RHI correlated with brachial flow mediated dilatation (FMD) measured with ultra-sound. Patients with a greater degree of cardiovascular disease have been reported to exhibit a lower score. In a prospective observational study, a low LnRHI <0.4 detected with EndoPAT was associated with a higher MACE rate compared to patients with a higher LnRHI >0.4 after a seven year follow-up period. One study reported that the addition of ezetimibe improved RHI measured by EndoPAT in patients with hypercholesterolemia. A few studies involving EndoPAT have shown an improvement in endothelial function as a result of lifestyle modification such as dietary change.

Although the test is non-invasive and easy to perform, it’s utility in clinical monitoring of endothelial function and in the modification of disease management remains under
investigation.\textsuperscript{123} There is some literature that raises doubt over the reliability of EndoPAT for monitoring changes in endothelial function. There is only limited information on the performance of the EndoPAT for repeated measurements in a relatively short time frame.\textsuperscript{169}

Moerland et al. conducted a series of investigations in order to determine the feasibility of the EndoPAT to evaluate acute changes in endothelial function with repeated noninvasive measurements and assessed its discriminating power in different populations.\textsuperscript{169} Surprisingly they found that endothelial function in renally impaired patients with known vascular disease and type 2 diabetic patients was not decreased compared to healthy volunteers.\textsuperscript{169} In addition they investigated the capability of the EndoPAT to detect changes in endothelial function induced by two acute interventions (oral glucose load and smoking) in healthy volunteers. Neither the oral glucose load nor the smoking intervention resulted in significant effects on endothelial function as detected by EndoPAT.\textsuperscript{169} The authors suggested that this could indicate that endothelial function as measured using the EndoPAT might be physiologically different from endothelial function as measured by conventional techniques.\textsuperscript{169} They also concluded that EndoPAT may not be useful to detect the effect of robust interventions on endothelial function and therefore, at present may not be suitable to assess changes in endothelial function and arterial stiffness in populations with sizes that are commonly employed in clinical studies.\textsuperscript{169}

Interpreting our study results in view of the above literature, it is feasible that the EndoPAT device may not be sensitive enough to detect improvements in endothelial function secondary to three months of apheresis ie. a relatively short intervention duration. Therefore, we cannot necessarily conclude that we have excluded the possibility that apheresis can improve endothelial function. In particular, we are unable to conclude that apheresis has no impact on micro-vascular function, since EndoPAT cannot provide a direct measurement of micro-vascular function. Although it is acknowledged that this is a contentious subject area, studies assessing the relationship between micro-vascular dysfunction secondary to syndrome X and stress-induced myocardial perfusion defects on CMR have shown that dysfunction of coronary microcirculation results in myocardial perfusion abnormalities.\textsuperscript{170,171} Hence, the positive treatment effect we have demonstrated on quantitative stress myocardial perfusion may potentially indicate that there is an
improvement in micro-vascular function with apheresis. We did however demonstrate a compelling treatment related improvement in carotid plaque regression, which may therefore indicate that it is primarily coronary macro-vascular function that is improved.

A more sensitive marker of macro-vascular endothelial function than EndoPAT may be necessary to accurately address whether apheresis can improve endothelial function. In pragmatic terms, the EndoPAT device was chosen at the planning stages of the study since it is non-invasive, convenient, relatively easy to perform and not time-consuming. We had opted not to perform alternative tests such as brachial FMD as it would have added further time to the already lengthy CMR protocol in which we were already assessing quantitative perfusion, carotids and a full cardiac functional study. This additional scanning time may have been prohibitive for patients and potentially could have led to poor compliance and withdrawals, especially as our protocol involved 4 such scans. Indeed, feedback we received from patients was that the CMR protocol used was the maximum duration that they felt would be feasible to undertake. Another possibility is that given that the average baseline LnRHI was within normal range in the trial sample group, the scope of improving upon normal endothelial function may have been limited. If the average baseline LnRHI had been abnormal or sub-optimal to begin with, potentially there may have been more scope for improvement with intervention. However, we would not be able to confirm this possibility unless we were to conduct a study involving patients with normal versus abnormal LnRHI at baseline to detect the impact of apheresis in both groups for comparison.

Our carotid imaging protocol involved measuring carotid distensibility on the left and right CCA i.e. measuring the cross-sectional area of the CCA at both systole and diastole to calculate the percentage change. However, we acknowledge as a limitation that to derive distensibility, correction for pulse pressure should have been performed and that therefore the equation we have used actually represents carotid wall strain. Interestingly, statistically significant improvements were detected on both sides. The mean left carotid distensibility (%) increased by 4.9 [95% CI, 0.6 to 9.2] during apheresis and similarly, mean right carotid distensibility (%) increased by 7.1 [95% CI, 3.3 to 10.9] during apheresis. In contrast, there was no improvement in carotid distensibility during sham. This implies that there may be some improvement in vessel wall elasticity or compliance detectable at the carotid arteries
with apheresis, with no change during sham. Other studies have also shown improvements in arterial stiffness following interventions. Ripley et al. demonstrated that in patients with essential hypertension and left ventricular hypertrophy, there was an improvement in aortic distensibility assessed with CMR after 6 months duration of anti-hypertensive therapy.\textsuperscript{172} In hindsight, it would have been interesting to have assessed the impact of apheresis on aortic distensibility, given some data has shown that a stiff aorta may be associated with reduced coronary blood flow (CBF).\textsuperscript{173} Using aortic mechanical indexes including central pulse-wave velocity (cPWV) and central pulse pressure (cPP), Leung et al. found that a stiff aorta is associated with a reduction in CBF, a lower hyperaemic CBF response, and may reduce the improvement in hyperaemic CBF following successful PCI.\textsuperscript{173}

7.5: Conclusion

In summary, the results of our randomised controlled cross-over trial indicate that in patients with refractory angina and raised Lp(a) treated with apheresis, statistically significant improvements occurred in the primary end point of quantitative CMR myocardial perfusion assessed with MPR; which was primarily driven by an increase in stress perfusion, with no significant change in rest perfusion. In comparison, there were no statistically significant changes in either stress or rest perfusion during sham treatment. In addition, regional analysis of the distribution and pattern of myocardial blood flow revealed that the percentage area of relatively hypo-perfused myocardium reduced substantially in stress conditions, and also to a lesser degree in rest conditions; implying that the ischaemic burden may be ameliorated by apheresis. Conversely, during sham there was a slight increase in the percentage area of relatively hypo-perfused myocardium, perhaps illustrating the progressive nature of coronary disease in untreated patients with refractory angina and raised Lp(a).

In addition, there were also statistically significant treatment related improvements or regression in the secondary end point of carotid atheroma burden assessed with quantitative CMR total carotid wall volume on both sides combined as well as on each side assessed separately. In comparison, to a lesser degree there was conversely some progression of carotid atheroma burden with a mild increase in total carotid wall volume on
both sides combined as well as on each side assessed separately during sham treatment. The treatment related improvement in atheroma burden was also accompanied with a statistically significant treatment related improvement in carotid distensibility measured on both sides, which did not change significantly during sham treatment.

Aside from these novel findings, which have not previously been evaluated in patients with refractory angina and raised Lp(a) undergoing apheresis; to the best of our knowledge, this is the first randomised controlled trial in which fully quantitative CMR perfusion and quantitative CMR assessment of carotid wall volume have been utilised as primary and secondary end points, representing a further research milestone towards advancing the application of quantitative CMR techniques in cardiovascular research.

Although, these promising findings need to be validated further with larger randomised controlled studies, ideally incorporating the effect of apheresis on hard clinical endpoints such as MACE; these positive impacts on myocardial perfusion and atherosclerotic burden suggest that lipoprotein apheresis deserves to be explored as a potential treatment option in patients with refractory angina and raised Lp(a).

Endothelial function, assessed peripherally via the finger-tips using the EndoPAT device did not change significantly during either apheresis or sham. However, there appears to be some controversy over the sensitivity of the test to detect changes in endothelial function secondary to therapies or interventions over relatively short periods of time. This raises the possibility that the test may not have had the capacity to detect apheresis related improvements in endothelial function particularly at the coronary level; which may have been detected with a more sensitive test.

CMR functional parameters were assessed before and after apheresis and sham including systolic and diastolic LV volumes as well as LV ejection fraction (LVEF). LVEF at rest did not change either during apheresis or sham. We had not anticipated that extra-corporeal lipoprotein removal would lead to improvement in myocardial contractility; hence this result is in keeping with our predictions.
Chapter 8: The impact of lipoprotein apheresis on lipid parameters, oxidised LDL and their antibodies and thrombogenesis in patients with refractory angina and raised Lp(a)

8.1: Introduction

It has been established that Lp(a) is a potent carrier of oxidised phospholipids,\textsuperscript{10} which may be one of the mechanisms by which Lp(a) contributes to atherosclerotic disease risk. To the best of our knowledge, to date experiments have not yet been performed to investigate the impact of lipoprotein apheresis on oxidised LDL and Anti-oxidised LDL antibodies before and after a period of treatment with apheresis, in patients with isolated raised Lp(a), in the absence of elevated LDL cholesterol. Therefore, our trial provided an ideal opportunity to further explore the link between Lp(a) and oxidised LDL and their antibodies and the impact that lipoprotein apheresis may have on these parameters.

Previously, in patients with familial hypercholesterolaemia, and therefore raised levels of LDL cholesterol, a study was performed in 18 patients to assess the levels of oxidised phospholipids (OxPL), Lp(a) and lipoprotein-associated phospholipase A2 (Lp-PLA2) before and after an apheresis session.\textsuperscript{174} The patients were subdivided into those with low, intermediate or high levels of Lp(a). They found that apheresis significantly reduced levels of OxPL and Lp-PLA2 on apoB and Lp(a) particularly in patients with intermediate and high Lp(a) levels.\textsuperscript{174}

The impact of apheresis on Lp(a) and the conventional lipid profile has previously been well documented.\textsuperscript{46,83,84,85} We measured all of these parameters in the trial in order to have a record of the impact of apheresis on standard lipid profiles and Lp(a) itself in patients with refractory angina and raised Lp(a).

Given that Lp(a) is felt to be pro-thrombotic via the inhibition of fibrinolysis\textsuperscript{5} with enhancement of clot stabilisation, as well as via enhanced coagulation,\textsuperscript{16,17} we assessed the impact of treating raised Lp(a) with apheresis on numerous markers of thrombosis, to
determine whether treatment can potentially help to reverse this risk. Thrombogenic markers assessed included the Global Thrombosis Test (GTT), as well as numerous assays testing thrombotic function described below.

