SYSTEMIC INFLAMMATORY DETERMINANTS OF LOWER EXTREMITY REVASCULARIZATION FAILURE

By

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To My Family – Janice, Max, and PJ – for all
their understanding and support during this process.

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Peripheral arterial disease (PAD) affects 10 million Americans. Many can be managed initially with risk factor modification, but for more advanced disease, lower extremity vein bypass grafting and percutaneous angioplasty/stenting are the mainstays of treatment. Technical success for these procedures is high, but their durability remains a vexing problem. Based on the developing association between inflammation and PAD, we hypothesized that the difference between success and early failure of lower extremity revascularization is dependent upon, and perhaps predicted by, the status of baseline immunity and/or the magnitude of the early post-surgical systemic inflammatory response.

Our research team comprises a multidisciplinary collaboration of clinicians, scientists, and statisticians. To study the relationship between systemic inflammation and clinical outcome, we developed a human translational functional genomic/proteomic initiative to study 300 symptomatic patients with PAD undergoing lower extremity revascularization with either angioplasty/stenting or vein graft bypass. This report focuses on the high throughput proteomic analysis of circulating cytokines as the initial step to characterize the perioperative systemic inflammatory profile. We assayed plasma cytokine concentrations taken at 7 time points: pre-operatively, at two hours and one day post-procedure, and then at 1 week, and 1, 6, and 12

months of follow-up - using the Luminex-100® 22-plex bead immunoassay system. We then analyzed normalized proteomic data over time, by type of procedure, and according to success or failure of the intervention. Significant time-dependent differences in cytokine profiles were seen for the entire study group following either treatment. The specific patterns of cytokine expression differed significantly between the two treatment options – angioplasty versus bypass. Finally, cytokine expression patterns were also significantly different between subjects with a successful intervention versus those that went on to failure. Representative examples of cytokine expression (IL-6, IL-8, IFNγ, TNFα, IP-10) are presented.

These findings indicate that alterations in both baseline immunity and the systemic inflammatory response to surgery, as evidenced by significant changes in circulating inflammatory cytokine concentrations, occur in PAD patients following lower extremity revascularization. Furthermore, we were able to identify cytokine expression profiles that were distinct between the two types of procedures with an early peak of lesser intensity for angioplasty/stenting, and a higher more persistent elevation for vein bypass. In addition, we were also able to discern distinct patterns of cytokine expression that correlated with the clinical outcome of revascularization. Taken together, these data serve as proof of principle for the development of class prediction models capable of forecasting success or failure of intervention based on the early components of the inflammatory response.

Ultimately, the combination of these proteomic data, together with genomic studies and clinical, functional, and quality of life outcome measures, will lead to new knowledge about the mechanisms of failure of vascular interventions and new strategies to improve approaches to lower extremity revascularization.

CHAPTER 1
INTRODUCTION

Clinical Relevance

Cardiovascular disease affects over 40 million Americans and remains the leading cause of death. Peripheral arterial disease (PAD) affects over 10 million people in the United States and, among this group, over 1 million arterial reconstructions are performed annually. As the population ages, continues to smoke, and suffers from an epidemic of metabolic syndrome and obesity, that number is increasing exponentially.[1] Although researchers have made significant progress in the understanding and treatment of coronary artery atherosclerosis, the same cannot be said for lower extremity PAD. As a result, outcomes following lower extremity revascularization for PAD continue to be disappointing. Conventional wisdom suggests 5-year patency rates (length of time the revascularization remains open without reintervention) of 60 to 80% for vein bypass grafting,[2-7] but more curent information suggests a 1-year primary patency rate of only 61%.[8] Outcomes are less well defined for angioplasty/stenting, but primary patency rates of 70 to 90% at 3 months that drop to 20 to 50% at 1 to 3 years have been described.[9-11] Furthermore, these results are continually being scrutinized in the context of ~80% symptomatic improvement in patients with intermittent claudication treated with conservative measures (i.e., smoking cessation, risk factor modification, and structured exercise).[12-14] Additionally, studies report poor functional and quality of life outcomes despite successful revascularization following vein bypass surgery.[15,16] Unfortunately, many aspects of the disease process of lower extremity PAD and its response to treatment are poorly understood. Clinicians need a better understanding of the arterial response to angioplasty, the vein graft response to arterial hemodynamics, and, finally, what metrics constitute the definition of success or failure of such interventions. Consequently, without a defined evidence-based approach to symptomatic lower extremity PAD,

practitioners frequently make management decisions without clear guidance of how to individualize the treatment to optimize patient outcomes.[17-19]

Role of Inflammation in Lower Extremity Arterial Disease

Overview

Basic research has characterized a role for inflammation in directing local responses to vascular injury at the time of intervention.[20] Such studies have established that temporally distinct blood vessel wall inflammatory events predict long term wall architecture,[21-24] and have confirmed that physical forces stand as the primary regulator of local vascular wall behavior.[25,26] For example, animal studies, have defined a specific causal links between tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) and intimal hyperplasia leading to vein graft failure. In addition, treatment with IL-10, an anti-inflammatory cytokine predicted to have a protective effect, fails to prevent intimal hyperplasia because it is downregulated in the same vein grafts. A critical link between these local events and systemic inflammation comes from the observation that vascular injury, as occurs with angioplasty/ stenting or vein bypass, results in the acute recruitment and adhesion of monocytes.[26]

Prior work in our labaoratory provides another example of this critical link between systemic inflammation and, in this case, therapeutic approaches to intimal hyperplasia. Thrombomodulin (TM) is an endogenous endothelial cell surface protein that inhibits the activity of thrombin, the primary mediator of hemostasis and clot formation at sites of vascular injury. Our work demonstrated that thrombin was a potent stimulus for vascular smooth muscle cell proliferation and migration, two key processes in the development of intimal hyperplasia. We went on to show that TM could inhibit these important processes through direct thrombin inhibition and inhibition of its downstream signaling pathways including G-protein-coupled receptor signaling, mitogen-activated protein kinase pathways, and intracellular calcium

trafficking in vitro.[27] Next, therapeutic application of TM in vivo, in a rabbit femoral artery injury model, showed significant inhibition of intimal hyperplasia and restenosis when TM was delivered intravenously.[28] However, subsequent attempts at more sophisticated local delivery including intrarterial infusion, adenoviral vector mediated in vivo transfection, in vivo electroporation, and development of a transduction recombinant TM protein all failed to reproduce the inhibition of intimal hyperplasia. [unpublished data] Reflection on these findings led us to conclude that systemic administration of TM likely produced its effect, not through local influences in the arterial wall, but through inhibition of systemic inflammation perhaps through an activated protein C dependent mechanism. Retrospective review of histology specimens supported this theory (Figure 1-1). Thus, the focus of this current investigation is the role of systemic inflammation in local restenosis and intervention failure.

C-Reactive Protein as a Potential Biomarker

Similar to our experience, other researchers over that last decade have shifted away from a focus on local mediators at sites of vascular injury as the cause of restenosis and intervention failure. Current theory holds that the blood vessel response to injury may be intimately linked to the host's systemic inflammatory response, and that intervention failure may be driven by these systemic factors.[29-34] Therefore, researchers have focused efforts on identifying a predictive, clinically useful, circulating biomarker closely associated with vascular disease. In patients with coronary atherosclerosis, some such global associations have been established. C-reactive protein (CRP) is a marker of systemic inflammation which has been associated with a history of myocardial infarction and prediction of future coronary events.[35-37] In PAD, however, results are conflicting as to whether these same biomarkers predict progression of PAD or have any relationship to the success of failure of its treatment. Some studies looking at CRP, often in combination with other potential biomarkers including fibrinogen, D-dimer, homocysteine, or

inflammatory cell adhesion molecule (ICAM)-1, have only been able to show a simple association with the presence of PAD. Most of the patient events described in these studies, however, reflect the relationship of CRP with coronary atherosclerosis. Furthermore, these and other studies have failed to define a consistent relationship between CRP and the pathogenesis of PAD, the severity of PAD, the progression of PAD, or the response to treatment for PAD.[38-40] One recent study suggested an association between pre-operative CRP levels and lower extremity vein bypass failure, however, the predominant association was again seen with post-operative coronary events.[41] Finally, none of these studies has serially studied CRP levels over time, nor have they incorporated CRP in any way into a system-wide approach to proteomic profiling.

Conclusions

Despite these important findings, decades of focus on local vascular wall events have failed to yield substantial progress toward more durable peripheral interventions,[8,19,30,42] and no predictive systemic biomarkers have emerged. These deficiencies likely reflect that, due to the complex and redundant nature of the innate immune response, characterization of a predictive systemic inflammatory profile may best be accomplished using a genome-wide transcriptome and/or system-wide multiplex proteomic approach.

