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Platelet Activity in Vascular Surgery and Cardiovascular Events

Protocol

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1. Synopsis

Peripheral artery disease (PAD) is a highly prevalent condition in the United States, and a significant cause of cardiovascular morbidity and all-cause mortality. Although vascular surgical procedures are commonly performed to improve patient survival and quality of life and to prevent vascular disease related complications, these procedures carry heightened risk of adverse perioperative cardiovascular events.

2. Background and Significance

Pathological and clinical studies have consistently demonstrated that abnormalities in thrombosis and hemostasis play a major role in the pathogenesis of atherosclerosis and atherothrombosis. Screening for abnormalities in thrombosis and hemostasis by measuring platelet activity, thrombin generation, markers of coagulation, and inflammatory biomarkers has been proposed to identify individuals at high-risk for cardiovascular events, however, it remains a research tool not ready for implementation in standard care. While many abnormalities in thrombosis and hemostasis have been widely studied in cardiac disease, there is a paucity of data in the setting of vascular surgery. It is unknown how vascular surgery affects different measurements of platelet activity, thrombin, coagulation and inflammatory markers. It is also unknown what the association of these markers is with perioperative and long-term cardiovascular and bleeding events.

Factors responsible for platelet activity are poorly understood. Recent advances in genomic and transcriptomic profiling technologies may offer the possibility of identifying novel biomarkers and pathways involved in platelet activity. Anucleate platelets lack genomic DNA, yet are known to retain a small amount messenger-RNA that can regulate protein expression, and have a diverse array of microRNA (miRNA). Comparison of platelet RNA and miRNA could potentially provide vital information on platelet function and disease states where platelet activity is affected, such as atherosclerosis. Combining descriptive analysis of platelet activity with novel RNA and DNA profiling may offer the unique opportunity to develop biomarkers for the diagnostic, prognostic, and predictive management of subjects undergoing vascular surgery.

The proposed study will add to the growing understanding of platelet activity and markers of coagulation in cardiovascular disease; examine a comprehensive battery of platelet activity markers, thrombin generation, markers of coagulation, and inflammatory biomarkers in subjects undergoing vascular surgery; and will provide important data on the mechanism of increased platelet activity using micro RNA, RNA and DNA expression profiling. The study design is prospective and the main outcome measures are platelet activity, coagulation markers and incident cardiovascular and bleeding events.

Previous research suggests that the incidence of dementia and Alzheimer's disease (AD) is higher in those with prevalent cardiovascular disease (CVD), and is also associated with increased platelet activity. Whether the platelet abnormalities are related to a common atherogenic process or rather represent different inflammatory pathways is unclear and needs to be examined. In addition to their role in CVD,

platelets have been implicated in the pathology of AD at several levels. First, abnormal platelet activation markers are frequently observed in AD patients when compared with age-matched cognitively normal controls. Additionally, results in experimental animal models and postmortem human brains show that platelet markers accumulate in perivascular lesions. Since platelets contain Aβ and tau, which are the components of amyloid plaques and neurofibrillary tangles found in AD lesions, it is possible that platelets are contributors to lesion formation at earlier stages of the disease.

We hypothesize that the platelet phenotypes (already measured) in patients with CVD are associated with cognitive decline and our preliminary studies suggest that this is the case. Using data from the Framingham Heart Study, we recently demonstrated that platelet aggregation in cognitively unimpaired middle age subjects is independently associated to the risk of incident dementia even after adjusting for potential confounders suggesting that the detection of platelet phenotypes in patients with CVD could be used for the detection of risk of cognitive decline in this population.

We designed the PACE clinical study initially to investigate platelet activity in PAD patients and the incidence of major adverse cardiac and limb events after lower extremity revascularization. PACE was set up to investigate the relationship between platelet activity and cardiovascular risk. Many studies suggest the overlapping nature of cardiovascular risk and dementia. Thus, we would like to extend findings that have established the link between heart disease and dementia to platelet activity and dementia. The comprehensive platelet characterization already performed would facilitate our study investigating the association between platelets and cognitive decline in this cohort of patients with established CVD. We will attempt to call all living participants and obtain telephone consent for the additional measure, assess vital status, and perform a validated survey of subclinical cognitive impairment in the PACE cohort with 2 previously validated neuropsychiatric tests that are frequently used in aging studies: TICS and Montreal Cognitive Assessment, MoCA. Both tests can be easily done remotely by trained personnel and are particularly good at detecting early subclinical impairment.