8.2: Methods

The methods used for sample collection and technical aspects of assay processing and analysis for the lipid and rheological tests, oxidised LDL and their antibodies and all of the thrombogenic parameters have been previously described in detail in Chapter 5, which covers the main methodology for the trial. All samples were processed in strictly blinded conditions with lab analysis performed by individuals who were not directly involved in conducting the trial, with no access to information about the collection time point of the samples and whether they were pre- or post-apheresis or sham.
8.3: Results

Table 12a: Change in lipid parameters during apheresis and sham shown as mean (lower 95% CI, upper 95% CI) or median [IQR].

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<tr>
<th>Variable</th>
<th>Apheresis</th>
<th>Sham:</th>
<th>P (between groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) Immage assay (mg/L)</td>
<td>-903 [-1474, -513]</td>
<td>-66 [-138, 170]</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lp(a) Ultra latex assay (mg/L)</td>
<td>-679.5 [-1102, -453]</td>
<td>-5.5 [-48.85, 51.5]</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>-1.7 [-2.2, -1.5]</td>
<td>0.1 [-0.25, 0.30]</td>
<td>0.0001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>-1.55 [-1.90, -1.17]</td>
<td>-0.03 [-0.04, 0.07]</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>-0.12 [-0.21, -0.04]</td>
<td>-0.002 [-0.08, 0.07]</td>
<td>0.006</td>
</tr>
<tr>
<td>TC:HDL ratio</td>
<td>-1.635 [-1.75, -1.24]</td>
<td>0.025 [-0.28, 0.35]</td>
<td>0.0002</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>-0.28 [-0.49, -0.07]</td>
<td>0.18 [-0.02, 0.37]</td>
<td>0.007</td>
</tr>
<tr>
<td>Apolipoprotein A (g/L)</td>
<td>-0.09 [-0.17, -0.00]</td>
<td>-0.01 [-0.06, 0.04]</td>
<td>0.074</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>-0.41 [-0.47, -0.34]</td>
<td>-0.04 [-0.11, 0.03]</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Table 12b: Change in fibrinogen, BNP and CRP during apheresis and sham shown as mean (lower 95% CI, upper 95% CI) or median [IQR].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apheresis</th>
<th>Sham:</th>
<th>P (between groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (g/L)</td>
<td>-0.93 [-1.15, -0.70]</td>
<td>-0.15 [-0.30, -0.02]</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>BNP (ng/L)</td>
<td>3.9 [-12.0, 19.8]</td>
<td>-3.7 [-23.5, 16.2]</td>
<td>0.57</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>-0.5 [-3.5, 0]</td>
<td>0 [-1.0, 0.5]</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Table 12c: Change in oxidised LDL (MDA-LDL) and their associated antibodies during apheresis and sham shown as mean (lower 95% CI, upper 95% CI) or median [IQR].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apheresis</th>
<th>Sham:</th>
<th>P (between groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-LDL Citrate (OD405nm)</td>
<td>-0.11 [-0.13, -0.09]</td>
<td>-0.01 [-0.04, 0.02]</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Anti-IgG MDA-LDL (OD450nm)</td>
<td>-0.14 [-0.19, -0.06]</td>
<td>-0.008 [-0.060, 0.03]</td>
<td>0.0036</td>
</tr>
<tr>
<td>Total IgG (g/L)</td>
<td>-2.45 [-3.71, -0.94]</td>
<td>-0.05 [1.05, 0.57]</td>
<td>0.0019</td>
</tr>
<tr>
<td>Anti-IgM MDA-LDL (OD450nm)</td>
<td>-0.15 [-0.25, -0.04]</td>
<td>-0.015 [-0.076, 0.055]</td>
<td>0.012</td>
</tr>
<tr>
<td>Total IgM (g/L)</td>
<td>-0.20 [-0.32, -0.08]</td>
<td>0.02 [-0.03, 0.01]</td>
<td>0.0009</td>
</tr>
</tbody>
</table>
Table 12d: Change in thrombogenic and haematological parameters during apheresis and sham shown as mean (lower 95% CI, upper 95% CI) or median [IQR].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apheresis</th>
<th>Sham:</th>
<th>P [between groups]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTT OT (Occlusion time) (s)</td>
<td>147.13 (97.86, 196.39)</td>
<td>-12.65 (-63.10, 37.81)</td>
<td>0.0002</td>
</tr>
<tr>
<td>GTT LT (Lysis time) (s)</td>
<td>-354.5 [-738.0, -88.5]</td>
<td>36.0 [-56.0, 204.0]</td>
<td>0.005</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>16.17 (11.94, 20.40)</td>
<td>-0.69 (-1.74, 0.36)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>PT (s)</td>
<td>1.45 [0.80, 2.35]</td>
<td>-0.1 [-0.6, 0.65]</td>
<td>0.0034</td>
</tr>
<tr>
<td>INR (ratio)</td>
<td>0.1 [0.1, 0.2]</td>
<td>0 [-0.1, 0.1]</td>
<td>0.004</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>-0.88 (-1.26, -0.50)</td>
<td>0.10 (-0.21, 0.41)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Platelets (10^9/L)</td>
<td>-49.65 (-65.91, -33.39)</td>
<td>-2.0 (-12.70, 8.70)</td>
<td>0.0003</td>
</tr>
<tr>
<td>PCV</td>
<td>-0.025 (-0.037, -0.013)</td>
<td>0.0045 (-0.006, 0.015)</td>
<td>0.0015</td>
</tr>
<tr>
<td>vWF (IU/dL)</td>
<td>-60.50 (-74.07, -46.92)</td>
<td>9.24 (-12.03, 30.50)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>D dimer (ng/ml)</td>
<td>3 [-40.5, 56]</td>
<td>14 [-26, 32]</td>
<td>0.88</td>
</tr>
<tr>
<td>TAT (µg/L)</td>
<td>0.32 (0.03, 1.54)</td>
<td>0.02 (-0.26, 0.70)</td>
<td>0.13</td>
</tr>
<tr>
<td>F 1+2 (pmol/L)</td>
<td>669.5 [140.58, 951.03]</td>
<td>0.96 [-75.82, 62.21]</td>
<td>0.0008</td>
</tr>
<tr>
<td>TGA LAG time (min)</td>
<td>0.05 [-0.44, 0.83]</td>
<td>-0.17 [1.34, 0.34]</td>
<td>0.13</td>
</tr>
<tr>
<td>TGA ETP (nM/min)</td>
<td>-4.42 (-114.42, 105.58)</td>
<td>-59.25 (-165.58, 47.07)</td>
<td>0.48</td>
</tr>
<tr>
<td>TGA peak (nM)</td>
<td>-7.40 (-37.62, 22.82)</td>
<td>-3.19 (-29.39, 23.01)</td>
<td>0.82</td>
</tr>
<tr>
<td>TGA tt Peak (min)</td>
<td>-0.28 [-0.89, 0.89]</td>
<td>-0.28 [-1.95, 0.78]</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Lipid parameters and fibrinogen, BNP and CRP

Lp(a) in mg/L using the nephelometric IMMAGE assay reduced by a median value of -903 (IQR -1474, -513) during apheresis from 1125 (IQR 793.50, 1775) to 240 (IQR 168, 447). During sham, it did not change significantly from 1275 (IQR 841.50, 1715) to 1180 (IQR 756, 1685) (P=0.0001 between groups) (See Table 12a).

Using the Lp(a) Ultra latex immunoassay, Lp(a) in mg/L reduced by a median value of -679.5 (IQR -1102, -453) during apheresis from 1001.5 (IQR 695.5, 1429.5) to 248.0 (IQR 171.5, 339.5). During sham, it did not change significantly with a median change of -5.5 (IQR -48.85, 51.5) from 942.5 (IQR 686.5, 1441.0) to 885.5 (IQR 633.5, 1467.0) (P=0.0001 between groups) (See Table 12a).
Total cholesterol (TC) in mmol/L reduced by a median value of -1.7 (IQR -2.2, -1.5) during apheresis from 3.67 ± 0.81 to 1.84 ± 0.39. During sham, it did not change with a median change of 0.1 (IQR -0.25, 0.30) from 3.78 ± 1.05 to 3.74 ± 0.73 (P=0.0001 between groups) {See Table 12a}.

LDL cholesterol in mmol/L reduced by a median value of -1.55 (IQR -1.90, -1.17) during apheresis from 1.99 ± 0.68 to 0.40 ± 0.27. During sham, it did not change with a median change of -0.025 (IQR -0.035, 0.065) from 2.14 ± 0.91 to 2.01 ± 0.63 (P=0.0001 between groups) {See Table 12a}.

HDL cholesterol in mmol/L reduced slightly by a mean value of -0.124 (95% CI -0.213, -0.035) during apheresis from 1.11 ± 0.28 to 0.99 ± 0.23. During sham, it did not change with a mean change of -0.0015 (95% CI -0.077, 0.073) from 1.14 ± 0.29 to 1.14 ± 0.27 (P=0.006 between groups) {See Table 12a}.

TC:HDL ratio reduced by a median value of -1.635 (IQR -1.75, -1.235) during apheresis from 3.42 ± 0.87 to 1.89 ± 0.39. During sham, it did not change with a median change of 0.025 (IQR -0.275, 0.350) from 3.46 ± 0.94 to 3.41 ± 0.77 (P=0.0002 between groups) {See Table 12a}.

Triglycerides in mmol/L reduced moderately by a mean value of -0.28 (95% CI -0.49, -0.07) during apheresis from 1.22 ± 0.48 to 0.94 ± 0.45. During sham, there was no significant change, with a mean change of 0.18 (95% CI -0.02, 0.37) from 1.18 ± 0.38 to 1.36 ± 0.48 (P=0.007 between groups) {See Table 12a}.

Apolipoprotein A (g/L) reduced very slightly by a mean value of -0.085 (95% CI -0.166, -0.004) during apheresis from 1.24 ± 0.24 to 1.15 ± 0.19. During sham, it did not change with a mean change of -0.01 (95% CI -0.055, 0.035) from 1.27 ± 0.19 to 1.26 ± 0.20 (P=0.074 between groups) {See Table 12a}.

Apolipoprotein B (g/L) reduced by a mean value of -0.41 (95% CI -0.47, -0.34) during apheresis from 0.80 (IQR 0.65, 0.90) to 0.4 (IQR 0.4, 0.4). During sham, it did not change
with a mean change of -0.04 (95% CI -0.11, 0.03) from 0.77 (IQR 0.70, 0.89) to 0.75 (IQR 0.68, 0.87) (P=0.0001 between groups) {See Table 12a}.

Fibrinogen (g/L) reduced by a mean value of -0.93 (95% CI -1.15, -0.70) during apheresis from 3.12 ± 0.68 to 2.20 ± 0.53. During sham, it did not change significantly with a mean change of -0.15 (95% CI -0.30, -0.002) from 3.14 ± 0.53 to 2.99 ± 0.54 (P=0.0001 between groups) {See Table 12b}.

