Summary/Abstract

Bypass graft and/or stent thrombosis resulting in loss of blood flow to the lower extremity is a leading cause of amputation in elderly patients (≥60 years of age), with an incidence rate between 5-17%. The most common etiology driving graft/stent thrombosis is hypercoagulability, an increased propensity for blood to thrombose. While current strategies to prevent graft/stent thrombosis rely on anti-platelet and anticoagulant medications, management of thromboprophylaxis in elderly patients presents a significant challenge as this patient population is at high risk of both life-threatening thrombotic and hemorrhagic complications. Hypercoagulable presentations in these patients after extremity revascularization surgery can be transient, so the current standard practice of anticoagulation initiation to combat temporary thrombotic propensity increases the risk of hemorrhagic events in the months post-procedure. At present, there is no way to accurately predict which elderly patients are at risk of thrombosis at a particular point in time post limb revascularization to guide targeted, safe anticoagulation administration practices. The overall goal of this application is to define objective, individualized metrics to identify elderly patients at high risk of thrombosis and guide thromboprophylaxis strategies to improve care of elderly patients. Based on our preliminary data we hypothesize that utilization of longitudinal coagulation assays (TEG/platelet mapping/thrombin generation) in conjunction with clinical variables in a risk prediction model will identify elderly patients at risk of graft thrombosis. Our specific aims are to: (1) Identify unique patterns of hypercoagulability amongst elderly patients at high risk of extremity graft/stent thrombosis; and (2) Develop and validate a novel and individualized risk prediction tool for extremity graft/stent thrombosis in elderly patients. This study will result in a novel, individualized risk prediction tool that can be used to identify elderly patients at high risk for graft/stent extremity thrombosis and guide clinical decision making in pursuit of the safest and most effective thromboprophylaxis strategies for this vulnerable population.

Public Health Relevance

Hypercoagulability is the most common etiological factor in bypass graft/stent thrombosis/clotting in elderly patients and may lead to limb amputation. The current “one size fits all” approach to anticoagulation to prevent graft/stent thrombosis increases the risk of bleeding *and* clotting events in the months post-procedure. The goal of this application is to define objective, individualized clotting factor metrics to identify elderly patients at high risk of thrombosis and develop and validate a novel, personalized risk prediction tool that can be used to identify elderly patients at high risk for graft/stent thrombosis and guide clinical decision making in pursuit of the safest and most effective anticoagulation strategies for this vulnerable population.

FACILTIES AND OTHER RESOURCES – The Massachusetts General Hospital/Harvard Medical School

Environment – Contributions to Success

Dr. Dua's laboratory is located in the Edwards Building within the Massachusetts General Hospital (MGH). The facilities, equipment, and other essential resources that are available to her, through her personal lab space and core facilities, are more than sufficient for the successful completion of her proposed experiments. Her research office is located in the same hallway as her laboratory space and the vascular research office, which is in close proximity to the clinical site from which patient samples will be collected. Furthermore, Dr. Dua and her research team have 24/7 access to her laboratory where the blood sample analyzers are housed. There are ample resources that will facilitate Dr. Dua's success as a researcher at the MGH. These include the Mass General Research Institute which is designed to promote, support and guide all research done at the Massachusetts General Hospital. With a budget of $1 billion in 2020, MGH is home to the largest hospital-based research enterprise in the USA. MGH ranks 1st among independent hospitals in the amount of annual funding received from NIH, and 9th among all institutions. The MGH comprises more than 8500 researchers working across more than 30 institutes, centers and departments. Together, the strong research environment will ensure the success of the proposed study and enable Dr. Dua to complete her aims successfully.

Institutional Commitment to Early Stage Investigator: The MGH, including the Department of Surgery and the Codman research center, is fully committed to supporting Dr. Dua, an early stage investigator. Upon her appointment as an Assistant Professor, Dr. Dua was given 30% protected time for research, a competitive starting salary, office space, lab space, and start-up funds to support a full-time coordinator and the necessary materials to produce the preliminary data for this application. Dr. Dua was able to leverage these resources to secure further funding from industrial partners. Dr. Dua was also provided with administrative support and given unrestricted access to departmental administrative core. The department also supports Dr. Dua's participation in an NIH grantsmanship workshop and access to grant submission assistance. Finally, Dr. Dua has the full support of her mentor, Dr. Joren Madsen who is director of the Mass General Transplant Center, Co-Director of the Center for Transplantation Science, Paul S. Russell/Warner-Lambert Professor of Surgery at HMS and Professor of Surgery at MGH; he has been continuously funded by the NIH since 1995. Dr. Madsen's area of research is complementary to Dr. Dua's, and he meets with her regularly and provides any guidance necessary for experimental design, execution, analysis, or general guidance of academic development.

Facilities

Laboratory: Dr. Dua's laboratory is located at MGH, on the 3rd floor of the Edwards Building and adjacent to the Codman research center. In total, her laboratory is comprised of 2500 square feet is divided into a main, general purpose lab and a shared office space. Minor equipment in her laboratory includes: fume hoods, ultra pure water system, refrigerators/freezers (4°C, -20°C, -80°C), bench top centrifuges (both cool and room temp.), bench top oven, and water baths. Specialized equipment particularly relevant to this project include: three TEG 6000s machine and refrigerators specifically calibrated to TEG cartridge storage. *This laboratory space was uniquely selected for Dr. Dua, with proximity to the clinical, operative and emergency department spaces within the hospital to support her research in arterial thrombosis by providing easy access to subjects for sample collection. As such, she is capable of completing the proposed experiments here in her laboratory at the MGH.*

Clinical: The clinical site for this proposal will be the MGH ICU and Emergency Department, operative suites, interventional radiology suites, and vascular surgery clinic. The MGH is a Level I Trauma Center serving approximately 4.8 million people in the greater Boston area and Northeastern USA. The MGH encompasses the building (Edwards) which houses Dr. Dua's laboratory. The MGH emergency room, operative area, and clinical spaces are connected via an access hallway. Dr. Dua and her staff have direct access to all clinical spaces of the MGH, which will allow feasibility for obtaining the clinical samples necessary for the proposed project and allow for quick transport back and forth between the hospital and the laboratory. The MGH is one of the oldest and most prominent hospitals in the USA with a large surrounding population and referral network. Furthermore, as Dr. Dua is the director of the vascular lab, associate director of the wound care center and co-director of the peripheral artery disease center, she is in direct contact with the study population sought after in this proposal. *This provides an ideal setting to execute future interventional studies that result from the proposed*

project. A dedicated data collection staff is available for use on projects funded through leveraged resources. Work-stations are available for the researchers both in the MGH as well as in the laboratory building itself. Investigators work closely with these data collectors to ensure that accurate and appropriate data is collected for analysis.

