Contrast-Enhanced Ultrasound Microbubble Uptake and Abnormal Plasma Biomarkers are Seen in Patients with Abdominal Aortic Aneurysms

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SPECIFIC AIMS

Aging is a major risk factor for abdominal aortic aneurysm (AAA). The incidence of AAA is highest in the elderly, and AAA rupture is the 13th leading cause of death among individuals aged 65–85 in the United States. AAA rupture is associated with high morbidity and mortality, particularly in elderly patients undergoing emergent aortic surgery. Ultrasound surveillance is currently recommended to follow progression of small AAA, but it is limited in its ability to accurately predict AAA growth or rupture. This is due to the unpredictable clinical behavior of AAA. In an autopsy study of 473 non-resected AAA, 40% of AAA between 7 cm and 10 cm had not ruptured, while nearly 13% of patients with aneurysms smaller than 5 cm had ruptured. The risk of AAA rupture is also higher in women, and tends to occur at a smaller diameter than men, raising the possibility of gender disparities in aortic wall vulnerability. These observations highlight the limitation of adopting diameter as a predictor of AAA instability, defined as risk for growth or rupture.

Attempts to image the aortic wall in order to predict AAA growth or rupture have been inconclusive. Existing imaging modalities include CT, MRI, and intravascular ultrasound, but these are limited by low accuracy, radiation exposure, availability, and safety issues. Recently, contrast ultrasound (CEUS) using microbubbles has emerged as a method for imaging adventitial vasa vasorum (VV) in carotid plaques. We have an active CEUS program and have previously correlated VV density with neovascularization in an animal model, and have imaged 850 patients with carotid CEUS. CEUS can also be used to image AAA, since neovascularization and VV are commonly seen on AAA wall histology. Both may be markers of hypoxia and weakening of the aortic wall, and can be quantified with CEUS using time–signal intensity curve analysis to detect VV density in different regions of interest in the AAA wall.

Our goal is to non-invasively study the metabolic processes within the aortic wall that are thought to explain AAA growth and rupture, and their variation with age and gender. This process also affects unstable coronary and carotid disease, where the progression from an asymptomatic plaque to rupture and thrombosis, also known as “vulnerable plaque”, is not

fully explained by the luminal diameter. Similarly, localized hypoxia, neovascularization, and inflammation of the aortic wall are thought to contribute to degeneration of AAA, accounting for growth and possibly rupture that are otherwise not explained by absolute AAA diameter. In addition, pro-inflammatory systemic biomarkers, such as interleukin, tumor necrosis factor-α; and matrix-degrading enzymes, are up regulated in patients with AAA suggesting a systemic inflammatory process. Non-invasively detecting AAA wall neovascularization using CEUS is a novel approach to evaluate aortic wall vulnerability. It could identify regional inflammation, and propensity for AAA wall weakening, enlargement or rupture. Our hypothesis is that increased neovascularization of the aortic wall correlates with AAA growth and rupture and is more pronounced in women. CEUS imaging of AAA, coupled with serum biomarker measurements, has the potential to identify the propensity for growth or rupture. The Specific Aims are:

Aim#1: Determine the relationship between AAA growth rate and VV density. Approach: prospective CEUS imaging of a cohort of patients with AAA 4-5.4cm in diameter at 6 month intervals for 18 months. Hypothesis: VV density on CEUS is increased in AAA at risk for growth.

Aim#2: To correlate CEUS findings with serum biomarkers. Approach: longitudinally measure AAA specific serum biomarkers with every CEUS. Hypothesis: Levels of pro-inflammatory serum biomarkers correlate with VV density and are more elevated in unstable AAA.

APPROACH

The study protocol was approved by the Institutional Review Board (IRB) of the University of Pittsburgh. The study was funded by the AGS GEMSTAR award from the Society of Vascular Surgery (SVS) foundation in addition to the National Institute of Health (NIH) through a R03 grant.

Study Design

The objective of this study was to non-invasively study the metabolic processes within the aortic wall that are thought to explain aortic growth. This was a single center prospective evaluation of patients with an AAA diameter of at least 4.0 cm. enrolled between December

2011 and December 2016, in the outpatient vascular clinic. Patients were followed up until October 2019.

Aneurysm diameter size was determined on duplex ultrasound. Patients were excluded if they had contraindications to the administration of Definity contrast, such as pulmonary hypertension, unstable cardiopulmonary conditions, known right-to-left cardiac shunting and pregnancy. Patients underwent CEUS and serum PIB testing at enrollment and every 6 months up for 18 months or until eventual repair. PIB testing was also done one year after AAA repair..

