Obtaining the Biomechanical Behavior of Ascending Aortic Aneurysm by using Novel Speckle Tracking Echocardiography

Mohammed Alreshidan MD.

Department of Experimental Surgery

McGill University, Montreal

April 2018

A thesis submitted to McGill University in partial fulfillment of the requirement of the degree of

Master of Science in Experimental Surgery

Table of Contents

List of figures:

List of Tables:

Acknowledgement:

I would like to express my great appreciation to my supervisor Dr Kevin Lachapelle for his valuable and constructive guidance during the planning and execution of this research work. Dr Lachapelle is an outstanding mentor who guided me in every step of my project. His willingness to give his time so generously has been very much appreciated.

I would also like to thank my co-supervisor Professor Richard Leask for his unlimited support. Prof Leask provided me with the lab space and he was always available to answer my questions

I would also like to thank my colleague at the lab Nastran Shahmansouri for her help in developing the Matlab programing protocol for the echo data and her great ideas over the time we spent at the lab.

Last but not least, I would like to thank my wife May and my sons, Alfaisal, Mishary and Raad for their understanding and non-conditional support. Without their help I would have not been able to succeed in this work.

Abstract

Introduction: Ex vivo measurement of ascending aortic biomechanical properties may help in the process of estimating the risk for rupture or dissection of dilated ascending aortas. A validated in vivo method that can predict aortic biomechanics does not exist. Speckle tracking transesophageal echocardiography (TEE) has been used to measure ventricular stiffness; we sought to determine if speckle-tracking echocardiography could be adopted to estimate aortic stiffness in vivo and compare these findings to those obtained by ex vivo tissue measurements.

Methods: 17 patients undergoing ascending aortic resection were recruited to be part of the study with the mean aortic diameter of 56.16 ± 15 mm. Intra-operative speckle tracking echocardiographic analysis was used to calculate aortic stiffness index using the following equation:

$\beta 2 = \ln(SBP/DBP)/AoS$ (β2 stiffness index; SBP: Systolic BP; DBP: Diastolic BP, AoS: circumferential strain). Ex vivo stiffness was obtained by mechanical tissue testing according to previously described methods. The aortic ring at the pulmonary trunk was divided into four equal quadrants

Results: the mean aortic diameter was. The in vivo stiffness index for the inner curvature, anterior wall, outer curvature and posterior wall were 0.0544 ± 0.0490, 0.0295± 0.0199, 0.0411± 0.0328 and 0.0502± 0.0320 respectively. The mean ex vivo 25% apparent stiffness for inner curvature, anterior wall, outer curvature and posterior wall were 0.0616 ±0.0758 MPa, 0.0352± 0.00992 MPa, 0.0405± 0.0199 MPa and 0.0327± 0.0106 MPa respectively. The patient matched ex vivo 25%

apparent stiffness and in vivo stiffness index were not significantly different ($p=0.8617$, two way ANOVA with repeated measures).

Conclusion: The use of speckle tracking TEE appears to be a promising technique to estimate ex vivo mechanical properties of the ascending aortic tissue.

Résumé:

Introduction: Déterminer les propriétés biomécaniques ex vivo de l'aorte ascendante pourrait aider à obtenir de meilleures estimations de risques de ruptures et dissections du tissu dans le cas de dilatations. Aucune méthode permettant la prédiction du comportement mécanique du tissu aortique in vivo n'a encore été validée.

L'échographie transoesophagienne de suivi des marqueurs acoustique est utilisée pour mesurer la rigidité du ventricule ; dans le cadre de cette étude, nous avons cherché à déterminer si une telle technique pouvait également être utilisée dans l'estimation de la rigidité de l'aorte in vivo en comparant les résultats mesurés à des données obtenues au préalable en ex vivo.

Méthodes: 17 patients ayant subi une ablation de l'aorte ascendante avec un diamètre moyen de 56.16 ± 15 mm ont fait parti de l'étude. L'analyse de l'échographie transoesophagienne preopératoire de suivi des marqueurs acoustique a permis le calcul de la rigidité aortique en utilisant l'équation suivante

$\beta 2 = \ln(SBP/DBP)/AoS$, (β2 rigidité; *SBP*: Pression systolique; *DBP*: Pression diastolique, *AoS*: Déformations circonférentielles). La rigidité du tissu a été obtenue ex vivo par des tests mécaniques en suivant des méthodes décrites dans de précédentes études. Enfin, l'anneau aortique au tronc pulmonaire fut divisé en quatre quadrants de tailles égales.

Résultats: Les rigidités in vivo de la courbure intérieure, paroi antérieure, courbure extérieure et paroi postérieure étaient de 0.0544 ± 0.0490, 0.0295± 0.0199, 0.0411± 0.0328 et 0.0502± 0.0320. Tandis que les moyennes ex vivo de la rigidité mesurée à

25% pour la courbure intérieure, paroi antérieure, courbure extérieure et paroi postérieure étaient de 0.0616 ±0.0758 MPa, 0.0352± 0.00992 MPa, 0.0405± 0.0199 MPa et 0.0327± 0.0106 MPa. Des résultats similaires ont été trouvés chez les patients entre les rigidités ex vivo (mesurées à 25%) et in vivo avec une différence non significative (p=0.8617, analyse ANOVA double avec des mesures répétées).

Conclusion: L'utilisation de l'échographie transoesophagienne de suivi des marqueurs acoustique semble donc être une technique prometteuse permettant l'estimation de propriétés mécaniques du tissu de l'aorte ascendante in vivo.

Contribution of Authors:

The role each author had in the preparation of this manuscript in this thesis is described.

Mohammed Alreshidan: participated in experimental design, performed mechanical experiments, analyses the data, wrote the manuscript, replied to reviewers.

Nastaran Shahmansouri: participated in experimental design of echo data analysis.

Jennifer Chung: participated in experimental design.

V. Lash: performed transesophageal echo.

Alexander Emmott: performed mechanical experiments.

Richard L. Leask: experimental design, reviewed data analysis, reviewed manuscript.

Kevin Lachapelle: assisted in coordination of tissue gathering at Royal Victoria Hospital, provided tissue from the patients at the time of surgery, reviewed data analysis, reviewed manuscript.

1- Introduction

Cardiovascular disease is the leading cause of death in the modern world [1]. It is well known that aging process and cardiovascular diseases can lead to functional and structural changes in the large arteries, which eventually may cause aortic wall stiffness. Aortic stiffness is a good predictor for cardiovascular morbidity and mortality related to ascending aortic aneurysm [2]. Aneurysms of the ascending aorta (AA) increase the chances of aortic dissection and rupture. Aortic dissection has been described as a catastrophic event as it may lead to acute aortic insufficiency, congestive heart failure, acute coronary occlusion, pericardial effusion, tamponade, stroke, syncope, limb ischemia, renal insufficiency, shock, rupture and eventually death. Thoracic aortic aneurysm is mainly an asymptomatic disease, with the first symptom often being death or a severe complication like dissection and rupture. Previous studies have shown that the risk of rupture, dissection, or death increases when the aneurysm reaches 6 cm in diameter. The current recommendation for surgical intervention to prevent rupture or dissection of the ascending aortic aneurysm (AscAA) is when the diameter reaches 5.5 cm. Despite that, the International Registry of Acute Aortic Dissection (IRAD) reported in 2007 that 60% of the patients with acute type A dissection, presented with aortic diameters of < 5.5 cm. Therefore, many clinical predictors developed not only to prevent dissection or rupture but also to determine the aortic diameter that justify the risk of surgical intervention of the ascending aortic aneurysms. Moreover, the most studied criteria in literature to understand the risk predication are ex-vivo mechanical properties of ascending aorta including stress/strain relationship,

apparent stiffness and energy loss. These mechanical metrics reflect the mechanical behavior of aorta and represent the pathology of the aorta. Our work aims to developed a minimally invasive medical image tool to identified the mechanical properties of ascending aorta using trans esophageal echo (TEE), and correlate the echo finding with actual mechanical tissue testing done ex-vivo.