BNP (ng/L) did not change with statistical significance during either apheresis or sham. BNP changed by a mean value of 3.9 (95% CI -12.0, 19.8) during apheresis and by a mean value of -3.7 (95% CI-23.5, 16.2) during sham (P=0.57 between groups) {See Table 12b}.

Similarly, CRP (mg/L) did not change with statistical significance during either apheresis or sham. It changed by a median value of -0.5 (IQR -3.5, 0) during apheresis and by a median value of 0 (IQR -1.0, 0.5) during sham (P=0.19 between groups) {See Table 12b}.

**Oxidised LDL and their antibodies**

MDA-LDL (OD405nm) reduced by a mean value of -0.11 (95% CI -0.13, -0.09) during apheresis from 0.37 ± 0.06 to 0.26 ± 0.04. During sham, it did not change significantly with a mean change of -0.01 (95% CI -0.04, 0.02) from 0.35 ± 0.07 to 0.34 ± 0.07 (P<0.0001 between groups) {See Table 12c}.

Anti-IgG MDA-LDL (OD450nm) reduced by a median value of -0.14 (IQR -0.19, -0.06) during apheresis from 0.61 ± 0.21 to 0.47 ± 0.20. During sham, it did not change significantly with a median change of -0.008 (IQR -0.060, 0.03) from 0.57 ± 0.21 to 0.55 ± 0.21 (P=0.0036 between groups) {See Table 12c}.

Total IgG (g/L) reduced by a median value of -2.45 (IQR -3.71, -0.94) during apheresis from 13.54 ± 3.89 to 11.13 ± 3.84. During sham, it did not change significantly with a median change of -0.05 (IQR -1.05, 0.57) from 13.68 ± 5.40 to 13.35 ± 3.96 (P=0.0019 between groups) {See Table 12c}.

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Anti-IgM MDA-LDL(OD450nm) reduced by a median value of -0.15 (IQR -0.26, -0.04) during apheresis from 0.66 ± 0.43 to 0.54 ± 0.36. During sham, it did not change significantly with a median change of -0.015 (IQR -0.08, 0.06) from 0.67 ± 0.39 to 0.67 ± 0.44 (P=0.012 between groups) {See Table 12c}.

Total IgM (g/L) reduced by a median value of -0.20 (IQR -0.32, -0.08) during apheresis from 0.77 (IQR 0.43, 0.98) to 0.61 (IQR 0.36, 0.68). During sham, it did not change significantly with a median change of -0.02 (IQR -0.03, 0.01) from 0.72 (IQR 0.47, 0.93) to 0.71 (IQR 0.48, 1.03) (P=0.0009 between groups) {See Table 12c}.

**Thrombogenic parameters**

The Global Thrombosis Test (GTT) produces 2 parameters, the occlusion time (OT) and the lysis time (LT), both measured in seconds.

GGT OT (seconds) increased significantly by a mean value of 147.13 (95% CI 97.86, 196.39) during apheresis from 576.32 ± 116.32 to 723.45 ± 141.63. During sham, it did not change significantly with a mean change of -12.65 (95% CI -63.10, 37.81) from 585.45 ± 142.10 to 572.81 ± 112.83 (P=0.0002 between groups) {See Table 12d}.

GGT LT (seconds) decreased significantly by a median value of -354.5 (IQR -738.0, -88.5) during apheresis from 1339.50 (IQR 1127.50, 1682.00) to 846.50 (684.50, 1301.50). During sham, GGT LT (seconds) increased slightly with a median change of 36.0 (IQR -56.0, 204.0) from 1097.50 (IQR 983.00, 1573.00) to 1247.50 (IQR 986.50, 1591.50) (P=0.005 between groups) {See Table 12d}.

Basic clotting function was checked including APTT, PT and INR. APTT (seconds) increased by a mean value of 16.17 (95% CI 11.94, 20.40) during apheresis from 33.28 ± 4.55 to 49.45 ± 11.10. During sham, APTT (seconds) did not change significantly with a mean change of -0.69 (95% CI -1.74, 0.36) from 33.49 ± 4.71 to 32.80 ± 4.28 (P<0.0001 between groups). PT (seconds) increased very slightly by a median value of 1.45 (IQR 0.80,2.35) during apheresis from 12.10 (IQR 11.35, 13.25) to 12.95 (IQR 12.70, 14.20). During sham, PT (seconds) did not
change significantly with a median change of -0.1 (IQR -0.6, 0.65) from 11.90 (IQR 11.20, 12.95) to 12.20 (IQR 11.40, 12.95) (P=0.0034 between groups). INR (ratio) did not change during apheresis from 1.1 (IQR 1.1, 1.15) to 1.1 (IQR 1.1, 1.2); or during sham from 1.0 (IQR 1.0, 1.1) to 1.0 (IQR 1.0, 1.1) {See Table 12d}.

Basic full blood count was also checked, including the haemoglobin (Hb), platelet count and haematocrit or packed cell volume (PCV). The Hb (g/L) dropped by a mean value of -0.88 (95%CI -1.26, -0.50) during apheresis from 13.48 ± 1.60 to 12.60 ± 1.22, whereas there was no significant change during sham from 13.32 ± 1.10 to 13.42 ± 1.38 (P=0.0011 between groups). The platelet count (10⁹/L) dropped by a mean value of -49.65 (95%CI -65.91, -33.39) during apheresis from 204.20 ± 36.07 to 154.55 ± 35.54; whereas there was no significant change during sham from 200.65 ± 30.56 to 198.65 ± 33.14 (P=0.0003 between groups). The PCV dropped slightly by a mean value of -0.025 (95%CI -0.037, -0.013) during apheresis from 0.40 ± 0.04 to 0.38 ± 0.04; whereas there was no significant change during sham from 0.40 ± 0.03 to 0.40 ± 0.04 (P=0.0015 between groups) {See Table 12d}.

In addition, more detailed assays testing thrombotic function were also analysed including Von Willebrand’s Factor (vWF), D dimer, Thrombin/Anti-thrombin III complex (TAT), Prothrombin fragment 1 + 2 (F 1+2) and the Thrombin generation assay (TGA).

vWF (IU/dL) reduced by a median of -60.50 (IQR -74.07, -46.92) from 149.45 (IQR 88.95, 164.25) to 64.15 (IQR 48.50, 89.75) during apheresis, indicating improvement in clotting risk. During sham, it did not change significantly with a median change of 9.24(IQR -12.03, 30.50) from 95.75 (IQR 86.15, 160.40) to 111.00 (IQR 99.45, 157.30) (P<0.0001 between groups) {See Table 12d}.

There was no significant net change in D-dimer or TAT. During apheresis, D dimer(ng/ml) did not change significantly with a net median difference of 3 (IQR -40.5, 56) from 107 (IQR 66, 132) to 118.5 (IQR 69.5, 173); or during sham with a net median difference of 14 (IQR -26, 32) from 82.5 (IQR 51, 137.5) to 101.5 (IQR 71.5, 138) (P=0.88 between groups). TAT (µg/L) increased slightly during apheresis by a median of 0.32 (IQR 0.03, 1.54) from 4.07 (IQR 2.91, 6.71) to 5.99 (IQR 3.26, 6.95), and did not change during sham with a median difference of
0.02 (IQR -0.26, 0.70) from 4.65 (IQR 3.18, 5.85) to 4.78 (IQR 3.21, 6.01); but this did not reach statistical significance (P=0.13 between groups) {See Table 12d}.

F 1+2(pmol/L) increased significantly during apheresis by a median of 669.55 (IQR 140.58, 951.03) from 158.16 (IQR 128.77, 232.09) to 795.12 (IQR 272.55, 1201.00); and did not change significantly during sham with a median change of 0.96 (IQR -75.82, 62.21) from 207.11 (IQR 128.72, 255.52) to 218.75 (IQR 152.31, 267.53) (P=0.0008 between groups) {See Table 12d}.

There was no significant net change in any of the 4 parameters of the thrombin generation assay (TGA) ie. lag time (TGA LAG time), endogenous thrombin potential (TGA ETP), peak thrombin concentration (TGA Peak), time-to-peak thrombin (TGA tt Peak). TGA LAG time (min) did not change significantly during apheresis with a net median difference of 0.05 (IQR -0.44, 0.83); nor did TGA LAG time (min) change significantly during sham with a net median difference of -0.17 (IQR 1.34, 0.34) (P=0.13 between groups). TGA ETP (nM/min) changed by a mean difference of -4.42 (95% CI -114.42, 105.58) during apheresis, and a mean difference of -59.25 (95% CI -165.58, 47.07) during sham; for which the net difference did not reach statistical significance (P=0.48 between groups). TGA peak (nM) did not change significantly during apheresis with a net mean difference of -7.40 (95% CI -37.62, 22.82); nor did TGA peak (nM) change significantly during sham with a net mean difference of -3.19 (95% CI -29.39, 23.01) (P=0.82 between groups). TGA tt Peak (min) did not change significantly during apheresis with a net median difference of -0.28 (IQR -0.89, 0.89); nor did TGA tt Peak (min) change significantly during sham with a net median difference of -0.28 (IQR -1.95, 0.78) (P=0.30 between groups) {See Table 12d}.

The IMUBIND® Total Tissue Factor Pathway Inhibitor (TFPI) ELISA was used to try and quantify TFPI levels pre- and post-apheresis and pre- and post-sham. However, quality control testing performed by the lab personnel who conducted the test felt that the results generated were not accurate or reliable based on poor reproducibility of the results, hence the results have not been included for the purposes of this thesis or publications.
8.4: Discussion

Lp(a) in mg/L using the nephelometric IMMAGE assay reduced by a median value of 80% of the baseline value after 3 months of apheresis, with no significant change during sham, with statistical significance (P=0.0001 between groups). Lp(a) in mg/L using the Ultra latex immunoassay reduced by a median value of 68% of the baseline value after 3 months of apheresis, with no significant change during sham, with statistical significance (P=0.0001 between groups). The reduction achieved in the Lp(a) Ultra latex immunoassay (which is considered an isoform insensitive method) is very consistent with previously documented reductions of Lp(a) via apheresis using dextran sulphate columns, in which Lp(a) was reduced by 67.4% +/- 11.6% immediately following apheresis.\textsuperscript{175} A recent review article stated that apheresis can acutely decrease Lp(a) by approximately 60-75%,\textsuperscript{176} which is also consistent with our data.