Functional Genomic/Proteomic Application to Human Vascular Disease

Overview

A paradigm shift has occurred recently, away from the focused study of local factors in the vessel wall towards the study of the influence of systemic inflammation on these local events that ultimately lead to revascularization failure. This new approach has been fueled in part by a broad based human initiative taking advantage of advances in the sequencing of the human genome and the development of high throughput genomic and proteomic analyses. This

approach opens further avenues of discovery. Researchers can start by analyzing gene expression profiles or molecular signatures in the interactome that are predicitive of successful or unsuccessful clinical outcomes. They can then identify single nucleotide polymorphisms (SNPs) with association to intervention failure. They can then use this information as reference for pharmacogenomic surveillance of the efficacy of anti-inflammatory treatments. Finally, multidisciplinary groups can apply these complex genotype-environmental interactions into systems biology approaches to predict outcome.[43] In this way, investigators are empowered to translate changes in basic building blocks (i.e., gene sequence), to changes in gene function (i.e., functional genomics, protein expression), and finally to changes in organ function or clinical phenotype (physiological genomics).[44]

Current Evidence

Genomics

Few studies have applied these methods to patients with symptomatic lower extremity PAD.[45,46] What is available is a number of observational studies, primarily genomic, that have linked a putative single nucleotide polymorphism (SNP) with some aspect of cardiovascular disease – most commonly hypertension or heart failure, or the response to a particular pharmacologic intervention. Genes associated with cardiovascular disease in these studies include myocyte enhancer factor-2 (MEF2A),[47] connexin 37 gene in men, PAI-1 and stromelysin genes in women,[48] 5-lipoxygenase activating protein,[49] leukotriene A4 hydrolase,[50] lymphotoxin-α gene,[51] HMG-CoA reductase and ADAMTS-1 metalloproteinase in statin therapy,[52,53] ß-adrenergic receptors with ß-blockade response,[54] and CYP2C9 and vitamin K epoxide reductase-1 in warfarin therapy.[55,56] The limitations to these studies and their findings lie in the fact that they often offer little biological or functional linkage from the specific gene to the disease process studied. Furthermore, they are single institution observational studies with no

subsequent confirmatory studies, validation, or intervention.[57,58] These underappreciated limitations emphasize the importance of a systems-wide approach to define genomic signatures and pattern recognition of genomic classifiers, with validation coming from the application of such classifiers to other populations (e.g., to related patient cohorts or between similar cohorts in a multicenter study design).[43]

Proteomics

Little information is available with respect to functional proteomics in vascular disease. Most of what is available is from in-vitro, ex-vivo or animal models studying small numbers of select proteins or protein families.[59] In one such example, 19 proteins were identified that were consistently overexpressed in a rat model of carotid artery stenosis, but these proteins correlated poorly with the parallel gene expression data analyzed.[60] High throughput proteomic technology (including 2D gel electrophoresis, high performance liquid chromatography, and tandem mass spectroscopy) is still in evolution and lags behind its genomic gene array technology counterpart,[61] but holds promise for future application to human clinical disease.[62] Complexity in post-translational modification of many proteins poses additional challenges to the reproducibility of the results from these methodologies.[45] For now, we are left with studies, similar to those described above, looking for single, predicitive biomarkers that characterize vascular disease.[63]

Clinical Trial Application

The CardioGene Study is an example of an ongoing investigation using comprehensive high throughput genome-wide molecular approaches to study clinical restenosis in bare metal stents used in the treatment of coronary artery disease.[64] The goal is to identify genetic determinants or predictors of inward remodeling and in-stent restenosis to explain the dichotomous outcome of failure following percutaneous coronary intervention. The study is a

collaborative initiative between the NHLBI and two clinical sites in the US, and plans to enroll 350 patients. Blood is sampled pre-intervention and then again at 2 weeks and 6 months following intervention. Clinical endpoints include symptomatic restenosis at 6 months and at 12 months. Genomic studies are performed on circulating leukocytes and mononuclear cells using the Affymetrix U133A GeneChip™ platform. Plasma proteomic studies are performed using multidimensional liquid chromatography and tandem mass spectroscopy. The investigators' initial focus is on gene regulatory regions and transcriptomes associated with modulation of gene expression. They are then planning a secondary genome-wide analysis to identify genes or clusters of genes related to in-stent restenosis and unfavorable outcomes. This will include investigation of candidate SNPs linked to stent failure. The investigators then plan a complex bioinformatics approach to define genomic biomarkers that would allow risk-stratification prior to intervention and may lead to development of new techniques to prevent coronary stent restenosis and failure. Results from this trial are not yet available, but are eagerly awaited due to the parallel nature of our study design for application of these methodologies to failure following lower extremity revascularization.

Summary

Overall, lower extremity PAD is a poorly understood disease lacking predictors for the arterial response to treatment such as angioplasty or vein bypass and therefore the metrics to define success or failure of such an intervention. Consequently, we currently make management decisions in patients with symptomatic lower extremity PAD based on soft criteria without a clear understanding of how to fit the treatment to optimize a patient's outcome.[17,18] Through advanced high throughput genomic and proteomic analyses, we plan to identify molecular evidence that a differential inflammatory response to vascular injury contributes to intervention failure and poor clinical outcomes. This will form the basis for what is currently unavailable –

an evidence-based approach to peripheral intervention and revascularization for symptomatic lower extremity PAD.

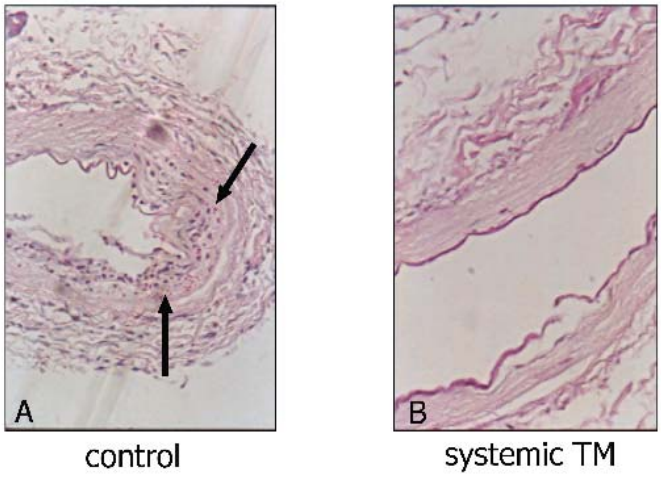

Figure 1-1. Effect of thrombomodulin (TM) on arterial inflammation following balloon angioplasty. An anti-inflammatory effect was only seen with systemic administration (B) compared to either control (A, dense inflammation indicated by arrows) or with local delivery (not shown).

CHAPTER 2
MATERIALS AND METHODS

Overall Study Design

Overview

A five-year study is underway in which 300 patients undergoing evaluation for symptomatic peripheral arterial disease (PAD) will be enrolled. The study cohort will be comprised of 50 patients treated with medical management alone to serve as a control group, 125 patients undergoing additional lower extremity angioplasty/stenting, and 125 patients undergoing additional lower extremity vein bypass (Figure 2-1). Data are collected prospectively with longitudinal evaluation to determine success or failure of the intervention with corresponding quality of life (QOL) measures. In parallel to the clinical assessment, blood sampling is performed for high throughput genomic and proteomic analyses (Table 2-1). Bioinformatics tools are then applied to reconcile the molecular data with clinical outcomes to arrive at molecular profiles that correspond to success or failure of intervention. All study patients sign informed consent under an Institutional Review Board approved protocol. An Access™ database (HIPAA-defined "limited data set") is currently in use to collect and store all study data.

Patient Selection

Eligible patients were identified amongst all patients being evaluated for symptomatic PAD in our current vascular surgical practice. The summary of specific inclusion and exclusion criteria are listed in Table 2-2.

Clinical Protocol

Evaluation of patients for symptomatic PAD follows current standards of practice.[65-67] Generally, patients with severe claudication or critical limb ischemia (Rutherford Grade I

Category 3 level disease or greater)[65] are considered candidates for arterial revascularization. In an effort to optimize patient outcomes[68,69] and standardize patients with respect to medications that likely influence systemic inflammatory response profiles, all patients are placed on antiplatelet therapy (at least 81 mg ASA daily) and statin (HMG-CoA reductase inhibitor) therapy (at least atorvastatin 10 mg daily). Statin therapy is adjusted according to the recent National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III revised national guidelines and recommendations.[70] Those with an LDL cholesterol level <70mg/dL will be maintained on 10 mg atorvastatin daily except in patients with documented intolerance to statins.

Patients requiring intervention then undergo lower extremity arteriography with treatment decisions guided by the TransAtlantic Inter-Society Consensus (TASC) I and II recommendations.[65,67] For patients undergoing percutaneous intervention, primary angioplasty is the preferred initial approach for superficial femoral and popliteal artery stenoses with subintimal recanalization and angioplasty for chronic total occlusions.[71-73] Primary angioplasty is also performed for infragenicular tibial artery stenoses or occlusions in patients with critical limb ischemia.[74-76] Selective stenting is indicated for unacceptable results following angioplasty (e.g., significant lesion recoil with residual stenosis or flow limiting dissection).[77] For patients undergoing vein bypass surgery, a non-reversed anatomic bypass using ipsilateral great saphenous vein is the approach of choice with other alternatives considered as the specific case warrants. No patients requiring synthetic bypass are eligible for this study. All patients are placed on an antiplatelet regimen consisting of ASA 81 mg daily with the addition of clopidogrel 75 mg daily for 30 days in patients undergoing angioplasty/stent procedures. Warfarin is

initiated for bypass grafts with compromised outflow,[78] and continued in all patients with preexisting indications.

The 50 planned control patients will have pre-operative blood sampling only at the time of evaluation for revascularization. If there is no indication for revascularization at that time, they will receive best medical therapy as delineated above and be followed clinically for progression of their symptoms/disease. This will constitute a type pf prevalence study to examine baseline immune system profiles with respect to progression of PAD. If these patients come to require revascularization, they will be considered for the interventional arm of the study.

