I am grateful for the funding support of the American Venous Forum received in the form of the American Venous Forum Foundation/JOBST Award for the period 1/1/2017-12/31/2019 for my project entitled “Modulation of Venous Thrombo-Inflammation by inhaled Carbon Monoxide and Heme Oxygenase-1.” As a junior faculty physician scientist focused on vascular disease, and in particular venous thrombosis, support from the AVFF-JOBST has helped me lay the foundation for a durable career in venous research. The overarching goal of my research program is to identify the molecular mechanisms underlying venous thrombo-inflammation to develop novel therapeutic targets for patients with venous thromboembolism (VTE). This project is focused on understanding novel mechanistic pathways in VTE, a disease that affects over 900,000 patients each year and kills >60,000 in the United States alone. The economic and societal burden are further amplified by long-term complications of post-thrombotic syndrome and recurrent VTE. Since 40% of patients with VTE have no identifiable cause, we have focused on identifying novel risk factors at the intersection of inflammation and coagulation. Chief among these is heme oxygenase-1 (HO-1), an inducible enzyme that degrades cytotoxic heme to release biliverdin, ferrous iron, and the endogenous biogas carbon monoxide (CO). CO is known in the lay press as a poisonous gas, but emerging data suggests it has critical functions in maintaining vascular homeostasis. *We hypothesized that deficiency of HO-1 would result in accumulation of heme, a known activator of inflammatory signaling, and subsequently exacerbate venous thrombosis in a murine model*. Our studies utilized HO-$1^{-/-}$ and haploinsufficient HO-$1^{+/-}$ mice. We determined that both HO-$1^{-/-}$ and HO-$1^{+/-}$ mice have exaggerated venous thrombosis in an IVC ligation model of DVT, which mimics venous stasis in patients. Interestingly, HO-1 deficiency did not alter venous thrombosis in an IVC stenosis model characterized by flow restriction, suggesting the HO-1 does not regulate platelet activation in VTE.

We then determined that administration of intermittent, low-dose inhaled CO (the heme degradation byproduct) conferred a statistically significant but minor protection from venous thrombosis in HO-1 deficient mice. We surmised that the limited bioavailability of CO during intermittent inhalation may prevent a durable effect on thrombosis, and hypothesized that injection of CO-releasing molecules (CORM) may be an alternate CO delivery platform. We delivered four unique CORM formulations at various doses by intraperitoneal injection to mice and noted no significant protection from venous thrombosis, which we believe can be attributed to the rapid degradation of CORM in biological systems. Interestingly, we were unable to measure detectable levels of CO in treated mice as therapeutic levels of CO in plasma are below the sensitivity limit for standard blood gas measurement systems which are calibrated to detect toxic concentrations of CO.

Further examination of stasis thrombi from HO-1 deficient mice revealed that thrombi are characterized by increased neutrophil recruitment to the thrombus:vessel interface. Neutrophils are known to undergo a particular form of cell death for host-defense in which chromatin unravels and is extruded into the extracellular space, decorated with antimicrobial proteins and highly charged particles. These neutrophil extracellular traps (NETs) have been recently found to play important roles in exacerbating venous thrombosis. We determined that HO-1 deficient neutrophils have enhanced capacity to form NETs compared with WT controls in cell-autonomous experiments *in vitro*, and in stasis thrombus lysate in vivo. As treatment with DNase, an enzyme that degrades NETs did not alter thrombosis, we hypothesized that NETs may be a surrogate for neutrophil activation and inflammation in HO-1 deficient DVT, and did not represent a treatment to abrogate HO-1 deficient thrombosis. HO-1 is a highly conserved enzyme across mammalian species. This is reinforced by the observation that no patients have

been identified with complete lack of HO-1, and patients with heterozygous mutations resulting in HO-1 deficiency are severe developmental and multi-organ inflammatory abnormalities. *We hypothesized that HO-1 modulates central inflammatory processes relevant in thrombosis*. We discovered that venous stasis thrombus lysates from HO-1 deficient mice had significantly increased interleukin-1β (IL1β), the prototypical inflammatory cytokine and pyrogen. The recent CANTOS trial demonstrated reduced arterial cardiovascular events in patients treated with an IL1β-blocking antibody. Recent studies published from my lab during this funding period (*JCI* 2019, *Nature Communications* 2019, *ATVB* 2019) identified this pathway as a viable target in treatment of venous disease. Although the funding period for this project has been completed, we will continue to explore whether IL1β blockade may be an effective VTE treatment in HO-1 deficient conditions. Once these studies are completed, we will prepare a manuscript for submission to peer review.

The scientific and technical insights from the studies funded by this Award have been instrumental in honing my investigative and laboratory skills to develop a robust venous research program. I have attended the previous three scientific sessions meetings of the AVF (2017-2019), presented my research findings (2018, 2019) and plan to attend the meeting again in 2020. Since the beginning of the funding period, and with mentorship from experts in thrombosis and vascular disease (Drs. Peter K. Henke, David J. Pinsky and Tom W. Wakefield), my venous research program has grown. My laboratory is now supported by multiple funding sources (AVFF-JOBST, Falk Research Trust, University of Michigan Biointerfaces grants for innovation in venous disease, NIH K08 award focused on mechanotransduction in veins, NIH R01 proposal on inflammation in VTE under review), with an expanded laboratory team (3 scientists, 6 students), and multiple manuscripts on molecular processes at the intersection of inflammation and coagulation published in high-impact journals (*JCI*, *Nature Communications*, *ATVB*, *JCI Insight*, *Circulation*). In addition to our discovery efforts, I am designing a clinical trial to test a pharmacologic intervention in patients with VTE to prevent recurrent thrombosis and PTS. These, and my future investigative endeavors will require continued guidance from the membership of the AVF. I firmly believe that the research support and advocacy of the AVFF/JOBST is imperative to the development of new treatments and advances in the care of patients with venous disease.

Relevant publications (2017-present, chronological order)

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