Program Overview
The 33rd annual Vascular Research Initiatives Conference (VRIC), presented by the Society for Vascular Surgery®, is designed to encourage interaction and collaboration between vascular surgeon investigators and scientists from other vascular biology-related disciplines. An additional objective is to stimulate interest in research among trainees who are aspiring academic vascular surgeons. The VRIC is a one-day conference, taking place on May 13, preceding the American Heart Association Vascular Discovery: From Genes to Medicine Scientific Sessions 2019, on May 14-16, 2019.

Learning Objectives
At the end of the following sessions, participants should be able to:

ABSTRACT SESSION I: VASCULAR REMODELING, THROMBOSIS, AND DISCOVERY SCIENCE FOR VENOUS DISEASE
1. Discuss the current use of animal models elucidating the pathophysiology of remodeling and thrombosis in both arterial and venous disease.
2. Identify new areas of basic and methodological research in vessel remodeling and thrombosis in both arterial and venous disease.
3. Describe new venues of current clinical and translational research in vessel remodeling and thrombosis in both arterial and venous disease.

ABSTRACT SESSION II: VASCULAR REGENERATION, STEM CELLS AND WOUND HEALING
1. Discuss the current use of animal, cellular, and mathematical models elucidating the pathophysiology of stem cells, wound healing and vascular healing and regeneration.
2. Identify new areas of basic and methodological research in stem cells, wound healing and vascular healing and regeneration.
3. Describe new venues of current clinical and translational research in stem cells, wound healing and vascular regeneration.

ABSTRACT SESSION III: AORTOPATHIES AND NOVEL VASCULAR DEVICES
1. Discuss the current use of animal models in understanding aortic disease.
2. Identify new areas of basic and methodological research in pathologic arterial remodeling and molecular targets of next stage vascular therapeutics.
3. Describe new venues of current clinical and translational research in the development of vascular devices to meet the varied needs of vascular patients.

ABSTRACT SESSION IV: ATHEROSCLEROSIS, ARTERIAL INJURY AND DIABETES
1. Discuss the novel dietary components of peripheral arterial disease (PAD), novel perfusion imaging of PAD, and the role of the microvasculature on skeletal muscle function in PAD patients.
2. Identify new mechanisms of basic and translational in PAD.
3. Describe mechanisms of arterial fibrosis and calcification in PAD.
TRANSLATIONAL SESSION
1. Discuss the physiologic disruptions that underlie calcification of cardiovascular tissue
2. Identify novel models and mechanisms in arterial calcification
3. Describe cutting edge technology and therapeutics to treat arterial calcification

Target Audience
The Vascular Research Initiative Conference brings together vascular surgeons, vascular biologists, physicians with an interest in vascular problems, vascular surgery trainees, research trainees in vascular surgery and vascular biology, and industry personnel with an interest in vascular disease.

Accreditation Statement
The Society for Vascular Surgery is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

Designation Statement
The Society for Vascular Surgery designates this live activity for a maximum of 6.25 AMA PRA Category 1 Credits™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.
MONDAY, MAY 13, 2019 8:00AM – 7:00PM

7:00 AM REGISTRATION AND CONTINENTAL BREAKFAST

8:00 AM INTRODUCTORY REMARKS

Luke Brewster, MD, Chair, Research and Education Committee
Michel S. Makaroun, MD, President, Society for Vascular Surgery
Edith Tzeng, MD, Chair, SVS Research Council

ABSTRACT SESSION I: VASCULAR REMODELING, THROMBOSIS, AND DISCOVERY SCIENCE FOR VENOUS DISEASE

Moderator: Areck Ucuzian, MD
Moderator: Naomi M. Hamburg, MD, MS

8:15 am Periprocedural Hydrogen Sulfide Therapy Impairs Vascular Remodeling and Improves Vein Graft Patency
Peter Kip*1, Ming Tao1, Kaspar M Trocha1, Michael R MacArthur2, Sarah J Mitchell2, Suzannah Patterson1, Jonathan J Jung2, Paul H. Quax3, Margreet R de Vries3, James R Mitchell2, C. Keith Ozaki1
1Brigham and Women's Hospital, Boston, MA; 2Harvard T.H. Chan School of Public Health, Boston, MA; 3Leiden University Medical Center, Leiden, Netherlands

8:27 am Microbial Colonization Restores Neointimal Hyperplasia Development after Arterial Injury in Germ-Free Mice
Edmund B Chen*1, Katherine E Shapiro1, Thomas Kuntz2, Betty Theriault2, Michael J. Nooromid1, Kelly H Wun1, Vanessa Leone1, Katharine Harris2, Qun Jiang1, Melanie Spedale2, Liqun Xiong1, Owen M Eskandari1, Eugene B. Chang2, Karen J. Ho1
1Northwestern Univ, Feinberg School of Med, Chicago, IL, 2Univ of Chicago, Chicago, IL

8:39 am Deletion Of Nr4a1 Is Associated With Increased Vein Wall Injury After Venous Thrombosis
Andrew Kimball, Andrea Obi, Cathy Luke, Qing Cai, Abigail Dowling, Farouc Jaffer, Katherine Gallagher, Peter Henke
8:51 am  **A Novel Design for Shear Rate Optimization of the Venous-End Anastomosis of an Arteriovenous Graft**
Dillon Williams, Mohamed Zayed, Guy Genin, Eric Leuthardt
Washington University School of Medicine, Saint Louis, MO

9:03 am  **Genetic Analysis Implicates LDL Cholesterol Reduction and Plasminogen Activator-inhibitor 1 Antagonism As Therapeutic Interventions For Venous Thromboembolism**
Derek Klarin¹, Emma Busenkell², Renae Judy³, Julie Lynch³, Krishna Aragam⁴, Mark Chaffin⁴, Mary Haas⁴, Themistocles L Assimes⁵, Jie Huang⁶, Kyung Min Lee⁷, Qing Shao⁷, Jennifer E Huffman⁸, Yunfeng Huang⁸, Yan V Sun⁸, Marijana Vujkovic², Danial Saleheen⁷, Donald R Miller⁷, Peter Reaven⁹, Scott DuVall³
¹Massachusetts General Hospital, Boston, MA; ²Univ of Pennsylvania School of Medicine, Philadelphia, PA; ³Salt Lake City Health Care System, Salt Lake City, UT; ⁴Broad Institute of Harvard and MIT, Cambridge, MA; ⁵Stanford University School of Medicine, Stanford, CA; ⁶Massachusetts Veterans Epidemiology Research and Information Center, Boston, MA; ⁷Edith Nourse Rogers Memorial VA Hospital, Bedford, MA; ⁸Emory University Rollins School of Public Health, Atlanta, GA; ⁹Phoenix Veterans Affairs Health Care System, Phoenix, AZ; ¹⁰State University of New York at Buffalo Schools of Medicine and Public Health, Buffalo, NY; ¹¹Boston VA Healthcare System, Boston, MA; ¹²VA Connecticut Healthcare System, New Haven, CT; ¹³School of Public Health, University of Washington, Seattle, WA

9:15 am  **Regulation of Vascular Smooth Muscle Cell Responses by IL-2/IL-2R alpha**
Victoria Wong¹, David Hu², John Matsuura³, Lucile Wrenshall¹
¹Wright State University, Dayton, OH; ²Univ of Cincinnati, Cincinnati, OH

9:27 am  **Inhibition of the Akt1-mTORC1 Axis Alters Venous Remodeling To Improve Arteriovenous Fistula Patency**
Arash Fereydooni¹, Xiangjiang Guo², Toshihiko Isaji³, Jolanta Gorecka³, Shun Ono³, Haidi Hu³, Shirley Liu³, Naiem Nassiri¹, Lan Zhang², Alan Dardik¹
¹Yale School of Medicine, New Haven, CT; ²Dept of Vascular Surgery, Renji Hosp, Shanghai Jiaotong University, Shanghai, China; ³Vascular Biology and Therapeutics Program, Yale School of Medicine, New Haven, CT

9:45 am  **BREAK**
ABSTRACT SESSION II: VASCULAR REGENERATION, STEM CELLS AND WOUND HEALING

Moderator: Bryan Tillman, MD
Moderator: Ngan Huang, MD

10:00 am  Inhibition of Xanthine Oxidoreductase Accelerates Diabetic Wound Healing
Kathy Gonzalez, Karim M Salem, Guiying Hong, Edith Tzeng
VA Pittsburgh Healthcare System, Univ of Pittsburgh, Pittsburgh, PA

10:12 am  Knockdown of TSP-1 and TSP-2 Decreases Intimal Hyperplasia in Rats after Carotid Balloon Injury
Furqan Muqri, Mohammed Kassem, Alex Helkin, David Bruch, Kristopher G Maier, Vivian Gahtan
SUNY Upstate Medical Univ, Syracuse, NY

10:24 am  Aging Impairs Wound Healing By Hematopoietic Stem Cell Autonomous Mechanism
Jinglian Yan, Guodong Tie, Amanda Tutto, Kate Hayes, Lyne Khair, Louis Messina
University of Massachusetts Medical School, Worcester, MA

10:36 am  Intracellular Notch1 Signaling Determines Fibroblasts-modulated Angiogenic Response In Diabetic Wounds
Hongwei Shao, Yan Li, Irena Pastar, Rochelle Prokupets, Sophia Liu, Marjana Tomic-Canic, Omaida C Velazquez, Zhao-Jun Liu
University of Miami, Miami, FL

10:48 am  Endothelial-to-Mesenchymal Transition is Regulated by Substrate Stiffness
Maedeh Zamani, Frank Charbonier, Ngan F Huang
Stanford University, Stanford, CA

11:00 am  Caspase-1 Mediates Muscle Fiber Typing And Functionality In Response To Ischemia
Ricardo J Ferrari, Xiangdong Cui, Abish Pius, Amrita Sahu, Sunita N Shinde, Fabrisa Ambrosio, Hong Liao, Melanie J Scott, Ulka Sachdev
University of Pittsburgh Medical Center, Pittsburgh, PA

ABSTRACT SESSION III: AORTOPATHIES AND NOVEL VASCULAR DEVICES

Moderator: Dai Yamanouchi, MD
Moderator: Sean English, MD
Moderator: Peter Henke, MD

11:15 am  Alternative Macrophage Activation Limits Experimental Abdominal Aortic Aneurysms
Baohui Xu1, Naoki Fujimura1, Hongping Deng1, Gang Li1, Yankui Li1, Xiaoya Zheng1, Fanru Shen1, Takahiro Shoji1, Jia Guo1, Shai Zhao1, Xiaofeng Chen2, Masaaki Miyata3, Alan Daugherty4, Hong S Lu4, Ronald L Dalman1
1Stanford University School of Medicine, Stanford, CA; 2Taizhou Hospital Wenzhou Medical University, Linhai, China; 3Kagoshima City Hospital,
A Retrievable Rescue Stent for Thoracic or Abdominal Traumatic Hemorrhage
Catherine Go, Jenna Kuhn, Moataz Elisy, Youngjae Chun, Bryan Tillman
University of Pittsburgh Medical Center, Pittsburgh, PA

Interleukin-6 Is Necessary But Not Sufficient For Abdominal Aortic Aneurysm Development
Jean Marie Ruddy, Randall T. Grespin, Nicholas Ward, Christine Couch, Rupak Mukherjee, Jeffrey A. Jones
Medical University of South Carolina, Charleston, SC

Changes in Systemic Inflammation are Associated with Frailty Phenotypes and Clinical Outcomes after Open Aortic Repair
Kerri A O’Malley, Jared Rozowsky, Grace Shan, Sarah Barbey, Qiongyao Hu, Thomas Huber, Scott Berceli, Salvatore Scali
University of Florida, Gainesville, FL

12:05 PM LUNCH WITH QUICKSHOT POSTER REVIEW

12:50 PM SVS FOUNDATION UPDATE AND AWARDS CEREMONY
R. Clement Darling, III, MD, Chair, SVS Foundation
VRIC Trainee Awards
Edmund Chen, MD
Peter Kip, MD
Constance Mietus, PhD
Thomas Sorrentino, BS

1:00 PM SPECIAL SESSION I
Presented by Recipients of the 2017 SVS Foundation Mentored Clinical Scientist Research Career Development Award
Karen Woo, MD
University of California, Los Angeles
K08 Project: Outcomes of Dialysis Vascular Access in the Elderly

Mohamed Zayed, MD, PhD
Washington University in Saint Louis
K08 Project: The Role of Phospholipogenesis In Diabetic Peripheral Arterial Disease
1:25 PM  RECOGNITION CEREMONY: Honoring the Work of Dr. Frank LoGerfo

Moderator: Karen Ho, MD
Moderator: Michel S. Makaroun, MD

Frank W. LoGerfo, MD
William V. McDermott Distinguished Professor of Surgery
Beth Israel Deaconess Medical Center
Harvard Medical School

ABSTRACT SESSION IV: ATHEROSCLEROSIS, ARTERIAL INJURY AND DIABETES

Moderator: Cynthia St. Hilaire, MD

1:55 PM  Perivascular Gene Targeted Therapy using Biodegradeable CLICK-Gelatin Hydrogels
Patric Liang¹, Marisa Sewall¹, Navneet Momi¹, David Mooney², Leena Pradhan-Nabzdyk¹, Frank LoGerfo¹
¹Beth Israel Deaconess Medical Ctr, Boston, MA; ²Harvard Univ, Boston, MA

2:10 pm  Diet, Nutrition And The PAD Patient: An Evaluation Of Dietary Factors Associated With Incident Peripheral Artery Disease Events Using The Uk Biobank Cohort Study
Elsie Ross, Atif Rana, Alyssa Flores, Daniela Zanetti, Eri Fukaya, Erik Ingelsson, Nicholas Leeper
Stanford University School of Medicine, Stanford, CA

2:21 pm  Circulating Exosomes in PAD Patients: Disease Severity Correlates with Effects on Vascular Cell Migration and miRNA Content
Thomas A Sorrentino*, Phat Duong², Laura Boucharaycha², Mian Chen¹, Allen Chung², Melinda S Schaller¹, Adam ZOskowitz², Robert L Raffai², Michael S Conte³
¹University of California, San Francisco, CA; ²San Francisco VA Medical Center, San Francisco, CA

2:32 pm  Arterial Spin Labeling Quantifies Regional Foot Perfusion During Sustained Toe Flexion
Joe Luis Pantoja, Fadil Ali, Jiaxin Shao, Donald Baril, Erik Dutson, John Paul Finn, Peng Hu, Peter F Lawrence
University of California, Los Angeles, CA