CEUS imaging protocol

Patients were positioned on their back in the non-fasting state. An intravenous line was placed by the research staff at the time of the office visit. The abdominal aorta was imaged initially without contrast administration. Following non-contrasted duplex imaging, CEUS was performed via intravenous administration of the ultrasound contrast agent, Definity® (Lantheus). 1ml of the contrast agent was diluted in 9ml of normal saline to achieve a concentration of approximately 10 microliters/kg. For each injection sequence, 0.5 mL contrast was injected over 2-3 sec during simultaneous burst replenishment real time imaging. Two injection sequences were typically performed. Each CEUS evaluation took approximately 20-30 minute to complete. Patient's heart rate, blood pressure and breathing were monitored during the examination and afterwards in the waiting area. The total monitoring time ranged between 30-90 minutes. Microbubble replenishment was analyzed using ImageJ (NIH) via manually drawn regions of the aortic wall and intraluminal thrombus (ILT) (Figure 1).

Ultrasound evaluation was performed using the GE US system (GE Healthcare, Chicago, IL, USA) with the standard curved array abdominal transducer. The contrast settings on the ultrasound system were set to a low mechanical index of 0.07 and a dynamic range of 50 with the focal zone placed underneath the posterior aortic wall.

Microbubble uptake was determined by the ultrasound technologist and the location was recorded on a study sheet. Uptake was then separately recorded by two of the investigators (RC, PC). Care was taken to correlate with color flow duplex ultrasound to avoid lumbar and mesenteric vessels as well as artifact. The visualization of the microbubbles within the

abdominal aortic aneurysm wall was defined as contrast uptake. This was determined by consensus agreement between the ultrasound sonographer and two investigators (The PI, RC and PC) for every single patient.

Uptake was quantified via video intensity analysis in a region of interest in the aortic adventitia. Background-subtracted peak video intensity, which has been shown to correlate with VV density, was derived and normalized to peak intensity in the lumen (Figure 2).[9]

Aneurysm growth, rupture and repair were recorded during the follow up period. Matched controls with no aneurysm disease underwent one-time CEUS and PIB testing using biomarker panels (Meso scale discovery) and Bioplex 100 analyzer (Bio-rad).

Biomarker testing

Blood samples were withdrawn from patients at each visit and one year following AAA repair. Blood was drawn in the Vascular Surgery clinic by the research staff. Blood samples were banked and used for the measurement of biomarkers. This is the list of plasma inflammatory biomarkers that were tested: Osteoprotegerin (OPGN), Galectin 3, Cystatin C, Neutrophil gelatinase-associated lipocalin (NGAL), Uromodulin (UMOD), C-reactive protein (CRP), Macrophage migration inhibitory factor (MIF), Intercellular adhesion molecule (ICAM1), Interferon γ (IFN-γ), Interleukin 10 (IL10), Interleukin 6 (IL6), Interleukin 8 (IL8), Tumor necrosis factor-a (TNF-a), Serum amyloid A (SAA), Vascular cell-adhesion molecule (VCAM1).

Statistical analysis plan: Sample size: No previous literature has reported the mean and standard deviation of AAA VV density measured in vivo by CEUS. Statistical analysis: Data will be summarized as mean and SD for continuous variables after testing for normality. Categorical data will be summarized with frequency and percentage. Correlation coefficients will be determined for the correlation of VV and microbubble density measured by CEUS, as well as serum biomarkers with AAA growth. We will use linear regression to test the significance of such higher VV and microbubble density by CEUS as predictor of AAA growth (Aim1). We will include serum biomarkers in the modeling process. Adjustment for any

demographics differences will be also attempted including age, gender and race. A p-value<0.05 will determine significance.

Anticipated findings and alternative approaches: We anticipate that this clinical research study will identify biomarker and CEUS differences in AAA adventitial VV density based on age, gender and AAA stability or growth. Our findings will support a larger clinical trial and future investigations of treatment paradigms of AAA based on biologic risk of growth or rupture instead of absolute diameter. If CEUS and biomarker differences are not detected during the study period in otherwise stable AAA, longer follow up with repeated imaging and biomarker testing at 6month intervals will be sought. If our hypothesis is not confirmed (i.e. differences in AAA wall neovascularization do not exist), imaging of AAA wall inflammation will be considered when available to human subjects using microbubbles targeted to either activated leucocytes or endothelial cell adhesion molecules that are upregulated in inflammation and mediate leukocyte recruitment and adhesion. If CEUS is not accurate given the depth of penetration of ultrasound, alternative imaging modalities such as IVUS or FDG-PET CT scanning will be explored. We anticipate that our results will identify active angiogenesis and ischemia of the AAA wall and biomarker predictors of growth, which may improve real time monitoring of AAA progression and response to systemic or targeted microbubble anti-angiogenesis therapies.