3. Specific Aims

Aim 1. To determine whether preoperative platelet activity measurements are independently associated with short-term cardiovascular events in PAD patients undergoing non-emergent lower extremity revascularization. We will characterize the platelet phenotype in 350 PAD patients before vascular surgery and use Cox proportional hazard models to determine the independent association of the platelet phenotype with risk of cardiovascular events in the first 30 days after surgery.

Aim 2. To determine whether postoperative platelet activity measurements are independently associated with long-term cardiovascular events in patients with established PAD. We will characterize the postoperative platelet phenotype following surgery and use Cox proportional hazards models to determine the independent association of the platelet phenotype with risk of long-term composite of myocardial infarction, stroke, or all-cause mortality with a mean follow-up of 2-years following vascular surgery.

Aim 3. To investigate mRNA-microRNA co-expression profiles in patients with and without elevated platelet activity measurements. We will establish the relationship between differentially expressed microRNAs and their target mRNAs related to platelet activity and thus identify new diagnostic markers and potential therapeutic targets of increased platelet activity.

Aim 4. To investigate whether the platelet phenotype of patients with PAD associates with measures of cognitive impairment. We will contact participants enrolled in PACE to conduct a screening test for cognitive function via telephone and/or video-call using the MoCA and/or TICS tests. We will correlate these measures with already collected platelet activity measures.

4. Research Design

a. Recruitment

350 non pregnant adults older than 21 years with peripheral artery disease undergoing planned lower extremity revascularization for lower extremity peripheral artery disease will be recruited from the inpatient setting at NYU Medical Center, Bellevue Hospital, the Manhattan VA, as well as the outpatient setting in the Faculty Group Practice. For the inpatients, health care providers, generally vascular surgeons and research coordinators will attempt to identify patients who present to one of the participating hospitals (mentioned above) and are scheduled to undergo vascular surgery. Once these patients are identified, one of the health care providers or a member of the research staff will make certain the patient fulfills the inclusion and exclusion criteria and will inform assigned health care provider who will obtain verbal consent for further contact by a member of the research staff prior to the surgery. For the outpatients, patients fulfilling inclusion and exclusion criteria will be identified by their health care professional, who will obtain verbal consent for contact by a member of the research staff. Vascular surgeons will not be involved in the consenting process due to their limited time but once consent for contact is obtained, research staff will approach subjects for consent of study participation. Subjects are not consented over the phone. Patients may be approached ahead of time to eliminate added stress of surgery and/or for ease of enrollment. The patient's vascular surgeon will contact patients by phone or in person prior to the surgery (e.g. pre-surgical testing or at the group practice), to obtain consent for contact by research staff and then the research staff would consent subjects in person. Only subjects that have the capacity to consent will be asked to consent. Subject's capacity is assessed by the research staff member performing the consent. The staff member will ask the subject if he/she understands the study procedures and consent form as well as answer subject questions to gauge capacity.

b. Informed Consent

If a subject wishes to participate, a member of the research team will review the informed consent documents, answer any questions, and obtain written consent for participation in the study and obtain

written consent for future analysis, contact, and sample storage. Stored samples will not undergo further genetic testing. Consenting will take place in the hospital (in the preadmission testing or in the pre-operative holding area) or in a private office (in the vascular surgery or medical practice). Subjects will be given the opportunity to be contacted for future studies that they may qualify for as well. All subjects will be given a copy of the consent. A member of the research team will conduct the baseline interview with the subject and review the clinical history, demographic information, diet questionnaire, lifestyle assessment, medication use and adherence, with the individual. A case report form is attached as an appendix. All information including the consent will be kept under double lock and key at the PI's office in NYU Skirball 9th floor