2:43 pm  Microvascular Pathology Influences Walking Performance in Patients with Peripheral Artery Disease
Constance J Mietus*, Matthew A Fuglestad, Timothy J Lackner, Gregory T Willcockson, Peter Karvelis, Heman Hernandez, Yue Gao, Kataryina Brunette, Holly Despiegelaere, Feng Xie, Thomas Porter, Iraklis Pipinos, George Casale
University of Nebraska Medical Center, Omaha, NE
2:54 pm  The Parathyroid Hormone Receptor Limits Arterial Fibrosis In Diabetic Vascular Disease
Abraham Behmann, Dalian Zhong, Su Li Cheng, Li Li, Megan Mead, Bindu Ramachandaran, Mohammad Goodarzi, Andrew Lemoff, Dwight A Towler
University of Texas Southwestern Medical Center, Dallas, TX

3:05 pm  Proteoglycan 4 Is Implicated In Osteo-chondrogenic Smooth Muscle Cell Differentiation During Vascular Remodelling And Intimal Calcification
Till Seime¹, Eva Karlöf², Asim C Akbulut³, Rick H van Gorp², Mariette Lengquist¹, Malin Kronqvist¹, Nuno Dias¹, Anton Razuvaev¹, Jacob Odeberg⁴, Jan H Lindeman⁵, Lars Maegdefessel⁶, Leon J Schurgers², Ulf Hedin¹, Ljubica Matic¹
¹Karolinska Instt, Stockholm, Sweden; ²Maastricht Univ, Maastricht, Netherlands; ³Skåne Univ Hosp, Malmö, Sweden; ⁴Royal Inst of Technology, Stockholm, Sweden; ⁵Leiden Univ Medical Ctr, Leiden, Netherlands

3:20 PM  BREAK

3:35 PM  SPECIAL SESSION II: ALEXANDER W. CLOWES DISTINGUISHED LECTURE
Moderator: Katherine Gallagher MD
New Concepts in Regulation and Bioengineered Therapies for Vascular and Valvular Calcification
Cecilia Giachelli, PhD
Professor and Chair
Hunter and Dorothy Simpson Endowed Chair in Bioengineering
Department of Bioengineering
University of Washington

4:05 PM  TRANSLATIONAL PANEL
Moderator: Shirling Tsai, MD
Moderator: R. Clement Darling, III, MD

HARD SCIENCE: CALCIFICATION AND VASCULAR SOLUTIONS
Raul Guzman, MD
Associate Professor of Surgery
Harvard Medical School

Elena Aikawa, MD, PhD
Associate Professor of Medicine
Director, Heart Valve Translational Research Program
Harvard Medical School

Dwight Towler, MD, PhD
J.D. and Maggie E. Wilson Distinguished Chair in Biomedical Research
Louis V. Avioli Professorship in Mineral Metabolism Research
Vice Chair, Research - Internal Medicine
UT Southwestern Medical Center
Course Director
Luke P. Brewster, MD, PhD, Emory University, Atlanta, GA

Faculty
Elena Aikawa, MD, Harvard Medical School, Boston, MA
Edmund Chen, MD, Northwestern University, Chicago, IL
R. Clement Darling, III, MD, The Vascular Group PLLC, Albany NY
Sean English, MD, Washington University-Barnes Hospital, St. Louis, MO
Arash Fereydooni, MS, Yale University School of Medicine, New Haven, CT
Ricardo Ferrari, PhD, PT, University of Pittsburgh, Pittsburgh, PA
Katherine Gallagher, MD, University of Michigan, Northville, MI
Cecilia Giachelli, MD, University of Washington School of Medicine, Seattle, WA
Catherine Go, MD, University of Pittsburgh, Pittsburgh, PA
Kathy Gonzalez, MD, University of Pittsburgh, Pittsburgh, PA
Raul Guzman, MD, Beth Israel Deaconess Medical Center, Boston, MA
Naomi Hamburg, MD, MS, Boston University School of Medicine, Boston MA
Peter Henke, MD, University of Michigan, Ann Arbor, MI
Ngan Huang, PhD, Stanford University, Stanford, CA
Andrew Kimball, MD, University of Michigan, Ann Arbor, MI
Peter Kip, MD, Harvard Medical School, Cambridge, Massachusetts
Derek Klarin, MD, Massachusetts General Hospital, Boston, MA
Patric Liang, MD, Beth Israel Deaconess Medical Center, Boston, MA
Zhao-Jun Liu, MD, PhD, University of Miami Health System, Miami, FL
Michel S. Makaroun, MD, University of Pittsburgh, Pittsburgh, PA
Furqan Muqri, MD, SUNY Upstate Medical University, Syracuse, NY
Kerri O’Malley, PhD, University of Florida, Gainesville, FL
Constance Mietus, BA, University of Nebraska Medical Center, Omaha, NE
Joe Pantoja, MD, Ronald Reagan UCLA Medical Center, Los Angeles, CA
Elsie Ross, MD, Stanford Hospital and Clinics, Stanford, CA
Jean Ruddy, MD, Medical University of South Carolina, Charleston, SC
Till Seime, MSc, Karolinska Institutet, Solna Sweden
Thomas Sorrentino, MD, University of California San Francisco, San Francisco, CA
Cynthia St. Hilaire, PhD, University of Pittsburgh, Pittsburgh, PA
Bryan Tillman, MD, PhD, University of Pittsburgh, Pittsburgh, PA
Dwight Towler, MD, PhD, UT Southwestern Medical Center, Dallas, TX
Edith Tzeng, MD, University of Pittsburgh, Pittsburgh, PA
Arek Ucuzian, MD, PhD, University of MD Surgical Associates, PA, Baltimore, MD
Dillon Williams, MENG, Washington University School of Medicine, St. Louis, MO
Karen Woo, MD, UCLA Gonda Vascular Center, Los Angeles, CA
Lucile Wrenshall, MD, Wright State University, Dayton, OH
Baohui Xu, MD, PhD, Stanford University, Palo Alto, CA
Yinglian Yan, MD, PhD, University of Massachusetts Medical School, Worcester, MA
Mohamed Zayed, MD, PhD, Washington University School of Medicine, St. Louis, MO
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Faculty and Committee Disclosures
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<td>Jayer Chung, MD</td>
<td>Planner</td>
<td>Lead Investigator</td>
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<td>Naomi Hamburg, MD, MS</td>
<td>Moderator</td>
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<td>Zhao-Jun Liu, MD</td>
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<td>Dwight Towler, MD</td>
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Periprocedural Hydrogen Sulfide Therapy Impairs Vascular Remodeling and Improves Vein Graft Patency


**Background:** Extending vascular reconstruction durability, including bypass vein graft (VG) patency, remains a clinical challenge. Modulation of the host response to vascular injury via dietary preconditioning has shown promise as a strategy to upregulate endogenous hydrogen sulfide (H₂S), a gaseous anti-inflammatory and cytoprotective molecule. Considering the challenges of installing even short-term lifestyle changes in the vascular surgery patient population, we hypothesized that direct periprocedural application of the H₂S donor GYY4137 enhances VG remodeling/patency.

**Methods:** All experiments were performed in C57BL/6 mice (male, 12-weeks old) on a 60% high-fat diet. Mice received carotid artery (CA) end-to-end bypass with caval veins from donor mice or received unilateral focal common CA stenosis. All mice received periprocedural 40% pluronic gel with GYY (250µM) or vehicle adventitally, applied before release of the vascular clamps or immediately after focal stenosis creation. Ultrasound was performed at post-op day (POD) 14 and 28, VG/CA were harvested at POD-28 and processed for histology.

**Results:** After periprocedural application of GYY/vehicle (A), ultrasound revealed increased VG patency at POD-14 (B, p<0.05) and POD-28 (C, p<0.05); histology demonstrated (D) decreased intimal area (E, p=0.05), intimal thickness (F, p<0.01), Intimal/Media+Adventitia area ratios (G, p<0.0001) and Intimal/Media+Adventitia thickness ratios (H, p<0.0001) in the GYY group. In the focal CA cohort, at POD-28 post-stenosis (I) the GYY group had improved patency (J, p<0.0001), decreased intimal thickness (K, p<0.01) and Intima/Media area (L, p<0.01) and thickness (M, p<0.01) ratios.

**Conclusions:** Periprocedural GYY therapy, both in post-stenosis arterial injury and a bypass VG model, impairs negative remodeling and improves patency, showing promise as a therapeutic to enhance vascular construction durability.
Microbial Colonization Restores Neointimal Hyperplasia Development After Arterial Injury in Germ-Free Mice

Edmund B Chen, Katherine E Shapiro, Northwestern Univ, Feinberg Sch of Med, Chicago, IL; Thomas Kuntz, Betty Theriault, Univ of Chicago, Chicago, IL; Michael J. Nooromid, Kelly H Wun, Northwestern Univ, Feinberg Sch of Med, Chicago, IL; Vanessa Leone, Katharine Harris, Univ of Chicago, Chicago, IL; Qun Jiang, Northwestern Univ, Feinberg Sch of Med, Chicago, IL; Melanie Spedale, Univ of Chicago, Chicago, IL; Liqun Xiong, Owen M Eskandari, Northwestern Univ, Feinberg Sch of Med, Chicago, IL; Eugene B. Chang, Univ of Chicago, Chicago, IL; Karen J. Ho, Northwestern Univ, Feinberg Sch of Med, Chicago, IL

Background: The role of gut microbiota in arterial remodeling is poorly understood. We previously showed that germ-free (GF) mice have less neointimal hyperplasia development that is associated with increased M2 macrophage infiltration and an altered cytokine profile compared to conventionally-raised (CONV-R) mice. To further understand the causative role of microbiota in neointimal development, we performed fecal transplantation in GF mice from CONV-R mice donors prior to performing arterial injury.

Methods: Fourteen-week-old C57BL/6 male GF mice underwent fecal transplantation using stool from donor CONV-R mice. Six weeks later, fecal transplanted GF (GF-FT) mice underwent left carotid artery ligation. Age- and sex-matched CONV-R and GF mice served as the comparison groups. Neointimal hyperplasia was assessed by morphometric analysis after four weeks. Total microbial load and composition in GF-FT and CONV-R groups was compared using 16s rDNA qPCR and next-generation sequencing of stool DNA.

Results: As previously reported, the GF cohort developed significantly less neointimal hyperplasia than the CONV-R cohort (mean neointimal area (NI): GF 0.005 ± 0.002 mm² vs. CONV-R 0.021 ± 0.004 mm²; p = 0.01). Fecal transplantation restored the arterial remodeling phenotype (NI: GF-FT 0.014 ± 0.003 mm²; p=0.8 vs. CONV-R, p=0.04 vs. GF). Microbial load at time of carotid ligation was similar between GT-FT and CONV-R groups (mean 16s rDNA gene copy number x 10⁵; GF-FT 4.2 ± 1.2 vs. CONV-R 2.3 ± 0.6; p=0.3). However, GF-FT and CONV-R microbial communities exhibited different alpha and beta diversity, suggesting that the presence of microbiota is sufficient to impact arterial remodeling despite divergent microbial composition.

Conclusions: We provide evidence that strengthens the proposed connection between gut microbiota and arterial remodeling after injury. Further investigation into the impact of bacterial colonization and the roles of specific microbes on arterial remodeling is warranted.
Deletion of Nr4a1 is Associated With Increased Vein Wall Injury After Venous Thrombosis

Andrew Kimball, Andrea Obi, Cathy Luke, Qing Cai, Abigail Dowling, Farouc Jaffer, Katherine Gallagher, Peter Henke, Univ of Michigan, Ann Arbor, MI

Introduction: Post-thrombotic syndrome is a chronic disabling sequelae of deep vein thrombosis (VT), for which no direct therapies beyond anticoagulation exist. Prior work has shown that monocyte/macrophages (Mo/MØ) are important for mid-term venous thrombosis resolution, in particular the pro-inflammatory Ly6C\textsuperscript{hi} cells and Ly6C\textsuperscript{lo} pro-healing Mo/MØ. We hypothesized that Mo/ MØ depletion would lessen vein wall fibrosis. Methods: We used CD11b-DTR mice to conditionally deplete Mo/MØ in the stasis venous thrombosis (VT) model (IVC ligation), two days after the VT was created. DTx was dosed every 48 hours to deplete >90% of circulating Mo. CCR2\textsuperscript{-/-} mice devoid of circulating Ly6C\textsuperscript{hi} Mo, and Nr4a1\textsuperscript{-/-} mice devoid of circulating Ly6C\textsuperscript{lo} Mo, were used to dissect the roles of the Ly6C\textsuperscript{hi} pro-inflammatory and Ly6C\textsuperscript{lo} pro-healing Mo/MØ, respectively. Tissue harvest at 14d assessed thrombus size, histology (trichrome, DDR2+ fibroblasts and CD68+ MØ) and immunologic assays were performed. All N > 4. Results: Conditional global Mo/MØ depletion was associated with a 37% decrease (p<.001) in vein wall thickness at 14 days, with a significant decrease in vein wall DDR2\textsuperscript{+} cells, and FSP-1, TGFb, VEGF1, and IL-6 levels. Interestingly, no difference in VT size was found. Nr4a1\textsuperscript{-/-} mice (no Ly6C\textsuperscript{lo} Mo) had no difference in VT size at day 14, but exhibited a 35% increase in vein wall thickness (p<0.001) and increased DDR2\textsuperscript{+} cells (P < .001). Conversely, day 14 CCR2\textsuperscript{-/-} mice (no Ly6C\textsuperscript{lo} Mo) had 40% smaller VT (p=0.007), but no change in vein wall thickness. Both Nr4a1\textsuperscript{-/-} and CCR2\textsuperscript{-/-} mice had an 85% decrease in CD68+ MØ in the vein wall at 14d (p<0.001). Conclusion: Mo/MØ drive post thrombotic vein wall fibrosis, possibly through fibroblast-mediated mechanisms. Deletion of Nr4a1, lacking Ly6C\textsuperscript{lo} Mo, exaggerated vein wall fibrosis but genetic deletion of CCR2 (Ly6C\textsuperscript{hi}) cells does not significantly lessen vein wall fibrosis, suggesting that Ly6C\textsuperscript{hi} are likely precursors for later Ly6C\textsuperscript{lo} conversion. Further study will elucidate this lineage change as well as identify targets to enhance Ly6C\textsuperscript{lo} Mo/MØ in vein wall post-thrombotic fibrosis.

A Novel Design for Shear Rate Optimization of the Venous-End Anastomosis of an Arteriovenous Graft

Dillon Williams, Mohamed Zayed, Washington Univ Sch of Med, Saint Louis, MO; Guy Genin, Washington Univ in Saint Louis, Saint Louis, MO; Eric Leuthardt, Washington Univ Sch of Med, Saint Louis, MO

Objective
Arteriovenous grafts used for hemodialysis have a high rate of failure due to stenosis at the venous-end anastomosis. We evaluated whether the anastomosis angle and graft end geometry can impact simulated blood shear rate on the adjacent venous wall.