Computer: Dr. Dua has been given two computers, a desktop in her office and a laptop for use off-site. All major equipment items in the laboratory have a dedicated Windows-based workstation for data acquisition and analysis including but not limited to the following software packages Microsoft Office, SigmaPlot 12.0, Graphpad Prism, Endnote, STATA, LabChart 7, Matlab, and ImageJ. Furthermore, all laboratory computers are linked via twin 1-TB servers for data storage/backup and remote-access for analysis and are equipped with telecommunication capabilities. The laboratory computers have access to internet and the Harvard Medical School Library, which houses thousands of electronic biomedical journal articles. *These technological resources reduce logistical barriers to completing the proposed research, handling and analyzing acquired data, and maintaining collaborations.*

Office: Dr. Dua's has two office spaces, in the Edwards building adjacent to her laboratory and her clinical office space housed in the Division of Vascular and Endovascular Surgery in the Wang building within the MGH. Her office is complete with a desk, filing cabinet, shelf, and dedicated phone and Ethernet connection. There are also two desks with computer availability within Dr. Dua's main lab. A shared office space houses 5 desks and 5 chairs with computers at which the technicians, postdocs, or research residents can work. An 10-15 person conference room is available in the clinical area as well as a 5-10 person conference room available within the Vascular Surgery research office which is in the same hallway as Dr. Dua's laboratory. More advanced conference facilities are available in the departmental administrative suite as well as elsewhere in the Harvard Medical School, with voice and video conference capabilities. *Such office space will enable Dr. Dua and her team to analyze data, write manuscripts, and maintain regular collegial lab meetings.*

Major Equipment & Other Resources: TEG® Hemostasis Analyzer Systems (3); Olympus Fluorescence Microscope; Nikon and Olympus Inverted Microscope; Beckman L8 ultracentrifuge; Beckman J21 centrifuge; Table-top centrifuge Beckman Spectrophotometer; Beckman Scintillation counter; Hypoxic Incubator; Blood Gas Analyzer and Computer operated Digital Microscope; Nikon E600 epiflourescent microscope

Research Cores: MGH Research Cores provide access to further research equipment and expertise for the investigators. Available cores include: Biostatistics Core, DNA Microarray Core, Electrophysiology Core, Flow Cytometry Core, Knockout Facility, Laser Capture Microscopy Core, Magnetic Resonance Imaging Core, Microscopy and Image Analysis Core, Model Shop (Prototyping and Fabrication Facility), Nucleic Acid Quantitation (RT PCR) Core, PET Core, and Transgenic Animal Core.

Harvard Catalyst Program: This is a pan-Harvard University enterprise to create a systematic way for investigators from disparate disciplines and institutions to find each other and form teams, to gain open access to tools and technologies, and to obtain seed funding to embark upon new areas of investigation, with the ultimate goal of enhancing the pace of translational research. It is a shared enterprise of Harvard University, its ten schools and its eighteen Academic Healthcare Centers (including MGH), as well as the Boston College School of Nursing, MIT, the Cambridge Health Alliance, Harvard Pilgrim Health Care and numerous community partners. The Catalyst program provides access to several core services, ranging from monoclonal antibody synthesis and RNAi screening to cellular and medical imaging, bioinformatics, biostatistics and a unique Harvard Catalyst - Laboratory for Innovative Translational Technologies that provides a myriad of enabling technologies for translational research. These services free for investigators at Harvard; Dr. Dua is an Assistant Professor of Surgery at Harvard and qualifies for all these advanced statistical services as a result. Dr. Chang, co-investigator and biostatistician on this proposal, is an active member and contributor to the Harvard catalyst statistical program. The study proposed here qualifies for support from Harvard catalyst and will draw on the statistical resources available at the Codman center for health outcomes research as Dr. Chang is the director of this institute.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Dua, Anahita

eRA COMMONS USER NAME (credential, e.g., agency login): ADUA11

POSITION TITLE: Assistant in Surgery Massachusetts General Hospital, Assistant Professor of Surgery Harvard Medical School

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Hampshire College, Amherst, MA	B.A.	06/2004	Organic Chemistry
University of Aberdeen Medical School, Aberdeen, UK	M.D.	08/2009	Medicine and Surgery
University of Texas-Houston, Houston, TX	Postdoctoral	06/2014	Resuscitation, limb salvage research
Barts and The London School of Medicine, London, UK	MSc	11/2013	Trauma Sciences
Western Governors University, Salt Lake City, UT	MBA	06/2014	Healthcare Management
Medical College of Wisconsin, Milwaukee, WI	Residency	06/2012, 06/2017	General Surgery
Stanford University Hospital, Palo Alto, CA	Fellowship	07/2019	Vascular Surgery
University of Washington, Seattle WA	Certificate	06/2019	Health Economics and Outcomes Research
Massachusetts Institute of Technology, Cambridge MA	Certificate	05/2020	Drug and Device Development

A. Personal Statement

I am a vascular surgeon at the Massachusetts General Hospital (MGH), with a specialty clinical and research focus in peripheral artery disease (PAD). I am Assistant Professor of Surgery at the Harvard Medical School, Director of the MGH Vascular Lab, Co-Director of the MGH Peripheral Artery Disease Center, Director of the MGH Lymphedema Center, and Associate Director of the MGH Wound Center. I am dually board-certified in Vascular Surgery and General Surgery, and my research centers on limb salvage and PAD.

As in the proposed project, my translational research focuses mainly on anticoagulation, emphasizing clotting pathway activation in post-revascularization of the vascular patient, which is the most common cause of graft loss, frequently resulting in amputation. My research aims to identify and prevent causes of thrombosis to

ensure and maintain graft/stent patency and prevent thrombosis – an area to which I bring extensive clinical and translational expertise. My 2-year dedicated postdoctoral research fellowship at UT-Houston focused on activation of the clotting cascade in vascular and trauma patients, and on associated patient-centered tailoring of resuscitation and anticoagulation while my 2 year clinical vascular surgical fellowship provided specialist training in advanced endovascular and open limb salvage surgical techniques and clinical management of this patient cohort. These training endeavors have provided me with an advanced understanding of coagulation assays, thrombotic propensity, and the clinical course of elderly PAD patients, making me an ideal investigator for the proposed project to develop new ways to improve surgeons' predictive capabilities for thrombosis in the elderly.

I am uniquely poised to lead my team of expert co-investigators (in hematology, thrombosis and coagulation, and biostatistics), as I have significant research experience in thrombosis *and* a large elderly PAD clinical practice from which to draw patients. Furthermore, my masters degree in trauma sciences was structured around development of health systems to improve population health and anticoagulation practice patterns, major concerns in the elderly population that drive my commitment to address this under-served clinical and public health need.