c. Methods

Following the interview and signed informed consent, subjects will have their blood drawn, and will be asked to provide a urine sample prior to surgery. Blood will be obtained from the patient using standard aseptic phlebotomy techniques. Blood will be collected from an arterial line placed for surgery (if available) or at the time of the intravenous line being placed for surgery or using a standard technique of blood drawing. A brief description of the standard technique follows: after cleansing of the venipuncture site with an alcohol wipe and removal of excess alcohol with sterile gauze, a tourniquet will be applied to the patient's bicep region. A 19G or 21G butterfly needle will be inserted into the antecubital vein, the tourniquet will be removed, and free-flowing blood will be collected with minimal trauma and stasis. The needle will be removed when blood collection is complete and sufficient pressure will be applied using sterile gauze at the puncture site until cessation of bleeding. A sterile band-aid will be applied to cover the venipuncture site. For each patient, no more than 60 ml (≈4 table spoons) of blood will be collected in gold (serum separator tube) top, lavender (EDTA anticoagulant) top, green (heparin), blue (sodium citrate anticoagulant) top and Pax gene tubes. Approximately 40 cc of blood will be used for the different measurements of platelet function, inflammation, coagulation and other biomarkers of cardiovascular risk and approximately 20 cc of blood will be used to purify platelet RNA, whole blood RNA and DNA and then stored in -80C for the RNA, microRNA, and DNA profiling. The samples will be placed in the PI's lab at NYU New Science Building- Cardiology 7th floor without any identifying information other than a code number. The code number will not be based on any information that could be used to identify the subject (for example, social security number, initials, birth date, etc). The master list linking names to code numbers will be kept in a locked file cabinet, separate from all research information in the PI's office at NYU Skirball 9th floor and will be accessed by the PI. The urine specimen will be collected to measure abnormalities in thrombosis, hemostasis and inflammation as well. At the end of the patient recruitment and measurement of platelet function (probably within 4 years time), based on ones level of platelet activity, thrombin generation, coagulation and inflammation we will measure RNA, microRNA, and DNA in patients with different phenotypes of vascular disease, markers of thrombosis/hemostasis, and events from the stored specimens.

There will be a follow-up component of this study that obtains blood and urine following surgery and at their first return visit following surgery. This will allow us to assess the change in platelet activity,

thrombin, coagulation and inflammatory markers during and following the perioperative period. This additional component would consist of a blood collection on postoperative day 2 (+/- 1 day). No more than 60 ml (≈4 table spoons) of blood will be collected at this time point. In addition, a blood collection will be obtained at the subjects' return visit following surgery to the vascular surgeon. No more than 60 ml (≈4 table spoons) of blood will be collected at this time point. The total blood drawn for the follow-up component would be no more than 120ml (8 tablespoons). This blood will be used for the different measurements of platelet activity, thrombin, coagulation and inflammatory markers. Data gathered from this component of the study will help understand the dynamic profile of biomarkers during and following the perioperative period.

Total amount of blood withdrawn will be no more than 180 ml (approximately 12 tablespoons or 40% of a standard blood donation). We believe this amount of blood is small and spread out over a month and therefore patient is at minimal risk. Blood collection at three different time points (before surgery, following surgery while still in the hospital, and at the subjects' first return visit to the vascular surgeon following surgery) will allow us to assess the dynamic change in platelet activity, coagulation and inflammation during the perioperative period. We believe that markers of clotting and bleeding will change during the course of surgery, and that some of these markers may be used to help predict the likelihood of developing a clotting or bleeding event following surgery. The long-term goal is to develop a clinically useful assessment of platelet activity, thrombin generation, coagulation and inflammation for risk stratification that may ultimately serve as a target for therapeutic intervention. The platform proposed will serve as a basis for future research and intervention, and hopefully, will have a great impact on subjects undergoing vascular surgery.

During surgery, clot and or part of the diseased vascular wall is sometimes removed. If available at the time of surgery, we will collect tissue samples that will be removed as a part of the surgery, which would otherwise be discarded. Preservation of this tissue does not involve additional risk to the subjects. A portion of this tissue will be preserved in formalin, and sectioned in order to study factors contributing to cardiovascular disease risk. Similar to the blood and urine collection, all samples will be stored under unique identifiers (as all samples taken in the study). Samples will be stored for up to 20 years. If subjects request the withdrawal of data and samples from the study, samples and all identifiers will be destroyed.

We *may* also implement an assessment of endothelial function prior to and following vascular surgery. Since measurement of peripheral vasodilator response as a measure for endothelial dysfunction is correlated with adverse cardiovascular events and measurements of this response using fingertip pulse amplitude tonometry (peripheral arterial tonometry-PAT) is a useful method for non-invasive assessment of vascular health. Peripheral arterial tonometry signals will be obtained using the EndoPAT 2000 device (Itamar Medical Inc., Caesarea, Israel), which has been validated and used previously to assess peripheral arterial tone in high-risk populations. This measurement would be done prior to and following surgery (same time points that blood is collected).