Methods
Using ANSYS Fluent computational fluid dynamics package an arteriovenous graft system was modeled and simulated with pulsatile blood flow. The system consisted of a tapered graft connecting an artery and vein. Venous anastomoses at 90°, 60°, 45°, 30°, 15°, and 13° angles of attachment and a novel graft design with micro-digit imprints were tested. Potential for thrombosis was measured as the area of the vein wall experiencing unhealthy shear rates (shear rates <50 1/s and >1000 1/s).

Results
A 90° anastomosis lead to the largest flow disruption and incidence of unhealthy high and low shear rate on the vein wall. Decreasing anastomosis angle improved the shear environment. Compared to the 90° anastomosis, a 13° anastomosis had the largest decrease in unhealthy high shear rate (by 97%; P<0.0001), and 30° anastomosis had the largest decrease in unhealthy low shear rate (by 94%; P<0.05). The novel graft design (Figure 1A) further reduced the high and low shear rates (P<0.0001 and P<0.05 respectively; Figure 1B).

Conclusions
Decreased arteriovenous graft venous anastomosis angle can dramatically improve the shear environment. Optimized geometry of the graft can further normalize the shear rates, and would be a novel method to potentially decrease the incidence of stenosis at the venous-end anastomosis. These findings can help inform future arteriovenous graft designs.

Figure 1A

Figure 1B

Genetic Analysis Implicates LDL Cholesterol Reduction and Plasminogen Activator-inhibitor 1 Antagonism as Therapeutic Interventions for Venous Thromboembolism

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Introduction: Venous thromboembolism (VTE) is a significant heritable cause of cardiovascular-related mortality, yet previously published GWAS have only identified 11 genome-wide significant (P<5x10^{-8}) risk loci to date. **Hypothesis:** Genetic variants affecting multiple biological pathways are associated with VTE risk and may reveal novel therapeutic targets. **Methods:** Using EHR data, we identified individuals with and without clinical VTE in the Million Veteran Program and UK Biobank participants. Individuals were genotyped on customized Affymetrix Biobank arrays, and we tested 13 million genotyped and imputed DNA variants for association with clinical VTE using logistic regression models adjusting for age, sex and population structure. We then meta-analyzed across the two datasets and set a P<5x10^{-8} for statistical significance. In downstream analyses, we examined genetic variant-plasma protein associations at P<5x10^{-8} generated from SOMAscan data quantifying 3,622 plasma proteins in 3,301 healthy participants from the INTERVAL study to identify VTE risk variants that alter protein concentrations in human plasma. We then performed a Mendelian randomization (MR) analysis to evaluate the causal role of lipids (HDL cholesterol/LDL cholesterol/triglycerides) on VTE risk using a 222-variant lipid genetic risk score and set P<0.016 (0.05/3 lipid fractions) for statistical significance. **Results:** We identified 26,066 VTE cases and 624,053 controls. Following meta-analysis, we identified 21 novel VTE loci implicating known VTE risk factors including body mass index (LMOD) and hypercoaguability (VWF, FX, PROC, PROS1). Four of the VTE risk variants were associated with changes in protein concentrations in plasma resulting in a pro-coagulant effect, including an increased expression of 1) Factor Xa, a current VTE therapeutic target, and 2) plasminogen activator-inhibitor 1, a possible novel therapeutic target. Through MR analysis, we provide evidence that LDL cholesterol, may be a causal risk factor for VTE (OR=1.17 per SD increase in LDL cholesterol, P=0.003). **Conclusions:** Here, we assembled the largest reported cohort of individuals with clinical VTE and genetic data and identify novel associations with therapeutic implications.

Restenosis from intimal hyperplasia remains a critical problem in the United States, impacting every intervention undertaken to treat atherosclerotic occlusive disease. In response to injury from interventions such as bypass grafting or endarterectomies, vascular smooth muscle cells (VSMC) undergo a phenotypic and functional switch from quiescent and contractile, to migratory and proliferative. While several endogenous factors are known to promote proliferation, far fewer have been identified that switch VSMCs back to quiescence. Data from our laboratory suggest that one previously overlooked factor affecting quiescence and proliferation of VSMC is the cytokine IL-2, which surrounds VSMC by binding to the extracellular matrix. In examining the potential impact of the co-localization of IL-2 and VSMC, we discovered that IL-2 knockout mice exhibit a systemic loss of smooth muscle cells over time, causing aortic aneurysms and ectatic esophagi. This observation led us to ask whether IL-2 affects VSMC through an IL-2R. We found that human VSMC express all three subunits of the IL-2R, α,β, γ. Localization of the IL-2Rα subunit, however, differed based on VSMC phenotype, with quiescent cells exhibiting a predominantly nuclear localization and proliferating cells a predominantly membrane-associated localization. This same pattern was also observed in Western blots of atherosclerotic versus normal arteries, where the nuclear IL-2Rα predominated in normal tissues (quiescent VSMC) and membrane-associated form predominated in atherosclerotic tissues (proliferating VSMC). Treating VSMC with IL-2 increased both nuclear localization of IL-2Rα and expression of the pro-survival transcription factor FOXO3a, suggesting a possible mechanism for the systemic loss of SMCs in IL-2 deficient mice. To determine the relevance of the IL-2Rα subunit to VSMC responses in vivo, we injured the carotid arteries of IL-2Rα knockout mice. The absence of IL-2Rα resulted in a hyperplastic response, suggesting that IL-2Rα inhibits VSMC proliferation and migration. In total, our results suggest that IL-2-IL-2Rα promote VSMC survival and suppress migration and proliferation, representing a here-to-fore unrecognized means of regulating VSMC responses to injury.

V. Wong: None. D. Hui: None. J. Matsuura: None. L. Wrenshall: None.
Inhibition of the Akt1-mTORC1 Axis Alters Venous Remodeling to Improve Arteriovenous Fistula Patency

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Introduction Arteriovenous fistulae (AVF) are the most common access created for hemodialysis, but up to 50% fail to mature, suggesting a need to improve AVF maturation. In a mouse model, reduced Akt1 expression reduces AVF wall thickness and diameter. We hypothesized that inhibition of the Akt1-mTORC1 axis alters venous remodeling associated with failure of AVF.

Methods A C57BL6/J mouse aortocaval fistula model was used (male, 9-12 weeks). Mice were injected with 100 μg of vehicle or rapamycin (intraperitoneal) daily. The AVF were harvested at days 3, 7 and 21 for comparison of wall thickness, macrophage markers and expression level of mTOR signaling proteins using Western blot and immunofluorescence intensity (IF). AVF patency and diameter were assessed with ultrasound measurements.

Results Rapamycin reduced AVF wall thickness at days 7 and 21 (p<0.05; n=6) with no change in AVF diameter (p=0.5). Rapamycin decreased proliferation rate of smooth muscle cells (SMC) and macrophages (p<0.05). Rapamycin also reduced both M1 (iNOS, TNF-α) and M2 (IL-10, CD206) macrophage markers at days 3 and 7 (p<0.05). Rapamycin treatment was associated with diminished phosphorylation of Akt1 and the mTORC1 pathway members mTOR (Ser2481), 4EBP1 and p70S6K (p<0.05; n=6), but not of the mTORC2 pathway members mTOR (Ser2448), PKC-α and SGK1 (p>0.4). With rapamycin treatment, there was decreased IF of p-Akt1 and p-mTORC1 in SMC and macrophages at days 3, 7 and 21 (p<0.05). After depletion of macrophages with clodronate liposomes injection, there was reduced wall thickness (p<0.01, day 21), SMC proliferation and p-mTORC1 IF in SMC and macrophages (p<0.05). At day 42, rapamycin treated AVF had similar p-Akt1 and p-mTORC1 IF in macrophages compared to control (p>0.5).

Conclusion Rapamycin improves AVF patency by reducing early inflammation and wall thickening through the Akt1-mTORC1 signaling pathway in macrophage and SMC. Macrophages are essential for SMC activation and AVF maturation. Rapamycin may be a translational strategy to improve AVF patency.

Inhibition of Xanthine Oxidoreductase Accelerates Diabetic Wound Healing

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Background:
Impaired diabetic wound healing, a source of significant disability, has been attributed to increased oxidative stress and overproduction of reactive oxygen species (ROS). While NADPH oxidase is thought to be the predominant source of ROS in wounds, our studies have demonstrated significant xanthine oxidoreductase (XOR) in wound beds. In normal mice, XOR appears to be required for normal wound healing. However, in diabetic wounds, where ROS formation is upregulated, XOR may contribute to the oxidative stress and impaired healing. We hypothesized that inhibition of XOR activity in diabetic wounds will improve diabetic wound repair.

Methods:
Anesthetized diabetic db/db mice underwent excisional wounding. The wounds were treated with either Aquaphor with 0.2% febuxostat (febux; N=6) or Aquaphor alone (N=3) and covered with a bioocclusive dressing. Febux is an inhibitor of XOR used in the treatment of gout. Wounds were photographed every other day for digital planimetry with ImageJ until post-operative day 12. Data were analyzed with Student's t-test.

Results:
Topical Aquaphor alone slightly improved wound healing in db/db mice. The application of Aquaphor + febux improved wound healing at all time points as compared with Aquaphor alone (Figure). By post-operative day 6, there was a significant difference in wound size between the two groups that persisted throughout the experiment. On post-operative day 12, wounds treated with Aquaphor + febux were approximately 50% smaller than controls (24.7±4.5% and 46.1±3.3%, respectively; p=0.017).

Conclusion:
The local inhibition of XOR activity in diabetic wounds with topical febux greatly improves healing. This suggests that XOR contributes to the oxidative stress in diabetic wounds and the poor healing. Further studies are needed to confirm the mechanisms involved but our findings support the potential use of topical febux to improve diabetic wound repair.

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Knockdown of TSP-1 and TSP-2 Decreases Intimal Hyperplasia in Rats After Carotid Balloon Injury

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Objective: Thrombospondins (TSP) are matricellular proteins involved in intimal hyperplasia (IH). TSP-1 and TSP-2 are causative agents, while TSP-5 is likely protective. Males are more likely to develop IH as compared to females. We hypothesized that siRNA mediated knockdown of THBS1 and THBS2 would decrease IH in rats after carotid artery balloon injury, while THBS5 knockdown would increase IH. We also hypothesized that female rats would show decreased IH as compared to males in all groups. Methods: Sprague Dawley rats [males (M) and ovariectomized females (F)] underwent carotid artery balloon injury using a Fogarty catheter (5 atm, 5 min). Then a 100 microliter solution of saline (M: N=9, F: N=10), adeno-associated virus (AAV) containing siRNA to THBS1 (M: N=9, F: N=9), THBS2 (M: N=9, F: N=9), THBS5 (M: N=8, F: N=8), or scrambled (scr) siRNA (M: N=8, F: N=9) was instilled intraluminally (30 min). At 14 days, rats were sacrificed and carotid arteries perfusion fixed. IH was measured using Intima/Media ratios. Data was analyzed using ANOVA with a Fisher’s post-hoc test, and \( p < 0.05 \) considered significant. Results: In males, THBS1 siRNA decreased IH by 36\% (0.22 vs 0.34) \( (p<0.05) \) as compared to scr-siRNA. THBS2 siRNA resulted in a 64\% (0.12 vs 0.34) decrease \( (p<0.05) \) in IH. No difference in IH existed between saline treated and scr-siRNA treated rats; there was also no difference in IH in THBS5 siRNA treated rats as compared to scr-siRNA. Female saline treated rats had 37\% (0.26 vs 0.41) \( (p<0.05) \) less IH as compared to male saline treated. Female scr-siRNA treated rats had 48\% (0.13 vs 0.26) less \( (p<0.05) \) IH compared to the saline treated. The female rats showed no difference across treatment groups. Conclusion: TSP-1 and TSP-2 are important mediators of IH and knockdown of the genes decreased IH in males. Knockdown of the TSP-5 gene had no effect on IH. These observations may be explained by similarities in the structure of TSP-1 and TSP-2, and the substantial difference in structure of TSP-5. Ovariectomized female rats showed less IH than males; additionally, there was no difference across all treatment groups in females. These distinct sex differences warrant further investigation. AAV mediated siRNA delivery may be an effective and safe therapy for IH in males.

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Aging Impairs Wound Healing by Hematopoietic Stem Cell Autonomous Mechanism

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Rationale: In the United States, chronic non-healing wounds affect more than 6.5 million patients yearly. While advanced age and diabetes are well recognized risk factors for non-healing wounds, little is known about the mechanism by which aging impairs wound healing. We have shown that diabetes impairs wound healing through a hematopoietic stem cell (HSC)-autonomous mechanism. Objective: This study tests the hypothesis that aging impairs wound healing through a hematopoietic stem cell (HSC) autonomous mechanism that disrupts the normal orchestrated immune cell response across the three phases of wound healing. Methods and Results: To test this hypothesis, cutaneous wounds were created in young (2-month-old) and aged mice (24-month-old). The wound closure rate of aged mice was almost two times longer than that in young mice (Young WT vs. Aged WT: 24 vs. 49). To determine if this difference is due to an HSC-autonomous mechanism, we generated chimeric mouse models by transplanting HSCs from either young or aged WT donor mice into lethally irradiated young WT mice. The wound closure rate of young WT mice reconstituted with HSCs of aged mice was similar to that in aged mice, consistent with an HSC-autonomous mechanism. To determine the effects of aging on HSC differentiation towards immune cells and thereby on wound healing, chimeric mouse models were generated by which young WT mice were reconstituted with HSCs from young or aged EGFP mice. In the cutaneous wounds of young WT recipient with HSCs from aged EGFP mice, neutrophils and proinflammatory Ly6Chi monocytes/macrophages were significantly increased during all three phases of wound healing. More than 90% of neutrophils and macrophages in cutaneous wounds were derived from donor HSCs. As for T cells, decreased CD4/CD8 ratio was observed during last phase (tissue remodeling phase) of wound healing, but no difference in CD3e+ T cells. Conclusion: This study shows for the first time that the HSC-derived immune cells are the major cell components dynamically responding to wound healing processes. Aging impairs wound healing by HSC autonomous mechanisms that dysregulates the normal immune cell response. These findings could lead to a novel HSC-based therapy to restore normal wound healing.

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Intracellular Notch1 Signaling Determines Fibroblasts-modulated Angiogenic Response in Diabetic Wounds

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Introduction. Neovascularization participates in the provisional granulation tissue formation and provide oxygen and nutrients to support wound repair. The assembly of the endothelial network and stabilization of neovessels is largely dictated by external signals from fibroblasts in granulation tissue. However, the molecular mechanisms determining the pro-angiogenic response of fibroblasts remains unknown. Here, we identify Notch1 signaling as a molecular determinant controlling the plasticity and function of fibroblasts in modulating angiogenesis and wound healing.