LDL cholesterol in mmol/L reduced by a median value of 78% of the baseline value after 3 months of apheresis, with no change during sham, with statistical significance (P=0.0001 between groups). This degree of reduction in fact supersedes the acute reductions in LDL cholesterol achieved with dextran sulphate based systems reported in the literature previously.\textsuperscript{175,177} There are 2 potential explanations for this discrepancy. Firstly, the acute reductions in LDL cholesterol reported in the literature using the same dextran sulphate based systems we used reported on LDL reduction after a single treatment;\textsuperscript{175,177} whereas in our study, we reported on the reduction of LDL cholesterol after 3 months of therapy. Therefore, the cumulative effect of 3 months of sustained treatment is likely to supersede the impact of a single treatment. Secondly, the baseline pre-treatment LDL cholesterol (mmol/L) in our study was intentionally relatively low at 2.16 ± 0.73. In comparison, the mean pre-treatment LDL cholesterol (mmol/L) reported in the literature was significantly higher at 4.81 ± 0.72,\textsuperscript{177} given that the majority of patients assessed had familial hypercholesterolaemia; hence it could be argued that LDL reductions quoted in the literature cannot be compared with the reduction achieved in our study due to incomparable baseline LDL levels.
Fibrinogen (g/L) reduced by a mean value of 30% of the baseline value after 3 months of
apheresis, with no change during sham, with statistical significance (P=0.0001 between
groups). This is very consistent with fibrinogen reduction quoted in the literature using
dextran sulphate systems which reports a reduction of -29.8 ± 14.7%\textsuperscript{178}. Plasma fibrinogen is
a vital component of the coagulation cascade, and a major determinant of blood viscosity
and blood flow.\textsuperscript{179} Several epidemiological studies suggest that raised fibrinogen levels are
associated with an increased risk of cardiovascular disease, including ischaemic heart
disease, stroke and other thromboembolic events.\textsuperscript{180,181} In the process of disruption of
unstable atherosclerotic plaques, which ultimately lead to spontaneous myocardial
infarction; platelets adhere to GPIIb/IIIa, which also binds fibrinogen, which then drives
platelet aggregation and thrombus growth.\textsuperscript{182} Extrapolating from this, it is possible that
fibrinogen reduction with apheresis may weaken this pathological process and thereby
reduce the chances of spontaneous myocardial infarctions arising from unstable plaques;
especially in the context of the improvement demonstrated in fibrinolysis with apheresis.
Accumulation of fibrin within the vasculature depends not only on the activity of the
coagulation cascade, but also the fibrinolytic pathway, which degrades fibrin.\textsuperscript{182} Hence the
improvement in fibrinolysis with apheresis may also contribute to a potential reduction in
the risk of the clinical sequelae of unstable plaques. On these grounds, it is possible that the
fibrinogen lowering effect of apheresis may contribute to the treatment benefit. However, it
is not yet possible to conclude this definitively, as treatments to selectively lower fibrinogen
have not yet been developed. Hence interventional trials to study the impact of lowering
fibrinogen levels on pathophysiological processes and overall cardiovascular risk profile
have yet to be performed,\textsuperscript{179} before we can confirm the clinical benefit of reducing it.

Oxidised LDL as measured by MDA-LDL reduced by a mean of 30% of the baseline value
after 3 months of apheresis, with no change during sham, with statistical significance
(P<0.0001 between groups). This could be due to a combination of reduced levels of its
most potent carrier Lp(a) with apheresis, and because it may be directly removed itself via
adsorption to the apheresis column.

All of the antibodies associated with oxidised LDL which we measured ie. Anti-IgG MDA-LDL,
Anti-IgM MDA-LDL, as well as total IgG and IgM reduced with statistical significance during 3
months of apheresis and did not change during sham. This could be due to reduced levels of their associated antigen (MDA-LDL) and also because the complexes they form with MDA-LDL may be directly removed via adsorption to the apheresis column.

It has been demonstrated that a significant amount of oxidised LDL accumulates in atherosclerotic plaques.\textsuperscript{183,184} As described previously, Matsuo et al. found MDA-LDL levels were associated with the presence of thin cap fibroatheromas as detected by optimal coherence tomography, suggesting that MDA-LDL could be regarded as a marker of plaque vulnerability.\textsuperscript{11} Therefore, albeit speculative, it is possible that reduction in MDA-LDL could represent yet another mechanism by which there may be reduction in the risk of spontaneous myocardial infarctions arising from unstable plaques. Furthermore, a positive relationship between raised levels of oxidised LDL and the severity of acute coronary syndromes has been demonstrated.\textsuperscript{185} Therefore, the fact that we have shown that oxidised LDL reduces with apheresis is potentially beneficial. The role of anti– oxidized LDL antibodies (anti-ox LDL antibodies) such as Anti-IgG MDA-LDL and Anti-IgM MDA-LDL is less clear. On the one hand, there is a correlation between the existence and titres of anti-ox LDL antibodies and the extent of atherosclerosis and cardiovascular disease.\textsuperscript{186} Conversely, experimental data indicate that anti-oxLDL antibodies may potentially be protective.\textsuperscript{186} Therefore, we cannot conclude whether it is beneficial that we have demonstrated a reduction in Anti-IgG MDA-LDL and Anti-IgM MDA-LDL with apheresis.

In terms of the thrombogenic markers, some of the parameters improved in accordance with our hypothesis and some did not. In particular, the GTT which is an ex-vivo whole blood test indicated clear improvements in thrombogenic risk, whereas the other assay based thrombogenic markers were less conclusive.

In retrospect, it may have been preferable to include a more limited selection of haematological tests for this thesis to focus on. Although, it is very difficult to conclude which of the tests performed are the most important and informative; it could be argued that the GTT tests were the most physiological and representative of whole blood activity, given that the test involves whole blood as opposed to assay based testing of frozen plasma samples, which was required for all of the other haematological tests. It was also very
unfortunate that the TFPI could not be tested due to concerns over the reproducibility and accuracy of the assay; given that inhibition of the function of TFPI is thought to be one of the mechanisms by which Lp(a) may enhance coagulation. Therefore, it is likely that testing of TFPI would have produced informative and meaningful findings.

The GTT OT increased with apheresis, with no change during sham (P=0.0002 between groups). This implies that the thrombus formation process of whole blood ex vivo took longer in patients following apheresis, indicating an improvement in clotting risk, with no change demonstrated during sham.

The GTT LT decreased with apheresis, with no significant change during sham (P=0.005 between groups). This implies that the lytic process of whole blood ex vivo took less time in patients following apheresis, indicating an improvement in fibrinolysis, with no change demonstrated during sham. As discussed above, this improvement in fibrinolysis could potentially lead to a reduction in the risk of spontaneous myocardial infarctions arising from the pathophysiological sequelae of unstable plaques. Although, during this short study there was no significant change in the qualitative visual assessment of new late gadolinium enhancement (LGE) or infarct burden, in the future it would be useful to explore whether longer term apheresis related improvements in fibrinolysis and oxidised LDL levels lead to differences in infarct burden determined with extent of LGE, compared to control subjects not treated with apheresis.

With regards to the assay based thrombogenic markers, vWF reduced during apheresis, with no significant change during sham (P<0.0001 between groups); which may indicate an improvement in thrombotic risk. However, vWF has been noted to reduce for up to 24 hours following extracorporeal membrane oxygenation (ECMO) according to prospective observational data. This may be due to increased shear stress, from an extra-corporeal circuit provoking degradation of high-molecular-weight von vWF multimers. A similar phenomenon may be occurring with apheresis, which also involves an extra-corporeal circuit.
There was no significant net change in D-dimer or TAT, nor any significant net change in any of the 4 parameters of the thrombin generation assay (TGA) ie. TGA LAG time, TGA ETP, TGA Peak, TGA tt Peak. A potential explanation of this is that contact activation from an extra-corporeal circuit may lead to an acute increase in thrombin generation.

Contrary to expectation, F 1+2 increased significantly during apheresis, with no significant change during sham (P=0.0008 between groups). As for the thrombin generation assays, a potential explanation of this could be contact activation from an extra-corporeal circuit which may have led to acutely increased thrombin generation. In addition, the half-life of F1+2 is approximately 90 minutes. Therefore, given that collection of the blood sample for testing of F1+2 was conducted approximately 1-2 hours after the final active apheresis session (when absence of plasma heparin was confirmed) it is almost certain that the acutely raised F1+2 was still detectable upon testing. To get a better impression of the general impact of apheresis on F1+2, testing should ideally have been performed at least 12 hours after the final treatment as this would have overcome the confounding impact of the acute rise in F1+2, which may have been caused by contact activation. Furthermore, serial testing of F1+2 at various time points in the first 24 hours after the final apheresis session would have enabled us to observe the trend in F1+2 levels following apheresis sessions. However, this detailed level of testing was beyond the scope and funding of this trial.

In retrospect, a potentially more conclusive assessment of the impact of apheresis on these thrombogenic parameters may have been obtained by deferring these tests until 24 hours after the completion of the final active treatment session as this may have excluded the possibility of acute contact activation from an extra-corporeal circuit confounding the results. However, in practical terms the study patients may not have been keen to do this as it would have involved extra hospital visits on Saturdays, as active/sham treatments were all conducted on Fridays. Also, limitations from research staffing and funding constraints may not have permitted this.

Although citrate was used as the regional extracorporeal anticoagulant, with the use of 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
around 5 minutes. Therefore, it is improbable that citrate was still lingering systemically when testing of thrombogenic parameters was performed 1-2 hours after finishing active apheresis.

The fact that there was a dramatic reduction in the Lysis time (LT) in the GTT, with a lesser degree of increase in the Occlusion time and essentially inconclusive impact on the other markers of thrombosis may suggest that the impact of apheresis in this cohort of patients relates more to an improvement in fibrinolysis rather than reducing thrombotic risk. In addition, we cannot be sure how much of this benefit, if any, is attributable to the lowering of raised levels of Lp(a). In order to address this question, we would have to conduct an experiment comparing the improvement of fibrinolysis with apheresis in patients with raised Lp(a) as opposed to those without raised Lp(a) at baseline. Until such an experiment is performed, it remains possible that the improvement in lysis time with apheresis is unrelated to the lowering of raised Lp(a).

There was a significant drop in both the Hb and the platelet count with apheresis, which has been observed in a previous observational study which examined the impact on full blood count parameters of apheresis with the same dextran-sulphate column used in our study. The treatment related drop in platelet count raises the possibility that the improvement in the GTT results, in particular the OT which reflects thrombin formation could be partially explained by platelet reduction. This uncertainty could be resolved by examining the impact of a specific Lp(a)-lowering treatment such as the novel anti-sense oligonucleotides on GTT derived OT, as this would not lower platelet counts; hence the true impact of lowering Lp(a) on the thrombotic process could be demonstrated.