As a recognized leader in clinical research, I am the national PI on the LimFlow, Inc. CLariTI Study, which is examining clinical progression of chronic limb-threatening ischemia (CLTI) and incidence of death, amputation, and revascularization attempts in the USA. I am also PI of the PROMISE II trial, which is evaluating percutaneous deep vein arterialization for treatment of late-stage chronic limb threatening ischemia. I am the also the PI and co-PI on multiple studies of wound healing and limb salvage. In my own lab at MGH, I have had 2 grants funded as PI: ***1)*** Haemonetics® Corp., *Utilization of Thromboelastography (TEG) to Identify Patients with Extremity Vascular Injury at Risk of Graft Thrombosis* (01/10/2020-02/10/2021; $50,000); and ***2)*** Executive Committee on Teaching and Education (ECOTE), *Development of Open Aortic Aneurysm Repair Simulation;* 09/01/2019-08/31/2020; $10,000; a study of methods for teaching advanced open aortic surgical skills. The industry grant support from Haemonetics® Corp. provided the preliminary data for the proposed study. My dedicated research lab at MGH includes 650 square feet, bench space, and key equipment including TEG devices, and a team including a research nurse, a part-time post-doctoral student and two part-time research residents, funded by the Department of Surgery.

Overall, my accumulated research aims to advance clinical therapy options for PAD, and innovative and mechanistic ways to optimize anticoagulation and wound healing, to improve success in revascularization and decrease amputation rates. I have authored over 125 peer-reviewed published articles, including ~86 as first or senior author, and edited 4 textbooks, including a comprehensive clinical guide to amputation. I also have completed an MBA with a focus in healthcare management to understand the logistics of PAD resources allocation, as this is intimately associated with access to care and limb salvage. These efforts and qualifications, prior and related roles as PI on national trials, and my roles and resources at MGH position me exceptionally well to achieve the aims of the proposed R21-funded research and extend it in planned R01-funded research.

B. Positions, Scientific Appointments and Honors

Positions and Employment

2019 - Present Assistant Professor of Surgery, Harvard Medical School
2019 - Present Assistant in Surgery, Massachusetts General Hospital

Honors

2005 FEZANA scholar for academic excellence and overall achievement, endowed scholarship
2009 Emad El-Omar Prize for Academic Development at the University of Aberdeen School of Medicine
2009 Ogsten Prize in Surgery
2012 Youth Without Borders scholarship for medical mission to Chad
2012 American College of Surgeons – Wisconsin Committee on Trauma (ACS-COT) – 1st place clinical paper presentation
2012 Earl Young Resident Paper Competition Western Trauma Association (WTA) – 3rd place podium presentation award

2012 Oriens Award for The Eastern Association for the Surgery of Trauma (EAST) – Honorable Mention award
2013 Eastern Vascular Society (EVS) Resident Award Winner
2013 1st place, Condon-Donegan Research Competition
2013 Society of Vascular Surgery (SVS) Travel Scholarship Award
2013 Best Poster Prize, Association of Surgeons of Great Britain and Ireland (ASGBI)
2013 Hitoshi Nikaidoh, M.D. Memorial Endowment Award
2014 Society of Vascular Surgery (SVS) Travel Scholarship Award
2014 Society of Vascular Surgery (SVS) Resident Surgical Skills Winner
2015 Society of Vascular Surgery (SVS) Resident Surgical Skills Winner
2016 South Asian American Vascular Society (SAAVS), Best Resident Research award
2017 American College of Surgeons Leadership and Advocacy Summit Scholarship
2018 Society of Vascular Surgery (SVS), Vascular Surgery Trainee Advocacy Scholarship
2018 Society of Vascular Surgery (SVS), Best Poster Award [PAD]
2018 Eastern Vascular Society (EVS), Traveling Scholarship
2019 Critical Limb Ischemia Course (CLIC) Traveling Scholarship
2019 Venous Symposium Fellows Scholarship
2019 Program for Advanced Limb Preservation (PALP) – 1st Place, Best Poster Prize
2019 Amputation Prevention Symposium (AMP) Fellow and Early Career Physician Activity Scholarship
2020 James and Linda Wong Endowed Visiting Professorship

Professional Memberships

2008 - Present Society of Vascular Surgery (SVS)
2017 - Present American College of Surgeons (ACS)
2018 - Present Eastern Vascular Society (EVS)
2018 - Present Society of Clinical Vascular Surgery (SCVS)
2019 - Present Association for Academic Surgery (AAS)
2020 - Present New England Society of Vascular Surgery (NESVS)

Inventions

2015 Dua Dissector - Item # FL0888.12 - WEXLER SURGICAL
Description – Dua Dissector – Strongly Curved serrated tips, Stainless Steel, 6.75" (17cm)
2016 Co-inventor on US Patent Application No. 15/224, 116, filed July 29th, 2016: Nucleic Acid Amplification Techniques and Methods for Detecting Bacterial Infection
2020 Patent No PCT/US20/16372, filed June 30th, 2020: Clot Removal System (Thrombolysis)

Leadership Positions

2009 - 2010 Founder of the Aberdeen Summer Research Studentship, University of Aberdeen http://www.abdn.ac.uk/acat/ugrad-research/ASRS/
2011 - 2012 Board of Directors – Medical Society of Milwaukee County
2011 - 2012 Wisconsin Resident Delegate to the American Medical Association (Resident Fellows Section)
2012 - 2013 Appointed Animal Welfare Committee Board Member – University of Texas-Houston
South Asian-American Vascular Society (SAAVS) – Co-chair, Young Surgeons Committee
2013 - 2014 Appointed Texas Medical Association (TMA) Representative **-** Committee on Blood and Tissue Usage
2017 - 2018 Society of Vascular Surgery (SVS) International Relations Committee
2017 - 2018 Society of Vascular Surgery (SVS) Post Graduate Education Committee; Moderator Hemodialysis Breakfast Session
2018 - 2019 Eastern Vascular Society (EVS) Ad-Hoc Center for Vascular Awareness V-Healthy™ Committee
2017 - 2019 Senior Research Associate - VascTrac **(**http://vasctrac.stanford.edu/education/**)**
2018 - 2019 Resident Safety Council
2018 - 2019 Association of Program Directors in Vascular Surgery (APDVS) Executive Council
2018 - 2019 Stanford Surgical Quality Council
2018 - 2020 Society of Vascular Surgery (SVS) Post Graduate Education Committee member (PGEC)
2019 Society of Vascular Surgery (SVS) – Moderator, Hemodialysis Breakfast Session

2019 Society of Vascular Surgery (SVS) – Organizer and Moderator, Tibial Workshop organizer
2019 Society of Vascular Surgery (SVS) – Moderator SVU-SVS joint session
2019 - 2020 South Asian American Vascular Society (SAAVS) Membership Committee Chair
2019 - 2020 Society of Vascular Surgery (SVS) Post-Resident and Student Outreach Committee member
2019 - present Associate Director of the Massachusetts General Hospital Wound Center
2019 - present Director of the Massachusetts General Hospital Vascular Lab
2020 - present Director of the Massachusetts General Hospital Lymphedema Center
2020 - present Co-director of the Massachusetts General Hospital Peripheral Vascular Disease Center

C. Contributions to Science

1. *Limb salvage and PAD* I have contributed significantly to this field, as first or senior author on multiple publications on physiological mechanisms that contribute to graft thrombosis, amputation and/or mortality in patients with PAD. My research findings have been incorporated into patient management guidelines, specifically the part of my findings revolving around preventing amputation in patients managed early with 1:1:1 resuscitation efforts, and the impact of this management on thrombotic status. My recent case report paper in the *New England Journal of Medicine* highlights the advanced endovascular and open technical skills and patient complexity I draw upon to set the gold standard for care in the vascular (aortic and PAD) arena.
Relevant Publications:

- **Dua A**, Sutphin PD, Siedner MJ, Moran J. Case 16-2021: A 37-Year-Old Woman with Abdominal Pain and Aortic Dilatation. *N Engl J Med*. 2021 May 27;384(21):2054-2063. doi: 10.1056/NEJMcpc2100278. PubMed PMID: 34042393.
- Latz C, Wang L, Boitano L, DeCarlo C, Sumpio B, Schwartz S, Lee C, **Dua A.** Contemporary Endovascular Outcomes for Critical Limb Ischemia are Still Failing to Meet Society for Vascular Surgery Objective Performance Goals. *Vasc Endovascular Surg*. 2021 Jan;55()1):33-38. PMID: 33030116.
- Latz C, Wang L, Boitano L, DeCarlo C, Pendleton A, Sumpio B, Schwartz S, **Dua A**. Unplanned Readmissions After Endovascular Intervention or Surgical Bypass for Critical Limb Ischemia. *J Vasc Surg*. 2020 Aug 27. PMID 32861862
- Ho VT, Gologorsky R, Kibrik P, Chandra V, Prent A, Lee J, **Dua A.** Open, percutaneous, and hybrid deep venous arterialization technique for no-option foot salvage. *J Vasc Surg*. 2020 Jun;71(6):2152-2160. doi: 10.1016/j.jvs.2019.10.085. Epub 2019 Dec 31. Review. PubMed PMID: 31901360.

2. *Thrombosis & vascular surgery outcomes* I have made key contributions to this field, via my translational work on the impact of freezing and thawing on clotting factors. As a post-doctoral research fellow, my research focused on thrombosis and anticoagulation in the lab of Charles Wade, PhD and John Holcomb, MD at the University of Texas-Houston/Center for Translational Injury Research (CeTIR). I analyzed mechanisms associated with clotting profiles in patients, and the factors in blood components that serve to inhibit or promote thrombosis. My basic science research on freezing-thawing of donated plasma yielded an article published in the *Journal of Trauma and Acute Care Surgery* that revealed never-frozen, liquid plasma repairs permeability of endothelial membranes as effectively as thawed fresh-frozen plasma. Those findings assist practitioners in making clinical decisions regarding blood component distribution and management in large vascular trauma centers. I also recently led a research term to identify that unexpected thrombotic events in the lower extremities may be a predictor of cancer, in patients who experience these events within 180 days of cancer diagnosis. This was a completely novel finding, as the time to event had never been quantified. With this research, we are building risk-prediction models to identify which patients may carry malignancies based on their coagulation profiles.
Relevant Publications:

- **Dua A***, Cao Y*, Matijevic N, Wang YW, Pati S, Wade CE, Ko TC, Holcomb JB. Never-frozen liquid plasma blocks endothelial permeability as effectively as thawed fresh frozen plasma. *J Trauma Acute Care Surg*. 2014 Jul;77(1):28-33; discussion 33. doi: 10.1097/TA.0000000000000276. PubMed PMID: 24977751.
- Png CYM, Wang LJ, DeCarlo CS, Latz CA, Sumpio BJ, Weinberg I, Eagleton MJ, **Dua A**. Effect of occult malignancy on femoropopliteal bypass graft thrombosis. *J Vasc Surg. 2021* Feb 16;. doi: 10.1016/j.jvs.2021.01.058. [Epub ahead of print] PubMed PMID: 33600933.
- Decarlo C, Boitano L, Sumpio B, Latz C, Feldman Z, Pendleton A, Chou E, Stern J, **Dua A**. Comparative Analysis of Outcomes in Patients Undergoing Femoral Endarterectomy Plus Endovascular (hybrid) or Bypass for Femoropopliteal Occlusive Disease. *Ann Vasc Surg*. 2020 Sep 11. PMID: 32927041

- Latz CA, Boitano L, Wang LJ, Pendleton AA, DeCarlo C, Sumpio B, Schwartz S, Srivastava S, **Dua A**. Contemporary Endovascular 30-Day Outcomes for Critical Limb Threatening Ischemia Relative to Surgical Bypass Grafting. *Vasc Endovascular Surg*. 2021 Feb 19;:1538574421989516. doi: 10.1177/1538574421989516. [Epub ahead of print] PubMed PMID: 33602047.*)*

3. *COVID-19-related thrombotic events* I have made significant contributions to advance understanding of the novel and critical problem of thrombotic events occurring in patients with COVID-19 that emerged during only the past 1.5 years of this pandemic, as clinicians came to recognize, and struggled to manage, thrombotic syndromes in patients with COVID-19. I published several key manuscripts delineating the relationship between clotting and COVID during the pandemic. One of these was a study I spearheaded published in *Annals of Hematology* detailing the lack of association between blood type and COVID-19 severity. This study received significant international and national media attention (CNN, New York Times), and served to quell concerns that certain blood types were associated with negative patient outcomes in COVID-19. I was also senior author on a study that determined the D-dimer level that correlated with microembolic or deep vein thrombosis in patients with COVID-19, which served to establish current national vascular lab guidelines that identify which patients require a confirmatory ultrasound scan to initiate anticoagulation. This study was published in the *Journal of Vascular Surgery: Venous and Lymphatics and* is the only national guideline that both optimizes access to anticoagulation and also decreases technologist exposure time to COVID-19 patients, by eliminating the need for unnecessary DVT scans. Aside from COVID, I publish extensively on associations between specific disease processes or co-morbid conditions and thrombotic events. Recently, I led a research team that evaluated unique peripheral arterial events that occur in pregnant patients, which is the largest, most comprehensive study reviewing thrombotic peripheral events in this patient cohort.

Relevant Publications:

- **Dua A**, Thondapu V, Rosovsky R, Hunt D, Latz C, Waller D, Manchester S, Patell R, Romero J, Ghoshhajra B, Eagleton M, Brink J, Hedgire S. Deep vein thrombosis protocol optimization to minimize healthcare worker exposure in Coronavirus disease-2019. *J Vasc Surg Venous Lymphat Disord*. 2020 Aug 11. PMID: 32795617.
- Latz C, Decarlo C, Boitano L, Png C, Conrad M, Eagleton M, **Dua A.** Blood type and outcomes in patients with COVID-19. *Ann Hematol*. 2020 Jul 12;1-6. doi: 10.1007/s00277-020-04169-1
- DeCarlo C, Boitano LT, Molina RL, Weinberg I, Conrad MF, Eagleton MJ, **Dua A**. Pregnancy and preeclampsia are associated with acute adverse peripheral arterial events. *Arterioscler Thromb Vasc Biol*. 2021 Jan;41(1):526-533. doi: 10.1161/ATVBAHA.120.315174. Epub 2020 Oct 15. PubMed PMID: 33054392.
- Latz CA, Boitano LT, Wang LJ, DeCarlo C, Pendleton AA, Waller HD, Lee CJ, **Dua A**. Perioperative outcomes for carotid revascularization on asymptomatic dialysis-dependent patients meet Society for Vascular Society guidelines. J Vasc Surg. 2020 Dec 17;. doi: 10.1016/j.jvs.2020.11.044. [Epub ahead of print] PubMed PMID: 33340696.