Methods. We assessed the Notch pathway activity in fibroblasts derived from human and murine diabetic wounds versus their non-diabetic counterparts and explored the role of the intracellular Notch1 pathway activity in fibroblasts in regulating angiogenesis and wound healing using novel mouse lines in which gain-of-function Notch1 signaling specifically occur in fibroblasts. We also addressed whether and how manipulation of the intracellular Notch1 pathway activity in fibroblasts alters their cross-talk with endothelial cells by which modulate angiogenesis using in vitro 3D angiogenesis model and in the FSP-1+/−;ROSA^LSL-N1IC+/+ mice.

Results. Intracellular Notch pathway activity is elevated in the fibroblasts from human diabetic foot ulcers (DFU) and murine diabetic wounds compared to non-diabetic controls. High Notch1 activity in fibroblasts suppressed their differentiation into myofibroblasts and diminished their role in modulating angiogenesis in a 3D angiogenesis by ~85%, P<0.001. Consistently there was an impaired wound angiogenesis (~30% less vessel density, P<0.05) and a delay in wound healing (~30% delay, P<0.001) in the FSP-1+/−;ROSA^LSL-N1IC+/+ mouse, in which Notch1 signaling is specifically activated in fibroblasts.

Conclusions. These findings suggest that intracellular Notch1 signaling determines the plasticity and function of fibroblasts in modulating angiogenesis and wound healing, unveiling intracellular Notch1 signaling in fibroblasts as potential target for therapeutic intervention in diabetic wound healing and therapeutic angiogenesis.

Approximately 40% of the vascular grafts fail within 2 years of implantation, mainly due to graft stenosis. Recent studies demonstrated the incidence of endothelial-to-mesenchymal transition (EndoMT) during graft stenosis progression. Despite evidence of the correlation between mechanical properties and EndoMT progression, the regulatory effect of substrate stiffness on progression of EndoMT remains poorly defined. The aim of this study is to investigate the effect of mechanical properties of the underlying matrix on progression of EndoMT and the mechanism underlying this process. We hypothesize that substrate stiffness modulates vascular endothelial cell (EC) phenotype and regulates the stimulatory effect of transforming growth factorβ (TGFβ) on progression of EndoMT. Human aortic ECs (HAECs) were cultured on hydrogels with tissue-like stiffnesses of 4 kPa or 100 kPa on tissue culture (TC) plates. The expression of endothelial, mesenchymal and EndoMT marker genes were performed for HAECs with/without exposure to TGFβ. RT-PCR analysis demonstrated that HAECs expressed mesenchymal (SM22, calponin, Twist, α-SMA) and EndoMT (SNAIL) markers in a stiffness-dependent manner after 6 days, where stiffer substrates induced more phenotypic transition. Interestingly, in the presence of TGFβ, a known stimulator of EndoMT, the cell culture on the hydrogels of physiological stiffness could partially abrogate the expression of mesenchymal markers compared to TC (Figure 1). Immunofluorescence staining of mesenchymal (SM22) and EndoMT (SNAIL) showed that with increasing the substrate stiffness, SM22 protein expression was notably increased, and that TGFβ-induced SM22 expression was significantly inhibited on lower stiffness substrates. HAECs on hydrogels expressed lower levels of SNAIL, compared to TC. Together, these results demonstrate the mediatory effect of both substrate stiffness and TGFβ treatment on the morphology and phenotype of HAECs.
Caspase-1 Mediates Muscle Fiber Typing and Functionality in Response to Ischemia

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Introduction: We have shown that chloroquine (CQ), a lysosomal inhibitor, increases inflammatory cleaved caspase-1 in myocytes, and improves ischemic muscle histology in mice. This is relevant to myopathy in peripheral arterial disease (PAD). Caspase-1 is protective in liver injury, but a similar role in muscle is unknown. We hypothesize that caspase-1 mediates muscle function in ischemia and is modulated by CQ, representing a novel target in PAD. Methods: C57Bl6/J wild type (WT) and caspase-1 knockout (KO) mice underwent unilateral femoral artery ligation (FAL) and CQ or PBS injections for 21d. In situ physiologic testing assessed time to peak contraction and ½ relaxation time (RT) to measure function. Myocyte cross sectional area (CSA) and caspase -1 expression was evaluated with H&E and immunofluorescence, respectively. Cleaved caspase-1 was detected by western blot (WB). % recovery of live muscle satellite cells (MuSc) was measured to determine resistance to ischemic stress. Laser Doppler perfusion imaging (LDPI) documented perfusion recovery. Results: CQ increased total caspase-1 expression by ≈25% in nonischemic and ischemic WT limbs (p<0.01) while genetic absence of caspase-1 significantly decreased detection of its cleaved, active form by WB. Caspase1KO myocyte CSA was smaller than WT (8811±1785um² vs. 23,410 ± 6487um²; p<0.05, N=4-6/group). Correspondingly, caspase1KO mice had shorter time to peak contraction by 20% (PBS) and 29% (CQ; p<0.01). ½ RT in CQ treated caspase-1KO mice was 42% faster than controls (p<0.05, N=5/group, ANOVA). Perfusion recovery in caspase-1KO mice was not significantly attenuated. Recovery of live muscle satellite cells from WT and caspase-1KO mice was similar. Conclusion: As a product of inflammasome activation, caspase-1 reflects inflammation, a precursor for healing. Lack of caspase-1 does not affect perfusion or MuSc viability, but may cause muscle fiber type switching to a smaller, fast twitch phenotype, with shorter contraction and relaxation times. Clinically, fast twitch fibers may not favor sustained exercise like walking in claudicants with PAD. Detrimental effects of caspase-1 deficiency were exacerbated by CQ, suggesting a link between lysosomal and inflammasome pathways in ischemic muscle.

Abstract Session III: Aortopathies and Novel Vascular Devices

19-A-381-AHA-VD

Alternative Macrophage Activation Limits Experimental Abdominal Aortic Aneurysms

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Objective: Macrophages are functionally heterogenous. IL-4 and IL-13 promote an anti-inflammatory macrophage phenotype (alternatively activated or M2 macrophages). We explored the significance of M2 macrophage activity in experimental abdominal aortic aneurysm (AAA) pathogenesis using genetic, cellular and pharmacological approaches. Methods: AAAs were created in myeloid IL-4Ralpha-deficient (CKO, functional defect in M2 macrophages) or wild type (WT) male mice via intra-aortic porcine pancreatic elastase (PPE) infusion. Alternative AAAs were created in additional WT mice via inoculation with an adeno-associated virus (AAV) expressing a gain-of-functional mutation of PCSK9, initiating a high fat diet and subcutaneous angiotensin II injections. IL-4/IL-4 mAb complex or in vitro IL-4 activated macrophages were administered to all mice on indicated days. Outcome was assessed via ultrasonography and histopathology. Results: Increased aortic enlargement was noted in IL-4Ralpha CKO as compared to WT mice following PPE infusion. Administration of IL-4/IL-4 mAb complex, however, attenuated aneurysm enlargement. Further progression of existing AAAs was reduced via transfer of in vitro IL-4-activated macrophages. Histologically, myeloid IL-4Ralpha deficiency augmented, whereas treatment with IL-4/IL-4 mAb complex or in vitro IL-4-activated macrophages attenuated, medial elastin and smooth muscle cell depletion, macrophages and T cell accumulation and mural neoangiogenesis. Additionally, myeloid IL-4Ralph deficiency increased aneurysm incidence, severity and mortality in AAV-inoculated hyperlipidemic mice following angiotensin II infusion. Conclusion: Promoting alternative macrophage activation negatively regulates experimental aneurysm formation and progression in complementary modeling systems. Pharmacologic manipulation of macrophage activation may provide a novel interventional pathway for AAA disease management.

Figure. Role of alternatively activated macrophages in experimental AAAs. (A): Infrarenal aortic diameter in WT and myeloid IL-4Ralpha-deficient mice following PPE infusion. (B): Infrarenal aortic diameter in WT mice receiving IL-4/IL-4 mAb complex (5 μg/30 μg) or vehicle every day starting 3 days prior to PPE infusion. (C): Infrarenal aortic diameter in WT mice intravenously injecting IL-4-activated macrophages on day 4 or days 7 & 10 (one million cells/injection) following PPE infusion. (D): Suprarenal aortic diameter in AAV-PCSK9-injected, high fat diet-fed WT and IL-4Ralpha deficient mice following subcutaneous angiotensin II infusion. All data are mean and standard deviation from 6-9 (A-C) and 10-12 (D) mice in each group. ANOVA followed by two sample comparison, 0.05<*P<0.1 and **P<0.01 compared to WT mice at same time point.
A Retrievable Rescue Stent for Thoracic or Abdominal Traumatic Hemorrhage

Catherine Go, Univ of Pittsburgh Med Ctr, Pittsburgh, PA; Jenna Kuhn, Moataz Elsisy, Youngjae Chun, Univ of Pittsburgh, Pittsburgh, PA; Bryan Tillman, Univ of Pittsburgh Med Ctr, Pittsburgh, PA

Background: Mortality after vascular injuries of the torso approaches 80%. Even Balloon Occlusion of the Aorta (REBOA) has failed to significantly improve survival, perhaps secondary to ischemia and retrograde hemorrhage. We hypothesized that a retrievable three-tier Rescue stent would offer superior hemorrhage control for both thoracic and abdominal injuries while preserving visceral, spinal cord, and distal perfusion.

Methods: Rescue stents were fashioned from nitinol wire with the top and bottom thirds covered by PTFE while the middle visceral third is bare metal. Fifteen anesthetized swine were divided into groups for either thoracic injury [REBOA (n=6) and Rescue-T (n=5)] or abdominal injury [Rescue-A (n=4)]. During the injury period, with the REBOA or Rescue stent in place, hemodynamic, neurophysiologic, and visceral flow monitoring was performed. After retrieval and permanent repair, animals were monitored until post-op day 2.

Results: Average blood loss was 3.7 ± 0.5 L (REBOA), 1.3 ± 0.4 (Rescue-T), and 0.45 ± 0.4 (Rescue-A) (P<0.001). Both Rescue-T and Rescue-A maintained significantly higher overall mean arterial pressure (p<0.01) and higher CVP (p<0.05) as compared to REBOA animals. Antegrade flow to all viscera was confirmed in both Rescue groups by angiogram (Figure) and by visceral flow measurements. All REBOA animals expired with profound malperfusion and loss of neurophysiologic responses. With the exception of one Rescue-T animal that expired from an arrhythmia post-op, all Rescue animals survived to post-op day 2 with normal clinical neuro exams (P<0.05).

Conclusions: Use of a Rescue stent is associated with significant improvement in blood loss, blood pressure, organ perfusion, spinal cord perfusion, and mortality when compared to REBOA. This study suggests that the three tier Rescue stent offers effective hemorrhage control with preserved visceral/distal perfusion in aortic injuries, whether in the thoracic or abdominal aorta.

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Interleukin-6 is Necessary but Not Sufficient for Abdominal Aortic Aneurysm Development

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Introduction: The gene expression profile of interleukin-6 (IL-6) signaling through the STAT3 transcription factor distinguishes it as a potential effector of macrophage accumulation and abdominal aortic aneurysm (AAA) growth. This project aims to demonstrate that IL-6 is an integral component of aortic macrophage accumulation and AAA development. Methods: C57Bl/6 and IL-6 knockout (IL-6KO) mice underwent induction of AAA by the application of peri-adventitial CaCl2 (0.5M) +/- implantation of an osmotic mini-pump delivering IL-6 (4.36μg/kg/day x 21 days). At the terminal procedure, aortic diameters (AoDs) were measured by digital microscopy and represented as percent change from baseline (n=4-7). The infrarenal aorta was harvested for immunoblot (pSTAT3/STAT3; n=3 ) or flow cytometric analysis of macrophage content (CD11b+/F4-80+ cells; n=2-3) and reported as a fold change from C57Bl/6. Treatment groups were compared by ANOVA. Results: IL-6 treatment or AAA induction alone significantly increased the AoD of C57Bl/6 mice (Figure 1), along with increased pSTAT3/STAT3 ratio (1.66+/−0.15 and 1.94+/−0.17 fold, respectively; p<0.05) and elevated accumulation of CD11b+/F4-80+ cells in the aorta (1.84+/−0.04 and 1.90+/−0.13 fold, respectively; p<0.05). In the IL-6KO mice, the change in AoD with AAA induction was attenuated (Figure 1), as was the change in pSTAT3/STAT3 and the accumulation of CD11b+/F4-80+ cells (p<0.05). However, when IL-6 was replaced in the IL-6KO mice, the change in AoD in response to AAA induction was restored (Figure 1), the pSTAT3/STAT3 ratio increased 3.74+/−0.37 fold (p<0.05), and the accumulation of CD11b+/F4-80+ cells was rescued (3.78+/−0.09-fold, p<0.05). Conclusion: By employing dual therapy with IL-6 infusion and AAA induction in IL-6KO mice, this project has demonstrated that IL-6 is necessary but not sufficient for AAA development, thereby emphasizing the therapeutic value of targeting this cytokine signaling pathway.

Figure 1. Percent Change in Aortic Diameter

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent Change</th>
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<tbody>
<tr>
<td>C57Bl/6 Control</td>
<td>0</td>
</tr>
<tr>
<td>C57Bl/6+IL-6</td>
<td>+</td>
</tr>
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<td>IL-6KO Control</td>
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Changes in Systemic Inflammation are Associated with Frailty Phenotypes and Clinical Outcomes After Open Aortic Repair

Kerri A Omalley, Jared Rozowsky, Grace Shan, Sarah Barbey, Qiongyao Hu, Thomas Huber, Scott Berceli, Salvatore Scali, Univ Florida, Gainesville, FL

Objective: The underlying biology of frailty, as it pertains to an individual's response following surgical insult, is poorly understood. Given the critical role played by the inflammatory system in the early response to injury, we hypothesize that patient frailty is a significant determinant of the dynamic inflammatory response following major vascular surgery. Specifically, we sought to define the relationship between frailty and the transcriptional response of circulating inflammatory cells following open AAA repair, and its impact on clinical outcome. Methods: Blood monocytes and neutrophils were isolated pre-op and at 1D, 7D, 28D following open AAA repair (n=20), and RNA-seq [50 million reads; 25,343 genes] performed to define gene expression profiles. Frailty assessment was performed using the Fried Frailty Score, and a composite outcome of any 30-day death, major complication, and reoperation were used as the clinical outcome. Results: Monocytes demonstrated a robust dynamic mRNA response pattern characterized by early augmentation at Day 1 with return to baseline by 1 month (Fig 1). Employing a stringent false discovery rate (FDR) selection criteria, a core set of genes were highly associated with baseline frailty and clinical outcome. In contrast, surgery had limited influence on neutrophil expression and no relationship to frailty or outcome. While a dampened D1/D7 monocyte response was observed in frail individuals, a hyperacute early response was associated with poor outcomes. Comparison to our previous studies in the trauma and vein bypass grafting underscores the deleterious influence of an accentuated early monocyte response (Fig 2).