8.5: Conclusion

In summary, in keeping with pre-existing literature, apheresis led to significant reductions in Lp(a), LDL cholesterol and fibrinogen. A more interesting and novel finding is that apheresis led to a significant reduction in oxidised LDL, which is potentially pathogenic, with no change demonstrated during sham therapy. It may be directly removed itself via adsorption to the apheresis column. We also demonstrated significant reductions in antibodies
associated with oxidised LDL ie. Anti-IgG MDA-LDL, Anti-IgM MDA-LDL; however, whether this is clinically beneficial is more debatable.

In terms of the impact of apheresis on haematological markers of fibrinolysis and thrombosis, a significant improvement in the fibrinolytic process was observed based on the significant reduction in LT with the GTT. However, although the GTT OT suggested a more modest improvement in the thrombotic process, this was not accompanied with clear improvement in thrombotic risk based on the other thrombogenic parameters we assessed. However, the possibility of contact activation related to the extra-corporeal circuit and the impact that this may have had on haematological factors poses a significant confounding effect. This may have been better assessed by deferring the testing of thrombogenic markers at least 24 hours after the final treatment. Furthermore, future experiments to isolate the impact of lowering Lp(a) on thrombosis and fibrinolysis should ideally involve treatments that selectively lower Lp(a), as coagulation factors themselves may be removed from the apheresis column, combined with the fact that apheresis improves plasma viscosity. 49,191
Chapter 9: The impact of lipoprotein apheresis on symptoms, quality of life and exercise capacity in patients with refractory angina and raised Lp(a)

9.1: Introduction

In addition to the quantitative imaging parameters and lipid and thrombogenic parameters we have assessed, at the trial design stage we felt it was imperative to include patient related outcomes such as exercise capacity and symptoms. Particularly in the assessment of patients with refractory angina, for whom the condition can be debilitating and adversely affect motor activity and quality of life. Also, a demonstrable impact on symptoms is more meaningful to patients themselves than imaging or lab parameters. Furthermore, we wanted to assess whether changes in the imaging and lab parameters correlated with potential impact of the treatment on angina symptoms, quality of life and exercise capacity.

To the best of our knowledge, previously no one has prospectively examined the impact of lipoprotein apheresis objectively on exercise capacity, angina symptoms and quality of life in patients with a combination of refractory angina and raised Lp(a), in the absence of raised LDL cholesterol; in the context of a randomised sham-controlled trial.

9.2: Methods

As previously described in detail in the main methods section, the 6MWT was used to evaluate exercise tolerance, SAQ was used to measure impact on aspects of angina and SF-36 was used to measure the physical and mental components of quality of life. For consistency and logistical reasons, these tests were performed during a visit to Harefield Hospital either 2 working days preceding the first treatment for pre-treatment tests or 2 working days following the final treatment for post-treatment tests. Patients remained strictly blinded to treatment allocation and order whilst completing all of these tests.
For both questionnaires, automated software was used to provide scores to enable quantitative calculation of scores to allow comparison of differences pre- and post-apheresis and pre- and post-sham.

9.3: Results

Table 13: Change in symptoms, quality of life and exercise endpoints during apheresis and sham, shown as mean (lower 95% CI, upper 95% CI) or median [lower quartile, upper quartile].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apheresis</th>
<th>Sham:</th>
<th>P (between groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAQ – Physical limitation</td>
<td>27.8 [16.7, 43.1]</td>
<td>-4.2 [-11.1, 6.9]</td>
<td>0.003</td>
</tr>
<tr>
<td>SAQ: Angina stability</td>
<td>17.5 (6.70, 28.3)</td>
<td>-3.75 (-17.1, 9.55)</td>
<td>0.016</td>
</tr>
<tr>
<td>SAQ – Angina frequency</td>
<td>35.0 [20.0, 50.0]</td>
<td>-5.0 [-20.0, 5.0]</td>
<td>0.005</td>
</tr>
<tr>
<td>SAQ – Treatment satisfaction</td>
<td>6.25 [0.0, 18.75]</td>
<td>0 [-3.125, 6.25]</td>
<td>0.14</td>
</tr>
<tr>
<td>SAQ – QoL</td>
<td>25.8 (17.5, 34.1)</td>
<td>4.6 (-6.1, 15.3)</td>
<td>0.005</td>
</tr>
<tr>
<td>SF-36 – Physical component score (PCS)</td>
<td>7.5 [5.0, 13.0]</td>
<td>-2.0 [-4.5, 1.0]</td>
<td>0.001</td>
</tr>
<tr>
<td>SF-36 – Mental component score (MCS)</td>
<td>6.4 (2.5, 10.2)</td>
<td>1.6 (-3.8, 7.0)</td>
<td>0.19</td>
</tr>
<tr>
<td>6MWT distance (m)</td>
<td>70.5 [41.5, 105.5]</td>
<td>3.5 [-15.1, 30.8]</td>
<td>0.001</td>
</tr>
<tr>
<td>6MWT Oxygen saturation (%)</td>
<td>0.70 (-0.32, 1.72)</td>
<td>-0.45 (-1.14, 0.24)</td>
<td>0.167</td>
</tr>
<tr>
<td>6MWT Borg dyspnoea score</td>
<td>-0.27 (-0.97, 0.42)</td>
<td>-0.15 (-0.95, 0.65)</td>
<td>0.417</td>
</tr>
<tr>
<td>6MWT Borg fatigue score</td>
<td>-0.42 (-1.32, 0.47)</td>
<td>0.03 (-0.72, 0.77)</td>
<td>0.333</td>
</tr>
</tbody>
</table>

Improvements occurred in all five domains of the SAQ, indicating amelioration of angina symptoms during apheresis, which did not occur during sham (See Table 13). SAQ-Physical limitation score improved by a median increase of 27.8 [IQR 16.7, 43.1] from 54.58 ± 21.91 to 83.61 ± 14.28 with a median change of -4.2 [IQR -11.1, 6.9] during sham from 59.86 ± 20.78 to 59.72 ± 16.70 (P=0.003 between groups). (See Figure 14) SAQ-Angina stability score improved by a mean increase of 17.5 [95% CI, 6.70, 28.30] during apheresis with a mean change of -3.75 [95% CI, -17.05, 9.55] during sham (P=0.016 between groups). SAQ- Angina frequency score improved by a median increase of 35.0 [IQR 20.0, 50.0] during apheresis from 50.0 [IQR 40.0, 60.0] to 90.0 [IQR 75.0, 100] with a median change of -5.0 [IQR -20.0, 5.0] during sham from 60.0 [IQR 40.0, 70.0] to 55.0 [IQR 40.0, 80.0] (P=0.005 between groups). (See Figure 14) SAQ- Treatment satisfaction score improved by a median increase
of 6.25 [IQR 0.0, 18.75] from 82.25 ± 20.88 to 91.88 ± 8.63 during apheresis with a median change of 0.0[IQR -3.125, 6.25] during sham from 85.63 ± 14.91 to 85.63 ± 14.91 (P=0.14 between groups). SAQ- Quality of life score improved by a mean increase of 25.8[95% CI 17.5, 34.1] from 41.67 [IQR 25.00, 58.33] to 70.84 [IQR 54.17, 79.19] with a mean change of 4.6 [95% CI -6.1, 15.3] during sham from 50.0 [IQR 37.50, 62.50] to 58.33 [IQR 33.33, 75.00] (P=0.005 between groups).

Assessing the raw angina frequency data, the average number of angina episodes per week was reported as “>3 episodes per week but not every day” pre-apheresis. Post-apheresis the average reported frequency was “less than once a week”. During sham, there was no change in the angina frequency which was reported as an average of “>3 episodes per week but not every day” both pre- and post-sham.

In terms of quality of life, there was an improvement in the SF-36 parameters during apheresis, which did not occur during sham {See Table 13}. SF-36 physical component summary (PCS) improved with a median increase in PCS score of 7.5 [IQR 5.0, 13.0] from 35.05 ± 7.76 to 43.50 ± 7.83 during apheresis and a median change of -2.0 [IQR -4.5, 1.0] during sham from 37.30 ± 7.70 to 35.85 ± 7.45 (P=0.001 between groups). {See Figure 14} SF-36 mental component summary (MCS) showed a mean increase of 6.4 [95% CI 2.5, 10.2] during apheresis from 45.40 ± 11.51 to 51.75 ± 9.67 and a mean change of 1.6 [95% CI -3.8, 7.0] during sham from 45.20 ± 10.86 to 46.80 ± 14.20, however this improvement did not reach statistical significance (P=0.19 between groups).

The favourable treatment effect on symptoms net of the effect of sham, also correlated with an improvement in exercise capacity which occurred similarly in active treatment; with no significant placebo effect {See Table 13}. The mean 6MWT distance(m) improved during apheresis from 400.69 ± 119.06 to 481.24 ± 124.54 with a median improvement of 70.5 [IQR 41.5, 105.5]; and did not change significantly during sham from 412.17 ± 131.02 to 422.10 ± 116.07, with a median change of 3.5 [IQR -15.1, 30.8] (P=0.001 between groups). {See Figure 14} In addition, prior to apheresis, 11 of the 20 subjects (55%) experienced chest pain during the 6MWT; in contrast following apheresis only 2 of the 20 subjects (10%) developed chest pain during the test. Pre-sham 10 of 20 (50%) developed chest pain during the 6MWT; with
no change post-sham when again 10 of 20 (50%) developed chest pain during the test. The oxygen saturation (%) measured with pulse oximetry immediately after completing the test did not change significantly during apheresis, with a treatment related difference of 0.70 [95% CI -0.32, 1.72]; nor during sham with a difference of -0.45 [95% CI -1.14, 0.24] (P=0.167 between groups). Although there was a significant treatment related improvement in the 6MWT distance, the 6MWT Borg dyspnoea score (scale 0-10) did not change significantly during apheresis, when compared to sham (P=0.417 between groups). Similarly, the 6MWT Borg fatigue score (scale 0-10) did not change significantly during apheresis, when compared to sham (P=0.333 between groups).

Figure 14: Graphs showing improvements during apheresis compared with sham in: distance walked on Six Minute Walk Test (top left); angina (top right); physical limitation (bottom left); overall physical wellbeing (bottom right).
In order to directly examine the individual improvements in the myocardial blood flow or MPR, carotid plaque regression and carotid distensibility with exercise capacity and angina frequency, the following correlative calculations were performed.

A correlation was performed between individual improvement in MPR with improvement in 6MWT distance(m) during apheresis \((r=-0.08, P=0.75)\), indicating no significant correlation on the individual level. Similarly, individual improvement in MPR did not correlate with improvement in SAQ angina frequency during apheresis \((r=0.06, P=0.80)\). In addition, individual reduction in LEFT + RIGHT combined total carotid wall volume did not directly correlate with improvement in either 6MWT distance(m) \((r=0.003, P=0.99)\), nor with improvement in SAQ angina frequency \((r=-0.08, P=0.75)\). Finally, there was also no significant correlation in the individual apheresis related change in left carotid distensibility with 6MWT distance(m) \((r=-0.23, p=0.33)\); nor in the individual apheresis related change in right carotid distensibility with 6MWT distance(m) \((r=-0.02, p=0.92)\).