URL to a complete list of published research:
https://www.ncbi.nlm.nih.gov/myncbi/1P1j_oq6fmqAsz/bibliography/public/

Budget Justification: Massachusetts General Hospital

The following budget reflects funding request is for a one year proposed, multidisciplinary, prospective study.

Personnel: The total personnel costs for requested for Year 1 (Y1) $21,110. The responsibilities of said personnel is also described:

A. Senior/Key Personnel:
Anahita Dua MD MS MBA

Responsibilities: Dr. Dua (vascular surgeon) will serve to guide the data collection and interpretation of results in this study. Dr. Dua, the PI, is not requesting funding from this grant as salary support.

B. Other Personnel:

Research Assistant (50% effort, 6.0 calendar months: Y1 $21,110)
Responsibilities: He/She will be responsible for patient screening, ensuring efficient patient enrollment, data collection, data entry and analysis.

C. Equipment: Not applicable to the current application.

D. Travel: A total of $1,500 per year is requested for travel expenses over this study period. This sum will cover the airfare, lodging and registration at the Eastern Vascular Society (EVS) meeting where this work shall be presented.

E. Participant/Trainee Support Costs: Not applicable to the current application.

F. Other Direct Costs:

a. **Materials and Supplies:** A total of $250 is requested per year for data collection. This includes, but is not limited to, data collection forms, materials to collect data (office supplies), record retrieval, server and storage space.

b. **Publication Costs:** Not applicable to the current application

c. **Consultant Services:** A total of $2,150 per year is requested for statistical support

d. **ADP/Computer Services:** Not applicable to the current application.

e. **Subaward/Consortium/Contractual Costs:** Not applicable to the current application.

f. **Equipment or Facility Rental/User Fees:** Not applicable to the current application.

g. **Alterations and Renovations:** Not applicable to the current application.

h. **Other Expenses:** Not applicable to the current application.

Budget (2021-2022)

Item	**Cost**
Statistical Support	$2,130
Materials	$250
Research assistant (data collection)	$21,110
Travel	$1,500
Total	**$24,990**

Preventing Graft Thrombosis in the Elderly through Development of a Risk Prediction Tool

A. ABSTRACT FOR RESEARCH PROPOSAL

Peripheral artery disease (PAD), caused by atherosclerotic chronic arterial occlusion of the lower extremities, is endemic in the elderly population, and its prevalence increases with age[1-4]. Over 50% of elderly patients are symptomatic, warranting extremity artery bypass or endovascular stenting to increase limb perfusion and relieve symptoms[1-4]. Thrombosis of bypass grafts or stents that results in impaired blood flow to lower extremities is a leading cause of amputation in elderly patients (aged ≥60 years)[5-7]. The incidence rate of early graft/stent thrombosis after extremity bypass is significant, ranging from 5% to 17%[5-7], and up to 50% of patients die within one year of amputation[5-7]. Therefore, accurate identification of elderly patients that are high risk for graft/stent thrombosis is critical to inform targeted, early interventions that will prevent graft/stent loss and amputation. *This study will identify a novel, objective, coagulation assay-based risk prediction tool to identify elderly patients at risk of graft/sent thrombosis.*

The most common etiology driving graft/stent thrombosis is hypercoagulability[5-7]. Elderly patients who develop graft/stent thrombosis demonstrate pronounced elevations of platelet reactivity and thrombin generation, with concomitant reductions in fibrinolysis[8-12]. Current strategies to prevent thrombosis rely on anti-platelet and anticoagulant medications but managing thromboprophylaxis in elderly patients is highly challenging as this patient population carries dual high risks of both life-threatening thrombotic *and* hemorrhagic complications. Therefore, targeted and timely anticoagulant prescribing is especially critical in the elderly. Also, after extremity revascularization surgery, hypercoagulability in such patients can be *transient*, such that anticoagulation initiated to combat temporary thrombotic propensity increases the likelihood of hemorrhagic events during post-procedure months[13-22]. Thus, the current practice of a "one size fits all" anticoagulation approach to thromboprophylaxis in elderly patients is inappropriate and dangerous. The key barrier to progress is our current lack of any way to accurately predict ***which elderly patients are at risk of graft/stent thrombosis at various time points*** after extremity revascularization surgery to guide targeted, post-surgical anticoagulation administration practices. The ***goal of this EVS award application*** is to define novel, objective, and individualized biometrics to identify elderly patients at high risk of extremity bypass graft/stent thrombosis and guide thromboprophylaxis strategies to improve the care and safety of elderly patients. Our **preliminary data from a prospective, observational pilot study** demonstrate that utilizing point-of-care coagulation assays can identify *transient* hypercoagulability that is amplified among elderly patients who develop extremity bypass graft/stent thrombosis. Specifically, we showed that whole blood thromboelastography (TEG) and platelet mapping (PM) coagulation assays were able to identify hypercoagulability *prior* to a thrombotic event in this population. These preliminary findings suggest we can advance the field beyond those of prior research by developing/implementing into clinical care a *new risk-scoring tool* that incorporates patient-specific longitudinal coagulation assay data and can enable accurate prediction of *1)* which elderly patients are at risk of graft thrombosis, and *2) when* they are at highest risk following extremity revascularization surgery. When combined with clinical variables, such a tool would *enable early, targeted anticoagulant-based intervention that can prevent clotting complications and risk of extremity amputation and death.*

Based on our preliminary data, we hypothesize that utilization of longitudinal coagulation assay data (TEG/PM/thrombin generation) in conjunction with clinical variables in a risk prediction tool will identify elderly patients at risk of extremity graft/stent thrombosis.

Specific Aim 1. Identify individual hypercoagulability patterns among elderly patients at risk of extremity graft/stent thrombosis. We will prospectively establish *individualized* coagulation profiles utilizing pre- and post-operative data from point of care coagulation testing (TEG/PM/thrombin generation) at a minimum of ten time points over a 6-month period. We will determine how clinical and pharmacological factors impact these coagulation assays *longitudinally* to identify individualized patient coagulation trends over time. This will identify *when* in the post-operative course individual elderly patients are at highest risk for graft/stent thrombosis and *what* coagulation assay values are predictive of a thrombotic event.

Specific Aim 2. Develop and validate a novel and individualized risk prediction tool for extremity graft/stent thrombosis in elderly patients. Using longitudinal coagulation testing and clinical variables, we will generate a personalized thrombosis risk prediction tool using multivariable analysis. We will test the validity of our model through bootstrap analysis by repeated, random resampling of the cohort [14].

Completion of the proposed aims will result in a novel, individualized risk prediction tool that can be used to identify elderly patients at high risk for graft/stent extremity thrombosis and ultimately guide clinical decision making in pursuit of the safest and most effective thromboprophylaxis strategies for this vulnerable population.