Abstract Session IV: Atherosclerosis, Arterial Injury and Diabetes

19-A-360-AHA-VD

Perivascular Gene Targeted Therapy Using Biodegradable CLICK-Gelatin Hydrogels

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Objective

Intimal hyperplasia (IH) at the prosthetic graft-native artery anastomotic interface consists primarily of proliferating smooth muscle cells (SMCs) from the native artery. Genes involved in IH have been found to be upregulated several weeks after bypass grafting. We developed a biodegradable CLICK-gelatin hydrogel to provide sustained targeted delivery of siRNAs and effective gene silencing. Here we investigated whether perivascular application of the hydrogel would result in effective transmural transfection.

Methods

siTSP2 was complexed with transfection reagent PEI and combined with 10% m/v CLICK-gelatin hydrogels. Human aortic SMCs (HAoSMCs) were transfected with siTSP2/PEI, eluted from degrading hydrogels at 4-72 hours and 1-week intervals. TSP2 gene expression was evaluated using qRT-PCR. In a rat model, CLICK-gelatin hydrogels containing siGAPDH/PEI were placed circumferentially around one common carotid artery (CCA) while the contralateral CCA served as an untreated control. Using qRT-PCR and IHC, GAPDH gene and protein expression relative to the control CCA was assessed at 48 hours and 1-week.

Results

In HAoSMCs, siTSP2/PEI complexes released from degrading hydrogels significantly reduced TSP2 expression up to 72 hours in vitro. In the rat model, siGAPDH/PEI released from the hydrogels resulted in 31% (P<0.001) and 52% (P<0.001) GAPDH gene knockdown at 48hrs and 1 week, respectively. No evidence of immunologic reaction to the hydrogel was found on histology. IHC revealed significant reduction of GAPDH protein expression across all layers of the arterial wall at both the 48hour and 1week timepoints.

Conclusions

siRNA/PEI complexes released from CLICK-gelatin hydrogels are functional and result in reduction in targeted gene expression. Applied external to an artery, this hydrogel system offers a stable platform for sustained transmural delivery of siRNA with successful gene and protein silencing.

Figure. GAPDH Expression in Rat Carotid Arteries After Treatment with siGAPDH/PEI Eluted from Degrading Hydrogels


Diet, Nutrition and the Pad Patient: An Evaluation of Dietary Factors Associated With Incident Peripheral Artery Disease Events Using the Uk Biobank Cohort Study

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Objective: To identify dietary and nutritional factors associated with symptomatic peripheral artery disease (PAD) in a large, well-phenotyped cohort. Methods: Data were derived from the UK Biobank study, a longitudinal cohort study of over 500,000 individuals aged 40-69 years from 21 centers in the United Kingdom. We performed machine learning analysis including Random Forest and Least Absolute Shrinkage and Selection Operator to identify diet and nutritional factors associated with peripheral artery disease death, hospitalization and surgical events. We then utilized multivariate Cox regression modeling to identify independent factors associated with PAD events. Results: A total of 202,576 individuals were included in our analysis, including 1,055 patients with PAD events. After controlling for age, sex, and body mass index, dietary factors associated with lower risk of PAD events included higher: fresh fruit intake (Hazard ratio 0.96, 95% confidence interval 0.95-0.99), cereal intake (HR 0.97, 95% CI 0.96-0.98), dietary Vitamin C intake (HR 0.99, 95% CI 0.998-0.999), dietary magnesium intake (HR 0.99, 95% CI 0.997-0.999), dietary Vitamin E intake (HR 0.97, 95% CI 0.96-0.998), glucosamine supplementation (HR 0.7, 95% CI 0.6-0.8), cod liver oil supplementation (HR 0.66, 95% CI 0.47-0.93). Factors associated with higher risk of PAD events included higher: coffee intake (HR 1.05, 95% CI 1.03-1.07), sugar intake (1.003, 1.002-1.005), dietary Vitamin B6 intake (HR 1.2, 95% CI 1.1-1.3), alcohol intake (HR 1.003, 95% CI 1.0005-1.006), and iron supplementation (HR 1.9, 95% CI 1.4-2.6). Interestingly, general fish oil supplementation was not associated with reduced risk of PAD events. Conclusions: Nutrition, an often overlooked factor in PAD prevention, plays an important role in development of PAD events. In particular cod liver oil and glucosamine supplementation but not general fish oil supplementation was associated with significant protective benefit. Conversely, higher dietary B6, coffee and alcohol intake were associated with increased risk. The mechanisms of these associations and further prospective study are warranted.

Circulating Exosomes in PAD Patients: Disease Severity Correlates with Effects on Vascular Cell Migration and miRNA Content

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Introduction:
Peripheral arterial disease (PAD) is a chronic condition characterized by inflammation. Emerging literature suggests that circulating exosomes and their miRNA contents may influence atherosclerosis and vascular remodeling. We hypothesize that PAD severity correlates with circulating exosome pro-inflammatory miRNA content and their effects on vascular cells.

Methods:
Exosomes (particle size 30-100nm) were isolated from plasma of healthy (n=6), mild PAD (mPAD, Rutherford 2; n=6) and severe PAD patients (sPAD, Rutherford 4; n=5). Exosome purity, size, and concentration were determined by western blot and nanoparticle tracking analysis. Exosome miRNA were isolated and assessed by qPCR. Human vascular smooth muscle cell (VSMC) and endothelial cell (EC) migration were assessed via wound closure assay.

Results:
There was no difference in overall exosome particle concentration or size between the three groups (ANOVA >0.05). Compared to exosomes from healthy subjects, exosomes from mPAD and sPAD subjects increased VSMC migration (1.0±.09-fold vs. 1.5±.09-fold vs. 2.0±.12-fold wound closure, ANOVA <0.0001) and decreased EC migration (1.8±.07-fold vs. 1.5±.04-fold vs. 1.3±.02-fold wound closure, ANOVA <0.01) in a step-wise fashion. Hierarchical clustering analysis of exosome miRNA revealed distinct clustering of vascular-active miRNA expression between the three groups. Several miRNA which influence inflammatory pathways in vascular cells were expressed at higher levels in exosomes from sPAD subjects (Fig 1).

Conclusion:
Plasma-derived exosomes from healthy, mPAD, and sPAD patients contain distinct signatures of immune-regulatory miRNA. In addition, circulating exosomes have in vitro functional effects on VSMCs and ECs that may negatively impact vessel remodeling. Together these data suggest that the pro-inflammatory function and miRNA cargo of circulating exosomes correlates with atherosclerosis progression in PAD patients.

Figure 1: Hierarchical cluster analysis of exosomal miRNA reveals distinct clustering of patients with sPAD and elevated levels of a number of immune-regulatory miRNA.

Arterial Spin Labeling Quantifies Regional Foot Perfusion During Sustained Toe Flexion

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Objectives: Tissue perfusion is a critical component to healing diabetic foot ulcers. However, tools to quantify tissue perfusion of the lower extremities are deficient, which contributes to the uncertainty in predicting ulcer healing potential. This study aims to quantify foot perfusion using arterial spin labeling (ASL) MRI. We hypothesize that ASL can detect regional differences in foot perfusion during sustained toe flexion. Methods: A group of 20 healthy volunteers were recruited. Each participant underwent a vascular exam and non-contrast MR angiography to ensure the absence of vascular disease. A non-contrast ASL MRI was then performed to measure absolute perfusion at the plantar aspect of the foot after 5 minutes of laying supine (rest) and after 2 minutes of sustained toe flexion. The perfusion maps of the plantar foot were divided into angiosomes (medial plantar, lateral plantar, medial calcaneal, and lateral calcaneal). Regional perfusion during rest and toe flexion was compared using linear mixed effects models. Results: During rest, there was a small variation in regional perfusion among angiosomes, with the only significant difference being between the medial and lateral plantar angiosomes (29.572 vs. 24.154 mL/100g/min, p= 0.031). Upon sustained toe flexion, there was an increase in overall foot perfusion compared to the resting state (43.90 vs. 27.26 mL/100g/min, p<0.001). The greatest increases occurred at the medial (64.29 vs. 29.57 mL/100g/min, p<0.001) and lateral plantar angiosomes (39.98 vs. 24.15 mL/100g/min, p<0.001). Conclusion: Differences in regional perfusion during rest and sustained toe flexion are quantifiable using ASL MRI, demonstrating the feasibility of using ASL MRI to measure foot perfusion. Its non-invasive nature facilitates incorporation into existing MR angiography protocols already used in the assessment of peripheral arterial disease. There are immediate clinical applications to this technology specifically in the diabetic foot ulcer patient population, whereby determining the perfusion deficit around an ulcer may more effectively guide revascularization therapies.

Microvascular Pathology Influences Walking Performance in Patients with Peripheral Artery Disease

**Constance J Mietus**, Matthew A Fuglestad, Timothy J Lackner, Gregory T Willcockson, Peter Karvelis, Hernan Hernandez, Yue Gao, Katyarina Brunette, Holly Despiegelaere, Feng Xie, Thomas Porter, Iraklis Pipinos, George Casale, UNMC, Omaha, NE

**Background:** Peripheral Artery Disease (PAD) is caused by atherosclerotic narrowing of the arteries supplying the legs. PAD-induced myopathy is characterized by myofiber degeneration, fibrosis, and alterations of microvascular architecture. As PAD severity advances, microvascular basement membrane thickness (BMT) increases and the inner BM diameter (IBMD) expands to accommodate increased numbers of pericytes. We tested the hypothesis that in PAD patients with intermittent claudication (IC), BMT and IBMD are associated with microperfusion deficits and diminished walking performance.

**Methods:** Calf muscle microvascular blood flow (MBF) was evaluated by post-occlusive contrast enhanced ultrasonography with continuous infusion of 5% Definity® in PAD patients with IC (n = 15). Walking performance was determined by the Walking Impairment Questionnaire (WIQ), and Gardner test for claudication onset time (COT) and peak walking time (PWT). Gastrocnemius biopsies were labeled with antibodies specific for Collagen IV and analyzed by quantitative fluorescence microscopy. Microvessel BMT and IBMD were measured. Correlations were determined by linear regression analysis.

**Results:** IBMD had stronger linear correlations with MBF, both at rest and after ischemic stress, than BMT. In patients with greater IBMD, resting MBF was lower (R = -0.76, p = 0.006). During reperfusion after ischemic stress, patients with greater IBMD exhibited increased blood velocity (R = 0.78; p = 0.005) in association with decreased blood volume. IBMD also was associated with calf muscle flow reserve (R = 0.65; p = 0.03). Patients self-reported walking shorter distance (WIQ-2, R = -0.55; p = 0.04), slower walking velocity (WIQ-3, R = -0.58; p = 0.03), and greater difficulty climbing stairs (WIQ-4, R = -0.58, p = 0.03) as IBMD increased. Importantly, increased IBMD was associated with more rapid onset of claudication pain (COT, R = -0.62; p = 0.03) and decreased walking duration (PWT, R = -0.68, p = 0.01).

**Conclusions:** Microvascular architecture is tightly linked to microperfusion in PAD muscle and contributes to PAD patient walking limitations. Pericytes may contribute to microvascular pathology by secreting BM collagen, promoting vasoconstriction, and impairing angiogenesis.

**C.J. Mietus:** None. **M.A. Fuglestad:** None. **T.J. Lackner:** None. **G.T. Willcockson:** None. **P. Karvelis:** None. **H. Hernandez:** None. **Y. Gao:** None. **K. Brunette:** None. **H. Despiegelaere:** None. **F. Xie:** Consultant/Advisory Board; Modest; Lantheus Medical Imaging Inc. **T. Porter:** Research Grant; Modest; Theodore F. Hubbard Foundation. Other Research Support; Modest; Bracco Diagnostics Inc. Consultant/Advisory Board; Modest; Lantheus Medical Imaging Inc. Other; Modest; equipment support from Philips Research North America. **I. Pipinos:** None. **G. Casale:** None.
The Parathyroid Hormone Receptor Limits Arterial Fibrosis in Diabetic Vascular Disease

Abraham Behrmann, Dalian Zhong, Su Li Cheng, Li Li, Megan Mead, Bindu Ramachandaran, Mohammad Goodarzi, Andrew Lemoff, Dwight A Towler, UT Southwestern Medical Ctr, Dallas, TX

Male LDL receptor-null (LDLR-/-) mice develop type 2 diabetes (T2D) and dyslipidemia with arteriosclerosis on high fat diets. The PTH receptor (PTH1R) is expressed in vascular smooth muscle (VSM), and we’ve shown that augmenting VSM PTH1R signaling -- either genetically or pharmacologically -- mitigates arterial fibrosis and calcification in LDLR-/- mice. To determine consequences of reduced VSM PTH1R signaling, we generated SM22-Cre;PTH1R(fl/fl) mice (PTH1R-VKO) on the LDLR-/- background. No differences in HFD-induced diabetes were observed between PTH1R-VKO mice and Cre-negative PTH1R(fl/fl);LDLR-/- controls (CON). Compared to CON, PTH1R-VKO cohorts exhibited increased aortic collagen but without increased calcification. VSM from PTH1R-VKO mice elaborated more collagen (53.6 +/- 4.9 ug collagen / 250K cells vs. 21.7 +/- 5.0 ug collagen / 250K cells, p = 0.01), and expressed higher levels of Col1a1, Col1a2, & Col3a1. Expression of VSM contractile markers were concomitantly reduced. To understand mechanisms whereby PTH1R signaling controls VSM collagen expression, we performed mass spectrometry on nuclear extracts from CON and PTH1R-VKO VSM. PTH1R deficiency resulted in a > 90% reduction in the Ets transcription factor Fli1 (p=0.01), an inhibitor of fibrogenesis deficient in systemic sclerosis, along with >50% reduction in Gata6 (p = 0.03), a GATA factor that modulates the contractile VSM phenotype. By contrast nuclear Prrx1, a profibrogenic homeobox factor, was increased with PTH1R deficiency (7-fold, p < 0.0001) and synergizes with Klf4 (up 2-fold) to activate Col1a1 transcription. Western blot, mRNA & immunofluorescence analyses confirmed reductions of VSM Gata6 and Fli1 expression with PTH1R deficiency. Gata6 and Fli1 suppress Col1a1/Col3a1 transcription, & PTH1R-dependent suppression maps to GATA-, Ets-, and CArG- box regulatory regions in the Col3a1 promoter. Thus, the PTH1R restricts expression and synthesis of collagen in VSM, mediated in part via Gata6 and Fli1 regulatory circuits that suppress collagen gene transcription. Strategies that augment normal VSM PTH1R signaling tone, reflected in sustained Gata6 / Fli1 activities, may mitigate arteriosclerosis and vascular fibrosis in diabetes and other dysmetabolic diseases.