9.4: Discussion
These results indicate significant improvements in exercise capacity, all domains of the SAQ, and the SF-36 PCS, overall confirming a beneficial effect of apheresis on patient related outcomes in the trial participants; thereby fulfilling our secondary hypothesis that apheresis may have a therapeutic effect on these parameters. In fact, the improvement in angina frequency was so substantial that 7 out of the 20 patients had an SAQ angina frequency score of 100 post-apheresis ie. had been rendered chest pain-free. Interestingly although a modest improvement was demonstrated in the MCS of the SF-36 during active treatment, to a lesser degree, a mild improvement occurred during sham; hence the net improvement did not achieve statistical significance \((P=0.19\) between groups). In comparison, improvement in the SF-36 PCS and 4 out of 5 domains of the SAQ improved during apheresis compared to sham with strong statistical significance between groups. This perhaps suggests that apheresis has a more substantial impact on physical symptoms and aspects of angina itself when compared to its impact on mental health, as one might expect.

In terms of interpreting the impact of treatment on the 6MWT, a review article suggested that the test is able to provide information regarding functional capacity, response to
therapy and prognosis across a range of chronic cardiopulmonary conditions and that a change in walking distance of more than 50m is clinically significant in most disease states.\textsuperscript{192} Taking this into consideration, the fact that the mean 6MWT distance(m) improved during apheresis from $400.69 \pm 119.06$ to $481.24 \pm 124.54$ with a median improvement of $70.5$ [IQR 41.5, 105.5] with no significant change during sham ($P=0.001$ between groups) would therefore imply that a clinically significant improvement occurred in the exercise capacity during active treatment. This is further supported by the fact that this was associated with improvements in all domains of the SAQ as well as the physical component of the SF-36 questionnaire. Although the 6MWT distance itself improved with apheresis, it should be noted that the oxygen saturation as well as the Borg dyspnoea and fatigue scores did not improve significantly with apheresis. It is acknowledged that cardiopulmonary exercise testing may have provided more detailed physiological information, such as VO\textsubscript{2} max; and may ultimately have been more informative that the 6MWT. However, for logistical reasons and due to funding constraints, implementation of cardiopulmonary exercise testing in this study was not feasible.

Thus, the improvement in myocardial perfusion and carotid atheroma burden has been demonstrated to be accompanied with improvements in patient related outcomes. However, when correlations were performed on the individual level, there were no statistically significant correlations between the degree of apheresis-related change in MPR, carotid plaque regression or carotid distensibility and exercise capacity or angina frequency. We had hypothesised that individual improvement in MPR with apheresis may correlate with the extent of improvement in exercise capacity, given that a study previously performed on patients with severe aortic stenosis had demonstrated that MPR was predictive of age and sex corrected peak VO\textsubscript{2}.\textsuperscript{193} Again, it is plausible that our sample size is insufficient to detect these correlations if they do indeed exist. A much larger study may be required to explore these correlations more definitively with correction of co-existing confounding variables to shed light on the mechanism of symptomatic improvement with apheresis. It is also possible that the mechanism of improvement in exercise capacity and symptoms is multi-factorial ie. improvement in perfusion, atheroma burden, arterial distensibility and endothelial function all contribute synergistically; therefore, making it difficult to independently isolate the impact of each variable.
Although these patient related outcomes are perhaps not perceived to be as quantitative as our primary and secondary imaging end points or our lab biomarkers, arguably the positive impact we have detected in these symptomatic parameters is more directly relevant to patient care. These results in particular help to convince us that apheresis has a truly positive impact on reducing the symptomatic burden of refractory angina.

Interestingly, the results indicate that there was little to no placebo effect during sham. In fact, 3 of the 5 SAQ domains i.e. physical limitation, angina stability and angina frequency, as well as the SF-36 PCS conversely showed a mild deterioration in scores during sham. No significant change occurred in the SAQ treatment satisfaction score and there was only mild improvement in the SAQ quality of life score during sham. There was no significant improvement in the 6MWT distance during sham. Prior to conducting the trial we had predicted that there could be a significant placebo effect, particularly on quality of life and symptoms, secondary to weekly contact with healthcare professionals. To the contrary, the mild deterioration in the physical limitation, angina stability and angina frequency domains of the SAQ during the sham phase appear to reflect the chronically progressive nature of refractory angina left untreated. The lack of placebo effect observed with sham therapy perhaps validates our trial findings further, confirming a statistically significant treatment benefit which has occurred net of any sham effect.

9.5: Conclusion

Our randomised controlled cross-over trial results indicate that in patients with refractory angina and raised Lp(a), apheresis led to statistically significant improvements in patient related outcomes such as exercise capacity determined by 6MWT; angina symptoms with significant improvements in 4 out of 5 domains of the SAQ including physical limitation, angina stability and frequency, angina-related quality of life; as well as physical aspects of quality of life according to the SF-36 questionnaire. These treatment related effects occurred net of any significant sham or placebo effect on either exercise, angina symptoms or quality of life; potentially validating them further. In addition, improvement in these patient related outcomes in conjunction with treatment related improvements demonstrated in myocardial perfusion, carotid atherosclerosis, lipid profiles and
thrombogenic parameters. This rationally indicates that improvement in pathophysiological parameters translates to improvement in patient symptoms, which are after all the most relevant and tangible outcomes in terms of patient welfare.

These patient related outcomes represent direct improvements in wellbeing in a cohort of patients who suffer from the burden of debilitating angina; raising the possibility that apheresis could be considered a viable treatment option for those with refractory angina and raised Lp(a).
Chapter 10: Genetics in patients with refractory angina and raised Lp(a)

10.1 Introduction

Previously Clarke et al. identified a common variant (rs10455872) at the LPA locus with an odds ratio for coronary disease of 1.70 (95% confidence interval [CI], 1.49 to 1.95) and another variant (rs3798220) with an odds ratio of 1.92 (95% CI, 1.48 to 2.49). Both variants were strongly associated with an increased level of Lp(a) lipoprotein, a reduced copy number in LPA (which determines the number of kringle IV–type 2 repeats), and a small Lp(a) lipoprotein size. Therefore, we thought it may be of interest to test all of the patients involved in the trial for the presence of these 2 variants. In addition, we performed sub-group analysis to ascertain whether the presence of having either variant present influences the response to therapy with apheresis, in terms of clinical endpoints measured in our trial specifically including impact on quantitative perfusion, exercise capacity assessed by 6MWT distance and angina frequency determined by the SAQ Angina Frequency score. In future, testing for these variants may provide a useful means of risk stratifying patients at a higher risk of developing problematic coronary disease, such that closer monitoring and primary prevention can be implemented prior to the onset of symptomatic progressive coronary disease. Furthermore, if it is demonstrated that the presence of these single nucleotide proteins (SNP) correlates with an improved response to apheresis, genetic testing could potentially be utilised as a tool to help guide us in terms of patient selection for treatment. Meaningful guides for appropriate patient selection for treatment will become increasingly necessary with the rising incidence of patients with refractory angina, in whom a substantial proportion may be revealed to have raised Lp(a) as a relevant underlying risk factor. Appropriate patient selection is critical in the current economic environment in which the healthcare funding available for provision of expensive therapies such as apheresis is likely to fall short of the demand.

10.2 Methods

Specific consent for bio-banking was obtained for all trial patients and whole blood samples were obtained. Genomic DNA was extracted from whole blood using the Qiagen EZ1 system
and PCR amplified using custom primers for SNPs rs10455872 and rs3798220. Amplicons were sequenced with Applied Biosystems 3500 and analysed with Sequencher software (Genecodes). Results were analysable for all 20 trial subjects.

10.3 Results
A pathological or alternative allele was found to be present for rs10455872 in 9 out of the 20 (45%) trial patients. A pathological or alternative allele was found to be present for rs3798220 in 2 out of the 20 (10%) trial patients. There were no patients with both genetic variants or single-nucleotide polymorphisms (SNPs) present. In total, 11 out of the 20 (55%) patients had either variant present.

Sub-group analysis was also performed to determine whether there was any interaction between the presence of either of these genetic variants and the degree of improvement in myocardial perfusion, exercise capacity and the frequency of angina.

In those with either rs10455872 or rs3798220 (n=11), MPR improved by a mean of 0.41 ± 0.29 during apheresis and deteriorated by a mean of -0.25 ± 0.41 during sham (P= 0.002 between groups). In those with neither rs10455872 nor rs3798220 (n=9), MPR improved by a mean of 0.55 ± 0.39 during apheresis and deteriorated by a mean of -0.05 ± 0.31 during sham (P= 0.02 between groups). Using mixed models analysis, there was no significant interaction between the presence or absence of genetic variants and the extent of improvement in MPR [p (interaction) = 0.58].

In those with either rs10455872 or rs3798220 (n=11), 6MWT distance (m) improved by a mean of 98.8 ± 64.8 during apheresis and improved by a lesser degree during sham by a mean of 24.6 ± 50.6 (P= 0.016 between groups). In those with neither rs10455872 nor rs3798220 (n=9), 6MWT distance (m) improved by a mean of 58.2 ± 35.7 during apheresis and deteriorated by a mean of -8.0 ± 28.5 during sham (P=0.01 between groups). Using mixed models analysis, there was no significant interaction between the presence or absence of genetic variants and the extent of improvement in 6MWT distance (m) [p (interaction) = 0.74].

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In those with either rs10455872 or rs3798220 (n=11), SAQ angina frequency score improved by a mean of 28.2±35.2 during apheresis and improved by a lesser degree during sham of 10.0±37.4 during sham (P=0.23 between groups). In those with neither rs10455872 nor rs3798220 (n=9), SAQ angina frequency score improved by a mean of 36.7±20.0 during apheresis and deteriorated by a mean of -14.4±18.8 during sham (P=0.0004 between groups). Using mixed models analysis, there was no significant interaction between the presence or absence of genetic variants and the extent of improvement in SAQ angina frequency score [p (interaction) = 0.23].

10.4 Discussion

Despite all of the trial patients by definition having Lp(a) levels > 500mg/L and progressive coronary disease with associated refractory angina, 9 out of the 20 patients (45%) did not have either genetic variant (rs10455872 or rs3798220). However, Clarke et al. had previously concluded that these SNPs explain only 36% of the variation in the Lp(a) lipoprotein level, which may suggest that other genetic variables may contribute towards Lp(a) levels. In future as the genetic characterisation of raised Lp(a) becomes more defined, more genetic tests may become available to enable us to genotype patients and guide risk stratification in a more focussed manner; such that high risk individuals can be identified prior to the onset of cardiovascular disease to allow implementation of preventative interventions.