B. SIGNIFICANCE OF RESEARCH

B.1 Risks & Limitations of Anticoagulation Therapy in the Elderly. Elderly patients on anticoagulants have increased mortality from bleeding compared with younger individuals[15]. Anticoagulation is commonly used to prevent venous and arterial thrombosis is elderly populations, but treatment is often complicated by the dual increased hemorrhagic *and* thromboembolic risk caused by age, comorbidities, and polypharmacy[15-19]. Furthermore, age-related modified pharmacodynamics of anticoagulants (especially vitamin K antagonists), the presence of comorbidities, and concomitant medications can impact an individual's coagulation profile *differently* at different points in time. Hence, physicians' ability to decipher longitudinal, *personalized* thrombotic risk in elderly patients and prescribe **optimal, timely anticoagulant medication is critical. Although basic risk prediction models for anticoagulation in the elderly exist, none are longitudinal in nature nor have they considered objective coagulation assay data or been validated**[18]. No objective, validated tool is thus available to accurately predict graft/stent thrombosis in elderly patients. To address this critical need and enable improved anticoagulant management in this vulnerable population, the proposed project aims to create and validate a risk prediction tool that includes objective coagulation assay data and clinical variables to accurately identify individualized graft/stent thrombotic risk in elderly patients.

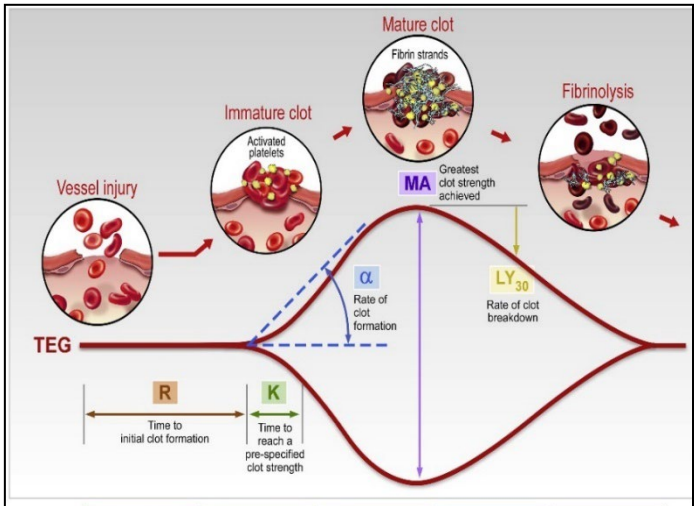
A common indication for anticoagulant therapy in elderly populations is to prevent graft/stent thrombosis and the possibility of amputation due to graft loss[9-12,20]. Amputation, resulting from loss of blood flow to the extremity, is a significant cause of death in elderly patients; mortality rates increase by 4% per year of age in patients who undergo amputation[17]. Early graft failure due to thrombosis is the most common cause of secondary amputation in the elderly and can be prevented by appropriate anticoagulation therapy[5-7]. Amputation rates due to early graft occlusion after arterial extremity revascularization range from 13%–30% depending on the anatomic location of vessel atherosclerosis[7] and the incidence rate of early graft thrombosis after extremity bypass is significant, ranging from 5% to 17%[5-7]. Most efforts to alter the natural history of graft failure have focused on modulating the intimal hyperplastic responses impacting mid-term and late failure[21,22], but there is little research and a paucity of clinical data focused on modulating factors that impact early graft or stent (≤6 months) thrombosis. The proposed project aims to overcome this knowledge gap.

Multiple interventions to salvage thrombosed grafts (e.g., lysis, thrombectomy, re-operation) impose significant cost on the healthcare system with a mean 5-year graft maintenance cost of $16,318[23] and nonetheless result in amputation[9-12,24-26]. Hence, regardless of the etiology, preventing early graft/stent thrombosis using anticoagulation/antiplatelet medications is crucial to decreasing amputation rates and healthcare costs in elderly patients. However, the current anticoagulation/antiplatelet standard of care poses significant challenges for the elderly due to lack of predictive tools to enable precise, targeted administration.

C. BACKGROUND INFORMATION

C.1 Concerns with Anticoagulation Standard of Care: The current standard of care is to place elderly patients on antiplatelet therapy (aspirin or clopidogrel) following bypass graft/stent procedures for peripheral arterial occlusive disease[27]. Unfortunately, there is no level 1A evidence to support antiplatelet/anticoagulation practices post revascularization surgery in the elderly [27]. Clopidogrel is more expensive but offers no evidence of superiority to aspirin and up to 25% of the population is resistant to it[28]. Use of anticoagulation after extremity bypass surgery is also highly controversial. The Dutch Bypass Oral Anticoagulants or Aspirin trial found that oral anticoagulation improved bypass graft patency versus aspirin alone, but the Veterans Affairs cooperative trial that randomized patients to warfarin and aspirin versus aspirin alone concluded that adding warfarin to aspirin failed to improve bypass patency, *but there was a significant added risk of hemorrhagic events in elderly patients*[29-31]. Owing to conflicting data and lack of sufficiently accurate predictive tools, the type and duration of anticoagulant/antiplatelet therapy is based entirely on **physician preference** in elderly patients after extremity bypass, relying on **subjective analysis** of thrombotic risk based on clinical variables, past medical history, and medications[27]. The current standard of care to prevent graft/stent thrombosis is thus administration of anticoagulant/antiplatelets based on clinical history and we predict it would be qualitatively improved by the addition of *objective, more accurate point of care (POC) blood coagulation tests*[26,27,32-34] as we propose. The current single-tier strategy is inadequate for managing the medically nuanced elderly patients as it leads to under and overdosing of anticoagulant/antiplatelet medications, which leads to thrombosis and hemorrhage, respectively [29-31]. Elderly patients would thus benefit significantly from *objectively* deciphering their anticoagulant needs in real time, and varying anticoagulant doses based on a risk prediction model that combines clinical factors *and* POC testing to determine their ***present*** coagulation profiles.

C.2 Hypercoagulability as Thrombotic Risk Determinant. Graft/stent thrombosis is frequently a product of hypercoagulability [5-8]. Based on the relationship between graft thrombosis and hypercoagulability, our proposed solution is to use objective coagulation assays to evaluate the combination of factors that yield hypercoagulable states (increased thrombin generation, increased platelet functionality, and decreased fibrinolysis) to provide a given patient's coagulation profile[35-38].



	R-time (R)	K-time (K)	Alpha angle (α)	Maximum Amplitude (MA)	Lysis % at 30 mins. (LY30)
Definition	Time to first deviation from baseline	Time for tracing to reach 20 mm amplitude	Angle between baseline and tangent line that intersects initial deviation	Maximum deviation of tracing from baseline	Decrease in curve amplitude (relative to MA) at 30 minutes
Controlling Pathways	Coagulation cascade	Fibrinogen cleavage Fibrin polymerization	Fibrinogen cleavage Fibrin polymerization	Fibrinogen activity Platelet count / quality	Fibrinolysis
Interpretation	↑ = hypocoagulable ↓ = hypercoagulable		↓ = hypocoagulable ↑ = hypercoagulable		
Therapeutic Implications	↑ = administer fresh frozen plasma	If K time is ↑ or alpha angle is ↓, then: administer cryoprecipitate or fibrinogen concentrate		↓ = administer platelets	↓ = administer tranexamic acid

Figure 1. Depiction of TEG parameters, clotting cascade and associated therapeutic implications (adapted from[64]).

