2 Abstracts

**Objectives:** Coronavirus-associated coagulopathy is postulated to be driven by systemic macrophage activation after severe acute respiratory syndrome coronavirus 2 infection and presents with an increased risk of thrombogenesis and hyperfibrinolysis. Previous work shows that the histone methyltransferase KMT2A/MLL is a key mediator of inflammatory signaling in monocytes and macrophages (Mo/Mes). In this study, we sought to identify the regulation of factors important in coronavirus-associated coagulopathy by MLL.

**Methods:** Mice with myeloid specific knockout of MLL (Cre') and littermate controls (Cre') underwent intranasal inoculation of 2 × 10^5 pfu of the murine coronavirus MHVA59, an established model which phenocopies severe acute respiratory syndrome coronavirus 2 infection. Splenic Mes (surrogate for circulating Mo/Mes) were isolated and RNA and protein levels of urorikase (Plau; probrinolytic), urorikase receptor (Plaur; probrinolytic), and tissue factor (F3/TF; procoagulant) were analyzed using quantitative reverse-transcriptase polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. Thromboelastography on whole blood and urorikase activity assays from mouse plasma were performed. Urorikase and tissue factor activity assays were performed on plasma from human samples.

**Results:** RNA (Figure, top panel) and protein (Figure, bottom) levels of Plau, Plaur, and F3 were suppressed in the splenic Meso harvested from Cre' animals (white bars compared with splenic Meso harvested from Cre- animals (blue bars Figure A). Cre' mice displayed a shortened reaction time as measured by thromboelastography (Figure, B) and elevated plasma urorikase activity levels (not shown). Hospitalized coronavirus disease 2019-positive patients displayed elevated plasma urorikase and tissue factor activity levels (Figure, C).

**Conclusions:** We identify a role for MLL for basal expression and for coronavirus-mediated induction of factors important for fibrinolysis and coagulation in murine Mo/Mes and in driving coagulopathy. Our results suggest that MLL blockade may be an attractive strategy to combat coronavirus-associated coagulopathy.

**Methods:** Fourteen- to 16-week-old wild-type (WT, C57Bl/6) or TSP5-null male and female mice underwent left femoral artery ligation and transection (n = 6–9/group). Laser Doppler data were collected preoperatively, after ligation, and at humane killing (day 14). Blood flow recovery was expressed as a ratio of ischemic/nonischemic limb. Immunohistochemistry was performed using anti-t-smooth muscle actin on the vastus lateralis to quantify angiogenesis and anti-CD31 on the gastrocnemius to quantify arteriogenesis. Arteriole and capillary density were determined by vessel counts/5 high-power field. Data were analyzed by analysis of variance with post hoc testing, with a P value of less than .05 being significant.

**Results:** TSP5-null mice had decreased blood flow recovery in males (WT 0.85 ± 0.04 vs TSP5 0.48 ± 0.06; P < .001) and females (WT 0.63 ± 0.07 vs TSP5 0.36 ± 0.04; P < .01). No difference in flow recovery was seen between male and female TSP5 nulls (male 0.48 ± 0.06 vs female 0.36 ± 0.04; P < .14). Arteriogenesis was impaired in male TSP5 null mice (WT 3.91 ± 0.24 vs TSP5 2.6 ± 0.42; P < .02) but increased in females (WT 2.5 ± 0.26 vs TSP5 4.35 ± 0.73; P < .01). Angiogenesis was decreased in TSP5-null males (WT 10.9 ± 0.93 vs TSP5 7.03 ± 0.84; P < .01), but not females (WT 6.1 ± 0.50 vs TSP5 5.6 ± 0.80; P < .9). In contrast, flow recovery, angiogenesis, and arteriogenesis were decreased in WT females compared with WT males (P < .05).

**Conclusions:** Our findings suggest that TSP5 is relevant to vascular remodeling following hindlimb ischemia with a divergence in mechanism between males and females. TSP5 is necessary in males for arteriogenesis and angiogenesis. TSP5 in females may be suppressive of arteriogenesis and has no effect on angiogenesis. Further investigation into these sexually dimorphic adaptive processes may lead to specific targeted CI treatments.