2- Literature Review:

2-1: Significant and burden of the Ascending Aortic Aneurysm:

Aneurysms of the aorta are defined by localized dilation of the aortic wall which is caused by segmental weakening of the vessel wall [1]. The most common form of aortic aneurysms is the infrarenal abdominal aortic aneurysm followed by the ascending aortic aneurysm [2]. The estimated incidence of thoracic aortic aneurysms is 6 per 100,000 persons in United State every year [3]. Aneurysms of the ascending aorta (AA) increase the chances of aortic dissection and rupture [4]. Aortic dissection has been described as a catastrophic event as it may lead to an acute aortic insufficiency, congestive heart failure, acute coronary occlusion, pericardial effusion, tamponade, stroke, syncope, limb ischemia, renal insufficiency, shock, rupture and eventually death [5]. Thoracic aortic aneurysm is mainly an asymptomatic disease, with the first symptom often being death or a severe complication like dissection and rupture [6]. Furthermore, aortic aneurysm can be only diagnosed with the aid of different imaging modalities [5]. It is important to know that thoracic aortic aneurysm is a slowly growing disease with an average of 0.10 to 0.12 cm diameter expansion per year [6, 7]. Even by knowing the already

established clinical risk factors ;like systemic hypertension and aortic aneurysms; identifying patients at risk of dissection and rupture is difficult. Moreover, patient with Marfan, Ehlers-Danlos syndrome, familial aortic aneurysm, or congenital bicuspid aortic valve; who are known to be at risk of dissection and rupture; often present with acute aortic syndrome [5].

2-2: Mechanical properties of ascending aorta:

A significant amount has been done ex-vivo to assess the mechanical properties by measuring the elastic modulus of aorta and linking it to different aortic disorders and pathologies for better understanding of the pathophysiological mechanisms of the disease. Others have tried to find the mechanical characteristics of tissue that are more prone to rupture or fail [1, 3, 4, 6, 8, 9]. The three mechanical concepts that frequently discussed in field of the mechanical testing of ascending aorta are stress, strain and stiffness (elastic modulus). A strain on a material can be defined as any change in the materials dimension, and any force acting on a material produces a stress [10]. Strain can also be expressed as, simply the ratio of the change in size to the original size [10]. Often the strain represent as a percentage; e = 0.1 indicates that each unit of length has extended by 10% [10].The stress is the force per unit area (a Newton per square meter), represented by the Pascal (Pa) or megapascal (MPa) [10]. Young's modulus of elasticity (E), also known as the elastic modulus, is the ratio between stress and strain and has the same units as stress [10]. For elastic martial, the relation between the stress and strain is linear and the elastic modulus is constant because the stiffness equals the slope of the curve. However, when we

look into the stress/strain relationship in hyperelastic viscous tissue such as the aorta, the relationship is no longer linear; and the stiffness is defined as the local slope at a given strain.

The simplest model that is used to estimate the stress on thin vessels wall is Laplace's law . [11]. Form Laplace's law, by knowing the vessel thickness, pressure and radius, the stress of the arterial wall (σ) can be calculated : (t: wall thickness)

$\sigma = Pr/t$ [11], (σ : stress, P: pressure, r: radius, t: wall thickness).

Laplace's law implies that the same stress is applied throughout the blood vessel wall thickness. However, in the aorta itself there is variation in the thickness between different regions and even the stress itself is not constant across the wall [8, 11]. Furthermore, even if the layers of the aortic wall have the same degree of Young's modulus, the stress applied across the wall thickness is not uniform. The inner surface will carry the highest level of stress and the stress will be reduced gradually towards the outer layer [8, 11].

The initial investigations on the mechanics of aortic dissection concluded that weakening of the intima; the most inner layer of the aorta; was responsible for dissection. Hypertension and the increase in aortic diameter lead to the increased overall stress of the aortic wall causing tears in the intima [4, 12]. This explanation was done considering the aorta has a uniform cylindrical shape without residual strain and this was proven to be incorrect from different animal data [4, 13]. The concept of residual strain is essential for better understanding of the aorta mechanics. A vessel with no residual strain will hold its shape when cut. However,

we know that when a vessel is cut, it recoils both along its length and circumferentially. This phenomenon is daily observed in the cardiovascular operating room. After resection of the ascending aorta, it becomes like an open ring. This is called an open angle and it is the result of zero stress states [4, 14, 15]. Alteration in residual strain is thought to be one of the factors that may lead to an abnormal distribution of stress throughout aortic layer. Furthermore, disproportionate stress on the outer layer could result in a primary mechanical failure [15]. Okamoto and his group studied the difference between the elastic properties, strength, and residual strain in three different groups of patients with ascending aortic aneurysms, Marfan, BAV, and TAV [4]. Their findings were significantly related to the age of the patient as the open angle test was higher in patients above 50 years. Furthermore, biaxial tensile testing of human aorta tissue has revealed a nonlinear relationship between stress and strain. In the group containing older patients, the circumferential and the axial stress increased rapidly with minimal strain[4]. Additionally, the strength was lower in the older patients as compared to the younger ones in all three groups [4]. Other studies have been performed to assess the tensile strength in circumferential and longitudinal orientation. Interestingly, the lesser curvature showed the maximum elastic modulus in the circumferential orientation compared to the longitudinal one. The tissue wall thickness was inversely related to the peak stress. On the other hand, the maximum elastic modulus was positively related to the peak stress [3]. Comparing the maximal tissue stiffness and the tensile strength between the ascending aorta aneurysm and the normal aorta in circumferential and longitudinal directions,

showed that there were less tensile strength by 29% and 34% in the longitudinal and circumferential orientations, respectively, as compared to the normal ascending aorta. From the stiffness point of view, ascending aortic aneurysm was 72% stiffer in the longitudinal direction and 44% in the circumferential than the normal aorta [9]. Comparing the mechanical behavior of ascending aortic aneurysms associated with different aortic valve type; tricuspid aortic valve (TAV) with bicuspid aortic valve (BAV); showed that TAV patients were stiffer in the circumferential direction with low strain. On the other hand, the region of the aorta was the main factor affecting the elastic modulus rather than the direction. The lateral wall carried the highest level of stiffness and the medial wall had the lowest compared to the other aortic regions [8]. Connecting the mechanical properties of ascending aortic aneurysm with structural changes in the wall, studies showed a good correlation between histopathological changes mainly elastic fragmentation with altered biomechanics [8]. Biomechanics of the ascending aortic aneurysms clearly demonstrate altered mechanics compared with the normal aorta. Ex-vivo biomechanical data to determine the clinical outcome of the ascending aortic aneurysm, is limited to testing after intervention. This limitation has urged the development of non-invasive assessment the in-vivo biomechanics by using medical imaging, mainly echo and MRI [16-19].