C.3 Advantages of TEG, PM, CAT Coagulation Assays. Early tests for hypercoagulability, such as the International Normalized Ratio (INR) or prothrombin time (PT), were not specific, did not guide treatment well[27,48] and were generally not available at POC. Therefore, to help predict hypercoagulability, we will use TEG, a novel and advantageous tool for assessing hyper- or hypocoagulation based on parameters correlated with fibrinogen, fibrinolysis, clot strength, and time to clot formation[49,50]. TEG has been used to evaluate both prior-existing and acquired hypercoagulable states in patients with coagulation factor disorders and acquired hyper- or hypo-coagulant states such as cirrhosis or cardiopulmonary bypass[39,40-55]. In hypercoagulative states, TEG parameters will depict a 1) shortened time to clot initiation (R time), 2) decreased time to reach clot strength (K time), 3) increased rate of clot formation (α angle) and clot strength (mA), and 4) decreased rate of clot breakdown (Lysis 30)[43,52] (**Fig. 1**). Platelet hypercoagulability appears in the TEG trace as both rapid clot development (high angle) and strong clot development (high maximum amplitude [mA]). TEG with PM (TEG-PM) is used to measure platelet function, specifically in patients taking antiplatelet medications[44,45,56]. TEG-PM provides real-time assessment of platelet function, and is *predictive* of arterial thrombotic events in surgical patients[49,57-60]. Thrombin mediates the conversion of fibrinogen into the insoluble fibrin clot, which can be measured by CAT, a kinetic assay that measures cleavage of a fluorogenic thrombin substrate[61-63]. ***The proposed research aims to improve clinical care of elderly patients by developing a novel risk prediction tool that combines longitudinal POC coagulation assays (TEG/PM/CAT) with clinical variables to accurately identify elderly patients at risk of graft/stent thrombosis enabling targeted anticoagulation therapy to prevent graft/stent loss and amputation.***

D. PRELIMINARY DATA *Pilot Study Design:* With Institutional Review Board (IRB) approval and informed consent, our team *prospectively* collected daily TEG and PM data on revascularized (extremity bypass and stenting) elderly patients (age ≥60 years) who had atherosclerosis pre-operatively and up to 7 days post-operatively. We aimed to determine 1) if TEG values were predictive of thrombosis in the 7 days post-procedure; and 2) if TEG and PM values differed between patients with and without peri-operative graft/stent thrombosis (up to 7 days post procedure). Results: Among 30 patients enrolled, five experienced thrombotic events (16.6%), of which two (40%) resulted in amputation of the limb in the post-operative period (up to 7 days). **Figure 2** depicts the mA values of a patient who developed graft thrombosis on post-operative day 4, resulting in amputation. We found *significant elevation in mA on post-operative day 3 vs. baseline mA **preceding** graft thrombosis, and the mA spike was **predictive** of this thrombotic event on post-operative day 4*.

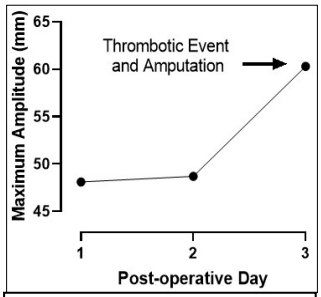


Figure 2. Clot strength (mA [mm] values) in patient with graft thrombosis spiked day 3, thrombotic event day 4.

Figure 3 shows that *patients with thrombosis had a lower mean R (time to clot formation) value than those without, indicating that those who thrombosed displayed faster clot formation.* Both the α angle (speed of clot formation) and mean mA (maximum clot firmness) values *trended* toward a difference, and we anticipate that with adequate power in the proposed study this difference will be significant.

Platelet Mapping: The most pronounced difference we saw between groups was in platelet-inhibition rates. All subjects were on anti-platelet medications (AA [aspirin] and ADP [clopidogrel]). However, elderly patients who had thrombotic events showed significantly *lower* levels of mean platelet inhibition in than did those who did not thrombose (p<0.05) (**Fig. 4**), indicating more platelets were active and contributed to thrombus formation in those who thrombosed. With enough power, we will set a benchmark therapeutic platelet inhibition percentage that will optimize thrombosis prevention in elderly patients with extremity revascularizations.

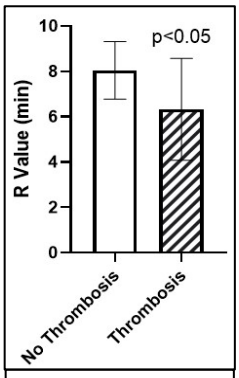


Figure 3. R value (min) difference in elderly patients with *vs.* without thrombosis.

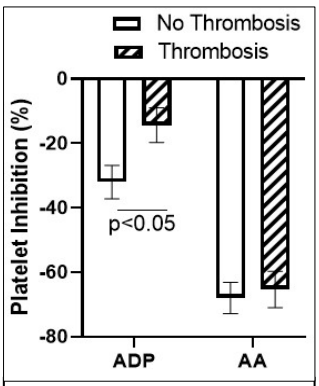


Figure 4. Difference in platelet inhibition (%) between elderly patients with and without thrombosis.

E. EXPERIMENTAL PLAN

Aim 1. Identify individual hypercoagulability patterns among elderly patients at risk of extremity graft/stent thrombosis.

Study Population. The target population includes all elderly patients with atherosclerosis undergoing revascularization at MGH/Harvard Medical School who meet the inclusion and exclusion criteria. Inclusion criteria are patients aged ≥60 years with a named vessel revascularization for atherosclerosis in the lower extremities (endovascular and/or open). Exclusion criteria are as follows: failure or refusal to provide written informed consent, aged <60 years or <50 kg in bodyweight if age is unknown, pregnant patients, and patients with contraindications to anticoagulation and/or antiplatelet agents. We aim to recruit 200 patients over a 10-month period including both genders and all ethnicities. The overall study design is depicted in **Fig. 5**.

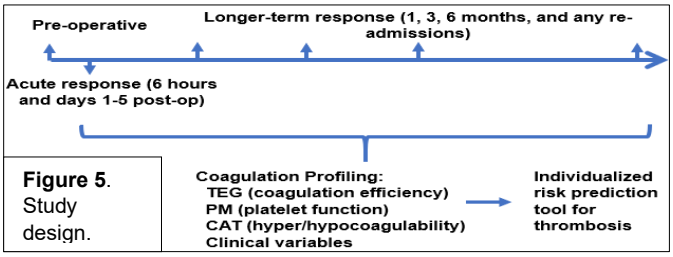


Figure 5. Study design.