For the 250 planned intervention subjects, we will collect clinical and laboratory data prospectively to determine preoperative risk factors and postoperative response to revascularization. The timing of assessment and data collection is summarized in Table 2-2 and is scheduled in accordance with current standard practice for surveillance following lower extremity intervention. As indicated, clinical evaluation includes review of symptoms, pulse exam with ABIs, and duplex ultrasound examination of the revascularized region. Laboratory evaluation includes standard perioperative hematology and chemistry panels, as well as a high sensitivity CRP level pre-operatively and 1 week and 1 month post-operatively. Intervention failure is defined as any evidence of narrowing (stenosis) of either the site of angioplasty/stenting or any segment of the vein bypass graft that leads to recurrence of ischemic symptoms, a decrease in ABI of ≥ 15%, or hemodynamic significance by duplex and CT angiography imaging according to the Society for Vascular Surgery Recommended Standards for Reports Dealing with Lower Extremity Ischemia (revised version).[66] Repeat angiography is performed selectively in those patients undergoing evaluation for re-intervention or salvage of their revascularization. Therefore, primary patency is defined as the length of time the revascularization remains open

without signs of clinical failure, without significant stenosis on imaging, and without requiring repeat angiography or reintervention.

Functional and Quality of Life Assessments

Functional exercise testing and QOL questionnaires are administered at the same time intervals indicated in Table 2-2. Quality of life instruments used in this study include both generic health and disease-specific QOL questionnaires. The Medical Outcomes Short Form-36 (SF-36)[79] serves as the generic health questionnaire and the Vascular Quality of Life (VascuQol)[80] questionnaire to measure specific elements of PAD to capture more subtle disease-specific effects of intervention. Results are compared to pre-intervention to determine the impact of intervention on QOL.

Molecular Analysis Overview

Molecular analyses are performed on peripheral venous blood and include evaluation of the transcriptome from an enriched monocyte population, as well as the proteome from the plasma fraction. Initial blood samples are obtained in the pre-operative holding area immediately before the procedure. Subsequent samples are taken 2 hours and 1 day postoperatively and then at 1 week, 1, 6, and 12 months of follow-up (Figure 2-2). At each time point, 15 mL of blood is sampled to establish genomic and proteomic inflammatory response profiles. All samples are de-identified and assigned a study-specific identification number to assure confidentiality and allow sample tracking. A 7 mL collection of EDTA anti-coagulated whole blood is obtained for flow cytometric analysis of the peripheral blood leukocyte phenotype, genomic analyses on the total leukocyte preparation, and proteomic analyses of the plasma fraction. Simultaneously, an 8ml whole blood is collected in a Becton-Dickinson CPT™ tube containing sodium citrate to be processed further for the isolation of an enriched blood monocyte fraction. Plasma and leukocyte RNA are also stored for additional future analysis if

needed. The actual protocols are detailed in two recent publications including a discussion of the advantages and limitations of these analytical approaches.[44,81] The protocols are also available through the Large Scale Collaborative Research Program (www.gluegrant.org).

Proteomics Pilot Study

The focus of our study is limited to the initial analysis of multiplex proteomic data from the first 20 human subjects enrolled in the overall study. The same hypotheses were considered, specifically, that differences in the baseline inflammatory state (as determined by baseline plasma cytokine levels), or an exaggerated inflammatory response to intervention (indicated by statistically higher circulating cytokine levels), can be detected and may differentiate responses to the different procedures. Such differences may then be predictive of clinical failure of lower extremity revascularization.

Plasma is collected from the blood samples processed for buffy coat analyses. After separation, each plasma sample is aliquotted into several tubes and stored at -70º C until analysis. Freshly thawed plasma samples are then batch analyzed using the Luminex® 100™ xMAP (Multi-Analyte Profiling)® System. This bead-based assay system is essentially a flow cytometric analysis employing novel fluorescent beads that are covalently linked (in the case of cytokine measurements) to antibodies specific for individual analytes. By coupling the specificity of antibody-based capture of specific cytokines using chromophore-labeled antibodies with flow cytometric analyses of individual reactions identified by unique fluorescent beads, the analytical system can multiplex the analysis, theoretically, of an unlimited number of cytokines simultaneously from a single sample. Using a two-laser system, the Luminex® technology simultaneously identifies the quantity of an analyte bound to a specific antibody, as well as its identity, critical for a multiplex approach. Our current working Luminex® platform (22-plex) determines simultaneously the concentrations of the following analytes: eotaxin, G-CSF, GM-

CSF, IFN-γ, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-18, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MIP1α, MCP1 and TNFα. Data output is presented in an Excel™ spreadsheet that is identified only by subject study number and sample number. Protein plasma concentration values are determined for each analyte in each sample. This spreadsheet is then readily transferable for statistical analyses. There are numerous advantages to this approach, including parallel analysis of several proteins for class prediction, conservation of precious materials, and a wide spectrum of proteomic analyses available.

Statistical Considerations

Clinical Data

A secure, de-identified Access™ database has been designed to track all aspects of this study from patient specific clinical information to specimen collection and processing. Clinical data is analyzed using standard approaches including Students' t-test for numerical data, Chi-square analysis for categorical data, and Kaplan-Meier life table analysis with the log rank sum test for time series analysis.

Molecular Data

Genomics

The genomic bioinformatics approaches planned for the overall study are beyond the scope of this thesis. Briefly, unsupervised approaches such as multidimensional scaling, cluster analysis and self-organizing maps are used in class discovery exercises to identify relationships among genes. Supervised approaches are then used to identify gene expression differences in predefined classes (e.g., angioplasty versus bypass) or subsequent groupings of the data (e.g., patients who develop failure of their revascularization versus those who do not). Unsupervised and supervised analytical approaches are not mutually exclusive and when used in conjunction with one another represent a very powerful method for identifying relationships among genes.

Finally, principal component analyses (PCA) as part of such a functional data analysis (clustering, pathway analysis) approach offers us the ability to capture subtle profile changes in patient response, and to uncover and describe temporal differences in the genomic profile of patients who have a successful outcome versus those that go on to failure.

Proteomics

For the proteomic data specifically analyzed in this study, analysis of variance (ANOVA) was the primary analytic approach. The raw data outcome from the Luminex® assay is internally normalized to baseline control concentration standards within the assay. Then, due to the wide variation in the magnitude of cytokine levels between different patients, the proteomic dataset is further normalized by conversion to a logarithmic scale which allows for meaningful ANOVA. Because we are primarily interested in proteomic profiling rather than simple association of a single cytokine/biomarker, we utilized split-plot ANOVA with repeated measures. In addition to analyzing the baseline pre-operative cytokine levels, this approach analyzes the "shape of the curve" of cytokine expression over the time points post-procedure. These curves, or profiles, can then be compared between groups of interest such as angioplasty versus bypass, success vs. failure, etc. Subsequently, although not performed here, additional sophisticated bioinformatics approaches described above (i.e., functional analysis, pathway analysis, PCA) can be applied as part of the overall molecular analyses.

Table 2-1. Clinical and biochemical timeline

	Pre-op	2 hrs	1 day	1 wk	1 mo	6 mos	12 mos
Clinical exam	√	√	√	√	√	√	√
- ABIs	√	√	√	√	√	√	√
- Exercise test	√				√	√	√
- Duplex	*			√	√	√	√
- CT angiogram	*			√	√	√	√
Quality of life							
-SF-36	√				√	√	√
- VascuQol	√				√	√	√
Molecular studies							
- Gene array	√	√	√	√	√	√	√
- Protein assay	√	√	√	√	√	√	√

*Pre-operative imaging will only be obtained if clinically necessary for decision making

Table 2-2. Study inclusion/exclusion criteria

Inclusion	Exclusion
1. Diagnosis of symptomatic PAD – Rutherford Grade I Category 3 or greater[65] confirmed by history and physical and non-invasive studies – planned for lower extremity revascularization 2. Male or female at least 18 years of age 3. Adequate arterial anatomy amenable to revascularization 4. Adequate autogenous vein conduit in patients undergoing bypass surgery	1. < 18 years of age 2. Existing medical condition(s) with resulting life expectancy less than one year 3. Documented intolerance or allergy to aspirin and clopidogrel 4. History of immunosupression on the basis of a preexisting medical condition or immunosuppressant therapy or chronic corticosteroid therapy to treat a preexisting condition 5. Documented active or quiescent autoimmune disorder 6. White blood cell count (WBC) <3.5 x 10^9/L 7. Platelets < 50 x 10^9/L 8. Any patient who has received experimental drug(s) (including experimental biologic agents) in the previous three months 9. Pregnancy 10. Patients receiving bypass using prosthetic, cryopreserved, or other non-autogenous conduits

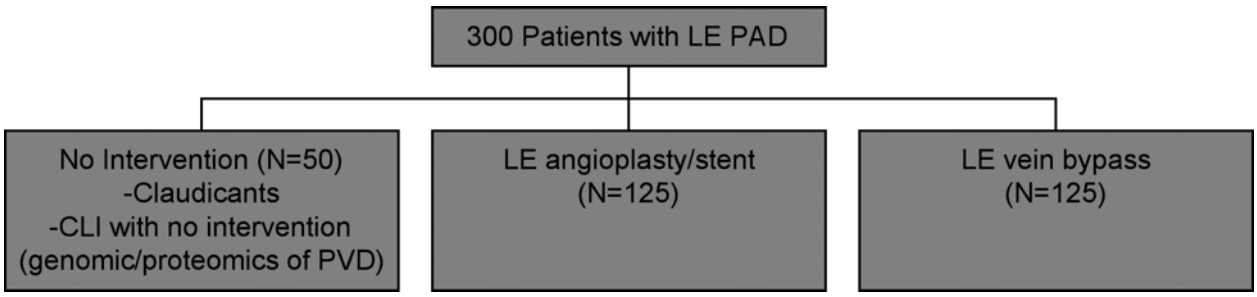

Figure 2-1. Overall study algorithm. 50 subjects receiving medical therapy alone will serve as a control prevalence group for comparison to the larger cohorts receiving additional lower extremity (LE) angioplasty or bypass.