2-3: Assessment of the Biomechanics of Ascending Aorta Non-invasively by Medical Imaging:

Studies to assess the elastic properties of the aorta used invasive techniques for obtaining the simultaneous pressure, aortic diameter and thickness [17]. Emerging noninvasive techniques are being developed to assess the elastic properties using ultrasound and blood pressure measurements. The drawbacks of these techniques are the inability to obtain aortic wall thickness measurements and to measure aortic wall stiffness indirectly [16, 17]. The most common way used; in the absence of aortic wall thickness; is a pressure/strain curve to measure the aortic stiffness. On the other hand, the variability in the wall thickness of the aorta may make this inaccurate [17, 19].

Another technique uses the trans-esophageal echo to assess the elastic properties of the descending thoracic aneurysm by obtaining aortic cross sectional area, wall thickness, and blood pressure simultaneously [17]. This technique used the following equations to calculate the elastic modulus and pressure-strain elastic modulus:

$$Ep = \frac{\Delta P}{\frac{D\,max}{D\,min} - 1} = \frac{\Delta P}{\sqrt{\frac{A\,max}{A\,min}} - 1}$$ [17], Ep =elastic modulus and measured by dynes/cm2 ΔP is pressure difference between systolic and diastolic pressure, D max and D min are the maximal and minimal aortic diameters, and A max and A min are the maximal and minimal aortic lumen areas. Using this technique showed a non-

linear correlation between the estimated elastic modulus and the age of the patients [17].

Furthermore, ultrasound has been used to assess the abdominal aortic distensibilty and stiffness β [20, 21]. Echo-tracking ultrasonographic system was used as a parameter in a cohort study to follow up patients with small abdominal aortic aneurysms. In that cohort, the ultrasound was used to measure the maximum diameter D max, rate of expansion Ep, and aortic stiffness β [20]. The following equations were used to measure Ep and β :

$$Ep\ (10^5\ N/m^2\ ) = \frac{133.3 \times (\ systolic\ BP - diastolic\ BP\ )}{(Dmax\ systolic - Dmax\ diastolic)/Diastoilc\ Diamster}$$ [20]

$$\beta\ (arbitaray\ units) = \frac{Natural\ logarithm\ (systolic\ BP/diastolic\ BP\ )}{(Dmax\ systolic - Dmax\ diastolic\ diameter)/Diastolic\ Diameter}$$ [20]

An interesting correlation was observed between the rate of expansion Ep and the maximum aortic diameter D max, but there was no correlation between the estimated measures and mortality. Despite this, ultrasound is a useful predictor for abdominal aortic expansion rate and a good tool to monitor patients with small abdominal aortic aneurysms. It can also help in predicting the time for elective surgery [20].

Furthermore, another study used the 2D strain echocardiography to measure the abdominal aortic stiffness β and correlate it with the patients` age and gender. It was noted that the aortic stiffness correlated strongly with an age above 50 in both groups and there was no relationship between the aortic stiffness and gender[21]. The author used the echo speckle tracking to calculate the circumferential strain,

and used the blood pressure as the surrogate marker for the stress following this equation:

$\beta = \ln(SBP/DBP)/Ao - S$ [21], (SBP is systolic blood pressure, DBP is diastolic blood pressure and Ao-S: is peak strain determined by the aortic circumferential strain curve).

Using the echo ultrasound to obtain the strain and indirectly calculating the stiffness by measuring the blood pressure is a great tool to estimate the biomechanics of ascending aortic aneurysm. However, the validity and accuracy of this techniques is still under investigation and the estimated stiffness was never been correlated with the measured ex-vivo to look into the accuracy and the attitude of the estimated stiffness.

3- Hypothesis and Objectives

We hypothesized that transesophogeal echocardiography (TEE) using speckle tracking, as is performed on the LV, could be used to measure the deformation of the ascending aorta during the cardiac cycle. This, coupled with the blood pressure, would allow us to estimate the in vivo stiffness index and compare these values with the ex vivo biomechanical properties. This is a first step to determine if echocardiography speckle tracking can be used to better stratify patients for ascending aortic replacement surgery.

4- Article: Journal of Thoracic and Cardiovascular Surgery 2017; 153: 781-788.

Preface:

As described in the introduction, we identified the limitations of assessing the mechanical behavior of ascending aortic aneurysm with biomedical imaging. We then used a novel method to calculate the biomechanics of ascending aorta using echo. Then we correlated the in-vivo calculated stiffness with ex-vivo measured stiffness to validated the accuracy of in-vivo stiffness. Our syatamatic protocol to collect the tissue from the patients at surgery has resulted in one of the largest series for this type of research. This study was finalist for C. Walt Lillehei Resident Forum at the American Association for Thoracic Surgery meeting in 2016.

We selected the Journal of Thoracic and Cardiovascular Surgery to submit our work to as it is the journal of our surgical association and the most important journal in the filed of cardiothoracic surgery.

The following article has now been published and we hope that our work will reach clinicians and prompt more research.

Obtaining the Biomechanical Behavior of Ascending Aortic Aneurysm by using Novel Speckle Tracking Echocardiography

Mohammed Alreshidan MD. MSc(C) [1,2], Nastaran Shahmansouri MSc[4], Jennifer Chung MD. MSc[1], V. Lash FRCPC.[3], Alexander Emmott MEng[4], Richard L. Leask PhD[4], Kevin Lachapelle FRCSC[1]

1 Cardiac Surgery Department, McGill University; 2 King Salman Heart Center, King Fahad Medical City, Riyadh, Saudi Arabia; 3 Anesthesia Department, McGill University; 4 Chemical Engineering Department, McGill University Montreal, Quebec, Canada

Word Count of the manuscript: 2969.

No discloser for the authors.

No funding is provided.