TEG/PM/CAT Collection and Analysis. After consent is obtained, all patients will have citrated tubes of blood drawn at each time point (pre-operative and the following times post-op: 6-h, 24-h, day 2, day 3, day 4, day 5, day 30, 3 months, 6 months, and at all readmissions) for the coagulation assays to establish an individual hypercoagulation profile pre- and post-operatively (up to 6 months) that integrates pre- and post-operative TEG, PM, and CAT (days 1–5, 3, 6 months and all readmissions) to determine when hypercoagulability peaks and when it trends to baseline. All PM samples will be analyzed on the TEG 6000s (Haemonetics®) machine using the platelet mapping cartridges. TEG samples will be analyzed in the principal investigator (PI) Dr Dua's lab at MGH using TEG 6000s. Samples will be analyzed within 15 minutes of collection. The CAT analysis will be performed on Thrombinoscope software to identify the peak of thrombin generation, time to achieve peak, area under the thrombin-generation curve (endogenous thrombin potential), and rate of thrombin generation.

Clinical Variable Collection and Analysis. Study subjects will receive standard of care at the clinical site. All clinical variables will be collected and reported in accordance with Society of Vascular Surgery (SVS) guidelines for Peripheral Artery Disease reporting (**Table 1**):

Table 1. TEG/PM/CAT value data points and clinical variables being collected to develop risk prediction tool	
TEG Values **CAT Values** **Platelet Mapping** **Collected at all time points*	R, K, α angle, mA, Lysis 30 CAT (thrombin generation, thrombin peak) ADP Aggregation, ADP inhibition; AA aggregation, AA inhibition
Patient Characteristics **Collected at baseline*	Demographics: gender, age, race/ethnicity
	Smoking Status
	Medical History: Diabetes, hypertension, renal status, hyperlipidemia, hypercholesteremia, coronary artery disease, functional status, clotting disease, cancer, thrombotic event history; vascular intervention history
Disease Information	Description of lesion (lesion length, degrees of stenosis, number of tibial run-off vessels)
	Location, name, and type (arterial) of bleeding vessels
Procedure Information	Intervention type (balloon angioplasty, stent, atherectomy, bypass)
	Procedure medications (including anticoagulation)
	Intra-operative complications (death, clotting event, excessive bleeding, estimated blood loss)
Resuscitation	Type of fluid, volume of fluid over 24 hours, blood transfusion (units)

Complications and resultant procedure	Thrombosis and resultant intervention (operative, interventional radiology, medical)
	Deep venous thrombosis (DVT), Pulmonary embolism (PE)
	Secondary overall complications (infection)
Hospitalization summary	Hospital duration (floor, intensive care unit)
	Complications *in hospital* (bleeding, clotting, death, reintervention)
	Blood products given *during hospitalization*
	Changes in anticoagulation/ antitherapy agents
Complications (**at 1M, 3M, 6M*)	Infections, Occlusion, Myocardial infarction (MI), DVT, PE, Death, Stroke
Limb status (**at 1M, 3M, 6M*)	Amputation, reinterventions, patency
Laboratory Values (**at 1M, 3M, 6M*)	INR, PT, Activated Partial Thromboplastin Clotting time, Complete Blood Count (Hemoglobin/Platelets), Comprehensive Metabolic Panel
Medications (**at 1M, 3M, 6M*)	Antiplatelet, anticoagulants, all other medications. **dose, frequency, and time of last administration*
Vascular Assessments (**at 1M, 3M, 6M*)	Ankle-Brachial Index, Rutherford class, Toe pressure

Power Analysis*:* The primary endpoint will be comparing the mean amplitude (mA) in subjects who did and did not have a thrombosis event as this trended toward significance in our preliminary study; subjects who had a thrombosis event had mean mA values of 66.1 at their thrombotic event and those who did not had a mean mA value of 64.1. The mA values for both groups had a pooled SD of 3.5. There were 16.6% of all subjects who experienced a thrombotic event. With 80% power and two-sided alpha of 0.05, a **sample size of 200 subjects** will be required to detect a statistically significant difference between subjects with thrombotic events versus those without a thrombotic event. In our pilot study, we averaged 6 enrollments per week from 11 eligible subjects. Hence, in our enrollment period of 10 months, we anticipate prospective enrollment of all subjects needed for this proposed study accounting for a 20% subject attrition rate (6 × 40 weeks = 240).

Statistical Analysis: Normally distributed data will be summarized using means and SDs; non-normally distributed data will be explored using the median and interquartile range. Nominal t-tests will be used to determine differences in TEG/PM/CAT values at different time points for categorical variables. Categorical variables will be summarized using counts and percentages. Unadjusted logistic regression models will be utilized to determine the association and predictive probability of continuous covariates and TEG/PM/CAT values on thrombosis. Associations of patient risk factors with presence of thrombosis will be tested by using logistic regression models. Associations will be summarized by the odds ratio for thrombosis and associated 95% confidence intervals. The candidate variables, the 30 variables with the lowest univariable p-values, will be included in a multivariable logistic regression model with thrombosis event as the outcome. Stepwise selection will be performed to obtain a final model. Criteria to enter the model will be P_{entry}=0.20 and to exit the model will be P_{stay}=0.05. Cox regression will be implemented to determine the effect of time dependency on thrombosis prediction.

Aim 2. Develop and validate a novel and individualized risk prediction tool for extremity graft/stent thrombosis in elderly patients.

Study Population. Aim 2 will use the same patient population and samples as Aim 1.

Data Collection and Analysis: Once we collect longitudinal data along with the clinical variables (**Table 1**), we will conduct univariate and multivariate analyses on clinical variables alone, TEG/PM/CAT values alone, and then as a combined model. Model development will be through purposeful selection of variables identified are significant on univariate analysis.

Statistical Analysis: *Model Development* We will use Net Reclassification Improvement (NRI) to summarize the improvement in classification when a predictor identified in the multivariable logistic model is added to the model. Patients will be classified as *predicted* to have thrombosis if their predicted probability from the model exceeds the mean overall thrombosis prevalence in the dataset. *Model Performance* will be assessed by the C-statistic for discrimination by calculating the area under the receiver-operating characteristic (ROC) curve and by plots of model calibration. Confidence limits for area under the ROC curve will be generated from the 2.5% and 97.5% percentiles from prediction on regression models generated on the bootstrap datasets to provide estimates of precision and internal validity. *Thrombosis Tool Scores* will be assigned to each predictor based on the number of 0.5 levels above the referent odds of 1.0, rounded to the nearest level. Individual risk estimates will be based on the sum of weighted scores for each variable. To create an easy-to-use two-tier risk stratification index, individual cumulative scores will be collapsed into low- and intermediate/high-risk categories based on the corresponding observed thrombosis rates. We will create a model predicting graft thrombosis using clinical data alone and a model with clinical variables *and* coagulation assay data; we will compare models using Akaike Information Criterion (AIC). The resultant models will be internally validated by bootstrap analysis (randomly resampling the cohort, with replacement, to create multiple training subsets)[14].

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