d. Genetic Profiling

The blood specimen will be analyzed upon completion of the study for mRNA, microRNA, and DNA profiling. We will perform detailed molecular profile study, describing genetic arrays with different phenotypes associated with platelet activity. We will be looking at specific transcripts associated with thrombotic and/or bleeding outcomes and different cardiovascular diseases. Platelet-rich plasma (PRP) will be isolated for platelet purification and subsequent genetic analysis will be performed. We will be analyzing those with high and low levels of platelet activity and other cardiovascular biomarkers as well as those with clotting/no clotting or bleeding/no bleeding. Biomarkers of cardiovascular disease will be shared as per NIH. The samples will be stored in the PI's lab, NYU New Science Building- Cardiology 7th floor, without any identifying information other than a unique code number. The code number will not be based on any information that could be used to identify the subject (for example, social security number, initials, birth date, etc.). The master list linking names to code numbers will be kept in a locked file cabinet, separate from all research information. Biomarker testing is separate from genetic testing. Genetic information will not be disclosed to anyone, including the participants and their physicians. Positive test result can be an indication that the individual may be predisposed to high platelet activity associated with peripheral vascular disease. There is no need for independent testing since patients are already diagnosed with this condition. No tests other than authorized shall be performed on the biological sample. Samples shall be destroyed at the end of the testing process.

e. Future Sample Storage

All patients will be offered an option to have their blood stored for future processing. If a patient does not agree to store his/her blood for future tests not related to this study, then his/her blood will be destroyed after all planned laboratory tests have been performed.

Blood samples will be assigned a unique code number and stored in the PI's lab without any identifying information other than a code number. The unique code number will not be based on any information that could be used to identify the subject (for example, social security number, initials, birth date, etc). The master list linking names to code numbers will be kept in a locked file cabinet, separate from all research information placed under double lock and key at the PI's office at NYU Skirball 9th floor. All confidential data will be stored with a unique code as an identifier and will be protected by a double electronic lock. All physical data will be kept under double physical lock. Access to the data will be given to the study personnel only.

Samples will be stored for no more than 10 years following completions of the study. Samples will be used for testing other potential biomarkers in patient plasma and/or serum related to other endpoints not specified in the protocol (e.g. cancer, metabolic disease, etc.). Genetic testing will not be conducted on stored samples. Results of the future research will not be shared with the subjects or their study doctor, and will not become part of the medical records.

Subjects can request for withdrawal of samples at any time by contacting PI Dr Berger in writing. His mailing address is Faculty Practice Tower 9 R, 530 First Ave NEW YORK 10016. Withdrawing

Authorization only affects uses and sharing of information after written request has been received, and subject may not withdraw his/her Authorization for uses or disclosures that have previously been made or must continue to complete analyses or report data from the research.

f. After Surgery and Follow Up

Starting on the day of surgery and continuing throughout hospitalization, a physician monitors the patient's course for clinically evident cardiovascular events (MI, stroke, TIA, major bleeding, kidney disease, or any surgical complication. Blood collections and/or ECG's that are performed as part of routine care and/or for study purposes will be recorded and may be used to help ascertain whether a cardiovascular and/or bleeding event has occurred. After hospital discharge, patients will be contacted by telephone (or during their routine clinical follow-up) at 30 days and every 6-month thereafter out to 5-years. At the patients' follow-ups they will be asked questions about their health, lifestyle, and medication use.

To ascertain vital status, we may use the social security index, medical record chart review, or contact the participant directly. If we learn that the participant is deceased, we may perform a chart review to check for any prior clinical diagnosis of cognitive impairment.

We will seek remote verbal re-consent for sub-clinical cognitive function testing (via TICS and/or MoCA test) from all living study participants. We will document in the study record whether a participant consents or declines to participate in the cognitive function testing. For participants who provide verbal consent, testing will be administered remotely by trained study personnel and will not require any in-person visits. If we identify sub-clinical signs of cognitive impairment based on testing, we will inform the patient and will offer them the possibility of writing a letter to their primary healthcare provider.

Participants with existing mild cognitive impairment: Given the advanced age of study participants, we will first ask participants if they have ever been diagnosed with dementia (including Alzheimer's) by their healthcare provider and record their response in the study record. In collaboration with the PI, our neuroscience experts, and our senior research coordinator, we will assess decision-making capacity while talking to the participant. If the participant has sufficient capacity to consent, we will proceed with seeking verbal consent for cognitive function testing. If the participant declines cognitive function testing but gives permission for us to record their history of cognitive impairment, we will record this information in the study record. If the participant appears unable to provide verbal consent, they will not undergo cognitive function testing.

g. Inclusion Criteria

1. Subjects undergoing non emergent lower extremity revascularization*
2. Age > 21 years of age

3. Able and willing to provide written informed consent for the study

*Subjects who participated in this trial may be re-enrolled if they undergo another lower extremity revascularization >90 days after the original procedure. *This modification is being done to increase enrollment and because the major aim is to look at peri-procedural events and thus would not interfere with the overall goal of the study.*

h. Exclusion Criteria

1. Use of any NSAID (ibuprofen, naproxen, etc.) within 72 hours
2. Thrombocytopenia (platelet count<100) or Thrombocytosis (platelet count>500)
3. Anemia (hemoglobin<8)
4. Any known hemorrhagic diathesis

