Morphometric Analysis of Elastic Matrix Structural Changes in Abdominal Aortic Aneurysm (AAA) Patients with Large Aneurysms

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ABSTRACT

Abdominal aortic aneurysms (AAAs) pose a significant health threat due to the risk of fatal rupture, primarily driven by the gradual expansion of the abdominal aorta. The pathogenesis involves the breakdown of elastin, a crucial extracellular matrix protein, attributed to the upregulation of matrix metalloproteinases (MMPs) and compensatory collagen synthesis. Despite current medical and surgical interventions, there are no effective strategies for regenerative repair of elastin, necessitating a comprehensive understanding of its morphological features in the AAA environment. This study focuses on advancing morphometric analysis beyond previous studies by quantifying critical parameters such as diameter, perimeter length, and tortuosity alongside the percentage of elastic fibers in tissue samples from AAA patients undergoing surgical repair. Utilizing a computer-based morphometry technique, aortic tissues from patients at the Cleveland Clinic Heart and Vascular Institute were processed and analyzed. The morphometric parameters were computed to assess variations associated with demographics such as gender, smoking history, thrombus presence, atherosclerosis, diabetes and chronic kidney disease. Notably, 81% of the tissue samples were from male patients aged between 55 and 85 years, revealing differences in various parameters, particularly in 82% of males with aortic dissection. These findings suggest a potential impact on elastin, emphasizing the need for further exploration. In conclusion, this research contributes crucial qualitative data for understanding tissue analysis in the context of AAA, laying groundwork for regenerative approaches in aortic injury. Ultimately, with the future direction of creating a database to predict when patients would need to undergo surgery with the data collected.

INTRODUCTION

- **AAA:** Localized, rupture prone expansions of abdominal aorta
- Caused due to chronic proteolytic activity owing to the increase in proteolytic enzyme called MMPs
- Proteolysis breaks down elastic matrix that provides vessel stretch and recoil
- Adult vascular cells are not able to regenerate elastin naturally
- **Goal:** to develop strategies for in situ regenerative repair of elastin
- What is the need? A quantitative method of elastin fiber morphometry assessment which gives information about the stage-wise elastic fiber changes in AAAs and its correlation to morphological and physiological aspects of AAAs.
- This would also enable prediction of elastic fiber fate and AAA rupture risk besides identifying targeted outcomes of regenerative therapies.
- This study employs a computer-based morphometric technique developed in our lab to quantify the critical parameters of elastic fibers in the tissue sample collected from AAA patients undergoing surgical repair.
- **Specific Aim:** to compare the critical morphometric parameters of elastic fibers like area, aspect ratio, minimum diameter, maximum diameter, perimeter length and tortuosity among different demographic criteria of the patients including age, sex, presence/absence of other conditions like atherosclerosis.
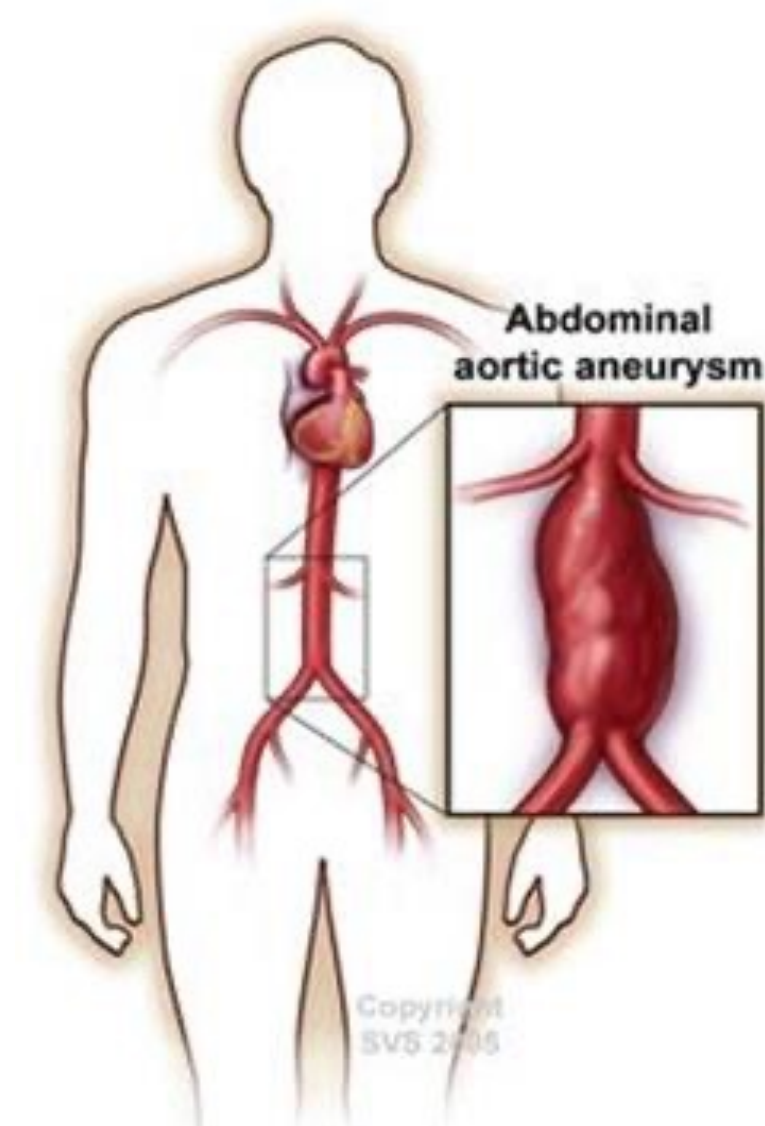
METHODS