Proteoglycan 4 is Implicated in Osteo-chondrogenic Smooth Muscle Cell Differentiation During Vascular Remodelling and Intimal Calcification

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Unstable atherosclerotic plaques are major cause of mortality, but knowledge of the underlying molecular processes is still incomplete. We reported enrichment of calcification associated genes in advanced carotid plaques from asymptomatic patients and in symptomatic patients on statin therapy, showing that calcification is a hallmark of atherosclerosis. Here, we investigated its key molecular signatures by associating macro-calcification estimated by computed tomography (CT) with gene expression profiles in human carotid lesions.

Microarray analyses of n=40 patients identified PRG4 as the most significantly upregulated transcript in calcified plaques, correlated to bone metabolism (via BMP2, SOX9, RUNX2, ACP5) and inhibition of inflammatory (CTLA4) pathways. Quantitative CT-image analyses confirmed a positive correlation of PRG4 with calcification (r=0.57, p=0.0001) and overall plaque burden (r=0.42, p=0.007), but negative with lipid-rich necrotic core volume (r=-0.32, p=0.0469). Immunostaining of human atheroprogression lesions, localised PRG4 protein in smooth muscle cells (SMCs) during early intimal thickening, while it was deposited within extracellular matrix (ECM) at later stages. In advanced lesions, PRG4 was detected in the ECM, localizing to OPN+ areas and SMA+, SOX9+, RUNX2+ and CD68+/TRAP+ cells surrounding macrocalcifications. In vivo, PRG4 was enriched in calcified plaques of warfarin treated ApoE−/− mice. An upregulation was also found in intimal hyperplasia response upon carotid artery ballooninjury in rats, correlating with chondrogenic (Sox9 r=0.62, p<0.0001; Bmp2 r=0.48, p<0.0001) and macrophage (CD68 r=0.64, p<0.0001) markers. While PRG4 transcript decreased in later phases of this model, the protein remained abundant in ECM. In vitro, PRG4 was induced in primary human SMCs exposed to differentiated macrophage medium or TGFβ. Stimulation with Pi or Ca, resulted in SMC calcification with elevation of PRG4 and Sox9.

Our results show that SMCs express PRG4 in association with osteo-chondrogenic transition during vascular remodeling and intimal macro-calcification. PRG4 may be a key component of ECM remodeling in response to mechanical and inflammatory stimuli, meriting further mechanistic studies.

Mitochondrial Dysfunction in a Novel, in vitro, Cell-based Model of Intermittent Claudication

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The leg muscles of claudicating patients with peripheral artery disease (PAD) experience ischemia during activity followed by reperfusion during rest. These effort-induced cycles of ischemia and reperfusion are a central component of the pathophysiology of PAD. We evaluated the mitochondrial function of primary human skeletal muscle cells (HSkMC) cultured under varying oxygen concentrations that mimic the O2 saturation data present in the legs of PAD patients during walking. HSkMC derived from calf muscle samples of PAD patients and healthy controls were incubated under serum-free conditions, and then exposed to constant normoxia (N: 20% O2), cycles of normoxia-hypoxia (NH: 5% O2), or cycles of normoxia-hypoxia-hyperoxia (NHH: 30% O2), with the OxyCycler C42. Mitochondrial respiration and glycolysis was then assessed using the Seahorse XFp Analyzer, which continuously measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Basal respiration was significantly higher in normoxic control cells compared to normoxic PAD cells (32.1±5.25 vs. 14.9±6.49, p<0.01) and PAD cells under normoxia-hypoxia cycles (32.1±5.25 vs. 13.3±1.91, p<0.01). Maximum respiration was reduced in normoxic PAD cells compared to normoxic control cells (20.9±1.68 vs. 31.2±7.11, p<0.05) as well as PAD cells under NH (11.1±1.81 vs. 31.2±7.11, p<0.05). Proton leak was significantly increased in PAD cells under NH compared to normoxic control (9.18±2.32 vs. 5.78±1.09, p<0.05) and significantly reduced in hypoxic PAD cells (9.18±2.32 vs. 3.20±0.91, p<0.005). ATP production was reduced in PAD cells under N, NH and NHH compared to control cells under normoxia (9.95±3.17 vs. 9.96±3.17 vs. 19.8±7.54, vs. 26.3±4.74, p<0.005). The ECAR was significantly increased in PAD cells under N, NH and NHH compared to control cells under normoxia (9.51±0.88 vs. 14.1±1.61 vs. 25.1±2.93, vs. 4.57±1.71, p<0.005). This study provides a novel, in vitro, human cells-based model for the evaluation of PAD pathophysiology. Our data suggest that HSkMC derived from PAD patients exhibit reduced mitochondrial respiratory capacity and increased reliance on glycolysis compared to control cells and this is further exacerbated by the introduction of hypoxia and hyperoxia.

Microvascular Stenosis in End-Stage Peripheral Artery Disease: Role of Partial Endothelial to Mesenchymal Transition


Peripheral artery disease (PAD) is a widespread and debilitating manifestation of atherosclerosis. Unfortunately, revascularization strategies are often precluded or unsuccessful, resulting in amputation. A major reason for treatment failure is likely co-existing abnormalities in the distal microvasculature. However, the specific microvascular defects present in end-stage PAD in humans remain unknown. To elucidate the microvascular landscape in PAD, we studied human tibialis anterior (TA, n=18) and gastrocnemius (n=13) muscles harvested from below-knee leg amputations of 10 individuals with end-stage PAD. Control muscles, from individuals without PAD, were obtained from TA (n=2) and vastus lateralis (n=4) samples. PAD but not control samples had myofiber damage features. Interestingly, capillary density, quantified from CD31-stained sections, was 2.1-fold higher in PAD tissue (p=0.005). Furthermore, the PAD capillaries were atypical, with a 1.7-fold increase in smooth muscle-α-actin-containing mural cells (p<0.001). Surprisingly, the arterioles (15-40 µm) in subjects with PAD were found to be stenotic, with 28% decrease in lumen area (p=0.0018). Moreover, 11% of the stenotic arterioles in the lower tertile of size were entirely occluded. Microvascular stenosis was EC-based, with pronounced nuclear rounding (p=0.03) and luminal encroachment. These aberrant ECs were CD31-positive but also expressed mesenchymal markers N-cadherin, S100A4, and SLUG, indicating partial endothelial to mesenchymal transition (EndMT). As well, the mural cells of PAD arterioles expressed higher TGFβ than control arterioles. Finally, using a novel microfluidic device with fully EC-lined micro-channels, we identified pronounced inward bulging and lumen encroachment of ECs when subjected to ultra-low flow and fluid shear stresses below 0.5 dynes/cm² (p<0.001).

Conclusions: These studies reveal EC-based microvascular stenosis as a previously unidentified feature of end-stage PAD. Ultra-low shear stress, TGFβ signalling, and partial EndMT are implicated in driving this novel micro-luminal obstruction process.

A Novel Machine Learning-Driven Clinical and Proteomic Tool for the Diagnosis of Peripheral Artery Disease

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Objective: PAD is often underdiagnosed and undertreated, which has been reported to have a high impact on population health and may be due to limitations in current noninvasive diagnostic tools. The aim of this study was to utilize a novel machine learning-driven clinical and proteomic approach to predict clinically significant PAD. Methods: We conducted a cross-sectional study of 131 outpatients (controls, 41; PAD, 90). PAD was diagnosed by a board-certified vascular surgeon based upon a comprehensive clinical assessment. Patients with CLI or a history of revascularization were excluded. Controls had no known clinical atherosclerotic disease and an ABI ≥ 0.9. Using 5 clinical variables and 35 plasma protein biomarkers, we identified a panel predictive of PAD using least angle regression and a final model was developed with LASSO. Results: The diagnostic panel consisted of 1 clinical variable (HTN) and 3 proteins: kidney injury molecule-1, pulmonary surfactant associated protein D, and interleukin-1 receptor antagonist. The model diagnosed PAD with a cross-validated AUC of 0.81 and an in-sample AUC of 0.84 (Figure). At optimal cutoff, the score had 63% sensitivity, 93% specificity, 95% PPV, and 54% NPV for PAD. Partitioning the score into five discrete risk levels resulted in a PPV of 100% and NPV of 82% in the highest and lowest levels, respectively. Restricting the analysis to only patients with diabetes (controls, 9; PAD, 30) resulted in an in-sample AUC of 0.84. Conclusion: This novel machine learning-driven clinical and proteomic diagnostic tool demonstrated accuracy in identifying PAD when compared to a comprehensive clinical assessment completed by a vascular surgeon. As technology’s role in assisting physicians continues to grow, machine learning may gain a more widespread role in aiding in the diagnosis of diseases such as PAD.
Stent Design Affects Femoropopliteal Artery Stenosis Rates

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Introduction: Femoropopliteal artery (FPA) stenting demonstrates poor durability, and severe FPA deformations occurring with limb flexion are likely involved. Recent studies demonstrate significant stent design effects on limb flexion-induced deformations, and we sought to relate them to the amount of stenosis after FPA stenting. Methods: Patients (n=19, age 64±7 yrs, range 51-80 yrs) had 22 limbs treated with 13 laser-cut (Absolute Pro, Everflex, Zilver PTX) and 9 wire-based (Supera, Viabahn, Tigris) devices. Of these, 55% were placed across the adductor hiatus or entirely in the popliteal artery. Patients had CT angiography of the lower extremities prior to intervention and at a median of 6.5±10 months after stenting. The cross-sectional areas of the FPA flow lumen were analyzed as a function of artery length to determine segments with >10% diameter reduction persisting for 5mm. Linear regression and nonparametric tests were used to determine predictors of stenosis defined as narrower lumen at follow-up compared with baseline. Patient demographics, risk factors, and stent characteristics were used as independent variables. Results: On the follow-up scan, 52% of patients had focal luminal narrowing either proximal to (10%), within (26%), or distal to (29%) the stented segment, and 6% of patients had >50% lumen reduction. Stent characteristics and patient factors did not have statistically significant effects on stenosis proximal to or within the stented segment. Distal to the stent 37% of variability in stenosis severity was explained by wire-based stent type (23%) and age (18%), with younger patients having more stenosis. Stents with poor ability to accommodate limb flexion-induced deformations due to high torsional stiffness (Supera, Viabahn, Tigris) were associated with higher stenosis rates (p=0.03). Discussion: Stent design appears to have stronger association with FPA stenosis than patient factors. Stents with poor ability to accommodate limb flexion-induced deformations may produce higher grades of stenosis, and disease often occurs distal to the stented segment where FPA deformations are most severe. The increased mobility of younger patients may lead to repetitive trauma from device-artery interactions contributing to stenosis.

**Increased Ceramide Content in the Peripheral Arterial Plaque of Patients with Diabetes Can Cause Endothelial Cell Dysfunction**

**Nikolai Harroun**, Mohamed Zayed, Chao Yang, Clay Semenkovich, Luis Sanchez, Washington Univ in St. Louis, St. Louis, MO

**Introduction:** Diabetes is an independent risk factor for peripheral arterial disease (PAD) and critical limb ischemia (CLI). Patients with diabetes are approximately 10 times more likely to have a major lower extremity amputation. We evaluated whether phospholipid profiles can impact peripheral arterial wall plaque disease severity in patients with diabetes. **Methods:** 21 patients (14 diabetic and 7 non-diabetic) receiving major lower extremity amputation were prospectively enrolled in a vascular surgery biobank to harvest minimally (Min) and maximally (Max) diseased peripheral arterial segments (PAS). Patient demographics were evaluated and PAS sphingomyelin and ceramide lipid content was evaluated using mass spectrometry. **Results:** We observed significantly higher C18:1/16:0 (C16) ceramide in the PAS of patients with diabetes (333.3% relative increase; P<.05). Max diseased PAS displayed higher C16 content than Min in both patients with diabetes (101.1% relative increase; P<.05) and without diabetes (323.8% relative increase; P<.05) as seen in Figure 1. Interestingly, Min diseased PAS of patients with diabetes also demonstrated higher C16 than patients without diabetes (P<.05). We also observed a significant correlation between C16 and the relative abundance of specific sphingomyelin phosphodiesterases (SMPDs) in PAS of patients with and without diabetes (R²=.52; P<.0001 and R²=.52: P<.05, respectively) as well as overall content of PAS sphingomyelins (R²=.27; P<.001). C16 introduced to HUVECs demonstrated decreased cell proliferation (P<.01), increased cell death (P<.05), and inactivation of autophagy machinery (P<.01). **Conclusions:** Patients with diabetes and CLI have increased C16 content in both Min and Max PAS. C16 may be a byproduct of sphingomyelins degraded by SMPDs, and appears to be deleterious to ECs. These findings suggest that C16 may be an important peripheral arterial tissue biomarker of disease severity in patients with diabetes.

![Figure 1](image)

**N. Harroun:** None. **M. Zayed:** None. **C. Yang:** None. **C. Semenkovich:** None. **L. Sanchez:** None.
Collagen Type I and III in Serum of Patients with Abdominal Aortic Aneurysm: Potential Biomarker of Risk Stratification?

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Objectives: The overall objective is a predictive risk assessment of abdominal aortic aneurysms (AAA) where decision for or against surgery is not clear with established methods. It has been shown that aortic wall properties correlate with degenerative processes, but it has not been evaluated so far how circulating serum markers are reflecting the inflammatory and proteolytic state of an aneurysmotic wall. We investigated the receptor-ligand complex CXCR4/CXCL12 and proteoglycans, as well as circulating structure proteins for their potential role as biomarkers of AAA progression. Methods: Tissue samples (n=83) from aneurysm patients were obtained from the aneurysm sac during open repair (n=29 from ruptured AAA (rAAA) and n=54 from elective repair (eAAA)), and compared to healthy aortas (n=8). Corresponding blood samples from these patients (n=9 from rAAA, n=47 from eAAA) were available for serum analyses. CXCR4, CXCL12, GAGs (glycosaminoglycans), HYP (hydroxyproline), Col1 (collagen type I) and Col3 (collagen type III) were analyzed on the protein level in tissue lysate and serum via enzyme-linked-immunosorbent-assay (ELISA). Data analysis was performed using the non-parametric Mann-Whitney U test and Spearman's rank correlation. Results: On the tissue level, significantly higher amounts of HYP (p<0.001), Col1 (p=0.001) and Col3 (p=0.02) were found in eAAA samples compared to healthy controls. In serum samples, the level of Col1 was increased in AAA samples compared to healthy controls (p=0.003) and for Col1 (p=0.001), Col3 (p<0.001) and CXCR4 (p=0.001) elevated levels were discovered in rAAA compared to eAAA. Furthermore positive correlation was found between tissue and serum levels for Col1 (rSp=0.380/p=0.003) and Col3 (rSp=0.262/p=0.043). Conclusion: Increased expression of collagen types 1 and 3 could be observed in tissue and serum of AAA patients, with a further increase in serum of patients with ruptured AAA. Together with the correlation between tissue and serum levels, this leads us to the conclusion that proteolytic processes may be reflected in the blood to a certain extent by collagen type 1 and 3. These circulating structure proteins could serve as potential biomarkers of AAA progression, although further studies are needed.