Corresponding Author:

Dr. Kevin Lachapelle MD. FRCSC

Program Director, Cardiac Surgery Department

Email: kevin.lachapelle@mcgill.ca

Tel: +1 514 934 1934 Ext. 36873

Fax: +1 514 843 1602

Royal Victoria Hospital

McGill University Health Center – Glen Site

1001 Boul. Decarie, C07 1284

Montreal, Quebec, H3A 3J1

4-1: Introduction:

An ascending aortic diameter greater than 5.5 cm is the main indication for aortic resection to mange the increased risk of rupture or dissection[6, 7, 22, 23]. There is epidemiologic evidence to suggest that there is a hinge point at 5.5 cm where the risk of aortic complication is markedly increased[6, 22, 23]. This hinge point is also supported by the study of biomechanical properties of ex vivo aortic tissue[1, 4, 8]. Tissue remodeling and medial degeneration occurs with increased diameter of aorta resulting in loss of the elastic properties and increased stiffness [24-27]. Moreover, aortic tissue remodeling has been noted as part of the aging process and is seen in patients with connective tissue disease and aortic aneurysms. It is this remodeling process which may make the aorta at risk for rupture or dissection. Our focus is to try to determine if the biomechanics of aortic tissue as measured ex vivo and in vivo can eventually help determine the risk of complications in-patient with tissue remodeling. Stiffness is a measure of the resistance offered by tissue to an applied force and can be calculated from the stress – strain relationship of the tissue using a tensile tester[3, 4, 8, 28]. Our group along with others in the field, have found that the biomechanics of aortic tissue may be a good predictor of the histologic integrity of the aortic wall[25, 27, 29]. Biomechanics, and therefore the integrity of the aortic wall, may be a better marker of risk than size alone especially given the observation that most dissections occur in aorta's less than 5.5 cm[5]. The major limitation to the use of biomechanics in risk prediction is the absence of a non-invasive method to measure the biomechanical properties of the aorta in vivo.

Recently, there has been an interest in estimating biomechanics in vivo by calculating tissue strain using echocardiography and magnetic resonance[16, 21, 30-34]. Speckle tracking is an echocardiographic imaging technique that can measure the motion of cardiac tissue by using the naturally occurring speckle patterns in tissue or blood created by ultrasound frequencies during cardiac imaging. The motion of the speckles during systole and diastole allows one to calculate strain. Much work has been done using speckle tracking to calculate LV strain [35-37]. The stress on the tissue can be estimated by recording the pressure during the cardiac cycle.

We hypothesized that transesophogeal echocardiography (TEE) using speckle tracking, as is performed on the LV, could be used to measure the deformation of the ascending aorta during the cardiac cycle. This, coupled with the blood pressure, would allow us to estimate the in vivo stiffness index and compare these values with the ex vivo biomechanical properties. This is a first step to determine if echocardiography speckle tracking can be used to better stratify patients for ascending aortic replacement surgery.

4-2:Methods:

4-2-1: Patient Population:

Patients undergoing ascending aortic resection were recruited to be part of the study. Patients with acute aortic dissection and connective tissue disease such as Marfan syndrome were excluded from the cohort. Patients who could not have a transesophageal study were also excluded. All ascending aortic samples were

obtained from operating room at the time of surgery at McGill University Health Center (MUHC) in Montreal, Canada, following informed consent.

4-2-2: Intra-operative Trans-Esophageal Echo (TEE) & In-Vivo Speckle Tracking Strain Imaging Analysis:

The TEE study was performed using Vivid 7, GE echo machine with simultaneous ECG tracking as routine preparation for the operation. After the patient was anesthetised, the TEE probe was inserted into the esophagus to the level of great vessels to obtain the short axis view of ascending aorta at the level of the pulmonary trunk, Figure 1. The measurements are taken with the patient off all inotropes/vasopressors and normotensive with a heart rate in the normal range. The blood pressure was recorded simultaneously by using radial artery line tracing. In preparation for echo, a swan ganz catheter was pulled back and the ventilation turned off to minimize the lung movement during capturing of the loop of the ascending aorta. A short axis view of the ascending aorta at the level of maximum dilatation or at the level of pulmonary artery was recorded by using the 2D echo image. The image was recorded during 5 heartbeats and the view optimized to guarantee that all walls of ascending aorta were visualized. A mid esophagus 120^{o} long axis view of ascending aorta was also obtained. The blood pressure during the study was recorded. The speckle echo tracking analysis was done for all ascending aortic loops post operatively using GE EchoPAC station. Strain mapping of the circumference of the aorta in short axis was done using advanced Q Analysis, 2d strain. Between 14 to 18 markers (depends on the aortic size) were set to demark

the inner wall of the aorta. Figure 2 is an explanatory cartoon showing the setup for the echo speckle tracking analysis. The minimum circumferential length of the aorta during the heartbeat was identified and set as L0. This length was used as a base to define the four quadrants of the ascending aorta (IC, Ant, OC, Post). The length of each region was assumed to be equal. The software tracked the changes in the length of the aorta with time during the heartbeat. The final length L_f and the original length L_o of each quadrant during the heartbeat were used to calculate the green strain, which we took as the AoS for each region.

Green strain $\in_{Green} = AoS = \frac{1}{2}\left(\frac{L_{f-}^2 L_o^2}{L_o^2}\right)$ (L_f: final length; L_o: original length)

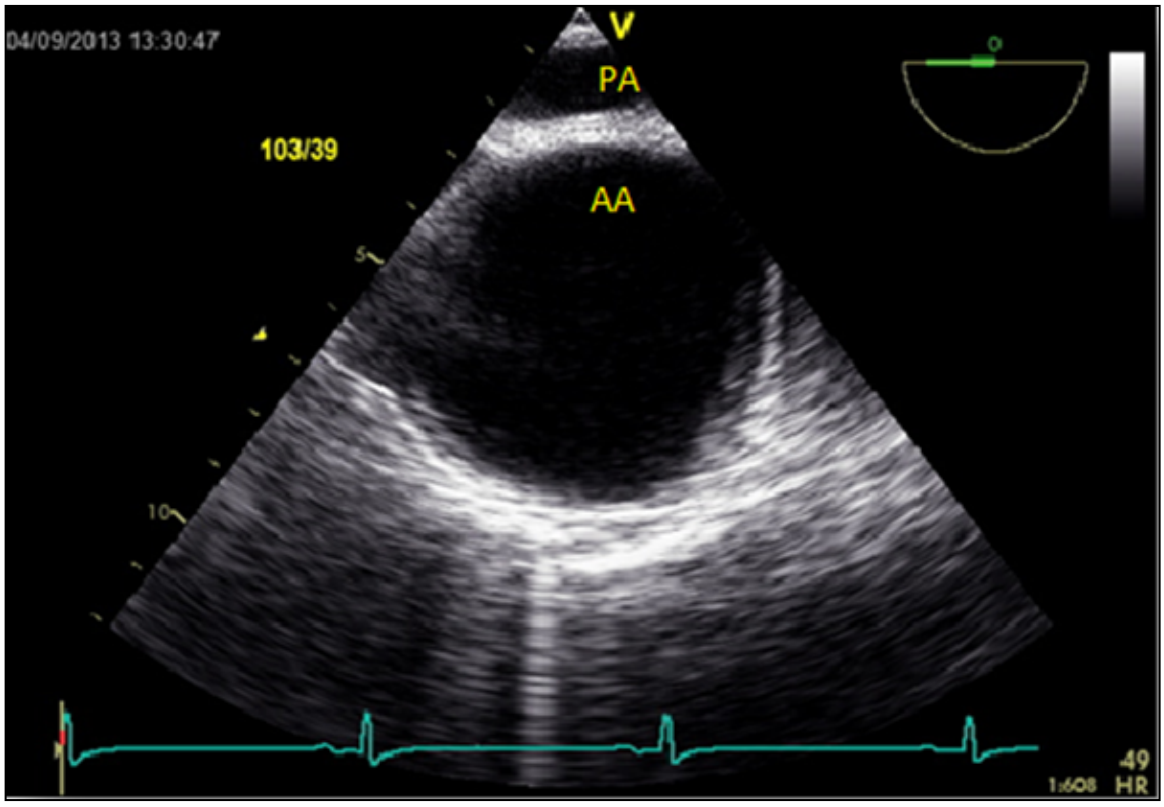


Figure 1: Short axis view of the ascending aorta using TEE at the level of maximal dilatation or the pulmonary artery. Blood pressure 103/39, AA: ascending aorta and PA: pulmonary artery.