- The aorta tissue was donated by the patients undergoing open AAA repair surgery at the Heart and Vascular Institute of Cleveland Clinic.
- Patient's demographic criteria was extracted using the Red Cap database.
- For morphometric analysis, the tissue was washed with PBS as received. 1/3rd of the tissue was fixed overnight in 4% PFA at 4°C.
- Next, the tissue was paraffin embedded and sectioned as 6 5 microns sections per slide and one slide per subject.
- The sections were then stained with modified Hart Stain. (1 volume of Wiegert's iron resorcin fuchsin and 9 volumes of 1% hydrochloric acid in 70% ethanol)
- The sections were scanned using a whole slide scanner and morphometry was done using Image Pro software
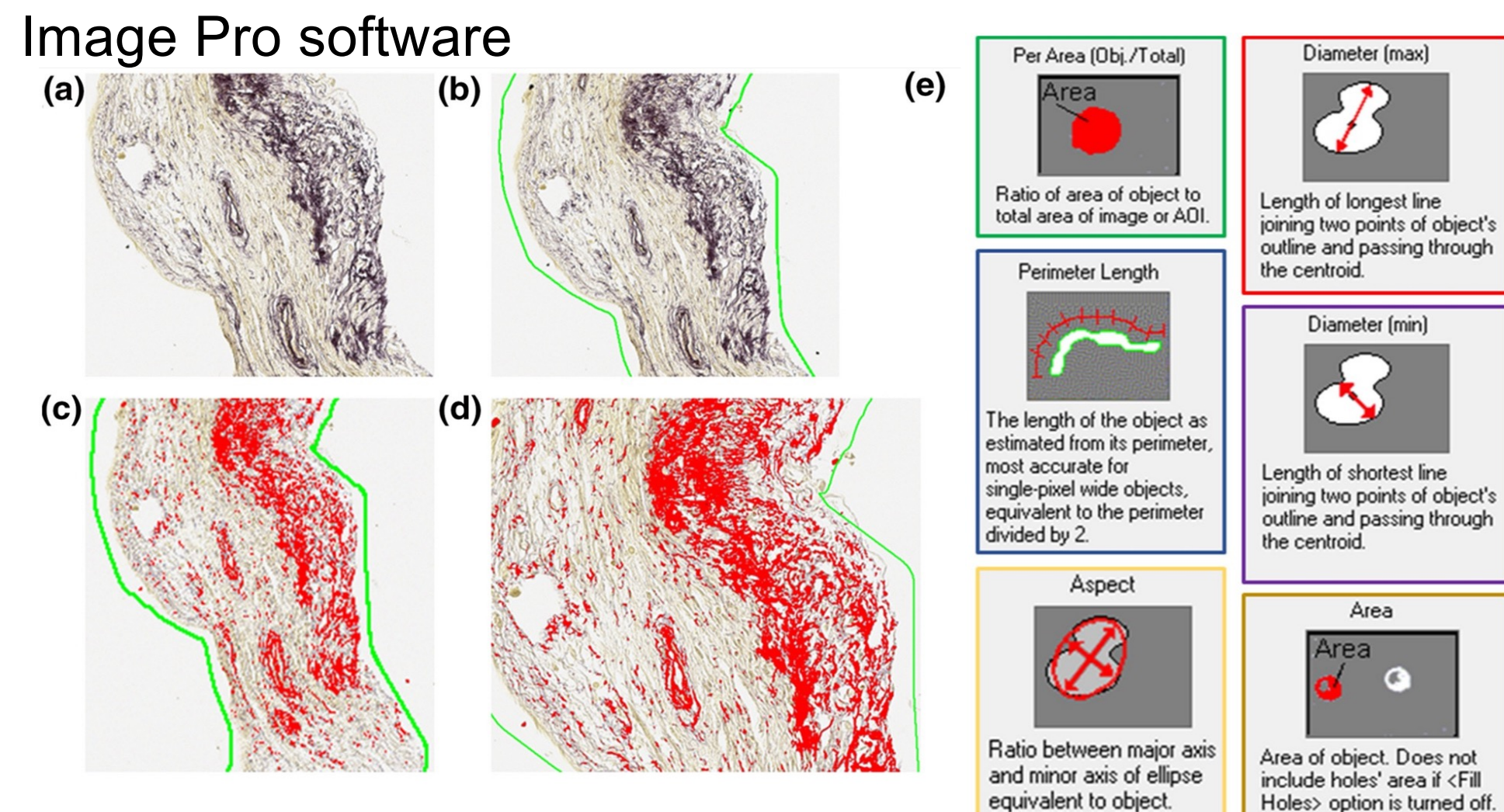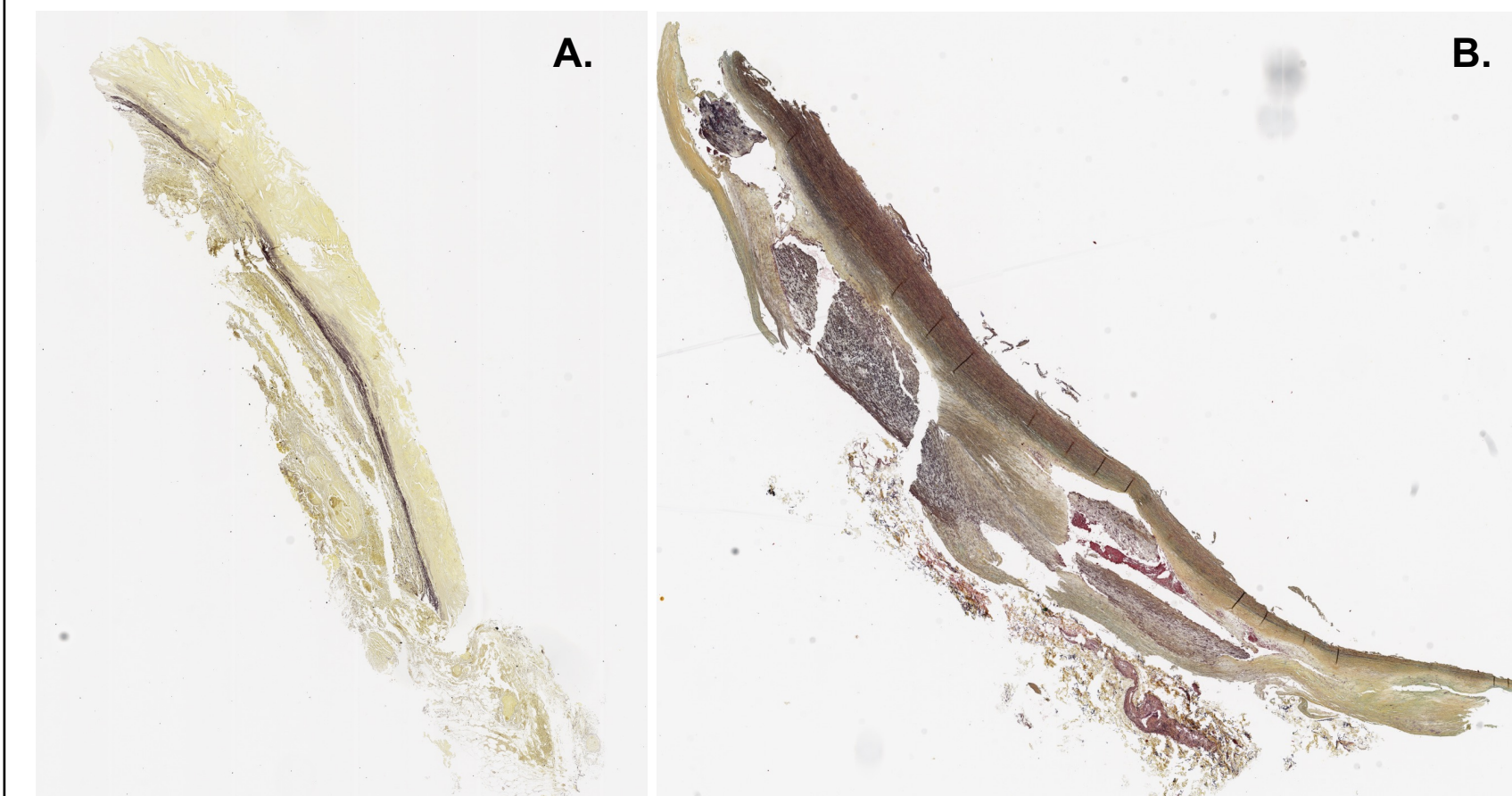