Subjects will be re-enrolled and contacted the same way any subject is enrolled in the study. If the subject is undergoing a lower extremity revascularization procedure and they are referred to the study by a health care professional taking care of the subject, they will be approached by a member of the research team. This does not give a benefit to the patient other than to help answer an important scientific question. This poses no additional risk to the patient.

i. Endpoint Assessments

At every visit after randomization, the study coordinator will ask participants if they have had any symptoms or a report from a healthcare provider consistent with an endpoint event since the last study visit. If at any time the subject refuses to continue with follow-up, every attempt will be made to continue contact by written communication, email, by proxy contact with family, friends, or allied health care providers, or record review to determine if outcome events have occurred, unless the subject specifically refuses such follow-up. An independent clinical event adjudication committee (CEC) will review and adjudicate primary and selected secondary endpoint events in a blinded fashion based on study definitions.

5. Potential Risks and Benefits

The potential risks of performed phlebotomy are pain, bruising, fainting, or small infection at puncture site. The use of the blood for future sample storage and genetic testing raises special issues of confidentiality. Genetic testing can generate information about a subjects' personal health risks and can cause or increase anxiety, damage family relationships, and/or compromise insurability, employability and can even lead to discrimination. All confidential data will be stored with a unique code as an identifier and will be protected by a double electronic lock. All physical data will be kept under double physical lock. Access to the data will be given to the study personnel only thereby greatly reducing the possibility of psychological or social risks that could arise from knowledge of this genetic information, such as risk for employability or insurability or the risk of discrimination.

Additionally, there is a potential risk for a breach of confidentiality for all confidential data. All data will be stored with a unique code that does not identify the subject. Furthermore, data will be saved using a double electronic lock. All physical data will also be kept under double physical lock. Access to the data will be given to the study personnel only.

There are no known risks associated with endothelial function assessment. The blood pressure cuff on the forearm may rarely cause pain or bruising. If a subject experiences pain, the cuff will be deflated.

5.1 Benefits

The proposed study will examine a panel of markers related to platelet function, inflammation, and coagulation and assess their ability to predict cardiovascular events following vascular surgery. The long-term goal is to develop a clinically useful assessment of platelet activity for risk stratification that may ultimately serve as a target for therapeutic intervention. The platform proposed will serve as a basis for future research and intervention, and hopefully, will have a great impact on subjects undergoing vascular surgery. There might be no direct benefit to enrolled subjects. The cognitive function follow-up screening will act as another clinically valuable measure of risk predicted by platelet activity. We aim to use the information as future clinical research goal and will not provide direct benefit to patients.

6. Cost

There is no cost or compensation to the study participants. There is also no direct benefit expected from the participation in this study. This study will provide potentially valuable information with minimal risk to study subjects. It is hoped that knowledge gained will be of benefit to others in the future.

7. Screening Log

During the study enrollment period, sites may collect de-identified data on all patients undergoing lower extremity revascularization. The goal of this effort will be to describe the characteristics of patients who are screened but not enrolled and to document the major reasons for exclusion. Examples of data elements to be collected, when available, include: (age [recorded for patients <90 years of age, recorded as 90 if >90 years of age], sex, race/ethnicity, basic medical history from medical chart (if available), in-hospital events (if available from the chart) and, if excluded from the study, reason(s) for exclusion will be recorded). Importantly, only de-identified health information will be recorded and stored in the PI's office at NYU Skirball 9th floor, under double physical lock and key.

8. Statistical Analysis

Power and sample size analysis was performed to investigate appropriate sample size for evaluating platelet activity markers in subjects with cardiovascular disease CVD versus age- and sex matched controls. The study is powered based on the analysis of association between platelet activity and cardiovascular events. Based on published and preliminary data, we expect the 30-day event rate to be 22%. The relationship between platelet activity and short- term cardiovascular events will be assessed using 2 co-primary measures: LTA and MPA. Based on preliminary data, LTA levels have skewed distribution and MPA levels are normally distributed. Consider a transformation (e.g. log- or Box-Cox) on LTA measures that achieves Normality. With 200 participants, two-sided Wilcoxon test will have 80% power at the 0.025 significance level to detect an association between platelet reactivity and short-term events corresponding to a 0.5*standard deviation difference in platelet

8.1 Data Analysis

All eligible subjects will be assigned a unique identification number upon enrollment in the study. Initial study data will be recorded on a printed form, which will be later converted to an IRB approved electronic database, REDCap. Only study personnel will have access to this database. All potential identifiers will be kept separately in a safe location at the PI's office in NYU Skirball 9th floor, where it will be placed under double lock and key and the PI will have access. After verification of entered data at completion of the study all potential identifiers will be stripped from the database.

