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Study Protocol and Statistical Analysis Plan

Acute Microvascular Changes with LDL Apheresis

NCT02388633

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Study Protocol

1. Protocol Title: Assessment of Changes in Tissue Perfusion after Plasma LDL Apheresis

2. Study Sponsor: PI-sponsored

3. Objectives: The objective of this study is to use contrast enhanced ultrasound perfusion imaging to explore benefits in microvascular perfusion produced by plasma apheresis in patients with severe hypercholesterolemia. We hypothesize that contrast-enhanced ultrasound (CEU) of the myocardium at rest, and in skeletal muscle at rest and during mild exercise stress will detect an improvement in the microvascular erythrocyte transit rate.

4. Background: Severe hypercholesterolemia produced by conditions such as heterozygous familial hypercholesterolemia is associated with multiple complications including premature atherosclerotic disease. There is evidence that microvascular perfusion, particularly flow reserve, in critical organs is limited due to abnormalities in plasma viscosity, abnormal RBC deformability, and an imbalance between vasodilators and vasoconstrictors. There is little is currently known about acute changes in microvascular blood flow and microvascular rheology that occur in response to plasmapharesis which is used in some patients to lower critically elevated cholesterol levels. Our research group has pioneered CEU methods for assessing myocardial and skeletal muscle perfusion, and has previously demonstrated in pre-clinical models that acute hyperlipidemia produces a reduction in microvascular RBC transit rate. In this study, we will assess acute changes in microvascular perfusion in patients undergoing clinically-indicated plasmapharesis.

5. Study Design: Subjects who are scheduled to have planned apheresis treatment for severe hypercholesterolemia will be recruited into the study. They will undergo a screening evaluation, including a medical history, physical examination, ECG, and limited echocardiogram to evaluate for exclusion criteria. Before the apheresis procedure, blood samples will be obtained for plasma markers of inflammation, erythrocyte deformability, and plasma viscosity. Contrast enhanced ultrasound perfusion imaging will be performed to evaluate blood flow in the myocardium at rest, as well as in the forearm skeletal muscle before and after mild isometric exercise (50% maximal grip, 0.2 Hz). Flow mediated vasodilation will be performed. The subjects will then undergo their planned apheresis procedure. Within 2 hours of completion of apheresis, blood collection and CEU will be repeated. Plasma lipids will be available as part of the standard apharesis protocol.

6. Study Population

a) **Number of Subjects:** 12

b) **Selection Criteria:**

Inclusion:

1. Hypercholesterolemia (LDL >200 mg/dL)
2. Clinically-indicated apheresis for hyperlipidemia
3. Age >18 y.o.

Exclusion:

1. Age <18 y.o.
2. Pregnant or lactating females
3. Hypersensitivity to ultrasound contrast agent (perflutren)
4. Evidence right-to-left, bi-directional, or transient cardiac shunt; unexplained pulmonary hypertension (PA systolic pressure >40 mm Hg) or more than mild reduction in left ventricular systyolic function identified on screening echo.
5. Currently on oral anticoagulants (Coumadin, thrombin-inhibitors, Factor X-inhibitors)

c) Vulnerable Populations: We will not include vulnerable populations, including children, pregnant women, decisionally-impaired adults, or prisoners.

d) Setting: All studies will be conducted in the Knight Cardiovascular Institute (KCVI) Clinical Research Room, 5th Floor, Multnomah Pavilion.

e) Recruitment Methods: Patients with documented hypercholesterolemia who are undergoing apheresis treatment will be recruited. These patients will be identified by Dr. Duell and Dr. Fazio who direct the lipid plasmapharesis program and the preventative cardiology programs, respectively, at OHSU. Only patients seen in the preventative cardiology clinic will be approached for participation in the study. These patients will be referred to Dr. Lindner, Dr. Davidson, or Dr. Wu who will contact the patients about participating in the study, discuss study details, and obtain consent.

f) Consent Process: Informed consent will take place either in the outpatient clinic or in the KCVI research room. Consent will be obtained by Dr. Lindner, Dr. Wu, or Dr. Davidson. All consents will be witnessed by a second party who will sign the consent form, to ensure the subjects' understanding. Translators will be asked to obtain consent for those in whom English is not the primary language. We will inform female patients of reproductive age that pregnancy tests will be obtained prior to performing the study. Minors will not be included as either subjects or witnesses.

- **Special Handling:** None
- **Circumstances and Procedures for Termination from Study:** Subjects will be informed that they can withdraw from the study at any time without consequence. Participants will be terminated from the study by the investigators for: (1) positive pregnancy test, (2) adverse event due to any portion of the study protocol. Patients will be informed verbally regarding subject termination from study.
- **Compensation to Subjects:** None
- **Estimated Costs to Subjects:** None
- **Estimated costs to Third Party Payers:** None

7. Procedures

a) Observations and Measurements

1. Contrast ultrasound measurement of myocardial blood flow at rest
2. Contrast ultrasound measurement of skeletal muscle blood flow at rest in the forearm flexor muscle groups at rest and during mild isometric exercise (50% maximal grip, 0.2 Hz).
3. Blood samples prior to and after apheresis to evaluate for plasma markers of inflammation, plasma viscosity, and RBC deformability.