22-VIRC-457-AHA-VD

**Microvessel Oxidative Stress Predicts Changes in Leg Function of Patients with Peripheral Arterial Disease after Supervised Exercise Therapy**

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**Objective:** To evaluate baseline oxidative stress in the microvessels of patients with peripheral arterial disease (PAD) (1) in association with heme oxygenase-1 (HO-1), a critical cytoprotective molecule for oxidative stress (2) as a predictor of the functional and pathophysiologic changes that occur in response to 6 months of supervised exercise therapy (SET). We hypothesized that accumulated oxidative stress in the microvessels of PAD at baseline would predict changes in walking performance, myophyler pathophysiology, and endothelial function. In an additional subset of patients, angio- and arteriogenesis were evaluated.

**Methods:** Twelve claudicating patients received six months of SET per the American College of Cardiology/American Heart Association guidelines. Before and after SET, patients were evaluated for leg biomechanics, overground walking capacity (6-minute walking distance [SMWD]), and maximum walking time on a treadmill. Subsequently, their more affected calf muscle was biopsied for quantification of HO-1 and oxidative stress (carbonyl content) in both myofibers and microvessels. We evaluated the association between HO-1 expression and carbonyl content with correlation and multiple regression.

**Results:** HO-1 expression and Carbonyl content were strongly associated in the microvessels (n = 1400, r = 0.98; P < .001). Increasing the carbonyl content in the microvessels of each patient was associated with increasing HO-1 expression. In a subset of patients, HO-1 expression increased more slowly with increasing carbonyl content. Pre-SET oxidative stress in microvessels was a significant predictor of SET-mediated change in the SMWD (r = -0.75; P = 0.012) and plantar flexion torque (r = 0.8571; P = .0065).

**Conclusions:** Carbonyl content (oxidative stress) in microvessels of each patient was positively associated with HO-1 expression. However, in a subset of patients, HO-1 expression increased more slowly with increasing carbonyl content. Baseline oxidative stress in the microvessels, which may be a function of the quality of HO-1 expression, was a significant predictor of SET-mediated change in the SMWD and plantarflexion torque. The data suggest that microvascular oxidative damage may contribute uniquely to calf muscle pathology and leg dysfunction in patients with PAD.

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**Thrombospondin-5 Is Necessary for Males But Not Females in Hindlimb Ischemia Recovery**

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**Objective:** To evaluate the role of thrombospondin-5 (TSP5) in hindlimb ischemia recovery.

**Methods:** Thrombospondin-5 (TSP5) is a secreted glycoprotein that belongs to the family of secreted proteins with multiple domains. It is produced by various cell types, including smooth muscle cells, endothelial cells, and macrophages. TSP5 plays a critical role in angiogenesis and arteriogenesis, and its deficiency results in impaired vessel growth and remodeling.

**Results:** In wild-type (WT) mice, TSP5-null (ΔTSP5) mice displayed impaired arteriogenesis and angiogenesis compared to WT mice. However, in female ΔTSP5 mice, arteriogenesis and angiogenesis were comparable to WT mice.

**Conclusions:** The results suggest that TSP5 is necessary for males but not females in hindlimb ischemia recovery.

**Authors:** M. M. Rangel: Nothing to disclose. C. Dunn: Nothing to disclose. X. Wang: Nothing to disclose. X. Ding: Nothing to disclose. K. Maier: Nothing to disclose. V. Gahtan: Nothing to disclose.

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Using Deep Convolutional Neural Networks to Automatize Classification of Carotid Plaques from Ultrasound Imaging

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Background: Stroke is a devastating consequence of plaque rupture from the carotid arteries. Current management of carotid plaques involves waiting for symptoms (eg, stroke or ministroke), intervention itself has risk of stroke and not all plaques are vulnerable to rupture. There is a need to better risk-stratify plaque that causes stroke. Carotid ultrasound (US) examination is a noninvasive and inexpensive visualization of plaques, but is limited by human interpretation. We hypothesize that convolutional neural networks will identify unique features of carotid plaques for automated risk stratification.