To obtain the stiffness index from echo calculation, we used the empirical formula defined by Hirai et al. [38] and adapted for 2D echocardiographic strain imaging by Oishi et al. [21] . Originally, Hirai et al. fit a semi-logarithmic equation to intraluminal pressures and the external diameter of the human abdominal aorta by defining the distension ratio (λ) as the arterial diameter (D1 at systolic pressure) at a given pressure (P systolic), normalized by the diameter (Do at diastolic pressure) at a standard pressure (P diastole). When they plotted the distention ratio against the logarithmic value of the relative pressure, a linear relation was observed in the physiologic range of pressure[38].

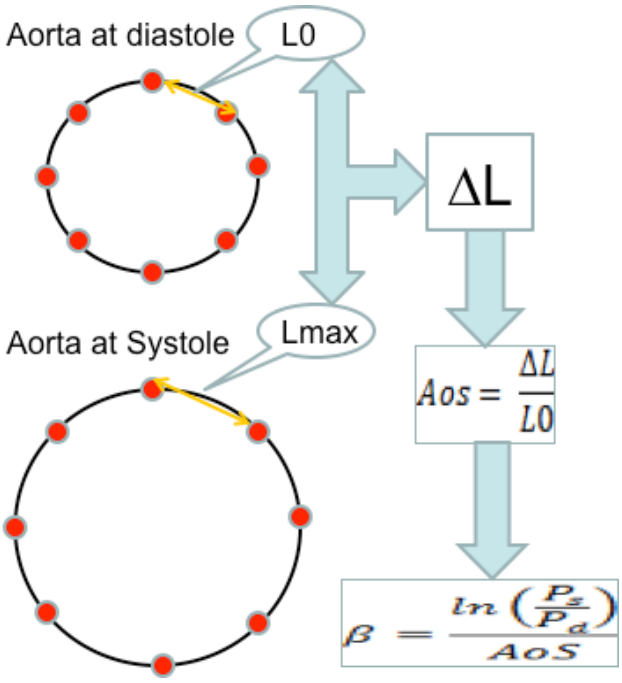
$\ln(SBP/DBP) = \beta 2\ ((\ diameter\ at\ SBP - diameter\ at\ DBP)\ /diameter\ at\ DBP)$

[38]. Oishi et al. adapted this metric for 2D echocardiography derived strain and defined the stiffness index as:

$\beta 2 = \ln(SBP/DBP)/AoS$ (β2 stiffness index; SBP: Systolic BP; DBP: Diastolic BP, AoS: circumferential strain) [21]

For our analysis, we defined the circumferential strain (AoS) as the maximum green strain, $\in_{Green}$ for each segment of the aorta.

A)



B)

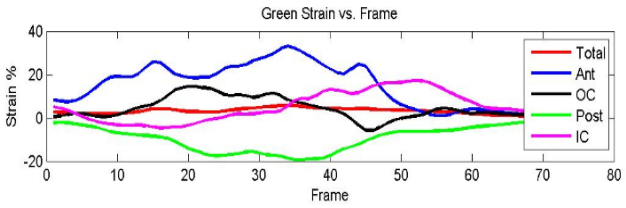


Figure 2: A) Cartoon of the in vivo echo strain calculation B) Echo-calculated strain mapping for each region of the aorta.

4-2-3: Tissue Preparation and ex-vivo aortic stiffness:

The tissue was obtained from the operating room as an intact ring with a single clip marker at the anterior wall of the aneurysm belly [25]. The tissue was kept in ice until we performed the mechanical testing. The mechanical testing was done within 24 hours from tissue collection. Sections of 1.5 × 1.5 cm were collected from each quadrant of the aortic wall (Inner curvature, Anterior, Outer curvature and Posterior). Each square underwent equibiaxial tensile testing at 37°C in a Ringer's Lactate Solution. After preconditioning, three cycles at 0.01 mm/s were used to capture the tissue stress-strain relationship (EnduraTEC Electro Force 3200 Biaxial Tensile Tester, Bose Corporation, Minnesota. USA).

The analysis of mechanical data was done using a Matlab R2012a (Math Works, Natick, Mass). We calculated the green strain, $\in_{Green}$, based on the displacement of the sutures securing the tissue during the biaxial loading. This data, along with the load applied and cross sectional area of the tissue was used to obtained the stress-strain curves, where the stress was defined as the second Piola-Kirchhoff stress: $S = \frac{FL_o}{A_o L} =$ (F: load; L_o : original length; L: final length; A_o : intial cross-sectional area). The apparent modulus of elasticity (slope of the average stress-strain curve) was calculated at 25% green strain, which approximates the physiological condition[39].

4-2-4: Statistical Analysis:

The statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, California). A difference in the means was considered significant for a *p*-value was less than 0.05. The regional variation between the ex vivo and in vivo strain index analysis was compared with a two-way analysis of variance (ANOVA) and a Bonferroni multiple comparison test. One-way ANOVAs were done to evaluate the regional variation of both the ex vivo apparent stiffness and in vivo stiffness index estimates.

4- 4: Results:

Table 1 summarize the demographics data for the patients involved in the study. Fifty seven percent of the patients were male and the mean aortic diameter of all patients was 56.16 ± 15 mm. The mean ejection fraction was 60 ± 10 % and 47% of the patients were hypertensive. Furthermore, approximately one third of the patients had a bicuspid aortic valve (41%) and 47% of the total cohort had aortic stenosis. In term of the blood pressure medication, 41% of the patients used calcium channel blocker and 53% were on beta-blocker.

Variable	%
Sex (male)	64 % (11/17)
Age (mean)	63.9 ± 15.9 years
Ejection fraction (mean)	60± 10 %
Hypertension	47% (8/17)
Diabetes mellitus	5.8% (1/17)
Dyslipidemia	23% (4/17)
Aortic diameter (mean)	56.16± 15 mm
Bicuspid aortic valve	41% (7/17)
Aortic stenosis	47% (8/17)
Aortic valve replacement	88% (15/17)
Bentall Operation	64% (11/17)
David Valve Sparing operation	5.8% (1/17)
Calcium channel blocker	41% (7/17)
Beta blocker	53% (9/17)

Table 1: patients demographics.

The estimated in vivo stiffness index was scaled by dividing it by 100 to be similar in magnitude to the ex vivo apparent modulus expressed in MPa. For the in vivo stiffness index obtained by TEE, the average β2/100, for the inner curvature, anterior wall, outer curvature and posterior wall were 0.0544 ± 0.0490, 0.0295± 0.0199, 0.0411± 0.0328 and 0.0502± 0.0320 respectively. The anterior wall had the lowest stiffness index and was significantly less than the inner curvature and posterior wall (p= 0.01 and p= 0.05 respectively, Bonferroni's Multiple Comparison Test), Figure 3.