In addition, sub-group analysis has not revealed a statistically significant interaction between the presence of either genetic variant, and the degree of improvement in either myocardial perfusion, exercise capacity or angina frequency determined by SAQ. The sample size of our study may not be adequate to detect this association if it does indeed exist. In a sample size of 20, it is probable that multiple confounding factors such as differences in the severity of coronary disease, level of baseline revascularisation, age, baseline physical mobility (to name just a few) would limit the ability to isolate the impact of these genetic variants on treatment effects. A study of a much larger scale would be necessary to investigate this more definitively.
10.5 Conclusion

In summary, genetic testing for the variants rs10455872 and rs3798220 which have previously been identified as being associated with an increased level of Lp(a) and risk of coronary disease,\textsuperscript{23} were found to be present in 11 out of the 20 trial subjects (9 had rs10455872 present and 2 had rs3798220 present). However, it is reported that these SNPs explain only 36% of the variation in the Lp(a) lipoprotein level,\textsuperscript{23} which may explain why a higher proportion of the trial patients did not have these variants despite all having Lp(a)>500mg/L.

In addition, sub group analysis in our trial subjects has not revealed a statistically significant relationship between the presence or absence of these variants and the treatment effect of apheresis on perfusion, exercise capacity or angina frequency. Our ability to address this question is limited by the sample size of the trial.
CONCLUSIONS

Chapter 11: General Discussion and Conclusions

11.1 General discussion

Refractory angina is a debilitating condition with limited treatment options that is increasing in frequency as more patients with CAD survive and experience angina despite excellent medical therapy and revascularization.63 There is a significant need to identify effective treatment options. A substantial amount of epidemiological evidence confirms that elevated Lp(a) is an independent cardiovascular risk factor and predictor of adverse outcome in atherosclerotic disease.3,4,21 There is some data suggesting that raised Lp(a) might be relevant in refractory angina.55,82 At present, lipoprotein apheresis remains the most effective treatment available for raised Lp(a),45 as currently no satisfactory pharmacotherapy is available for Lp(a). Existing evidence exploring the role of apheresis in raised Lp(a) in the context of coronary disease is very limited and consists mainly of a few observational studies,83,84 which are suggestive of a beneficial effect. Very few prospective randomised studies 41,85 have been performed which suggest that apheresis may have a positive impact in patients with raised Lp(a) and stable coronary disease. Certainly, prior to this research, a randomised controlled trial had not yet been conducted evaluating the role of apheresis in refractory angina and raised Lp(a).

Although our trial has a relatively small sample size, strengths of our trial design include the fact that it is a randomised sham-placebo controlled study, such that we were able to exclude any significant placebo effect. In addition, the cross-over design eliminated any randomisation errors that otherwise would have been likely to occur with a small sample size; as well as improving the statistical power of the study by effectively doubling the sample size. Overall, the compliance of trial subjects to the trial protocol was excellent, with only 2 patients ultimately withdrawing from the trial. We succeeded in completing the trial protocol with minimal deviations with the required number of patients based on our power calculations.
The results clearly demonstrate significant apheresis related improvement in our primary end point of quantitative myocardial perfusion, as well as the majority of the secondary end points including quantitative atheroma burden, exercise capacity, multiple aspects of angina symptoms, the physical component of QoL, lipid parameters including oxidised LDL, as well as some of the thrombogenic parameters. This implies that apheresis may lead to widespread and multi-factorial improvements in patients with refractory angina and raised Lp(a), including symptomatic benefits. Specifically, although the improvement detected in myocardial perfusion, carotid atheroma and distensibility and lipid biomarkers and some of the thrombogenic parameters was accompanied with improvements in the patient related outcomes; statistical analysis did not demonstrate significant correlations at the individual level between improvements in perfusion, carotid wall volume and distensibility and the patient related outcomes of exercise capacity and angina frequency. The sample size of the trial may be insufficient to detect the relationship between these parameters; which may be explored in more detail with a larger study.

Due to the trial design, we can be fairly confident that the treatment related benefits occurred net of any placebo effect. Perhaps surprisingly and contrary to our expectations, we did not detect any significant placebo effect in any of the trial end points during sham therapy; which may in fact further validate the positive impact detected in favour of apheresis.

This is also the first RCT to use fully quantitative perfusion CMR and quantitative carotid wall volumes as trial endpoints, representing additional research milestones in the form of progress in the use of CMR as a valuable tool in cardiovascular research.

Although genetic testing for the variants rs10455872 and rs37982220 yielded positive results in only 11 out of the 20 trial subjects, this may potentially be explained by the fact that these SNPs are thought to explain only 36% of the variation in the Lp(a) lipoprotein level.23
11.2 Limitations of this research

This trial is limited by its small sample size and ideally a larger study in patients with refractory angina and raised Lp(a) incorporating the impact of apheresis on MACE would help to validate the findings. A trial of a much larger scale would also be required to make the genetic studies more meaningful and may have enabled us to determine the significance of having a genetic variant associated with raised Lp(a) and whether genetic testing could be utilised to aid risk stratification and prediction of response to therapy. However, despite the small sample size we can be relatively confident that we have successfully fulfilled the main objectives of the study and can conclude with robust statistical significance that there was a positive treatment related effect on our primary end point, as well as the majority of our secondary end points. Also, it was challenging to recruit such a specific cohort of patient, as we wanted to remain confident that the participants strictly fulfilled all of the inclusion criteria and genuinely suffered with true refractory angina. Hence in real terms it would have been even more challenging to recruit a larger sample and we may have needed to extend it to a multi-centre trial in order to achieve this. Running a trial of this nature in a multi-centre context would have posed additional challenges in terms of maintaining good quality control and consistency of the complex and highly specialised investigations we performed, as well as practical issues such as transporting patients from remote locations to the multiple hospital visits.

It is acknowledged that the test-retest reproducibility of MPR and quantitative total carotid wall volume measurement using our chosen methods in our patient cohort was not formally assessed, and therefore has to be regarded as a limitation in terms of interpreting the results. Establishing test-retest reproducibility of MPR using our local methods should ideally have been performed prior to starting the trial would have produced a more informative power calculation.

The fact that 19 out of 20 of our trial subjects were male also deserves mention, as this may potentially limit extrapolation of these findings to female patients. However, there was absolutely no gender bias at the recruitment stage, hence the male preponderance may simply be a reflection of the fact that being male is itself a risk factor for coronary disease.\textsuperscript{194}
In addition, previously a large prospective epidemiological study showed that an elevated level of Lp(a) was an independent predictor of stroke, death from vascular disease, and death from any cause in men but not in women, implying that raised Lp(a) may carry a lower risk profile in women compared to men and may potentially explain why our trial patients were predominantly male.

Ideally the duration of treatment periods in the study could have been longer to determine the impact of a more sustained period of treatment. However, aside from funding constraints, the trial had to be completed within a realistic time frame for a PhD. In addition, in practical terms we were only able to treat 6 patients concurrently at any time due to a limited number of apheresis machines and limited availability of time and trained staff to perform the apheresis. Also given the high risk profile of the patients and the nature of their underlying condition, it is highly probable that if the treatment duration was six months or longer, a significant proportion may have had myocardial infarctions or required revascularisation within the treatment period; which would require withdrawing such patients, as otherwise these events would have a significant confounding impact on the results.

Questions remain over the mechanism of the treatment effect remain unresolved as the apheresis technique used was not Lp(a) specific. The DX21 DHP system utilises dextran sulphate to covalently bind Apo-B containing lipoproteins to remove them directly from whole blood. This is effective for Lp(a) but also lowers LDL cholesterol. This leaves open to interpretation whether the effect is primarily mediated by a reduction in Lp(a) or through LDL reduction or both. Further trials could address this issue through the use of Lp(a) specific apheresis columns, which were not available to us at the planning stages of the trial, or alternatively the technology of anti-sense oligonucleotide knock-down of Lp(a) mRNA. In addition apheresis has been shown to remove from blood multiple factors other than lipoproteins, including fibrinogen, coagulation factors, thrombogenic factors, complement factors, inflammatory factors and adhesion molecules. This may mediate reduced coagulation and improvements in viscosity and endothelial function that may be beneficial beyond lipoprotein removal alone. In addition, utilising an Lp(a) specific treatment such as anti-sense oligonucleotide therapy, would enable us to conduct more meaningful
assessments of the mechanistic impact of pure plasma Lp(a) reduction on thrombogenesis and oxidised LDL as coagulation factors and oxidised LDL itself would not be removed directly via apheresis columns.

There appears to be some controversy over the sensitivity of the EndoPAT test we used to detect changes in endothelial function, especially when applied to assess response to therapies or interventions over relatively short periods of time. Therefore, although endothelial function, assessed peripherally via the finger-tips using the EndoPAT device did not appear to change significantly during either apheresis or sham, it is possible that the test lacked the sensitivity required to detect a change. In hindsight, a more sensitive indicator of endothelial function may have been more informative.

Angiographic assessment of the coronary arteries using novel quantitative techniques such as infra-red spectroscopy may have added valuable information about coronary atherosclerosis and plaque composition in response to apheresis. However these are invasive procedures involving radiation which would pose a higher risk of complications for patients than CMR scans. Also, in their feedback patients retrospectively stated they would have been reluctant to participate in a trial involving invasive coronary angiographic assessment, particularly as their underlying condition already predisposes them to multiple revascularisation procedures.

In hindsight, measuring the thrombogenic parameters, in particular the assay tests at least 24 hours after finishing the final treatment may have yielded more meaningful results. In the present study the assays were measured approximately 2-3 hours after finishing apheresis or sham therapy, hence it is possible that the immediate impact of completing an extra-corporeal treatment could produce significant changes in haematological factors related to contact activation. It was impossible for us to predict this in the planning stages of the study, as previously, to the best of our knowledge these assays have not been performed following apheresis. Also, in practical terms, given treatments were always performed on Fridays, this would have involved an additional appointment on a Saturday, which many patients may not have been willing to undertake. Potentially, experiments involving pure Lp(a) reduction such as anti-sense oligonucleotide technology would remove
the confounding impact of extra-corporeal treatment on activation of coagulation and thrombogenic assays; and give a clearer indication of the impact of Lp(a) reduction on the thrombogenic process.