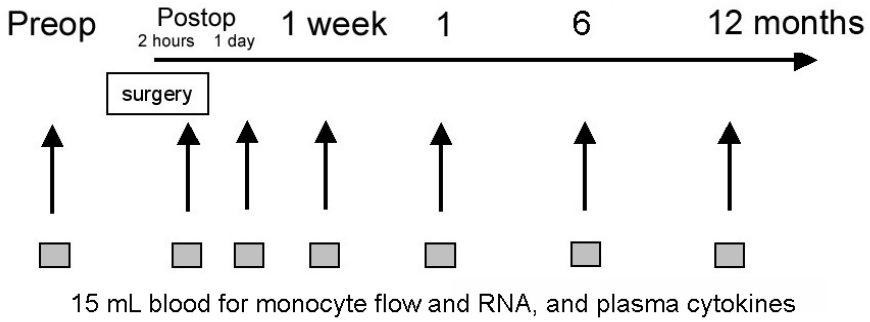

Figure 2-2. Timing of peripheral blood sampling. At each time point, 15 mL peripheral blood is sampled for the isolation of monocytes for flow cytometry and RNA isolation, and for the collection of plasma for cytokine analysis.

CHAPTER 3
RESULTS

Clinical Results

A total of 51 subjects entered the overall study by November 2008. Clinical analysis performed on this entire cohort shows no statistical differences by Student's t-test or Chi-squared analysis between the three patient cohorts - those patients receiving medical therapy alone, those undergoing additional angioplasty/stenting, or those having vein bypass surgery - with respect to age, gender, race, coronary artery disease, hypertension, hypercholesterolemia, diabetes, renal insufficiency, or smoking. Although there were no statistical differences, the angioplasty group was slightly older with a higher proportion of white subjects and a higher proportion of hypercholesterolemic subjects. The bypass group had a slightly higher proportion of diabetic subjects. The indication for intervention between the angioplasty and vein bypass groups, however, was significantly different with a preponderance of claudicants in the angioplasty group and patients with critical limb ischemia (CLI) in the vein bypass group (P = .008) (Table 3-1).

Kaplan-Meier life table analysis was performed for primary patency for all subjects receiving intervention (Figure 3-1) and limb salvage for the entire cohort (Figure 3-2). Overall primary patency was 73% at 7 months after which the standard error exceeded 10% (Figure 3-1, A). There were no early failures in the angioplasty group and primary patency for the vein bypass procedures was 85% at 4 months after which the standard error exceeded 10% (Figure 3-1, B). Limb salvage was 93% for the entire cohort. Due to the small numbers in each treatment group and the overall small number of events, multivariate analysis did not reveal any demographic factors to be predictive of bypass failure.

Proteomic Results

Luminex® multianalyte cytokine analysis was performed on plasma samples from the first 20 study subjects (5 control, 6 angioplasty/stenting, 9 vein bypass). Pre-operative samples (P0) for all subjects were evaluated, and then 2 hour (2H), 1 day (1D), 1 week (1W), and 1 month (1M) samples were analyzed for all interventions. Cytokine levels were below the sensitivity of the assay precluding further analysis for the following cytokines: IL-5, IL-12p70, IL-13, and IL-15. The remainder of the cytokines were analyzed statistically with results summarized in Tables 3-2 and 3-3. More detailed data and statistical analyses are provided in Appendix A.

Several cytokines demonstrated significant post-procedural changes over time for all subjects undergoing intervention. The analysis of cytokine levels separated by procedure type showed that pre-operative values did not differ between patients who subsequently underwent angioplasty/stenting compared to those who underwent vein bypass. However, several cytokines were significantly differentially expressed post-operatively discriminating between procedure groups. Two distinct cytokine expression patterns emerged. For the angioplasty/stenting group there appeared to be an early transient peak of moderate level increased expression, whereas for the bypass group, the magnitude of expression was larger with a more delayed but prolonged expression (see Figures 3-3 to 3-5, B). These intuitively follow the magnitude of the procedure, but warrant further analysis.

The analysis of cytokine levels based on outcome (success vs. failure) was restricted to the vein bypass group since there were no early failures in the angioplasty/stenting group. Increased expression levels of several inflammatory cytokines were found to be significantly associated with bypass failure supporting our hypothesis that an exaggerated inflammatory response is associated with poor outcome. Importantly, we found significant differences in pre-operative cytokine levels alone between the success and failure groups suggesting the ability to predict

failure based on pre-operative sampling alone. Furthermore, we found that these cytokine levels continued to differ over time post-intervention with temporal changes further discriminating between success and failure outcomes. All failures occurred later than the 1-month time point suggesting that identification of these early changes could allow for intervention that still avert failure. Examples of cytokine expression that are associated with either procedure or outcome differences, both, or neither are shown in figures 3-3 to 3-7.

Table 3-1. Study Cohort Demographics

Character	Control (N=13)	Angioplasty (N=12)	Vein Bypass (N=26)
Age (mean ± SD)	62.1 ± 7.4	66.7 ± 6.8	61.4 ±8.3
Male Gender (%)	100	92	85
White Race (%)	77	92	73
CAD (%)	54	50	54
HTN (%)	100	100	92
Hypercholesterolemia (%)	62	92	69
DM (%)	46	33	58
Smoker (%)	92	100	89
Indication – Claudication (%)	46	83*	15*
Indication – CLI (%)	54	17*	85*

*Chi square P = .008 for difference in indications between treatment groups

Table 3-2. Summary of proteomic results by procedure

Significance over time	Significance by procedure	No significance by procedure
EOTAXIN	EOTAXIN	IL-12p40
GMCSF	IFNγ	IP-10
IFNγ	IL-1β	MCP-1
IL-1α	IL-6	MIP-1α
IL-1β	IL-8	RANTES
IL-6	TNFα	GMCSF
IL-8		IL-10
IL-10		IL-1α
TNFα		

Table 3-3. Summary of proteomic results by outcome

Significance over time	Significance with failure	No significance with outcome
EOTAXIN	EOTAXIN	IL-12p40
GMCSF	GMCSF	IP-10
IFNγ	IFNγ	MCP-1
IL-1α	IL-1α	MIP-1α
IL-1β	IL-1β	RANTES
IL-6	IL-6	
IL-8	IL-8	
IL-10	IL-10	
TNFα	TNFα	

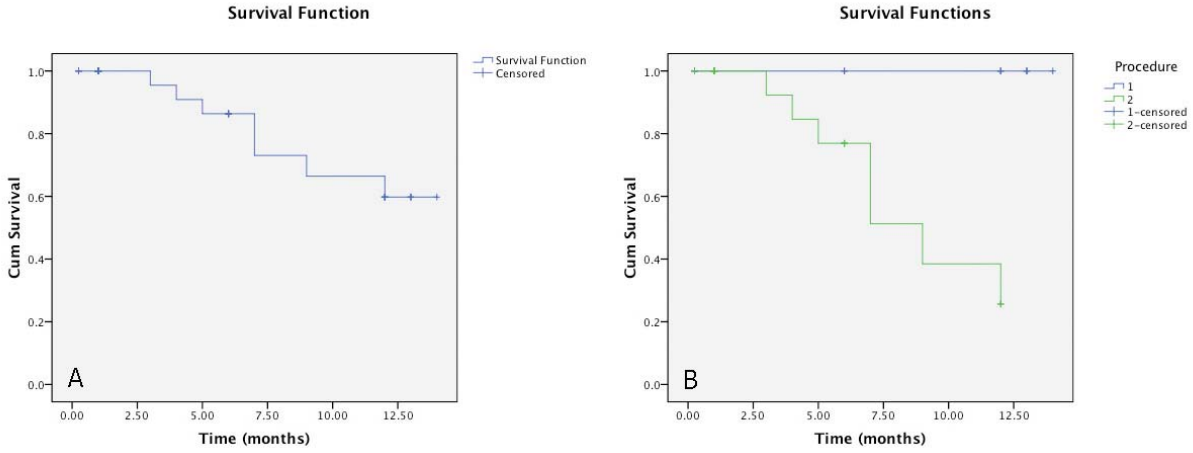

Figure 3-1. Kaplan-Meier life table analysis of primary patency rates of revascularization. Overall patency for all subjects undergoing intervention (N=38) is depicted in panel A, and patency separated by procedure type (Procedure 1 = angioplasty, N=12; Procedure 2 = bypass, N=26) in panel B.

