Localized quantification of geometry, hemodynamics, and histology in a rat model of abdominal aortic aneurysm

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Introduction

Abdominal aortic aneurysm (AAA) is a deadly disease with few clinical options. Although it has been proposed that slowing the rate of expansion of an AAA could delay or even mitigate surgical intervention [1], to date there is no prescription for achieving this goal. It is a wait and watch approach that leads to pre-emptive surgical repair in the best circumstances and rupture in the worst. The estimated mortality rate of the former is 5% [2] while the latter can be as high as 90% [3].

Given the high mortality rate associated with AAA rupture, the focus of most scientific investigation has been on events related to rupture and therefore necessarily the solid mechanics of the vessel wall. It is hypothesized, however, that fluid mechanics plays an important role, and perhaps the dominant role, in the initiation and progression of AAA disease. Furthermore, preclinical and clinical data suggest that there is pertinent interaction between geometry, hemodynamics, and the pathological state of the vessel wall, i.e. inflammatory infiltrate. For example, it has been shown in a rodent model of AAA that there is an inverse relationship between the amount of blood flow through the aneurysm and the growth of the aneurysm [4] as well as the number of macrophages present in the vessel wall [5]. A limitation of prior hemodynamic studies in rodent models of AAA is that the data was acquired using invasive methods and was only obtained at a single location in the infrarenal aorta.

The current study combined time-of-flight magnetic resonance angiography (TOF-MRA), phase-contrast MRI (PCMRI), and computational fluid dynamics (CFD) to quantify the three-dimensional geometry and hemodynamic conditions *in vivo* in a rat model of AAA. Histological endpoints were compared to geometric and hemodynamic results obtained from the MRI and CFD data.

Materials and Methods

All experiments were performed with local IACUC approval. Male 8-12 week old Sprague Dawley rats were utilized. The Anidjar/Dobrin model of AAA was used [5]. Briefly, animals were anesthetized and a segment of the infrarenal aorta was isolated while 10U/mL of porcine pancreatic elastase (n = 6) or saline (n = 3) was infused for 1 hour.

MRI was performed at 4.7T (Inova console, Varian, Inc., Palo Alto, CA) using a 6cm inner diameter RF volume coil. Animals were anesthetized using 2% isoflurane in 1L/min of O_2. Body temperature was maintained between 36-37C. Twenty-eight days after creation of the AAA, *in vivo* geometry of the aorta was visualized using 2D TOF-MRA (TR/TE 40/5ms, FOV $(5.5\text{cm})^2$, matrix 256^2, slice thickness 1mm, NEX 6). A saturation pulse was placed distal to the acquisition slice in order to null signal from venous blood. Three dimensional geometric models were constructed from the TOF-MRA data using custom software [6]. Briefly, centerline paths through the vessels of interest were defined and segmentations perpendicular to these paths obtained using thresholding. Solid models were then lofted, unioned, and discretized for flow simulations. For each animal, an inlet flow boundary condition for CFD simulations was specified based on velocity data obtained using a through-plane PCMRI sequence (TR/TE 13/3.2ms, FOV $(6\text{cm})^2$, flip angle 30°, matrix 128^2 zero-filled to 256^2, slice thickness 2mm, NEX = 8, 12 frames, venc = 200cm/sec, ECG gated and 'respiratory compensated', 1st order baseline corrections). Twenty-four hours after imaging, aortic specimens were prepared for histology (pressure perfusion *in situ* for 30 minutes at 100 mmHg) and excised. Sectioning was performed in approximately the sagittal plane to allow for quantification along the length of the aneurysmal vessel on both the anterior and posterior surfaces. Inflammation was quantified using ED1 immunohistochemical staining for macrophages. Elastica masson (EM) staining was performed to visualize and quantify the number of remaining elastin fibers.

Results

Representative results from a CFD simulation for a rat infused with elastase to create an AAA are shown in Figure 1. Mean wall shear stress (WSS) averaged over the cardiac cycle is illustrated in Figure 1A while the velocity field at approximately peak systole is shown in Figure 1B. The anterior and posterior surfaces are labeled for reference. Low mean WSS and reverse flow are evident along the anterior surface of the AAA. Diameter measurements from the same animal (blue line) and histological measurements of ED1+ cells (Figure 2A) and the number of remaining elastin fibers (Figure 2B) along the anterior (pink circles) and posterior (blue diamonds) surfaces of the vessel are shown. Corresponding locations of interest (labeled A, B, and C) are depicted in both figures for comparison of geometry, hemodynamics, and pathology. The maximum number of ED1+ cells along the anterior surface often corresponded with a location at or near the maximum AAA diameter; whereas the maximum number of ED1+ cells along the posterior surface never did. The number of remaining elastin fibers along the anterior surface was dramatically reduced or zero. This was in contrast to the posterior wall where even in the largest AAAs a number of elastin fibers remained intact.

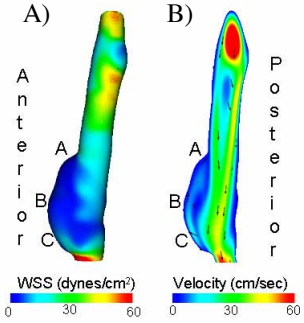

Figure 1. CFD results from an animal in which an AAA was created. A) Plot of mean WSS; B) Plot of velocity field at peak systole.

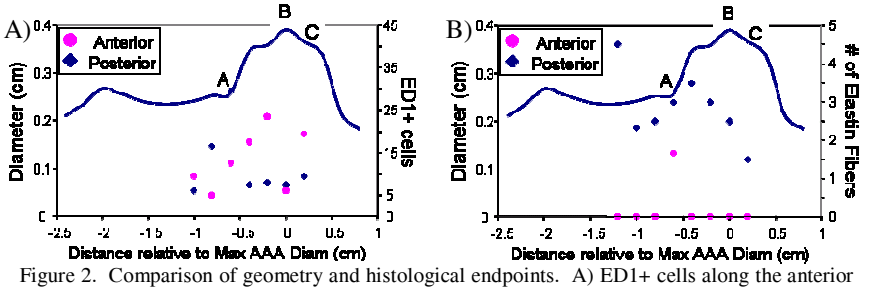

Figure 2. Comparison of geometry and histological endpoints. A) ED1+ cells along the anterior and posterior surfaces of the aneurysmal vessel. B) The number of remaining elastin fibers along the anterior and posterior surfaces of the aneurysmal vessel.

Discussion

The combination of MRI and CFD has allowed non-invasive quantification of the three dimensional geometry and hemodynamics in a rat model of AAA. In addition, this work provides spatially resolved information about the inflammatory state and number of remaining elastin fibers along the anterior and posterior surfaces of aneurysmal vessels. Comparison of the location of ED1+cells to geometric and hemodynamic results suggests that mean WSS and the direction of blood flow relative to geometry, such as inflection points in the vessel, contribute to local inflammatory content. With the creation of the AAA via infusion of elastase during complete cessation of flow through the aorta, it is reasonable to assume that there is axisymmetric distribution of the elastase to all parts of the isolated vessel segment and therefore equal opportunity for elastin degradation. The significant difference in the number of elastin fibers remaining on the two surfaces of the vessels suggests that an unknown process after re-perfusion contributes to preferential degradation of the elastic lamellae on the anterior surface. One potential candidate for the unknown process is increased wall motion on this surface of the vessel, as has been shown in a porcine model [7] and preliminarily suggested in this rodent model (data not shown). Understanding the role of biomechanical forces in aneurysm growth in rodent models may provide insight into the role of such factors in human AAA initiation, growth and rupture.

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