Autophagy is Impaired in Thoracic Aortic Aneurysms

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Objectives: Genetic variants in the transforming growth factor beta (TGF-β) signaling pathway are associated with aortic enlargement and dissection in patients with thoracic aortic aneurysm (TAA). Its mechanistic role in aortic aneurysm development, however, remains controversial, and current models of the disease are incomplete. Histologic studies in TAA show increased accumulation of TGF-β ligands, loss of contractile proteins and accumulation of proteoglycans. TGF-β has been implicated as a negative regulator of autophagy via mTOR complex activation, suggesting that autophagy may further clarify the relationship of TGF-β and TAA. Methods: Human vascular smooth muscle cells (VSMCs) isolated from TAA and healthy aortic tissue were used to evaluate the effect of TGF-β on autophagy-related genes. Autophagic flux was assessed by LC3-LAMP1 immunofluorescent staining and western blot. Chloroquine prevented lysosomal degradation for evaluation of vacuole contents. Results: TAA tissue and affected VSMCs show increased lipidation of LC3 compared to controls, increased number and size of intracellular vacuoles and lack colocalization of LAMP1 and LC3B—suggesting impaired autophagic flux, formation of autolysosomes and degradation of cell products. Treatment with chloroquine revealed SM22α and lysosomes within the vacuoles, implicating increased breakdown of this contractile protein in TAA, which is exacerbated by TGF-β. Application of recombinant SM22α in TAA VSMCs improved cytoskeletal structure and mitigated impairment of autophagic flux. Conclusion: We show that autophagy is impaired in VSMCs from human TAA tissue. The effect appears to be mediated and exacerbated by TGF-β signaling and modulated by loss of cytoskeletal integrity. The mechanism of impairment and the role of TGF-β signaling in autophagy, and the sometimes paradoxical relationship between TGFβ and aneurysmal disease, however, requires further investigation.

Figure 1. Treatment of VSMCs with TGFβ was associated with the formation of dilated irregular vacuoles in VSMCs from TAA patients. At baseline, TAA VSMCs have larger vacuoles compared to that of controls. There remains lack of colocalization of LAMP1 and LC3B in affected cells. The addition of Chloroquine inhibits lysosomal degradation and matrix visualization lysosomes and SM22α within vacuoles, suggesting degradation of contractile proteins (SM22α).

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AT1a Receptor Deficiency Attenuates Thoracic Aortic Aneurysm Progression in FBN1<sup>C1041G/+</sup> Mice

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Objective:
Angiotensin receptor type 1 (AT1 receptor) activation has been implicated in thoracic aortic aneurysms (TAAs). Losartan, an AT1 receptor antagonist, has been consistently shown to attenuate TAAs in multiple animal models. However, recent studies concluded that losartan’s attenuation of Marfan syndrome associated TAAs is unrelated to AT1 receptor antagonism. To resolve this discrepancy, we determined the effects of AT1a receptor deletion on TAAs in the fibrillin-1 haploinsufficient (FBN1<sup>C1041G/+</sup>) Marfan syndrome mouse model.

Methods and Results:
Aortas from wild type and FBN1<sup>C1041G/+</sup> littermates, that were AT1a receptor +/+ or -/-, were imaged from 1 to 12 months of age using a rigorously standardized ultrasound protocol and verified by direct visualization at termination. Male FBN1<sup>C1041G/+</sup> mice had increased aortic diameters at 1 month compared to wild type littermates (Ascending: 1.39±0.06mm vs 1.16±0.07mm; p=0.04. Root: 1.63±0.05mm vs 1.35±0.06mm; p<0.001). Dilation at 1 month was not attenuated by AT1a receptor deletion. Subsequent expansion of both the ascending aorta and the aortic root in male FBN1<sup>C1041G/+</sup> mice was attenuated by AT1a receptor deletion. This difference in FBN1<sup>C1041G/+</sup> mice with AT1a receptor +/+ vs -/- could be detected as early as 3 months (Ascending: 1.51±0.04mm vs 1.28±0.06mm; p=0.002. Root: 2.05±0.06mm vs 1.79±0.08mm; p=0.03) and persisted to termination. Conversely, aortic diameters in 12 month old female FBN1<sup>C1041G/+</sup> mice compared to their wild type littermates were minimal (Ascending: 1.50±0.06mm vs 1.36±0.06mm. Root: 2.06±0.13mm vs 1.77±0.13mm), limiting analysis of AT1a receptor deletion in female mice.

Conclusions:
Deletion of AT1a receptors attenuates TAA progression but not initial development in male FBN1<sup>C1041G/+</sup> mice. Minimal aortic expansion in female FBN1<sup>C1041G/+</sup> mice highlights the need to perform sex-specific analyses of TAAs.

Noninvasive Spect Imaging Using a Novel Formyl Peptide Receptor Ligand Can be Used to Diagnose and Monitor Abdominal Aortic Aneurysms

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Objective: Our previous studies showed that neutrophil infiltration and activation plays an important role in the pathogenesis of abdominal aortic aneurysms (AAA). However, there is a lack of noninvasive, inflammatory cell-specific molecular imaging methods to provide early diagnosis of AAA formation. We hypothesized that use of cFLFLF, a PEGylated peptide ligand that binds chemotactic formyl peptide receptor 1 (FPR1) on activated leukocytes, would permit accurate, noninvasive diagnosis of AAA via single photon emission computed tomography (SPECT) imaging. Methods: 8- to 12-week old male C57BL/6 (WT) mice were treated with topical elastase (0.4 U/ml type 1 porcine pancreatic elastase) or heat inactivated elastase and aortic diameter was measured by video micrometry. Separate group of animals were injected with ⁹⁹ᵐTc-c-FLFLF (1 mCi given i.v.) two hours prior to SPECT imaging on day 14. We also performed near-infrared fluorescence imaging using c-FLFLF-Cy7 probe on days 7 and 14 post-elastase treatment and measured fluorescence intensity ex vivo. Co-expression of neutrophils (Ly6G-FITC) and c-FLFLF-Cy5 was performed by immunostaining on aortic tissue on day 14. Groups were compared using ANOVA followed by Bonferroni post hoc test. Results: Aortic diameter was significantly increased in the elastase group compared to controls (159.7% ± 47.3% vs 3.7% ± 9.0%, P< 0.0001). SPECT imaging demonstrated a 3.6-fold higher ⁹⁹ᵐTc-cFLFLF signal intensity (SUVmax) in mice with AAA versus controls (n=5 mice/group; p<0.05). Moreover, a significant increase in fluorescence intensity was also observed in elastase-treated mice on days 7 (approximately 2-fold increase) and 14 (3-fold increase) compared to respective controls using fluorescence imaging (n=5 mice/group; p<0.001). Immunostaining of c-FLFLF-Cy5 demonstrated peptide specificity as it was co-expressed with neutrophils in aortic tissue on day 14. Conclusions: SPECT imaging using ⁹⁹ᵐTc-cFLFLF, a novel FPR1 ligand, enables quantifiable, noninvasive diagnosis and progression of AAAs. Clinical application of cell-specific, molecular imaging probes i.e. cFLFLF may permit early diagnosis of AAA formation, enabling targeted therapeutic interventions and preventing impending aortic rupture.

Characterization of Endovascular Abdominal Aortic Aneurysm Repair Surveillance Using Vascular Quality Initiative and Medicare Claims Linked Data

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Introduction/Objective Practice guidelines for endovascular aortic repair (EVR) recommend follow up CT scan at 30 days and yearly thereafter. We sought to characterize surveillance failure of patients in the Vascular Quality Initiative (VQI) using longitudinal data from Medicare claims. Methods We identified EVR patients within the VQI for years 2003-2015 and this information was linked to Medicare claims. Surveillance failures were defined as the absence of an abdominal imaging study during any 15-month interval after the index operation. A combination of clinical and claims-based independent variables of interest were assessed for univariate association with surveillance failure. Cox regression was then used to identify factors independently associated with surveillance failure. Survival analysis was used to identify the rate of surveillance over time and this was stratified by dual eligibility in both Medicare and Medicaid. Results The analytic cohort included 9,723 patients who underwent EVR. By 4.19 years after EVR, 50% of at-risk patients had an imaging failure. By 6.35 years, 75% of patients had an imaging failure. As shown in Figure 1, patients dually eligible for Medicare and Medicaid are more likely to have an imaging failure compared with those patients eligible for Medicare alone (72% vs 61% at 5-years, log-rank p < 0.0001). Patients who underwent re-intervention related to EVR or had cancer were less likely to experience an imaging failure (HR 0.74 [0.67-0.83] and 0.89 [0.80-0.99] respectively). Conclusion More than half of EVR patients experience a failure in surveillance imaging within five years of their surgery. Insurance status was especially influential in determining which patients experience surveillance failure. Surgeons must remain vigilant in encouraging follow up, especially among high-risk groups.

Figure 1: Kaplan-Meier Plot of Freedom from Imaging Surveillance Failure by Dual Medicare and Medicaid Eligibility Status

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c-Cbl Augments Ischemia Induced Angiogenesis in Hindlimb Ischemia Model in Uremic Milieu

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Objectives: Chronic kidney disease (CKD) constitutes a potent and independent risk factor for peripheral arterial disease (PAD), yet little is known about ways to abate the severity of PAD in these patients. Casitas b-cell lymphoma (c-Cbl) is a RING-finger containing E3 ubiquitin ligase regulating several pro-angiogenic mediators such as beta catenin and PLC-gamma. We hypothesized that loss of c-Cbl activity is likely to augment ischemia-induced angiogenesis in normal and uremic conditions.

Methods: c-Cbl +/- mice on C57BL/6J background were obtained from the National Cancer Institute, and the line was re-derived. Unilateral hindlimb ischemia (HLI) model was performed in 8-12 week old c-Cbl +/- and c-Cbl +/- female mice under non-CKD and CKD conditions using the adenine-induced CKD model. Laser Doppler imaging was used to determine perfusion recovery over time. CD31 staining was used to determine capillary density and quantitated using Image J. Capillary leakage was detected by dextran infusion and quantitated as integrated density. P value of < 0.05 was considered significant.

Results: Appropriate reduction in c-Cbl mRNA and protein levels were confirmed in several tissues in c-Cbl+/- mice. Under non-CKD conditions, compared to c-Cbl +/+, reperfusion after HLI in c-Cbl +/- was 2.17 fold higher at day 14 (p=.008) and 2 fold higher at day 21 (p=.02). This was corroborated with significantly increased capillary density c-Cbl +/- mice (1.6 ±.06 capillaries/myocyte) compared to c-Cbl +/- CKD mice (1.2 ± 0.11 capillaries/myocytes, p=.03). This pattern of augmented angiogenesis in c-Cbl+/- was also observed in CKD mice. At the end of 7 days, c-Cbl+/- CKD mice showed significantly higher perfusion (0.25± 0.05) compared to c-Cbl+/- CKD mice (0.35±0.06, p = 0.013). CKD exposure increased capillary permeability in the ischemic limb in c-Cbl+/- mice compared to non-CKD c-Cbl+/- mice (p = 0.05). Conclusion: We demonstrate that the reduction of c-Cbl activity results in enhanced angiogenesis by increasing capillary density and capillary leakage. These features persisted under the profound stress of uremia. These results support further exploration of c-Cbl as a therapeutic target for diseases such as PAD in both CKD and non-CKD states.

Epigenetic Modifications Influence Macrophage-mediated Inflammation in Abdominal Aortic Aneurysms

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**Objective:** Abdominal aortic aneurysms (AAA) are characterized by pro-inflammatory macrophage (Mφ) infiltration and vascular remodeling. The mechanisms regulating Mφ polarization during AAA development remain unknown. There is increasing evidence that epigenetic enzymes direct Mφ phenotypes and inflammation. Here, we examine if epigenetic modifications influence macrophage-mediated inflammation during AAA formation.

**Methods:** Male C57BL/6 mice (n=60) were injected intraperitoneally with adeno-associated vector containing either a null vector or a mouse PCSK9 gain-of-function mutation (D377Y) and fed a saturated fat-enriched diet. Two weeks after AAV infection, mice were infused with AngII or saline for 4 weeks. AAA maximum diameters were quantified and Mφs (CD11b+,CD3-,CD19-,Ly6G-) were sorted. mRNA abundance of *IL-1β*, *IL-12*, *IL-23*, *NOS2*, and *JMJD3* were determined by qPCR. Chromatin immunoprecipitation (ChIP) was used to analyze H3K27 methylation on inflammatory gene promoters. Statistical significance was determined using Student’s t-test or ANOVA.

**Results:** Aortic tissue and bone marrow derived macrophages from AngII-induced AAAs displayed upregulation of histone demethylase, JMJD3, and increased mRNA abundance of inflammatory genes (*IL-1β*, *IL-12*, *IL-23*) during AAA development (p<0.05). To determine the impact of JMJD3, we evaluated histone methylation in peripheral monocytes and found decreased levels of the repressive histone methylation mark, H3K27me3, on inflammatory gene promoters in AngII-induced AAAs, compared to controls. Inhibition of JMJD3 in vitro using siRNA significantly reduced Mφ inflammatory cytokine expression (p<0.05). To determine the concordance of these data to human pathology, surgically acquired AAA tissues were examined and JMJD3 was significantly overexpressed in human AAAs compared with control (non-AAA) tissues.

**Summary:** These results suggest an important role for JMJD3 in regulating macrophage-mediated inflammation and identify a potential target for the treatment of chronic inflammation in AAAs.