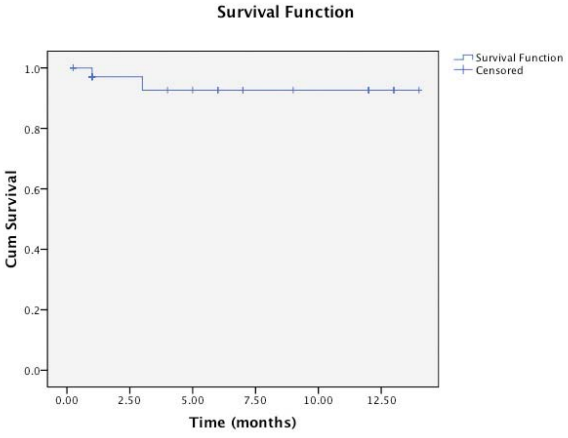

Figure 3-2. Kaplan-Meier life table analysis of limb salvage. Results for the overall cohort

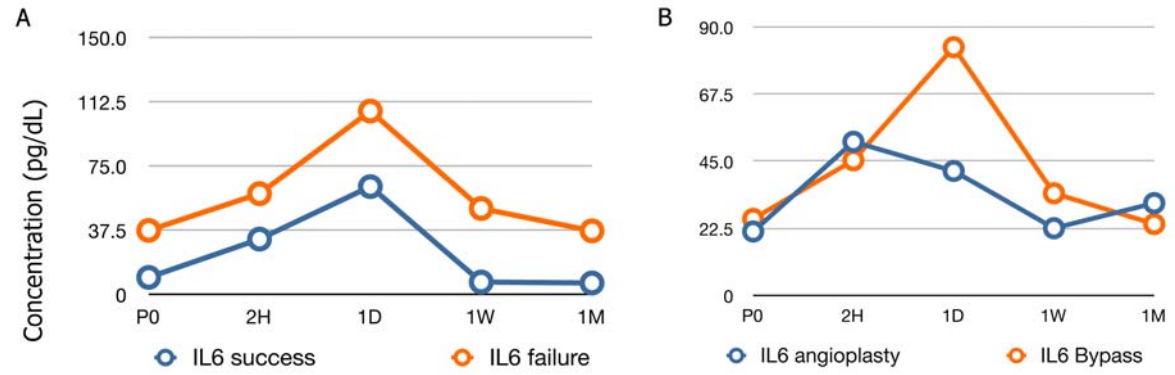

Figure 3-3. IL-6 plasma levels. IL-6 demonstrated differences that were significantly different over time and that correlated with both procedural (A, P = .047) and outcome (B, P = .0001) differences.

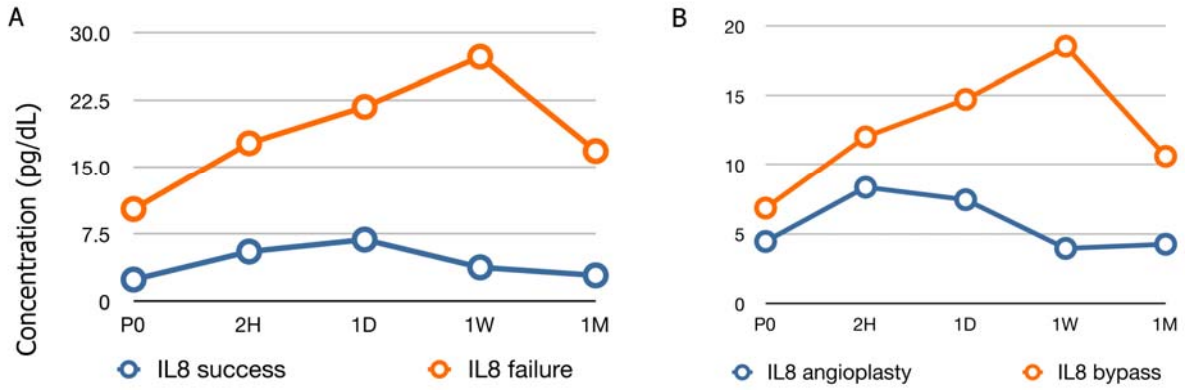

Figure 3-4. IL-8 plasma levels. IL-8 also demonstrated differences that were significantly different over time and that correlated with both procedural (A, P = .035) and outcome (B, P = .0001) differences.

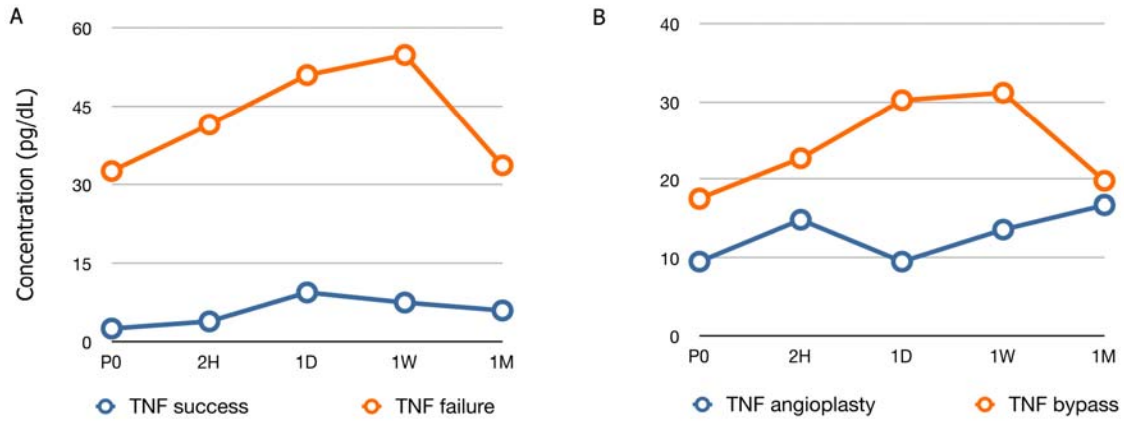

Figure 3-5. TNFα plasma levels. TNFα also demonstrated differences that were significantly different over time and that correlated with both procedural (A, P = .010) and outcome (B, P = .019) differences.

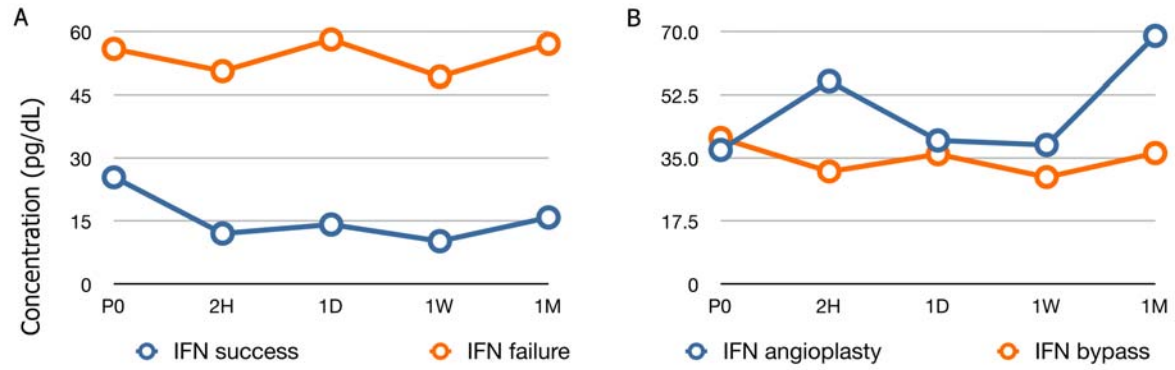

Figure 3-6. IFNγ plasma levels. IFNγ demonstrated differences that were significantly different over time, but that correlated only with outcome differences (B, P = .019). IFNγ levels did not differ by procedure (A).

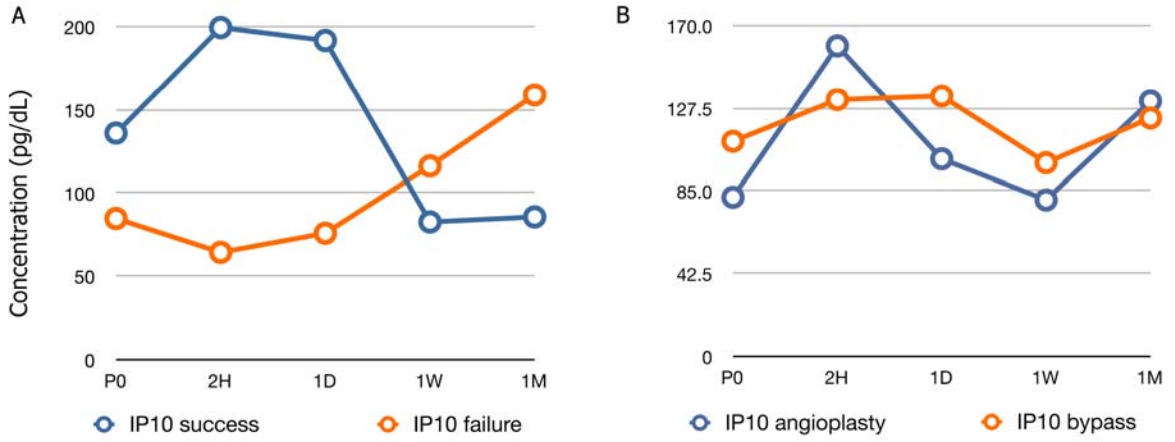

Figure 3-7. IP-10 plasma levels. IP-10 expression did not demonstrate significant differences with any of the comparisons [time, procedure (A), or outcome (B)]. The distinct difference in curve morphologies between outcome groups (A) warrants further evaluation.