Descriptive statistics, including frequency tables, measures of center and variability, and graphical displays will present baseline subject characteristics. Similar analyses will present values of platelet activity measures.

Primary Platelet Activity Measures: LTA and MPA To test the hypothesis that platelet activity measurements are independently associated with 30-day (Aim 1) and long-term (Aim 2) cardiovascular events in patients undergoing lower extremity revascularization we will employ the following analysis. For Aim 1, platelet activity will be measured preoperatively and the main outcome will be 30-day cardiovascular events. For Aim 2, platelet activity will be measured 48 hours postoperatively and the main outcome will be long-term (mean follow-up ~ 2 years) cardiovascular events. We will perform univariate analysis to investigate the association between LTA and MPA platelet activity measures and cardiovascular events. Next, we will examine differences in sociodemographic/clinical factors between participants with and without a cardiovascular event using two-sided tests of proportions for categorical variables (chi-square, Fisher's exact) and t-tests or non-parametric alternatives (e.g., Wilcoxon test) for continuous measures.

The analysis plan outlined above for the primary platelet activity measures will be applied to the set of secondary measures including thrombin. In addition, the analysis plan outlined above for the primary outcome measures will be applied to the secondary outcome measures including major bleeding.

To identify novel mRNA-miRNA target pairs involved in platelet activation, we will computationally inversely correlate miRNA expression with expression of gene targets using established prediction databases

8.2 Data Safety Monitoring Plan

There are no primary or secondary endpoints. Patients undergoing vascular surgery as part of their routine clinical care will have a blood collection before and after surgery to help identify future markers in their blood related to clotting and bleeding episodes. The proposal is deemed to be low risk for subjects. Safety information will be collected via telephone at 30 days after the surgery, case report form, and/or scheduled follow-up visits as part of standard of care.

PI will conduct interim monitoring of accumulated data from research activities to assure the continuing safety of participants, relevance of the study question, appropriateness of the study, protocol compliance and integrity of the accumulating data. The PI in conjunction with NYU IRB will be responsible for monitoring the data and conducting safety reviews at a minimum of every 12 months when annual re-approval is sought. Any unanticipated serious adverse event will be reported to IRB within 72 hours. During the review process, the PI and NYU IRB will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment.

9. Confidentiality:

Study is for research purposes only. Individual result will not be given back to study participant. This will include information from final results of the study, interim results of the study and incidental findings. Research result will never occur in medical records. Confidentiality will be preserved to the fullest extent by the research team. All data will be stored with a unique code that does not identify the subject. Furthermore, data will be saved using a double electronic lock. All physical data will also be kept under double physical lock. Access to the data will be given to the study personnel only.

10. Appendix A: <u>Schedule of study related procedure</u>

	Pre-op Period	POD 2 (+/- 1 day)	Discharge	Post-op Appointment follow-up	Long-term follow-up
Eligibility	X				
Informed consent	X				
Demographics	X				
Medical history/status	X	X	X	X	X
Medication use	X	X	X	X	X
Vital signs	X	X	X		
Standard lab results	X	X	X	X	X
Troponin	X	X			

ECG	X	X			
Blood draw	X	X		X	
Urine sample	X			X	
Endothelial dysfunction assessment	X	X		X	
QOL assessment	X			X	X
Diet questionnaire	X				
Lifestyle assessment	X			X	X
Medication adherence	X			X	X
Hospitalization assessment			X	X	X
Endpoint assessment		X	X	X	X
Cognitive Function Test					X

1. Pre-operative period
   1.1. Determine eligibility
   1.2. Obtain informed consent
   1.3. baseline interview with the subject and review the clinical history, demographic information, lifestyle assessment, QOL assessment, medication use and adherence
   1.4. Vital signs
   1.5. Obtain ECG
   1.6. Measure troponin
   1.7. Urine sample
   1.8. Blood sample
   1.9. Endothelial function assessment