Figure 1. Graphical methods of morphometric analysis. An example of a modified Hart's stained transverse section tissue (A-D). Green line around the tissue section in B shows the area of interest (AOI) drawn around the tissue section. Red in C indicates elastin deposits automatically selected as objects, using Image-Pro Plus® software. A higher magnification view of image C is shown in D, and indicates individual objects. Parameters calculated from morphometric analysis include density (Per Area), diameter (max and min), perimeter length, aspect ratio, area (E), and tortuosity (defined as the ratio of perimeter to maximum diameter).

RESULTS
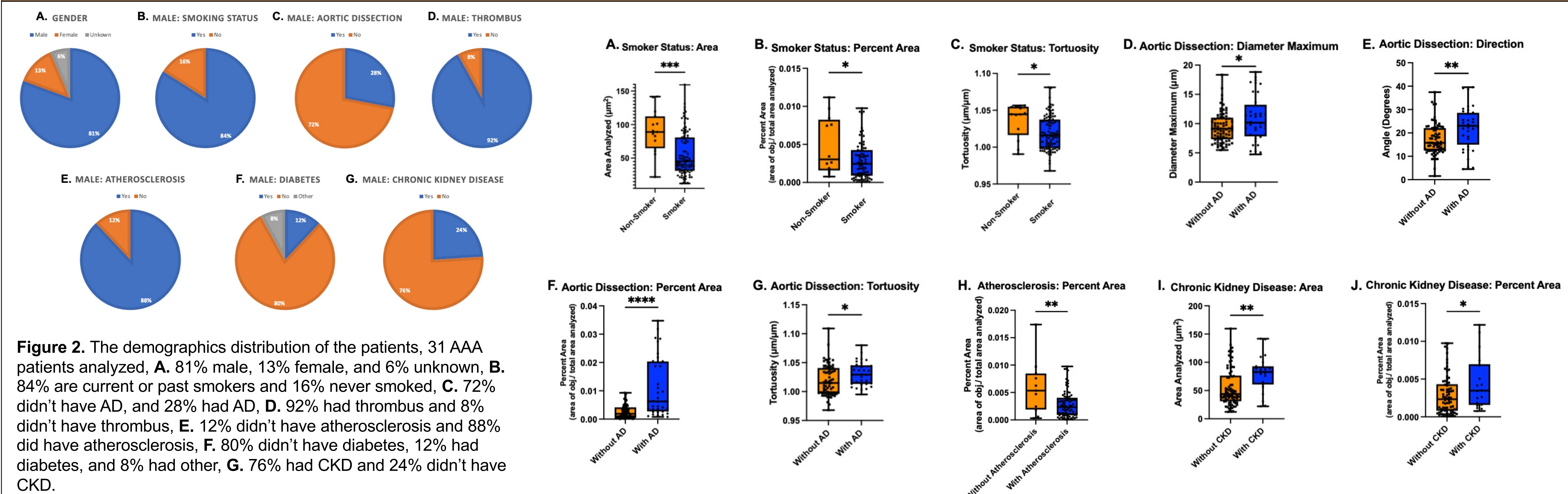
Figure 2. The demographics distribution of the patients, 31 AAA patients analyzed, A. 81% male, 13% female, and 6% unknown, B. 84% are current or past smokers and 16% never smoked, C. 72% didn't have AD, and 28% had AD, D. 92% had thrombus and 8% didn't have thrombus, E. 12% didn't have atherosclerosis and 88% did have atherosclerosis, F. 80% didn't have diabetes, 12% had diabetes, and 8% had other, G. 76% had CKD and 24% didn't have CKD.