CHAPTER 4
DISCUSSION

Summary and Significance of Results

This report contains the first significant pilot study as part of our larger overall comprehensive functional genomic/proteomic translational program in vascular disease. As such, the study provides important early confirmation that the research design is sound, and it delivers several critical proof-of-principle findings upon which subsequent study and analysis will be based.

Our early clinical results are consistent with previously reported experiences. Our 73% overall short-term primary patency is acceptable, and our 93% limb salvage rate is exceptional. The fact that the angioplasty/stenting procedures have not experienced failure is interesting in that angioplasty is typically felt to be less durable than bypass. Our success likely represents the small number of cases and our early selection of favorable subjects, and, with both more follow-up time and more broad inclusion, we will begin to see angioplasty/stenting failures and more representative results. This will then allow us to extend our analyses correlating inflammatory response to outcome to this treatment arm as well. Moving forward, we anticipate making our clinical dataset more robust both by adding more subjects, but also by adding analyses of the functional and QOL endpoints. This will ultimately allow us to strictly define comprehensive clinical outcomes as mandated in the aims of the study and will lead to far more sophisticated correlative molecular analyses.

At the outset, we had concerns that peripheral angioplasty, being a percutaneous procedure with significantly less operative stress than open surgical bypass, may incite only minimal inflammatory response that could fall below the sensitivity of the analyses to detect it. If this were to be the case, these studies may either have provided no meaningful results, or may have

given a false impression that there is no significant systemic inflammatory response to angioplasty. The sensitivity of the Luminex® assay however has provided interpretable data for all but four cytokines that fell below its minimal detection level. These cytokines were consistent between treatment groups, and therefore, it is unclear whether this means that those four cytokines are uninvolved in the inflammatory reaction to revascularization or that their changes were small and undetectable. What is clear, however, is that we can reliably detect and analyze an inflammatory profile following angioplasty/stenting and that it appears to be distinctly different than that seen with vein bypass. This serves as important proof of principle that procedure-specific analyses will be meaningful and that potential exists to individualize patient assignment to their optimal procedure based on their immune profile.

Finally, clear inflammatory profiles are emerging from our early proteomic data that are associated with, and perhaps predictive of intervention failure. Enrolled subjects demonstrated significant alterations in both baseline immunity and the systemic inflammatory response to intervention that predicted outcome. Such evidence is currently present for vein bypass surgery in the data presented here, and we are optimistic that, as the angioplasty/stenting experience unfolds, similar patterns will hold true for both procedure types. This serves as further proof of principle for ongoing studies focused on the development of class prediction models to determine whether failure of these surgical interventions can be predicted early by selected components of the inflammatory response. The real potential of this would rest in the eventual ability to refine patient-specific procedure choices based on pre-operative molecular testing, or to design post-operative pharmacologic (e.g., anti-inflammatory) strategies to optimize favorable outcomes. This system-wide, high throughput platform would facilitate the conduct of such pharmacoproteomic studies to survey and refine such treatment strategies.

Study Limitations

The primary limitation to this study is its small sample size. We chose to analyze the first 20 subjects data and only their proteomic data out to one month. This, however, allowed us to analyze a manageable number of samples from a reasonable number of patients to both validate our research protocol as well as to provide initial proof of principle that our study design and hypotheses have validity. The next steps will include analyzing additional patient samples to establish reproducibility and validity of the currents results. In addition, the analysis of samples out past one month will answer an important question – whether these vascular patients return to their pre-operative baseline immune status, or whether revascularization adjusts their overall immune system as reflected in new baseline values being established.

Another limitation in the overall study design is the lack of randomization of subjects to procedure type. Without randomization, the control, angioplasty, and bypass arms of this study were similar demographically. However, because subjects were not randomized to procedure type, the two treatment groups differed significantly with respect to the indication for intervention. Since the clinical indications for each procedure differs, subjects are not equally eligible for the two procedures. Therefore, randomization is not readily possible in this setting because procedural decisions are currently made based on angiographic distribution of disease, patient operative risk, availability of bypass conduit, a prevailing dogma that bypass is superior for advanced critical limb ischemia (i.e., rest pain, ulceration, gangrene), and that bypass is infrequently offered for claudication. One future solution would be to restrict entry to subjects within one symptom group, or subjects with strictly defined angiographic findings such that randomization to angioplasty/stenting versus vein bypass was possible. This approach, however, would likely hinder enrollment, make subject accrual difficult, and make the length of the study

prohibitive for a single clinical center. This may require extending this study model to a multicenter design.

Finally, one obvious limitation is the lack of parallel genomic data to further characterize the inflammatory response to revascularization. This will be discussed further in future directions, but reflects a delay in genomic analysis awaiting new cutting edge technology.

Future Directions

The original research protocol delineated genomic studies using the Affymetrix® human U133 Plus2 microarray chip which is the standard commercially available chip in use today for human gene expression analysis. Since the inception of our study, through collaboration with the Glue Grant [Inflammation and the Host Response to Injury, National Institute of General Medical Sciences (NIGMS)] investigators, we now have access to the GG-H2 microarray chip designed by Affymetrix® specifically for and through collaboration with the Glue Grant. This chip is an extraordinary advance in technology in that it incorporates all the standard expression analysis probe sets for the human genome, plus it contains exon array probe sets, probe sets for non-coding genomic regions, and single nucleotide polymorphism probe sets all on a single chip. This amounts to upwards of 7 million probe sets and produces a data file 750 MB in size. The obvious potential for this chip is the enormity and complexity of data it provides. The down side is that bioinformatics tools are currently lacking to analyze this amount and complexity of data. We anticipate that using this new platform for our genomic analyses moving forward will result in significant important advances and we look forward to being involved in the process for developing and validating novel bioinformatics tools.

More globally, this approach represents a paradigm shift in human investigation into the role of the systemic inflammatory milieu that will yield new knowledge that will significantly impact patient selection and the development of novel therapies for PAD intervention. The

framework established through this project will stand as a validated foundation for understanding the mechanistic impact of specific therapeutic interventions in peripheral vascular disease. The platform developed here can then be applied to other modalities of lower extremity revascularization including balloon cryoplasty, drug-eluting stents, catheter-based atherectomy, and excimer laser. Our approach will provide critical insight into patient selection and risk stratification when considering these alternative therapies. Information obtained will also lead to the development of improved interventional technology and/or pharmacologic adjuncts (e.g., anti-inflammatory, immune modulating therapies) to further impact the durability of lower extremity revascularization. Furthermore, once established, this platform can also be applied to other vascular disease processes such as the management of elective and ruptured abdominal aortic aneurysms with both open and endovascular treatment options, carotid endarterectomy versus carotid stenting, and perhaps treatment of chronic venous insufficiency. Finally, the knowledge and understanding gained through this project will likely be broadly applicable to understanding systemic cardiovascular disease.

APPENDIX
THE SPSS OUTPUT DATA AND STATISTICS

Table A-1. Eotaxin descriptive statistics

Procedure	Time	Outcome	Mean	Standard deviation	N
Angioplasty	lnP0	Success	3.7438	.43792	12
		Total	3.7438	.43792	12
	lnH2	Success	4.1292	.65921	12
		Total	4.1292	.65921	12
	lnD1	Success	3.6653	.35727	12
		Total	3.6653	.35727	12
	lnW1	Success	3.7476	.56530	12
		Total	3.7476	.56530	12
	lnM1	Success	3.9709	.59721	12
		Total	3.9709	.59721	12
Bypass	lnP0	Success	3.8473	.16609	6
		Failure	3.8830	.43259	10
		Total	3.8696	.34899	16
	lnH2	Success	3.5424	.41741	6
		Failure	3.6287	.59218	10
		Total	3.5963	.51995	16
	lnD1	Success	3.3841	.35913	6
		Failure	3.7332	.69637	10
		Total	3.6022	.60367	16
	lnW1	Success	3.1863	.10066	6
		Failure	3.4233	.49114	10
		Total	3.3344	.40267	16
	lnM1	Success	3.3766	.40555	6
		Failure	4.1638	.36424	10
		Total	3.8686	.53792	16

Table A-2. Eotaxin repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	889.982	1	889.982	997.060	.000
	Outcome	.000				
	Error	9.819	11	.893		
Bypass	Intercept	981.130	1	981.130	2639.976	.000
	Outcome	1.677	1	1.677	4.512	.052
	Error	5.203	14	.372		

Table A-3. IL-6 descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	2.6106	1.01568	12
		Total	2.6106	1.01568	12
	ln2H	Success	3.4992	1.10958	12
		Total	3.4992	1.10958	12
	ln1D	Success	3.3388	1.00879	12
		Total	3.3388	1.00879	12
	ln1W	Success	2.8617	.80438	12
		Total	2.8617	.80438	12
	ln1M	Success	2.8909	1.05519	12
		Total	2.8909	1.05519	12
Bypass	lnP0	Success	2.0628	.78437	6
		Failure	3.3836	.72500	10
		Total	2.8883	.97804	16
	ln2H	Success	3.4348	.34473	6
		Failure	3.9438	.55433	10
		Total	3.7529	.53735	16
	ln1D	Success	3.7918	.94357	6
		Failure	4.3848	.84502	10
		Total	4.1624	.90174	16
	ln1W	Success	1.9830	.35783	6
		Failure	3.7280	.65887	10
		Total	3.0736	1.03168	16
	ln1M	Success	1.8539	.48877	6
		Failure	3.3958	.71525	10
		Total	2.8176	.99040	16