Although the treatment effect of apheresis appears apparent in refractory angina and raised Lp(a), this needs to be weighed up against the fact that it is costly and requires specialised training and expertise for service delivery and that accessibility to treatment is generally limited. Although the cost of approximately £1,000–£1,200 per session is significant, it is estimated that the annual cost of lipoprotein apheresis currently represents less than 1% of the amount spent annually in the UK on haemodialysis.197 Cost-effectiveness analysis ideally in the context of a much larger study involving the incidence of MACE and hospital admissions in treatment and control arms versus the cost of therapy needs to be performed.

11.3 Implications of this research in clinical management

Although the sample size of this trial is small, statistically significant changes were detected in favour of apheresis in terms of patient related outcomes such as angina symptoms, quality of life and exercise capacity; which also correlated with objective and physiologically important parameters such as quantitative myocardial perfusion and carotid atherosclerosis. This does imply that lipoprotein apheresis yields significant clinical improvement in this difficult to treat patient group with refractory angina and raised Lp(a), and is a welcome and much needed novel treatment option.

Refractory angina is a growing problem worldwide due to improving survival rates owing to improved revascularisation techniques, causing an expanding population of patients with treatment resistant angina,59,63 The healthcare burden of this condition is significant and the management of affected patients is challenging.63 Previous studies have evaluated neurostimulation (transcutaneous electrical nerve stimulation and spinal cord stimulation), enhanced external counterpulsation (EECP) therapy, laser revascularization, gene therapy and newer procedures such as extracorporeal shockwave myocardial revascularization,64
however further evidence to support widespread use of these treatments is required. The use of apheresis offers a new avenue to explore in refractory angina patients. Ideally the findings of this trial should be validated further with a larger scale study that specifically involves patients with refractory angina in the context of raised Lp(a), that examines the risk reduction of MACE as well as cost effectiveness analysis. A larger scale study of this nature may provide rationale to implement change in clinical practice and establish lipoprotein apheresis in management guidelines as a recognised treatment option for patients with refractory angina and raised Lp(a). Our trial has provided a robust and scientifically important platform as a catalyst to stimulate further research in this field.

Aside from the limited sample size, there are additional uncertainties that remain with regard to implications of these results. One is the proportion of patients with refractory angina that may benefit. The prevalence of raised Lp(a) in refractory angina is reported in one study as 60%. This suggests that the proportion of patients with refractory angina that could benefit from this novel treatment is substantial, but it is not known whether there is a lower threshold of Lp(a) than 500mg/L which would yield clinical benefit by apheresis and increase applicability. Further trials to examine this issue may be stimulated by the current findings. In addition, for consistency a dextran sulphate based apheresis method was used for the purposes of this trial. However, there are numerous alternative methods of lipoprotein apheresis including heparin-induced extracorporeal LDL-cholesterol precipitation (HELP), immunoabsorption, double filtration plasmapheresis (DFPP) and direct adsorption of lipoproteins (DALI), as well as Lp(a)-specific apheresis. Therefore, although it is likely that these alternative methods may lead to similar benefits in refractory angina and raised Lp(a); we cannot conclude this with absolute certainty.

Currently, Lp(a) is not being measured routinely in clinical practice, and yet 60% of the patients with refractory angina screened for this study had raised Lp(a) >500mg/L. Our findings suggest that we should at least consider screening for raised Lp(a) in patients with refractory angina, which may be a causal risk factor. In addition, two observational cohort studies demonstrated a significant reduction in MACE in patients with established CAD and raised Lp(a) in whom regular apheresis was instituted; implying that we should infact consider screening all patients with established CAD for raised Lp(a); who may potentially
benefit from secondary preventative treatment of raised Lp(a). It may be that we are significantly underestimating the pool of patients that may benefit from Lp(a) reduction.

### 11.4 Future Directions

The promising findings of this trial are likely to stimulate further research on the subject of raised Lp(a) and its role in CHD, and the benefits of treating it.

As alluded to earlier, a bigger randomised multi-centre phase III trial to validate these findings including assessment of MACE incidence reduction and cost-effectiveness analysis in patients with refractory angina and raised Lp(a) is ideally required. A trial of this nature would be justified by the present findings and would to help to convince clinicians with added certainty that lipoprotein apheresis improves clinical outcomes for patients with refractory angina and raised Lp(a), and will determine whether this treatment option is financially justifiable. Leebman et al. performed a prospective observational multi-centre study in Germany which 170 patients were investigated who commenced lipoprotein apheresis for Lp(a)-hyperlipoproteinemia and progressive cardiovascular disease. In their trial, 142 MACE occurred in the 2 year period before lipoprotein apheresis versus 31 MACE during the 2 year lipoprotein apheresis period, which then translated into a number needed to treat of 3 to prevent 1 MACE per patient per year. Extrapolating from this, ideally a multi-centre phase III RCT should involve a minimum of 200 patients, in order for sufficient MACE to occur in the treatment arm; although to convince clinicians of the therapeutic impact of apheresis a trial involving at least 500 patients may be necessary. Recruiting patients and delivering a trial of this scale would be challenging, as a relatively limited number of centres perform apheresis. In addition, in Germany, which performs the most apheresis per capita with more than 2000 patients currently receiving apheresis according to a recent report from the German Apheresis Working Group, the ethical committees will not grant approval for RCTs; owing to the substantial number of patients already established on long-term apheresis for raised Lp(a). Therefore, a multi-centre phase III RCT would have to be limited to the remainder of European centres (excluding Germany), and the United States. Given that it is estimated that worldwide there are ≈2500 patients receiving apheresis
treatment, with a large majority of treatment recipients in Germany and less than 100 patients currently undergoing treatment in the UK;\textsuperscript{199} it is clear that conducting a large scale phase III trial will not be feasible with current treatment capacity, unless substantial resources are devoted to the expansion of apheresis centres and the specialist training of staff required to perform apheresis. Ideally, the primary outcome assessed in a large phase III RCT should be the incidence of MACE in the treatment arm compared to a sham-control group, in order to have maximum impact in terms of informing clinical practice. Secondary endpoints could include symptomatic parameters such as exercise testing, angina and quality of life assessments according to questionnaires, as we assessed in our study. Quantitative assessment of perfusion or atheroma burden with imaging modalities such as CMR may be prohibitive in terms of cost with a large scale study, and it would be difficult to achieve consistency across imaging protocols in an international multi-centre trial. Performing a Phase IIb study may be useful in order to establish whether there are significant differences in clinical outcomes if apheresis is performed weekly compared to a fortnightly regime (both of which are currently used in clinical practice); and would certainly help to inform the ideal treatment frequency that should be applied in performing a phase III trial.

In addition, although apheresis was generally well tolerated in this trial; it must be taken into consideration that apheresis is invasive and can lead to side effects or complications in 5-10\% of treatments.\textsuperscript{149} Therefore a larger phase III trial should also objectively evaluate the clinical benefit and net improvement in quality of life associated with treatment versus the potentially adverse impact on quality of life associated with the invasive nature of apheresis and the associated side effects or complications.

Ideally further trials need to be conducted using Lp(a) specific treatments such as Lp(a) specific apheresis columns,\textsuperscript{53} or the novel anti-sense oligonucleotide agents which target Lp(a) mRNA.\textsuperscript{56} This would help to isolate the role of reducing Lp(a) in cardiovascular risk reduction and may provide more mechanistic information about the potential reversal of Lp(a)’s pathological role towards atherogenesis, endothelial dysfunction, thrombosis, inflammation and the interaction with oxidised LDL. As discussed earlier, apheresis has many additional benefits beyond pure lipoprotein reduction, and dextran sulphate based
columns which were similar to the columns used in our trial were previously shown to remove numerous additional proteins other than Apo-B containing lipoproteins;\textsuperscript{196} which may be contributing to the treatment effect. Eventually Lp(a) specific treatments may need to be directly compared with lipoprotein apheresis in randomised controlled studies to ultimately determine the best treatment approach in terms of improving clinical outcomes.

Also, this trial has addressed the role of apheresis specifically in patients with refractory angina secondary to advanced coronary disease with elevated Lp(a). We should now consider taking a step back and assess the role of treating raised Lp(a) in the primary and secondary prevention of CAD. These questions could potentially be addressed with two further trials: one involving patients identified with raised Lp(a) prior to any cardiovascular event and another trial involving patients after their first cardiovascular event, in whom raised Lp(a) has been detected.

Also, large scale epidemiological studies will help us to examine the role of Lp(a) screening and the testing of genetics variants implicated in raised Lp(a) and CAD risk such as rs10455872 and rs3798220.\textsuperscript{23} Such studies will help to determine whether future strategies should involve identifying high risk patients prior to the onset of MACE, and whether prophylactically treating raised Lp(a) helps to reduce the incidence of MACE.

Potentially further larger scale studies may reveal that a vast population of patients with coronary disease and raised Lp(a) may benefit from apheresis, for whom the costs of treatment will exceed available public funding. A focus for further research should also include the identification of predictors of those who are likely to be the “best responders” to treatment, to allow appropriate selection of patients who will benefit most from therapy and avoid wastage of resources for those unlikely to benefit. Good quality cost effectiveness analysis will also help to inform this process. Significant healthcare planning and changes in infra-structure and specialist training will be required to enable delivery of treatment in practical terms, especially as current access to apheresis services is generally limited. Existing lipoprotein apheresis units may need to be expanded and new units developed to meet an increase in demand. Access to lipoprotein apheresis varies significantly between countries which may reflect differences in remuneration criteria utilised by health providers.
and insurance companies. Estimates regarding access to treatment range from 1.2 per 100,000 persons in Germany, to 0.13 per 100,000 in North America and 0.06 per 100,000 in the UK.

Although lipoprotein apheresis is generally considered a safe and reasonably well tolerated treatment, it is invasive and involves regular weekly or fortnightly treatments lasting 2-3 hours in a hospital setting, which some patients may regard as inconvenient. In the future, if novel pharmacological agents that target Lp(a) show non-inferiority when compared against lipoprotein apheresis, they may provide a more convenient and potentially cheaper therapeutic option for patients in the long term.

To conclude, our research concerns patients with refractory angina, a condition which is increasing in incidence worldwide and causes significant burden for patients; for which current treatment options are limited. Raised Lp(a) is a strong independent cardiovascular risk factor which is likely to affect a significant portion of those with refractory angina, for which effective pharmacological treatments are not yet established in clinical practice. We have been the first to conduct a randomised controlled trial in patients with refractory angina in the context of raised Lp(a) to try and determine the therapeutic role of lipoprotein apheresis. In such patients, our trial has shown that lipoprotein apheresis leads to significant improvements in myocardial perfusion, carotid atheroma, oxidised LDL and associated antibodies, certain thrombogenic parameters, as well as patient related outcomes such as angina burden, QoL and exercise capacity. Therefore, apheresis represents a promising and much needed therapeutic option in this patient cohort. The findings of our study are likely to stimulate further research to address additional questions that remain on this subject.
Chapter 12: References


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