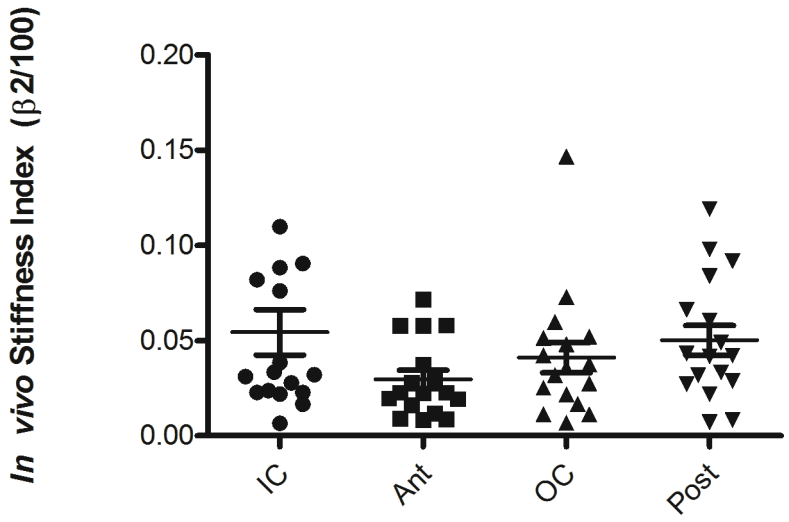


Figure 3: In vivo estimated stiffness index mean and standard deviation for all patients. (IC: inner curvature, Ant: anterior wall, OC: outer curvature, Post: posterior wall)

For the ex vivo stiffness as measured by tissue biomechanics, there was a similar pattern of apparent stiffness as in vivo samples, with the outer curvature the stiffest, however, there was much less variation in the data. The mean ex vivo 25% apparent stiffness for inner curvature, anterior wall, outer curvature and posterior wall were 0.0616 ±0.0758 MPa, 0.0352± 0.00992 MPa, 0.0405± 0.0199 MPa and 0.0327± 0.0106 MPa respectively. Figure 4.

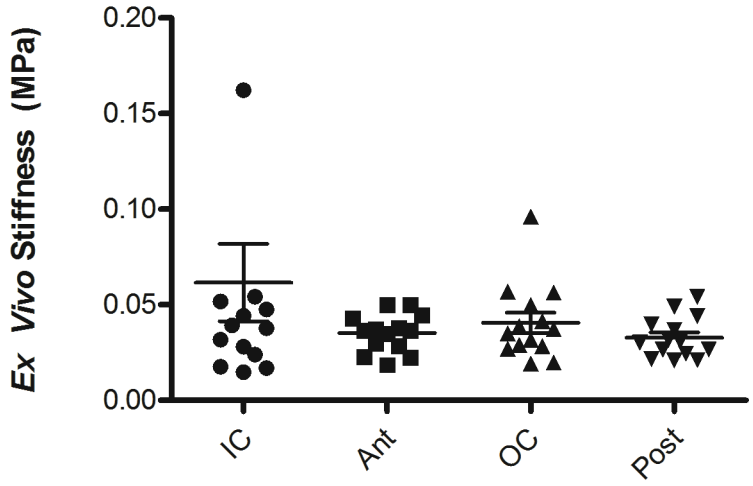


Figure 4: Ex-vivo calculated stiffness mean and standard deviation for all patients. (IC: inner curvature, Ant: anterior wall, OC: outer curvature, Post: posterior wall)

A two way ANOVA was used to evaluate if there was a significant difference between the mean values of the ex vivo stiffness and in vivo stiffness index, and if the results were dependent on region. The patient matched ex vivo 25% apparent stiffness and in vivo stiffness index (β2/100) were not significantly different (p=0.8617, two way ANOVA with repeated measures). The results were dependent on location (p=0.0372, two way ANOVA with repeated measures), Figure 5.

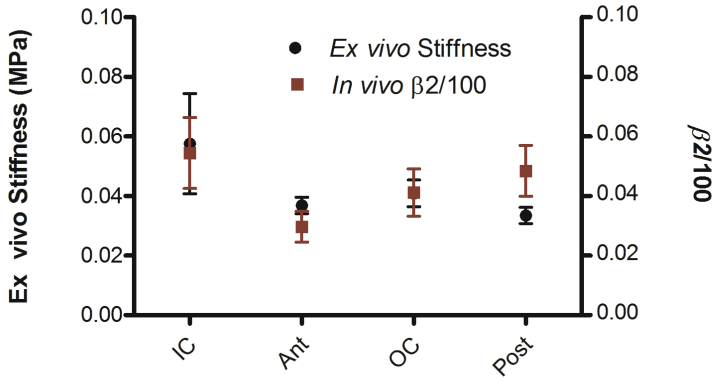


Figure 5: Ex vivo stiffness and In vivo stiffness index (β/100) regional wall variation. (IC: inner curvature, Ant: anterior wall, OC: outer curvature, Post: posterior wall)

4-5: Discussion:

Our main goal in this study was to determine if the speckle tracking performed from TEE data could be used to estimate the biomechanical features of the ascending aorta. To our knowledge, this is one of the first studies of its kind comparing estimates of a stiffness index obtained in vivo during TEE to those obtained ex vivo on the same aortic tissue. This is a pilot study to determine if echocardiography speckle tracking can reliably estimate the biomechanical tissue properties of aneurysmal tissue in our patient population. This is the first step in validating if such measures correlate with the degree of tissue remodelling and ultimately help to better stratify patients for ascending aortic replacement surgery. We found no significant difference in the mean values obtained by the two methods. The results suggest there is some merit in the in vivo stiffness index estimate as a measure of

aortic tissue biomechanics, but much more work is needed to make this method a reliable and predictable clinical tool.

Speckle tracking echo, was used initially to calculate the LV strain and deformation [40]. Speckle-tracking echo detects the deformation of tissue such as the left ventricle and allows for estimating the deformation in the three different directions, longitudinal, radial and circumferential[40]. The circumferential strain of the LV has been defined as shortening of LV myocardial fibre along the circular perimeter observed in a short-axis view [40].

To calculate the in vivo stiffness index we adapted the methods used to calculate the stiffness index in the abdominal aorta by Oishi et al. [21]. In their work, Oshi et al. used similar equipment and the entire circumference to define the peak aortic strain (AoS) over three cardiac cycles and compared the results with M-mode ultrasonographic estimates of stiffness as defined by Hirai et al. [38]. Oishi et al. showed β2 stiffness index to increase with age and increased dramatically with advanced age (>50 years). In normal individuals, they found the stiffness index to be linearly related to age.

Our results showed no correlation of in vivo stiffness index or ex vivo apparent stiffness with age (data not shown). This is not surprising as our patient population was exclusively aneurysmal and at risk of rupture and dissection. The cohort presented by Oshi et al. was of consecutive patients undergoing routine health checkups.