Table A- 4. IL-6 repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	554.576	1	554.576	155.082	.000
	Outcome	.000				
	Error	39.336	11	3.576		
Bypass	Intercept	766.194	1	766.194	840.458	.000
	Outcome	24.449	1	24.449	26.819	.000
	Error	12.763	14	.912		

Table A-5. IL-8 descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	1.1641	.78314	12
		Total	1.1641	.78314	12
	lnH2	Success	1.6914	.89514	12
		Total	1.6914	.89514	12
	lnD1	Success	1.3496	1.08354	12
		Total	1.3496	1.08354	12
	lnW1	Success	1.2699	.48094	12
		Total	1.2699	.48094	12
	lnM1	Success	1.3705	.46846	12
		Total	1.3705	.46846	12
Bypass	lnP0	Success	.7072	.69991	6
		Failure	2.1678	.57900	10
		Total	1.6201	.94752	16
	lnH2	Success	1.5141	.73124	6
		Failure	2.6136	.75184	10
		Total	2.2013	.90532	16
	lnD1	Success	1.7139	.80769	6
		Failure	2.6007	.90027	10
		Total	2.2681	.94887	16
	lnW1	Success	1.1765	.70719	6
		Failure	2.5606	1.17291	10
		Total	2.0415	1.21288	16
	lnM1	Success	.8920	.73095	6
		Failure	2.6368	.65173	10
		Total	1.9825	1.09270	16

Table A-6. IL-8 repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	112.466	1	112.466	55.376	.000
	Outcome	.000				
	Error	22.340	11	2.031		
Bypass	Intercept	258.996	1	258.996	163.222	.000
	Outcome	32.431	1	32.431	20.438	.000
	Error	22.215	14	1.587		

Table A-7. TNF alpha descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	1.9715	.78266	12
		Total	1.9715	.78266	12
	lnH2	Success	2.4654	.74789	12
		Total	2.4654	.74789	12
	lnD1	Success	2.0328	.70592	12
		Total	2.0328	.70592	12
	lnW1	Success	2.2507	.84234	12
		Total	2.2507	.84234	12
	lnM1	Success	2.2305	1.0397	12
		Total	2.2305	1.0397	12
Bypass	lnP0	Failure	2.4501	1.4100	10
		Success	.69338	.90851	6
		Total	1.7913	1.4965	16
	lnH2	Failure	2.8977	1.2840	10
		Success	1.3063	.44592	6
		Total	2.3009	1.2994	16
	lnD1	Failure	2.9576	1.4150	10
		Success	1.9618	.86138	6
		Total	2.5842	1.3025	16
	lnW1	Failure	3.1093	1.5044	10
		Success	1.8897	.57604	6
		Total	2.6520	1.3566	16
	lnM1	Failure	2.8896	1.2710	10
		Success	1.7816	.21754	6
		Total	2.4741	1.1366	16

Table A-8. TNF alpha repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	287.814	1	287.814	101.753	.000
	Outcome	.000				
	Error	31.114	11	2.829		
Bypass	Intercept	360.927	1	360.927	76.396	.000
	Outcome	33.382	1	33.382	7.066	.019
	Error	66.142	14	4.724		

Table A- 9. IFN gamma descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	3.3278	.83455	12
		Total	3.3278	.83455	12
	lnH2	Success	3.7693	.76727	12
		Total	3.7693	.76727	12
	lnD1	Success	3.3484	.87515	12
		Total	3.3484	.87515	12
	lnW1	Success	3.3008	.82089	12
		Total	3.3008	.82089	12
	lnM1	Success	3.6067	1.0692	12
		Total	3.6067	1.0692	12
Bypass	lnP0	Success	3.1695	.40254	6
		Failure	3.8113	.67395	10
		Total	3.5707	.65538	16
	lnH2	Success	2.3426	.66800	6
		Failure	3.5134	.88178	10
		Total	3.0744	.97874	16
	lnD1	Success	2.4423	.78340	6
		Failure	3.6012	.91533	10
		Total	3.1666	1.0213	16
	lnW1	Success	2.2951	.33028	6
		Failure	3.6058	.77113	10
		Total	3.1143	.90698	16
	lnM1	Success	2.4910	.79138	6
		Failure	3.9331	.50529	10
		Total	3.3923	.93908	16

Table A-10. IFN gamma repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	722.697	1	722.697	240.784	.000
	Outcome	.000				
	Error	33.016	11	3.001		
Bypass	Intercept	730.333	1	730.333	485.382	.000
	Outcome	24.576	1	24.576	16.333	.001
	Error	21.065	14	1.505		

Table A- 11. IP-10 descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	4.3198	.44483	12
		Total	4.3198	.44483	12
	lnH2	Success	4.9684	.50133	12
		Total	4.9684	.50133	12
	lnD1	Success	4.4301	.65487	12
		Total	4.4301	.65487	12
	lnW1	Success	4.3053	.43268	12
		Total	4.3053	.43268	12
	lnM1	Success	4.6940	.69197	12
		Total	4.6940	.69197	12
Bypass	lnP0	Success	4.8071	.49473	6
		Failure	4.2850	.55858	10
		Total	4.4808	.58046	16
	lnH2	Success	4.7805	1.21319	6
		Failure	4.0804	.46849	10
		Total	4.3430	.86303	16
	lnD1	Success	4.7647	1.26787	6
		Failure	4.1614	.62228	10
		Total	4.3876	.92692	16
	lnW1	Success	4.1913	.81478	6
		Failure	4.6150	.54991	10
		Total	4.4561	.66903	16
	lnM1	Success	4.2072	.87781	6
		Failure	4.9945	.40820	10
		Total	4.6992	.71538	16

Table A-12. IP-10 repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	1238.617	1	1238.617	4123.860	.000
	Outcome	.000				
	Error	3.304	11	.300		
Bypass	Intercept	1511.134	1	1511.134	1041.962	.000
	Outcome	.283	1	.283	.195	.665
	Error	20.304	14	1.450		

Table A-13. IL-10 descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	-.0368	.82410	12
		Total	-.0368	.82410	12
	lnH2	Success	.4076	.75182	12
		Total	.4076	.75182	12
	lnD1	Success	.1589	1.08101	12
		Total	.1589	1.08101	12
	lnW1	Success	.0839	.63720	12
		Total	.0839	.63720	12
	lnM1	Success	.2024	.94069	12
		Total	.2024	.94069	12
Bypass	lnP0	Success	-.3314	.70427	6
		Failure	.6440	1.07767	9
		Total	.2538	1.04184	15
	lnH2	Success	.5127	.07188	6
		Failure	.8742	1.10691	9
		Total	.7296	.85766	15
	lnD1	Success	-.0366	1.10474	6
		Failure	1.2086	1.01418	9
		Total	.7105	1.19262	15
	lnW1	Success	-.6955	.33865	6
		Failure	1.4318	1.54251	9
		Total	.5809	1.60134	15
	lnM1	Success	-.6531	.30968	6
		Failure	.8226	1.17978	9
		Total	.2323	1.17878	15

Table A-14. IL-10 repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	1.598	1	1.598	.522	.485
	Outcome	.000				
	Error	33.660	11	3.060		
Bypass	Intercept	10.273	1	10.273	3.931	.069
	Outcome	27.543	1	27.543	10.539	.006
	Error	33.976	13	2.614		

Table A-15. IL-12 descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	4.7469	.60598	12
		Total	4.7469	.60598	12
	lnH2	Success	5.1484	.87530	12
		Total	5.1484	.87530	12
	lnD1	Success	4.8832	.76977	12
		Total	4.8832	.76977	12
	lnW1	Success	4.8690	.88359	12
		Total	4.8690	.88359	12
	lnM1	Success	5.0817	1.12857	12
		Total	5.0817	1.12857	12
Bypass	lnP0	Success	4.5017	.22259	6
		Failure	4.9110	.48533	11
		Total	4.7666	.45094	17
	lnH2	Success	4.3215	.33945	6
		Failure	4.8342	.63647	11
		Total	4.6532	.59411	17
	lnD1	Success	4.4889	.50527	6
		Failure	4.9808	.74108	11
		Total	4.8072	.69408	17
	lnW1	Success	4.3947	.20643	6
		Failure	5.0771	.71651	11
		Total	4.8362	.66871	17
	lnM1	Success	4.2889	.34558	6
		Failure	5.1643	.34316	11
		Total	4.8553	.54487	17

Table A-16. IL-12 repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	1467.685	1	1467.685	494.649	.000
	Outcome	.000				
	Error	32.638	11	2.967		
Bypass	Intercept	1712.526	1	1712.526	3222.871	.000
	Outcome	6.857	1	6.857	12.905	.003
	Error	7.970	15	.531		