Methods: Our workflow is illustrated in Figure A. A total of 141 B-mode US images of carotid arteries were included. 64 high-risk with symptomatic carotid plaques and 75 low-risk with no significant plaque. Data were cropped and divided into training (70%) and holdout test (30%) subsets. During model training, an ensemble of ResNet-18 convolutional neural networks learned classification of low-risk and high-risk cases using five-fold stratified cross-validation and was used to predict on the holdout test set. The model was evaluated using area under the receiver operating characteristic curve (AUC) and sensitivity. Saliency maps were used for model interpretability to highlight relevant pixels for model decisions.

Results: The cross-validation AUC was 0.995 ± 0.010. The testing AUC was 0.909 and class-wise sensitivities were 0.88 (low risk) and 0.79 (high risk). The density plot (Figure B) shows that the classifier correctly identifies both classes with confidence. Model interpretability using saliency maps (Figure C) shows pixels corresponding to carotid artery vessel edges and in high-risk cases, carotid plaques.

Conclusions: Using this proof-of-concept model, carotid US long axis images are sufficient to identify high-risk plaques in symptomatic patients. We now need to determine whether we can identify high-risk plaques before symptoms to prevent devastating stroke caused by carotid disease.


22-VIRC-453-AHA-VD

Transforming Growth Factor-beta Signaling Mediates Endothelial-to-mesenchymal Transition during Arteriovenous Fistula Remodeling in the Chronic Kidney Disease Environment

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Objectives: Arteriovenous fistulae (AVF) are the optimal conduit for hemodialysis access in patients with end-stage chronic kidney disease (CKD) however, AVF have high rates of failure due to aggressive neointimal hyperplasia. Neointimal hyperplasia in the CKD environment is characterized by endothelial-to-mesenchymal transition. We hypothesized that endothelial transforming growth factor (TGF)-ß signaling promotes endothelial-to-mesenchymal transition to cause AVF failure.

Methods: Aorto caval AVF were created in C57BL/6J mice, with control mice having sham procedures. Three weeks before AVF, CKD was created via 5/6 nephrectomy (Nx); five groups were tested, including sham, sham+5/6Nx, AVF+0/6Nx, AVF+3/6Nx, and AVF+5/6Nx. Endothelial cell (EC)- and smooth muscle cell (SMC)-specific TGFßII knockout (KO) mice were treated with tamoxifen 2 weeks before AVF; ultrasound examination was used to determine AVF diameter and patency at postoperative days 7, 14, and 21. AVF were harvested at days 7 or 21 and examined with histology. Immunofluorescence and Western blot.

Results: There was no difference between mice treated with 0/6Nx or 3/6Nx regarding AVF diameter, AVF wall thickness, or accumulation of extracellular matrix components fibronectin, collagen 1, and collagen 3. However, AVF diameter was significantly decreased in mice treated with 5/6Nx compared with 0/6Nx or 3/6Nx, whereas there was increased AVF wall thickness as well as immunoreactivity of fibronectin (P < 0.01), collagen 1 (P = 0.02), collagen 3 (P = 0.005), and TGFß-II (P = 0.02) compared with 0/6Nx. ECs in the AVF of mice treated with 5/6Nx had significantly increased immunoreactivity of the mesenchymal markers notch3 (P < 0.0001), vimentin (P < 0.0001), and FSP-1 (P = 0.02) compared with 0/6Nx. However, blocking TGFß signaling using either EC-KO or SMC-KO mice had significantly decreased immunoreactivity of mesenchymal markers and wall thickness (P < 0.0001), consistent with reduced endothelial-to-mesenchymal transition and an increased diameter (P = 0.035). Interestingly, the AVF wall thickness in EC-KO mice was thinner than in SMC-KO mice. Both EC- and SMC-specific TGFß signaling promote endothelial-to-mesenchymal transition and wall thickening during venous remodeling in the CKD environment. These data suggest that the TGFß signaling pathway may be a potential therapeutic target to prevent AVF failure.


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Altered Macrophage Response Associated with Impaired Skeletal Muscle Regeneration in a Murine Model of Limb Ischemia

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Introduction: Skeletal muscle regeneration in the ischemic limb is a complex process dependent on coordinated intercellular...