2. Post-operative day # 2 (+/- 1 day):
   2.1. review medical status
   2.2. Medication use
   2.3. Vital signs
   2.4. Review standard lab tests
   2.5. Obtain ECG
   2.6. Measure troponin
   2.7. Blood sample
   2.8. Endothelial function assessment
   2.9. Endpoint assessment

3. Day of discharge
   3.1. review medical status

3.2. Medication use
3.3. Vital signs
3.4. Review standard lab tests
3.5. Hospitalization assessment
3.6. Endpoint assessment

4. Post-op Appointment follow-up):
   4.1. review medical status
   4.2. Medication use and adherence
   4.3. Review standard lab tests
   4.4. Blood sample
   4.5. Urine sample
   4.6. Endothelial function assessment
   4.7. Lifestyle and QOL assessment
   4.8. Hospitalization and assessment
   4.9. Endpoint assessment

5. Long term follow up (q 6months [+/- 2 months]):
   5.1. review medical status
   5.2. Medication use and adherence
   5.3. Review standard lab tests
   5.4. Lifestyle and QOL assessment
   5.5. Hospitalization and assessment
   5.6. Endpoint assessment
   5.7. Cognitive function test (will be assessed once)
   5.8. Question regarding history of dementia (will be assessed once)
   5.9. Vita status assessment

11. References:

1. Berger JS, Hiatt WR. Medical Therapy in Peripheral Artery Disease. Circulation 2012;126:491-500. PMID: 22825411
2. Berger JS, Becker RC, Kuhn C, Helms MJ, Ortel TL, Williams R. Hyperreactive platelet phenotypes: Relationship to altered serotonin transporter number, transport kinetics and intrinsic response to adrenergic co-stimulation. *Thromb Haemost*. 2013;109:85-92. PMID: 23223800
3. Chu S, Becker RB, Berger PB, Bhatt DL, Konkle B, Mohler ER, Reilly M, Berger JS. Mean Platelet Volume as a Predictor of Cardiovascular Risk. *J Throm Haemost* 2010;8:148-156. PMID: 19691485
4. Devereaux PJ, Goldman L, Yusuf S, Gilbert K, Leslie K, Guyatt GH. Surveillance and prevention of major perioperative ischemic cardiac events in patients undergoing noncardiac surgery: a review. CMAJ. 2005;173:779-788.
5. Eikelboom JW, Hankey GJ, Thom J, Bhatt DL, Steg PG, Montalescot G, Johnston SC, Steinhubl SR, Mak KH, Easton JD, Hamm C, Hu T, Fox KA, Topol EJ. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid: determinants and effect on cardiovascular risk. Circulation. 2008;118:1705-1712.
6. Eraso LH, Fukaya E, Mohler ER III, Xie D, Sha D, Berger JS. Peripheral Arterial Disease, Prevalence and Cumulative Risk Factor Profile Analysis. *Eur J Prev Cardiol* 2012; [Epub ahead of print] PMID: 22739687
7. Gawaz M. Platelets in the onset of atherosclerosis. Blood Cells Mol Dis. 2006;36:206-210.
8. Gurbel PA, Becker RC, Mann KG, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. J Am Coll Cardiol. 2007;50:1822-1834.
9. Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, Hiratzka LF, Murphy WR, Olin JW, Puschett JB, Rosenfield KA, Sacks D, Stanley JC, Taylor LM, Jr., White CJ, White J, White RA, Antman EM, Smith SC, Jr., Adams CD, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Hunt SA, Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of

Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. Circulation. 2006;113:e463-654.