Aerobic Exercise Attenuates Arterial Wall Remodeling in a Smooth Muscle Cell LRP1-Deficient Mouse Model

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The LDL receptor-related protein 1 (LRP1) is an endocytic receptor that maintains the structural integrity of blood vessels. Conditional smooth muscle cell knock out of LRP1 (smLRP1-/-) causes fragmentation of the elastic lamina and medial thickening and leads to spontaneous aortic and superior mesenteric artery (SMA) aneurysms. Histologically, these changes mimic those seen in humans with aneurysmal disease. The objective of this study was to investigate the role of exercise in modulating aneurysm development secondary to smooth muscle cell LRP1 deficiency. smLRP1-/- mice were employed as an animal model for our studies. The vasculature from sedentary WT and smLRP1-/- mice was compared to exercised smLRP1-/- mice. An exercise protocol of moderate intensity was initiated at 4 weeks of age. All animals were euthanized at 21-28 weeks of age. Micro-computed tomography identified an increased diameter of the SMA of smLRP1-/- sedentary mice compared to their sedentary WT littermates. Histologic analysis of the vasculature revealed abnormalities in the elastic lamina and increased thickness of the media and adventitia, which is most pronounced in the SMA. Mild aerobic exercise reversed these changes with a significant reduction in the number of SMA aneurysms and a significant reduction in the wall thickness of the ascending aorta, descending thoracic aorta, and SMA (P<0.05) with improvement in the elastin architecture. Current studies aim to investigate the proteomic and genomic profiles of sedentary and exercised mice to identify key pathways that are altered after mild-aerobic exercise. These may uncover therapeutic targets to slow the progression of aneurysmal degeneration. In summary, these data demonstrate that genetic deletion of smLRP1 has a significant effect in vascular remodeling that is ameliorated by mild aerobic exercise.

Figure: Vessel morphology showing reduced wall thickness in exercised smLRP1-/- animals as compared to their sedentary littermates. Additional reductions are seen in the thickness of the media of the ascending and descending thoracic aorta and the adventitia of the superior mesenteric artery (all P<0.05).

The BD2 Domain of BRD4 is a Determinant in EndoMT and Vein Graft Neointima Formation

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**Background:** Vein-graft bypass is commonly performed to overcome atherosclerosis but is limited by high failure rates, principally due to neointimal wall thickening. Recent studies reveal that endothelial-mesenchymal transition (EndoMT) is critical for vein-graft neointima formation. BETs are a family of Bromo/ExtraTerminal domains-containing epigenetic reader proteins (BRD2, BRD3, BRD4). They bind acetylated histones through their unique tandem bromodomains (BD1, BD2), facilitating transcriptional complex formation and cell-state transitions. The role for BETs, including individual BRDs and their unique BDs, is not well understood in EndoMT and neointimal formation.

**Methods:** In vitro, loss-of-function (siRNA, dominant-negative domain expression) and gain-of-function (adenoviral expression) were performed. In vivo, transgene was introduced to the vascular wall by ex vivo lentiviral transduction followed by grafting the transduced vein to a carotid artery using an improved cuff technique.

**Results:** Our previous data showed that repression of BRD4 expression abrogated TGFβ1-induced EndoMT, with greater effects than BRD2 or BRD3 knockdown (ATVB meeting abstract#254, 2018). Here we further found that an inhibitor selective for BD2 in all BETs, but not that for BD1, blocked EndoMT. Moreover, expression of a dominant-negative BRD4-specific BD2 fully abolished EndoMT. Concordantly, BRD4 knockdown repressed TGFβ1-stimulated increase of ZEB1 protein - a transcription factor integral in EndoMT. In vivo, lentiviral gene transfer of either BRD4 shRNA or dominant negative BRD4-specific BD2 mitigated neointimal development in rat jugular veins grafted to carotid arteries.

**Conclusions:** The results reveal the BD2 domain of BRD4 as a determinant driving EndoMT in vitro and neointimal formation in vivo. Our findings provide new insight into BET biology, while offering prospects of specific BET domain targeting as an approach to limiting neointima and extending vein graft patency.

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Pattern of Elastic Matrix Alteration Differentiates Adaptive Arterial Diameter Growth in Arteriogenesis From Pathologic Growth in Aneurysm

**Objectives:** Extracellular matrix (ECM) proteins and proteoglycans provide structure and the nonlinear elastic behavior necessary for the arterial system to function. They also confine the maximum diameter. For outward diameter expansion to occur beyond vasodilation, whether adaptive or pathological, the constraints on diameter from the ECM must be released. Two critical components of matrix structure are collagen and elastin. Loss of elastin and fragmentation of the internal elastic lamina (IEL) are seen in both arteriogenesis (adaptive) and aneurysm (pathological). We sought to compare the ECM structure for vessels of similar diameter growth reached via either an elastase perfusion model of aneurysm or a modified arteriogenesis model. **Methods:** All procedures are performed in Sprague-Dawley rats. Common and profunda femoral arterial tissues were harvested after 2 weeks. Tissues were imaged with multiphoton or confocal microscopy. **Results:** We observed in each group a similar increase in arterial diameter of 2-3 fold and a flattening/loss of wrinkled topology of the IEL (E,F). In arteriogenesis, fenestrations in the elastic structure expand, leaving the IEL with a mesh-like appearance which maintains load-bearing properties (A2, C,c). In aneurysmal tissues the internal elastic laminae were lost entirely to degradation and the collagen content demonstrated a stress loaded configuration in the adventitia (D,d). Notably, reduced exposure to elastase created a similar pattern of IEL expansion to arteriogenesis (A3). In both models, lysyl oxidase expression was increased (E,F). **Conclusions:** Arterial diameter expansion occurs through alteration of the ECM in both adaptive and pathologic models, but it is not simply degradation. Here we show that a key differentiating feature of this diameter growth is the maintenance of IEL load bearing with adaptive remodeling.
Correlation of Clinical Risk Scores for Stroke with Carotid Plaque Gene Expression Profiles in Atherosclerotic Patients


Carotid stenosis (CS) accounts for 20% of ischemic strokes (IS), with known modifiable and non-modifiable elements as risk factors for atherosclerotic plaque development. ABCD2 clinical risk score predicts the risk of stroke within 2, 7 and 90 days after a transitory ischemic attack (TIA), while Carotid Artery Risk (CAR) score estimates the rate of 5-year ipsilateral IS in recently symptomatic patients. Our aim was to determine whether ABCD2 and CAR correlate to global plaque transcriptomic profiles and patient clinical parameters.

Patients undergoing endarterectomy for symptomatic CS were included in the study based on the availability of ultrasound, clinical/blood variables and plaque microarray data (n=85). CAR and ABCD2 scores were calculated by an iPhone application assessing age, gender, smoking, comorbidities (diabetes), therapy, degree of stenosis, time from last event to surgery, most severe ipsilateral event, duration of TIA, hypertension, peripheral vascular disease or myocardial infarction and plaque ulceration. Patients were selected into low, intermediate and high risk groups and multivariate statistical analyses performed followed by plaque immunohistochemistry. CAR and ABCD2 scores were overall well-correlated to each other (r=0.5, p<0.0001). There was an inverse correlation of both scores to patient’s p-HDL levels. CAR was positively correlated to serum creatinine, fibrinogen and WBC count, while negative correlation was found to Hb. Microarray comparisons of high vs. low risk plaques revealed that coagulation, immune response, extracellular matrix organization and smooth muscle cell (SMC) contraction were enriched pathways in high-risk groups and ATP-binding cassette sub-family B member 5 (ABCB5) was the most upregulated gene. ABCB5 upregulation was validated in extended microarrays comparing plaques vs. normal arteries (n=127+10) and plaques from symptomatic vs. asymptomatic patients (n=87+40). ABCB5 protein was localized to SMCs in the normal media and abundantly in plaque necrotic core.

Here, molecular processes previously related to plaque vulnerability were associated with high clinical risk scores. ABCB5 involved in cellular drug efflux, was implicated as a key novel gene related to unstable plaques.

Objectives: Approximately 30% of human vein grafts fail within the first year of implantation secondary to neointimal hyperplasia and adverse remodeling. This process has been difficult to study in animal models because of lack of a similar pathology. Therefore, we created a novel xenograft model where human saphenous vein was implanted into the infrarenal aorta of Rowett nude rats. Methods: Weekly ultrasound was performed before pressure perfusion fixation on postoperative day 28. Paraffin-embedded cross-sections were analyzed for human vs rat cells (using human-specific antibodies to mitochondria and Ku80) and for changes in wall thickening and extracellular matrix (ECM; Movat’s stain and antibodies to versican and aggrecan). Results: Successful human vein grafts (n=4) were ~150% larger than the native rat aorta as documented by photomicrograph (Figure 1A) and ultrasound (Figure 1B top) and showed appropriate arterial Doppler waveforms (Figure 1B bottom). Particularly robust neointimal, but also medial and adventitial, hyperplasia was observed in Movat’s stained cross-sections of the vein (Figure 1C). The neointimal layer was primarily ECM with relatively few cells. Surprisingly, the neointima stained poorly for versican, but was strongly positive for aggrecan. While neither human-specific antibody stained all cells, human cells were noted throughout the vein wall in similar numbers seen in human saphenous vein controls. Conclusions: This novel human vein xenograft model will be suitable for testing therapeutic strategies for decreasing neointimal hyperplasia leading to human vein graft failure. The novel observation of aggrecan in the neointima suggests new functions for aggrecan in the vascular setting.
Single-cell Profiling of Atherosclerotic Tissue Identifies T Cell Subsets Associated with Cerebrovascular Events


Atherogenesis is driven by the infiltration of immune cells into the arterial wall where inflammation occurs and promotes plaque growth. While some plaques are stable, others are vulnerable and may rupture and lead to stroke or myocardial infarction. The contribution of specific immune cell types to plaque stability is obscure. In our present study, we aim to characterize the immune cell repertoire of human atherosclerosis using innovative single cell methods. We used mass cytometry (CyTOF) to study plaque tissue and paired blood from symptomatic (TIA or stroke < 6 months) or asymptomatic (no TIA or stroke) patients undergoing carotid endarterectomy. Using unbiased MetaLouvain clustering analysis, we characterized cell populations from two independent cohorts to (1) define major immune populations (cohort1; n=15), and (2) detail the adaptive immune compartment (cohort2; n=23), which was predominant in cohort 1. Moreover, we used single cell RNAseq to obtain the transcriptional signatures of immune infiltrates of atherosclerotic tissue. We found that T cells dominate advanced plaques. Atherosclerotic tissue was enriched in CD8+ Effector Memory (EM) T cells that were CD69hi, CCR5hi and PD-1hi vs. their blood counterparts. Our transcriptional profiling confirmed that T cells were the majority of all immune populations, and that T cells expressed genes that represented an activated and largely exhausted phenotype. Our CyTOF results identified differences in T cell subsets between patient types. Symptomatic patients had a higher frequency of a subset of CD4+ EM T cells (CD69hiCCR5hiPD1hiCD27loCD28lo), and displayed a more differentiated phenotype of CD27loGZMBloCD38loCD8+ EM T cells. Our study is the first to provide a single cell immune atlas of advanced atherosclerotic lesions and to identify specific subsets associated with clinical outcomes of atherosclerotic disease. This study will be valuable for the future design of precise tissue-targeted immunotherapies.

Dual-Ligand Modified Liposomal Nanoparticles Multifunctionalized for Spatially Controlled Delivery of Gene Therapeutics

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Objectives: Nanoparticles designed to localize to areas of vascular perturbations could provide a targeted delivery system for gene therapeutics aimed at improving intervention outcomes. PEGylated liposomes (PLPs) are potential delivery vectors, and PEG residues provide a scaffold for multifunctional surface modifications. We have previously developed PLPs functionalized with collagen targeting peptides (CTP-PLPs) that preferentially bind collagen IV, an abundant sub-endothelial matrix protein exposed during vascular intervention. Here we present dual ligand-modified liposomes with multifunctional potential, building on our CTP-PLP platform by simultaneously incorporating cell-penetrating peptides (R8) to enhance liposomal cell association and nucleic acid delivery. Methods: PLPs were formed with DOPC-PEG + 30mol% cholesterol + 0.1mol% Rhodamine-DOPE, and siRNA loaded via EtOH injection. CTP-PLPs were formed as PLPs + 5mol% CTP-modified-DSPE, as previously reported. Dual-ligand modified liposomes (R8-CTP-PLPs) incorporated CTP concurrently with 5-10mol% sterylated-R8. Vascular smooth muscle cells (VSMC) were treated for 24hr at 100uM lipid for cellular association, quantified via fluimetry, or at 400nM siRNA encapsulate for gene silencing, quantified via qPCR. Results: While CTP-PLPs increased VSMC association by 1.6-fold compared to PLP, R8-CTP-PLPs increased association by 3.6-fold (5mol%R8) and 10.4-fold (10mol%R8; Fig1A). Likewise, siRNA delivery via R8-CTP-PLPs resulted in enhanced gene silencing compared to CTP-PLPs and non-treated controls (Fig1B). Conclusions: R8-CTP-PLP nanocarriers established here show promise as the framework for a spatially controlled drug delivery platform for targeted vascular therapeutics. Ongoing studies aim to elucidate R8-CTL-PLP vessel wall binding and targeted gene silencing in a dynamic living environment via ex vivo vessel perfusion and in vivo rodent models of vascular injury.
Measurement of Transcutaneous Oxygen Pressure in Patients With Post-thrombotic Syndrome and Possible Clinical Applications

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Background: Post-thrombotic syndrome (PTS) is the commonest long-term sequela of deep venous thrombosis. Severe PTS reduces importantly the quality of life of patients, and undoubtedly tissue hypoxia is associated with ulceration. Transcutaneous oxygen pressure (tcPO2) provides information about the delivery of oxygen through the microvascular circulation. Measuring tcPO2, we could detect values at which patients with PTS will ulcerate. We studied PTS patients measuring tcPO2 and comparing these values with a control group.

Methods: A transversal study was performed at the National Institute of Medical Sciences and Nutrition in Mexico city. Periflux 5000 monitor was used to measure tcPO2 in patients with mild-moderate, severe PTS and control groups. Twelve patients and 13 patients with PTS and control group, respectively, were enrolled. In patients with ulcer, two measures were taken, the first one around the ulcer and the second one 10 cm away from the ulcer. Categorical data were analyzed with Fisher’s exact test. A p value <0.05 was considered statistically significant.

Results: The mean tcPO2 measurement in PTS group was 35.5 mm Hg, while in the severe PTS was 23.2 mm Hg. All severe PTS patients had measurements <40 mm Hg, tcPO2 value in control group was >50 mm Hg in all patients, with a mean value of 58.2 mm Hg. During comparison of severe PTS subgroup versus control group, we found that patients with venous ulcers had <40 mm Hg in tcPO2 measurement and all control group patients had >50 mm Hg (p<0.0001).

Conclusions: Our results demonstrate that severe PTS patients have significantly lower TcPO2 values compared to a control group and non-severe PTS (p=0.0001). Further research is necessary to establish accurate cut points of tcPO2 in PTS.