We adjusted our in vivo stiffness index by a factor of 100 to allow for similar magnitude as our ex vivo apparent stiffness expressed in MPa. The in vivo stiffness index as derived by Hirai et al.[38] is dimensionless. The ex vivo apparent stiffness depends on the units of stiffness, in our case, MPa. Due to the nonlinear nature of human aortic tissue biomechanics, the apparent stiffness changes with the level of strain. In our study, we have chosen to use 25% green strain, which is representative of our in vivo strain values.

The average in vivo stiffness index for all our patients was 4.3± 3.0, which is lower than the values found by Oishi et al. for the abdominal aorta [21]. Oishi et al. found in patients <50 years old the average stiffness index was 8±4, while patients over 50 had a stiffness index 29±20 [21]. The difference between the abdominal and ascending aorta stiffness index and our ascending aorta values are due to the structure of the aorta, which changes along its length. It has been shown that the aorta becomes less elastic with increasing distance from the heart [41-43]. The variation in mechanical properties along the aorta is partly due to changes in the tissue composition. The elastin content decreases along the length of the aorta [44].

The in vivo calculated stiffness index showed a significant difference between anterior, inner curvature and posterior wall, Figure 3. The ex vivo calculations showed a similar but non-significant trend. The difference in the in vivo stiffness index may be partially explained by the anatomical differences when comparing the anterior wall with inner curvature and posterior wall. The posterior wall and inner curvature of the aorta are supported by various structures such as pulmonary

artery, left atrium and the trachea; limiting the motion of these parts of the aortic wall. Epi-aortic ultrasound has shown the movement in the anterior and posterior aneurysmal ascending aortic wall to be limited[45].

When comparing in vivo and ex vivo values a few caveats must be taken into consideration. First, in-vivo , evaluation is effected by the surrounding structures such as trachea, left atrium and pulmonary artery, which can affected the strain values, as demonstrated by Modak, R.K., et al [45]during motion evaluation of the aortic wall using epiaortic ultrasound. On the other hand ex-vivo mechanical testing is performed on a area of aortic tissue without the effect of the sournding anatomical strcutrures or the whole intact aorta. Second, the calcualation methodolgy was different between the 2 modalities. The in vivo stiffness index is not the same mathematical definition as the apparent stiffness. We have artificially adjusted our in vivo estimate to be of similar magnitude as the ex vivo measured apparent stiffness. We have estimated, based on literature values, a representative in vivo strain [39] and used the stress-strain relationship to calculate the ex vivo stiffness. Because the mechanical stress-strain curve of ascending aortic tissue is non-linear [4, 8, 25], selecting one arbitrary strain to estimate the in vivo stiffness index is very simplistic. Furthermore, the in vivo estimated stiffness index depends mainly on the blood pressure of the patient, and varies between patients. Despite these simplifications, we found good agreement between the in vivo stiffness index ($\beta 2/100$) and the 25% ex vivo apparent modulus, Figure 5. Paired analysis between the in vivo and ex vivo data show the stiffness index $\beta 2$ is similar to ex vivo values.

More work needs to be done to improve the variability of the in vivo stiffness index, and investigate more reliable metrics of aortic biomechanics.

Ex vivo measured mechanical properties have shown very good correlations with histological changes that happen with ascending aortic aneurysms [25]. Our group found the energy loss to be a more robust mechanical metric for predicting aortic remodeling than stiffness [25]. The energy loss is calculated from the integral of the stress-strain curve and uses the full cycle stress strain data, as opposed the stiffness, which is described at one point in the curve. The strain mapping from 2D echocardiograms provide the full cardiac cycle strain data, which could be combined with the aortic pressure waveform. In fact, we have now started to collect in vivo strain data throughout the cardiac cycle allowing us to construct a more complete stress-strain curve for each aorta. This will be more precise then a single estimate of the strain –stress relationship, which is what was performed in this study. Moreover, we will move forward to start perform a transthoracic echo to calculate the aortic strain and compare that with transesophageal echo speckle tracking to make this method as less invasive as it goes.

This study is limited by a relatively small sample size. Moreover, we have grouped both bicuspid and tricuspid valves and have no normal tissue controls. Our in vivo measurements had much more variability then our ex vivo measures and this may reflect operator technique or selection of speckle points. Furthermore, this program was originally construct to be used in the left ventricle strain calculation and we used to calculate the strain in the aortic wall. Issues with the view of the aorta and

interference of surrounding tissue might be overcome by dedicated software and routines for the ascending aorta.

4-6: Conclusion:

The use of speckle tracking performed during transesophageal echocardiography appears to be a promising technique to estimate ex vivo mechanical properties of the ascending aortic tissue. In this study, mean stiffness values obtained in vivo were similar to those measured ex vivo. More work is needed to make TEE derived strain mapping a reliable method for stratifying ascending aortic aneurysm patients for surgery.

4-7: Future directions:

Our group is developing now a new model, were they used the blood pressure tracing as stress curve and the strain curve is obtained by echo speckle tracking. Then plot the stress against the strain and obtained the energy loss. Then we will correlate the echo energy loss with mechanical energy loss to validate the echo energy loss. This after all will help to highlight the aortas that are more prone to complications.

5- References:

1. Okamoto, R.J., et al., *The influence of mechanical properties on wall stress and distensibility of the dilated ascending aorta.* J Thorac Cardiovasc Surg, 2003. **126**(3): p. 842-50.
2. He, R., et al., *Characterization of the inflammatory and apoptotic cells in the aortas of patients with ascending thoracic aortic aneurysms and dissections.* J Thorac Cardiovasc Surg, 2006. **131**(3): p. 671-8.
3. Khanafer, K., et al., *Determination of the elastic modulus of ascending thoracic aortic aneurysm at different ranges of pressure using uniaxial tensile testing.* J Thorac Cardiovasc Surg, 2011. **142**(3): p. 682-6.