Figure 3. Stains of tissue from patients with AAA. A. Modified Hart Stain is used to show the elastin, the darker regions of the tissue sample. B. Movat Stain is used to determine thrombus, the dark red clusters in the tissue sample.

Figure 4. Morphometric analysis comparing AAA patients with and without Aortic Dissection(AD). (F) Elastin quantity was significantly higher in patients receiving an AD.
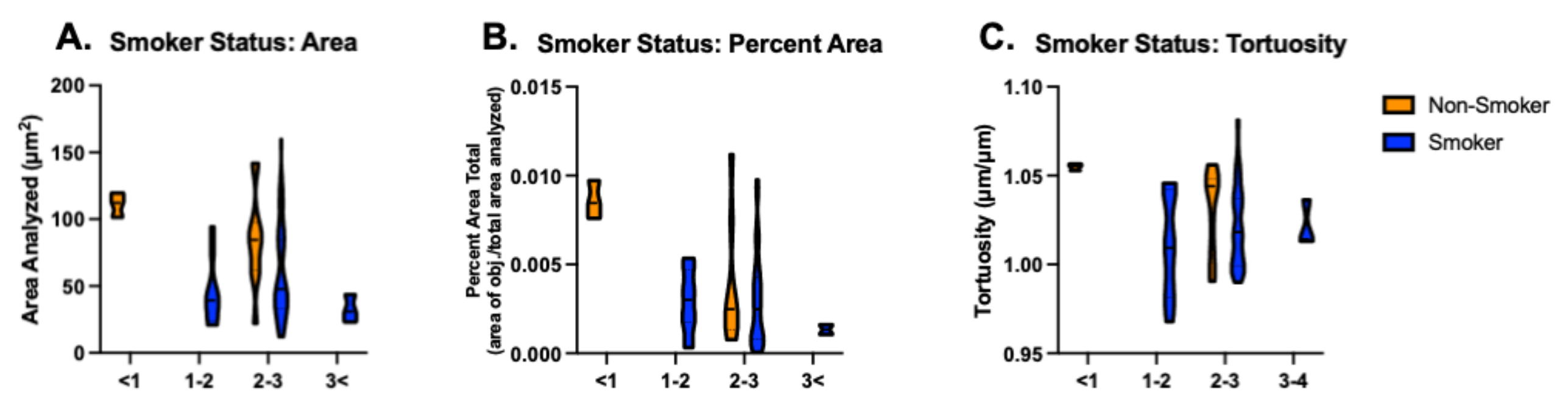
Figure 5. This groups each patient depending on each of the demographic criteria such as; atherosclerosis, aortic dissection, thrombus, diabetes, CKD, and smoking status then groups them based on smoker and non-smoker.

DISCUSSION

- Demographics data suggests that male patients are definitively more vulnerable to develop AAAs than females and smoking is a major contributing factor for the development of AAA pathology because a total of 84% of patients have smoking history either present or past.
- Atherosclerosis in 88% patients is in agreement with literature describing pathophysiological progression of AAA since atherosclerotic plaques blocks the entry of nutrients to the cells leading to cell death, elastin degradation and progression of AAAs.
- Studies show that progression of CKD and AAA is attributed to common matrix metalloproteases like MMP-2,8,92. Hence, it is more likely that CKD can lead to AAA or vice versa.
- We compared the morphometry data between the patients with and without AD in this work because AAA is followed by AD in many patients because severe aortic bulge increases the risk of a tear in the aortic lining, creating a false lumen and leading to AD3. So we wanted to see these changes in elastic fiber with increasing severity of aneurysm.
- Increased % area of elastin (Fig 4F) suggests more fragmented elastin in the patients with AD. While no difference in mean was seen in other parameters), the data trend shows higher variability of critical parameters like Diameter Max and Perimeter Length suggesting some pathological changes in the elastic matrix with the presence of AD.

FUTURE WORK

- Collect tissue samples from patients at St. Luke's.
- Analyze future data.
- Create a database that allows the demographics to be inputted and to predict how degraded the elastin is based on our parameters and current data.

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