10. Michelson AD. Methods for the measurement of platelet function. Am J Cardiol. 2009;103:20A-26A.
11. Rajagopalan S, McKay I, Ford I, Bachoo P, Greaves M, Brittenden J. Platelet activation increases with the severity of peripheral arterial disease: implications for clinical management. J Vasc Surg. 2007;46:485-490.
12. Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, et al. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. European Heart J 2010;31:1142-8.
13. Schneider G, Rockman C, Merolla M, Nardi M, Hu L, Berger J. Platelet Activation Increases in Patients Undergoing Vascular Surgery. J Am Coll Cardiol 2012;59:1699.
14. Sharma G, Berger JS. Platelet Activity and Cardiovascular Risk in Apparently Healthy Individuals: A Review of the Data. J Thromb Thrombolysis 2011;32:201-8. PMID: 21562837
15. Trip MD, Cats VM, van Capelle FJ, Vreeken J. Platelet hyperreactivity and prognosis in survivors of myocardial infarction. N Engl J Med. 1990;322:1549-1554.
16. Yee DL, Bergeron AL, Sun CW, Dong JF, Bray PF. Platelet hyperreactivity generalizes to multiple forms of stimulation. J Thromb Haemost. 2006;4:2043-2050.
17. Newman, A. B. et al. Dementia and Alzheimer's disease incidence in relationship to cardiovascular disease in the Cardiovascular Health Study cohort. J Am Geriatr Soc 53, 1101-1107, doi:10.1111/j.1532-5415.2005.53360.x (2005).
18. Rafnsson, S. B., Deary, I. J. & Fowkes, F. G. Peripheral arterial disease and cognitive function. Vasc Med 14, 51-61, doi:10.1177/1358863X08095027 (2009).
19. Gul, F. & Janzer, S. F. in StatPearls (2021).
20. Roher, A. E. et al. Intracranial atherosclerosis as a contributing factor to Alzheimer's disease dementia. Alzheimers Dement 7, 436-444, doi:10.1016/j.jalz.2010.08.228 (2011).
21. Gottesman, R. F. et al. Association of Intracranial Atherosclerotic Disease With Brain beta-Amyloid Deposition: Secondary Analysis of the ARIC Study. JAMA Neurol 77, 350-357, doi:10.1001/jamaneurol.2019.4339 (2020).
22. Sevush, S. et al. Platelet activation in Alzheimer disease. Arch Neurol 55, 530-536, doi:10.1001/archneur.55.4.530 (1998).
23. Cortes-Canteli, M., Zamolodchikov, D., Ahn, H. J., Strickland, S. & Norris, E. H. Fibrinogen and altered hemostasis in Alzheimer's disease. J Alzheimers Dis 32, 599-608, doi:10.3233/JAD-2012-120820 (2012).
24. Zamolodchikov, D. et al. Biochemical and structural analysis of the interaction between beta-amyloid and fibrinogen. Blood 128, 1144-1151, doi:10.1182/blood-2016-03-705228 (2016).

25. Zamolodchikov, D., Renne, T. & Strickland, S. The Alzheimer's disease peptide beta-amyloid promotes thrombin generation through activation of coagulation factor XII. J Thromb Haemost 14, 995-1007, doi:10.1111/jth.13209 (2016).
26. Mezzapesa, A. et al. Increased levels of the megakaryocyte and platelet expressed cysteine proteases stefin A and cystatin A prevent thrombosis. Sci Rep 9, 9631, doi:10.1038/s41598-019-45805-9 (2019).
27. Elaib, Z. et al. Platelet Functions are Decreased in Obesity and Restored after Weight Loss: Evidence for a Role of the SERCA3-Dependent ADP Secretion Pathway. Thromb Haemost 119, 384-396, doi:10.1055/s-0038-1677033 (2019).
28. Morel-Kopp, M. C. et al. The association of depression with platelet activation: evidence for a treatment effect. J Thromb Haemost 7, 573-581, doi:10.1111/j.1538-7836.2009.03278.x (2009).
29. Burkhart, J. M. et al. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. Blood 120, e73-82, doi:10.1182/blood-2012-04-416594 (2012).
30. Kniewallner, K. M., Foidl, B. M. & Humpel, C. Platelets isolated from an Alzheimer mouse damage healthy cortical vessels and cause inflammation in an organotypic ex vivo brain slice model. Sci Rep 8, 15483, doi:10.1038/s41598-018-33768-2 (2018).
31. Drummond, E. et al. Proteomic differences in amyloid plaques in rapidly progressive and sporadic Alzheimer's disease. Acta Neuropathol 133, 933-954, doi:10.1007/s00401-017-1691-0 (2017).
32. Pomara, N. & Murali Doraiswamy, P. Does increased platelet release of Abeta peptide contribute to brain abnormalities in individuals with depression? Med Hypotheses 60, 640-643, doi:10.1016/s0306-9877(02)00380-8 (2003).
33. Allen, N. et al. Circulating monocyte-platelet aggregates are a robust marker of platelet activity in cardiovascular disease. Atherosclerosis 282, 11-18, doi:10.1016/j.atherosclerosis.2018.12.029 (2019).
34. Newman, J. D. et al. Gene Expression Signature in Patients With Symptomatic Peripheral Artery Disease. Arterioscler Thromb Vasc Biol 41, 1521-1533, doi:10.1161/ATVBAHA.120.315857 (2021).
35. Dann, R. et al. Platelet-Derived MRP-14 Induces Monocyte Activation in Patients With Symptomatic Peripheral Artery Disease. J Am Coll Cardiol 71, 53-65, doi:10.1016/j.jacc.2017.10.072 (2018).