Table A-17. IL-1alpha descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	5.1398	.59605	12
		Total	5.1398	.59605	12
	lnH2	Success	5.3448	.60824	12
		Total	5.3448	.60824	12
	lnD1	Success	4.9656	.62261	12
		Total	4.9656	.62261	12
	lnW1	Success	5.0264	.49231	12
		Total	5.0264	.49231	12
	lnM1	Success	5.2151	.62969	12
		Total	5.2151	.62969	12
Bypass	lnP0	Success	5.2152	.44141	6
		Failure	5.5154	.65314	10
		Total	5.4028	.58603	16
	lnH2	Success	4.4663	.35426	6
		Failure	4.8938	.64905	10
		Total	4.7335	.58333	16
	lnD1	Success	4.5276	.62515	6
		Failure	5.0790	.50688	10
		Total	4.8723	.60036	16
	lnW1	Success	4.3380	.08824	6
		Failure	5.0948	.46655	10
		Total	4.8110	.52572	16
	lnM1	Success	4.3502	.10899	6
		Failure	5.5267	.52698	10
		Total	5.0855	.71877	16

Table A-18. IL-1alpha repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	1584.152	1	1584.152	1278.353	.000
	Outcome	.000				
	Error	13.631	11	1.239		
Bypass	Intercept	1801.266	1	1801.266	2034.288	.000
	Outcome	7.739	1	7.739	8.741	.010
	Error	12.396	14	.885		

Table A-19. IL-1beta descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	2.6441	1.1706	12
		Total	2.6441	1.1706	12
	lnH2	Success	3.1567	1.2106	12
		Total	3.1567	1.2106	12
	lnD1	Success	2.5302	1.1475	12
		Total	2.5302	1.1475	12
	lnW1	Success	2.7097	.93291	12
		Total	2.7097	.93291	12
	lnM1	Success	2.9904	.87415	12
		Total	2.9904	.87415	12
Bypass	lnP0	Success	1.6155	1.1996	6
		Failure	2.4782	1.0901	10
		Total	2.1547	1.1742	16
	lnH2	Success	1.4224	1.3449	6
		Failure	2.3850	1.1471	10
		Total	2.0240	1.2744	16
	lnD1	Success	1.6671	1.9690	6
		Failure	2.4866	1.2685	10
		Total	2.1793	1.5574	16
	lnW1	Success	1.3667	.63856	6
		Failure	3.0214	1.2869	10
		Total	2.4009	1.3469	16
	lnM1	Success	1.8338	.90716	6
		Failure	2.9803	.58829	10
		Total	2.5504	.90032	16

Table A-20. IL-1beta repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	472.495	1	472.495	93.954	.000
	Outcome	.000				
	Error	55.319	11	5.029		
Bypass	Intercept	338.895	1	338.895	67.063	.000
	Outcome	22.244	1	22.244	4.402	.055
	Error	70.747	14	5.053		

Table A-21. MCP-1 descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	2.9314	.54276	12
		Total	2.9314	.54276	12
	lnH2	Success	3.5284	.54968	12
		Total	3.5284	.54968	12
	lnD1	Success	3.0708	.60240	12
		Total	3.0708	.60240	12
	lnW1	Success	2.9302	.27768	12
		Total	2.9302	.27768	12
	lnM1	Success	3.1265	.56930	12
		Total	3.1265	.56930	12
Bypass	lnP0	Failure	3.3827	.49730	10
		Success	2.9014	.15019	6
		Total	3.2022	.46241	16
	lnH2	Failure	3.3661	.63994	10
		Success	3.5512	1.28763	6
		Total	3.4355	.89830	16
	lnD1	Failure	3.3881	.79115	10
		Success	3.2051	.42579	6
		Total	3.3194	.66660	16
	lnW1	Failure	3.5669	.64820	10
		Success	2.8120	.23112	6
		Total	3.2838	.64214	16
	lnM1	Failure	3.6230	.47466	10
		Success	2.8188	.33877	6
		Total	3.3214	.57888	16

Table A-22. MCP-1 repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	583.117	1	583.117	654.519	.000
	Outcome	.000				
	Error	9.800	11	.891		
Bypass	Intercept	797.813	1	797.813	729.328	.000
	Outcome	3.116	1	3.116	2.848	.114
	Error	15.315	14	1.094		

Table A-23. MIP-1alpha descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	3.7359	.59364	12
		Total	3.7359	.59364	12
	lnH2	Success	4.0090	.70237	12
		Total	4.0090	.70237	12
	lnD1	Success	3.6890	.56477	12
		Total	3.6890	.56477	12
	lnW1	Success	3.7619	.48043	12
		Total	3.7619	.48043	12
	lnM1	Success	3.9784	.58446	12
		Total	3.9784	.58446	12
Bypass	lnP0	Failure	4.1037	.30018	10
		Success	3.9427	.45337	6
		Total	4.0433	.35925	16
	lnH2	Failure	3.6339	.68569	10
		Success	3.3371	1.23172	6
		Total	3.5226	.89991	16
	lnD1	Failure	3.9175	.47048	10
		Success	3.7584	1.45891	6
		Total	3.8579	.92120	16
	lnW1	Failure	3.9907	.47582	10
		Success	2.9964	.77403	6
		Total	3.6178	.76335	16
	lnM1	Failure	4.0529	.35802	10
		Success	3.1699	.97950	6
		Total	3.7218	.76919	16

Table A-24. MIP-1alpha repeated measures ANOVA

Source	Type III sum of squares	df	Mean square	F	Sig.
Intercept	2003.455	1	2003.455	1267.998	.000
Procedure	3.104	1	3.104	1.965	.173
Outcome	4.666	1	4.666	2.953	.098
Procedure Outcome	.000				
Error	39.500	25	1.580		

Table A-25. RANTES descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	4.6419	.43316	12
		Total	4.6419	.43316	12
	lnH2	Success	5.0041	.69442	12
		Total	5.0041	.69442	12
	lnD1	Success	4.7095	.53157	12
		Total	4.7095	.53157	12
	lnW1	Success	4.6406	.52622	12
		Total	4.6406	.52622	12
	lnM1	Success	4.9280	.73824	12
		Total	4.9280	.73824	12
Bypass	lnP0	Failure	4.9500	.38953	10
		Success	5.1620	.24185	6
		Total	5.0295	.34896	16
	lnH2	Failure	4.6410	.50533	10
		Success	4.6485	.43405	6
		Total	4.6438	.46479	16
	lnD1	Failure	4.9349	.48090	10
		Success	4.8782	.48189	6
		Total	4.9137	.46580	16
	lnW1	Failure	4.9528	.65038	10
		Success	4.4568	.45791	6
		Total	4.7668	.62062	16
	lnM1	Failure	5.3435	.30333	10
		Success	4.3593	.47699	6
		Total	4.9744	.61092	16

Table A-26. RANTES repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	1373.664	1	1373.664	2635.477	.000
	Outcome	.000				
	Error	5.733	11	.521		
Bypass	Intercept	1751.628	1	1751.628	5924.245	.000
	Outcome	1.302	1	1.302	4.402	.055
	Error	4.139	14	.296		

Table A-27. GMCSF descriptive statistics

Procedure	Time	Outcome	Mean	Std. deviation	N
Angioplasty	lnP0	Success	2.3686	.80764	12
		Total	2.3686	.80764	12
	lnH2	Success	2.7466	.47656	12
		Total	2.7466	.47656	12
	lnD1	Success	2.3382	.83185	12
		Total	2.3382	.83185	12
	lnW1	Success	2.5827	.51127	12
		Total	2.5827	.51127	12
	lnM1	Success	2.5840	.73907	12
		Total	2.5840	.73907	12
Bypass	lnP0	Success	1.4180	.94333	6
		Failure	2.8671	1.19722	10
		Total	2.3237	1.29677	16
	lnH2	Success	1.3398	.90119	6
		Failure	3.0766	1.29444	10
		Total	2.4253	1.42483	16
	lnD1	Success	2.2542	.81722	6
		Failure	3.1292	1.37555	10
		Total	2.8011	1.24471	16
	lnW1	Success	2.1474	.37197	6
		Failure	3.2883	1.39400	10
		Total	2.8605	1.23996	16
	lnM1	Success	2.0837	.34335	6
		Failure	3.0881	1.24768	10
		Total	2.7115	1.10705	16

Table A-28. GMCSF repeated measures ANOVA

Procedure	Source	Type III sum of squares	df	Mean square	F	Sig.
Angioplasty	Intercept	382.242	1	382.242	198.731	.000
	Outcome	.000				
	Error	21.158	11	1.923		
Bypass	Intercept	457.289	1	457.289	105.485	.000
	Outcome	28.888	1	28.888	6.664	.022
	Error	60.692	14	4.335		

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BIOGRAPHICAL SKETCH

Peter R. Nelson was born in Framingham, MA, on September 18, 1966. He attended high school at Framingham North High School and graduated as a member of the National Honor Society. He received his dual BS degree in Biology and Classics from Tufts University graduating summa cum laude and Phi Beta Kappa. He received the Thomas and Emily Carmichael Award in Physiology for research performed as an undergraduate. Peter then attended Medical School at the University of Massachusetts and remained there for general surgery residency. During residency he sought specialty research training in the Harvard-Longwood Vascular Research Fellowship. Following residency, he then completed his clinical Vascular Surgery Fellowship at Dartmouth College in 2001. Peter's first faculty position was at the University of Massachusetts as Assistant Professor of Surgery and Cell Biology. He then moved to his current position as Assistant Professor of Surgery at the University of Florida College of Medicine in 2004. His research is supported by a K23 Mentored Patient-Oriented Research Career development Award from the National Heart Lung and Blood Institute of the National Institutes of Health. He currently resides in Gainesville with his wife Janice, and their two sons Maxwell (13) and Peter ("PJ", 4).