4. Flow-mediated vasodilation (brachial artery diameter measurement before and after inflation of a blood pressure cuff for 3 min) performed before and after plasmapharesis.

b) Drug Therapy Dose/Device Determination: None. All medical therapy will be at the discretion of the healthcare providers.

c) Description of the Study Design

- Subjects who are scheduled for apheresis for hypertriglyceridemia will be recruited.
- Subjects will undergo a screening echocardiography, history, and physical examination.
- Subjects will undergo contrast ultrasound perfusion imaging of the myocardium and forearm flexor muscle. This will involve:
  1. Assessment of myocardial blood flow
  2. Assessment of forearm skeletal muscle blood flow at rest and during stress.
  3. Flow mediated vasodilation
  4. Blood draw (<20 mL at each of the two stages) Blood drawn will not exceed the lesser of 50 ml or 3 ml per kg and the collection will occur only twice during the one-day study participation.
  5. Subjects who have already been studied may be asked to have blood draws repeated at a separate apheresis session if complete analysis is not possible with the samples taken on the primary study day. Patients will be re-consented for this second blood draw.

d) Duration of participation: The study duration will generally be one day, performed before and immediately after plasmapharesis. Additional bloodwork may be drawn on a subsequent time point before and after a scheduled apheresis session which are usually scheduled every 2 or 4 weeks apart.

8. Data and Specimens

a) Sharing of Results with Subjects: Results will not be shared with subjects

b) Previously unrecognized cardiovascular abnormalities found on echocardiography will be shared with the patient's primary care providers.

c) Data and Specimen Banking: Specimens will not be used for future genetic research. Specimens or data will not be sent to a separate repository. Specimens will not be banked in a repository for future use as part of this protocol.

9. Data analysis: All data analysis will be conducted at OHSU. Data will be compared before and after a planned intervention of the same subject using either a paired Student's t-test or a Wilcoxon test depending on whether data are normally distributed or not. Comparisons between the change in perfusion parameters (microvascular blood flow, microvascular RBC transit rate, functional capillary blood volume) and blood markers will be made using linear regression and determinants of perfusion will be made using logistic regression analysis and/or mixed models.

10. Privacy, Confidentiality and Data Security: All data will be de-identified and subject identification will be a study subject number assigned by order of recruitment. Dates of birth will not be recorded. All written data will be stored in a locked cabinet in a secure office, and de-identified digital data will be stored in

a database behind the OHSU firewall. All blood samples will be disposed of immediately after testing for blood viscosity, erythrocyte deformability, and plasma markers for inflammation (all on the same day as the imaging study or on the subsequent apheresis session at which blood is drawn).

11. Risks and Benefits

a) Risks to Subjects:

1. Risks related to IV placement and blood withdrawal: discomfort and bleeding.
2. Risks related to ultrasound contrast agent administration (Definity). These include minor risk of flank pain, dyspnea, headache, flushing (<5%) which resolve rapidly with discontinuation of the infusion. Serious reactions (pseudo-anaphylactic reacions) occur in 1 in 10,000 administrations and are manifest as severe dyspnea, wheezing/bronchospasm, edema, tachycardia, chest pain, hypotension. If any of these occur, the infusion will be stopped, the study will be terminated, and appropriate medical treatment will be given.
3. Risks related to flow-mediated vasodilation: none reported except brief arm discomfort during inflation of blood pressure cuff.
4. Loss of confidentiality

b) Benefits to Subjects: No direct benefit.

12. Results from Previous Research

a) Our laboratory has demonstrated in pre-clinical models that dogs infused with intralipid to produce hypertriglyceridemia have a reduction in microvascular RBC transit rate on CEU imaging of the myocardium that is commensurate with the change in plasma viscosity (Rim SJ, et al., Circulation 2001;104:2704.

b) Our laboratory has demonstrated in patients that mild exercise stress perfusion imaging with contrast ultrasound can be used to elucidate abnormal flow responses that occur due to either mild-modest PAD or to functional abnormalities in the microcirculation caused by diabetes mellitus (Lindner JR, et al., J Am Coll Cardiol Cardiovasc Imag, 2008;1:343; Womack L, et al., J Am Coll Cardiol 2009;53:2175.

c) Although investigators have used contrast ultrasound techniques to study atherosclerosis and vascular changes in hyperchholestrolemic animal models, but to our knowledge no group has looked at the rheologic and flow changes in humans with hyperlipidemia.

d) Safety of ultrasound contrast agents has been well established with studies involving hundreds of thousands of patients (Wei K, et al., J Am Soc Echocardiogr 2008;21:1206; Main M, et al., Am J Cardiol 2008;102:1742).