4. Okamoto, R.J., et al., *Mechanical properties of dilated human ascending aorta.* Ann Biomed Eng, 2002. **30**(5): p. 624-35.
5. Pape, L.A., et al., *Aortic diameter >or = 5.5 cm is not a good predictor of type A aortic dissection: observations from the International Registry of Acute Aortic Dissection (IRAD).* Circulation, 2007. **116**(10): p. 1120-7.
6. Elefteriades, J.A., *Indications for aortic replacement.* J Thorac Cardiovasc Surg, 2010. **140**(6 Suppl): p. S5-9; discussion S45-51.
7. Coady, M.A., et al., *Surgical intervention criteria for thoracic aortic aneurysms: a study of growth rates and complications.* Ann Thorac Surg, 1999. **67**(6): p. 1922-6; discussion 1953-8.
8. Choudhury, N., et al., *Local mechanical and structural properties of healthy and diseased human ascending aorta tissue.* Cardiovasc Pathol, 2009. **18**(2): p. 83-91.
9. Vorp, D.A., et al., *Effect of aneurysm on the tensile strength and biomechanical behavior of the ascending thoracic aorta.* Ann Thorac Surg, 2003. **75**(4): p. 1210-4.
10. Khanafer, K., M.S. Schlicht, and R. Berguer, *How should we measure and report elasticity in aortic tissue?* Eur J Vasc Endovasc Surg, 2013. **45**(4): p. 332-9.
11. Richardson, P.D., *Mechanical factors in bioresorbable grafts.* Bull N Y Acad Med, 1988. **64**(2): p. 132-44.
12. Robicsek, F. and M.J. Thubrikar, *Hemodynamic considerations regarding the mechanism and prevention of aortic dissection.* Ann Thorac Surg, 1994. **58**(4): p. 1247-53.
13. Humphrey, J.D., *Mechanics of the arterial wall: review and directions.* Crit Rev Biomed Eng, 1995. **23**(1-2): p. 1-162.
14. Chuong, C.J. and Y.C. Fung, *On residual stresses in arteries.* J Biomech Eng, 1986. **108**(2): p. 189-92.
15. Sundt, T.M., 3rd, *Residual strain in the aorta.* J Thorac Cardiovasc Surg, 2006. **131**(6): p. 1420-1; author reply 1421-2.
16. Imura, T., et al., *Non-invasive ultrasonic measurement of the elastic properties of the human abdominal aorta.* Cardiovasc Res, 1986. **20**(3): p. 208-14.
17. Lang, R.M., et al., *Measurement of regional elastic properties of the human aorta. A new application of transesophageal echocardiography with automated border detection and calibrated subclavian pulse tracings.* Circulation, 1994. **90**(4): p. 1875-82.
18. Mugge, A., et al., *Usefulness of a new automatic boundary detection system (acoustic quantification) for assessing stiffness of the descending thoracic aorta by transesophageal echocardiography.* Am J Cardiol, 1992. **70**(20): p. 1629-31.
19. Nichols, W.W., et al., *Enhanced external counterpulsation treatment improves arterial wall properties and wave reflection characteristics in patients with refractory angina.* J Am Coll Cardiol, 2006. **48**(6): p. 1208-14.
20. Hoegh, A. and J.S. Lindholt, *Basic science review. Vascular distensibility as a predictive tool in the management of small asymptomatic abdominal aortic aneurysms.* Vasc Endovascular Surg, 2009. **43**(4): p. 333-8.
21. Oishi, Y., et al., *Aortic stiffness is strikingly increased with age >/= 50 years in clinically normal individuals and preclinical patients with cardiovascular risk factors: assessment by the new technique of 2D strain echocardiography.* J Cardiol, 2011. **57**(3): p. 354-9.
22. David, T.E., *Surgical treatment of ascending aorta and aortic root aneurysms.* Prog Cardiovasc Dis, 2010. **52**(5): p. 438-44.
23. Hiratzka, L.F., et al., *2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease:*

a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. Circulation, 2010. **121**(13): p. e266-369.
24. Carlson, R.G., C.W. Lillehei, and J.E. Edwards, *Cystic medial necrosis of the ascending aorta in relation to age and hypertension.* Am J Cardiol, 1970. **25**(4): p. 411-5.
25. Chung, J., et al., *Energy loss, a novel biomechanical parameter, correlates with aortic aneurysm size and histopathologic findings.* J Thorac Cardiovasc Surg, 2014. **148**(3): p. 1082-8; discussion 1088-9.
26. El-Hamamsy, I. and M.H. Yacoub, *Cellular and molecular mechanisms of thoracic aortic aneurysms.* Nat Rev Cardiol, 2009. **6**(12): p. 771-86.
27. Schlatmann, T.J. and A.E. Becker, *Histologic changes in the normal aging aorta: implications for dissecting aortic aneurysm.* Am J Cardiol, 1977. **39**(1): p. 13-20.
28. Tremblay, D., et al., *A comparison of mechanical properties of materials used in aortic arch reconstruction.* Ann Thorac Surg, 2009. **88**(5): p. 1484-91.
29. Pisano, C., et al., *Histological and genetic studies in patients with bicuspid aortic valve and ascending aorta complications.* Interact Cardiovasc Thorac Surg, 2012. **14**(3): p. 300-6.
30. Allen, B.D., et al., *Time-resolved three-dimensional phase contrast MRI evaluation of bicuspid aortic valve and coarctation of the aorta.* Eur Heart J Cardiovasc Imaging, 2013. **14**(4): p. 399.
31. Burk, J., et al., *Evaluation of 3D blood flow patterns and wall shear stress in the normal and dilated thoracic aorta using flow-sensitive 4D CMR.* J Cardiovasc Magn Reson, 2012. **14**: p. 84.
32. Firmin, D.N., et al., *The application of phase shifts in NMR for flow measurement.* Magn Reson Med, 1990. **14**(2): p. 230-41.
33. Markl, M., W. Wallis, and A. Harloff, *Reproducibility of flow and wall shear stress analysis using flow-sensitive four-dimensional MRI.* J Magn Reson Imaging, 2011. **33**(4): p. 988-94.
34. Yamashita, S., et al., *Visualization of hemodynamics in intracranial arteries using time-resolved three-dimensional phase-contrast MRI.* J Magn Reson Imaging, 2007. **25**(3): p. 473-8.
35. Voigt, J.U., et al., *Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging.* J Am Soc Echocardiogr, 2015. **28**(2): p. 183-93.
36. Marwick, T.H., et al., *Myocardial strain measurement with 2-dimensional speckle-tracking echocardiography: definition of normal range.* JACC Cardiovasc Imaging, 2009. **2**(1): p. 80-4.
37. Levy, P.T., et al., *Reference Ranges of Left Ventricular Strain Measures by Two-Dimensional Speckle-Tracking Echocardiography in Children: A Systematic Review and Meta-Analysis.* J Am Soc Echocardiogr, 2016. **29**(3): p. 209-225 e6.
38. Hirai, T., et al., *Stiffness of systemic arteries in patients with myocardial infarction. A noninvasive method to predict severity of coronary atherosclerosis.* Circulation, 1989. **80**(1): p. 78-86.
39. Duprey, A., et al., *In vitro characterisation of physiological and maximum elastic modulus of ascending thoracic aortic aneurysms using uniaxial tensile testing.* Eur J Vasc Endovasc Surg, 2010. **39**(6): p. 700-7.

40. Mondillo, S., et al., *Speckle-tracking echocardiography: a new technique for assessing myocardial function.* J Ultrasound Med, 2011. **30**(1): p. 71-83.
41. Learoyd, B.M. and M.G. Taylor, *Alterations with age in the viscoelastic properties of human arterial walls.* Circ Res, 1966. **18**(3): p. 278-92.
42. Han, H.C. and Y.C. Fung, *Longitudinal strain of canine and porcine aortas.* J Biomech, 1995. **28**(5): p. 637-41.
43. Rouleau, L., et al., *Regional variations in canine descending aortic tissue mechanical properties change with formalin fixation.* Cardiovasc Pathol, 2012. **21**(5): p. 390-7.
44. Fry, P., et al., *Mechanical properties of tissues of lathyritic animals.* J Physiol, 1962. **164**: p. 77-89.
45. Modak, R.K., et al., *Ascending aortic aneurysms: asymmetrical differences in aortic cross-sectional wall motion detected by epiaortic echocardiography.* J Cardiothorac Vasc Anesth, 2010. **24**(5): p. 776-9.