Energy Loss, a Novel Biomechanical Parameter,

Correlates with Aortic Aneurysm Size and Histopathology

Jennifer Chung, MD

Department of Experimental Surgery

McGill University, Montreal

April, 2015

A thesis submitted to McGill University in partial fulfilment of the requirements of the degree of Master of Science.

Table of Contents

List of Figures

List of Tables

Acknowledgements

First of all, I would like to acknowledge the guidance and support of my two supervisors, Dr. Richard Leask and Dr. Kevin Lachapelle. I am grateful for their advice and encouragement. The many thought-provoking and insightful conversations helped me overcome the challenges that came along the way in completion of this project.

This work could not have been accomplished without the help of many individuals. From the lab, Evan Wener was a tremendous help in introducing me to the experimental set-up and a great partner in data collection. The tissue collection would not have been possible without the support of all of the surgeons at the Montreal Heart Institute and the Royal Victoria Hospital, as well as the generosity of the patients. I would like to thank Marie-Elaine Clavet (Montreal Heart Institute) for her assistance with the histology work. Also, I would like to acknowledge Dr. Richard Fraser for his contributions in providing tissue specimens and for review of the pathology component of the final manuscript.

Finally, I would like to thank my husband for his support as well as my parents and brother.

Abstract

It is not well understood which ascending aortas will develop complications. Biomechanics may provide insights beyond the consideration of size alone, and improve risk prediction. Energy loss is a biomechanical parameter describing the relative amount of energy absorbed by the aorta during the cardiac cycle. We aim to correlate energy loss with ascending aortic aneurysm size and histopathology to elucidate the pathophysiology of aneurysm complications.

Aneurysmal ascending aortic specimens were obtained during surgery. Controls were obtained from autopsy and organ donors. Biaxial tensile tests were performed on the four quadrants of the aortic ring. Energy loss was calculated using the integral of the stress-strain curve during loading and unloading. It was compared to size and the traditional biomechanical parameter, stiffness (apparent modulus of elasticity). Elastin, collagen and mucopolysaccharide content was quantified using Movat pentachrome staining of histology slides.

Forty-one aortas were collected (34 aneurysmal, 7 control). Aneurysms exhibited increased stiffness ($p<0.0001$) and energy loss ($p<0.0001$) compared to control. Energy loss correlated significantly with aortic size ($p<0.0001$, $r^2=0.60$). A hinge point was noted at a diameter of 5.5cm, after which energy loss rose rapidly. The relationship between energy loss and size became strongly linear once size was indexed to body surface area ($p<0.0001$, $r^2=0.78$). Energy loss correlated with histopathology, especially the collagen to elastin ratio ($p=0.0002$, $r^2=0.49$). High energy loss distinguished patients with pathological histology from others of similar diameters.

As ascending aortas dilate, they exhibit greater energy loss, rapidly rising after 5.5cm. This mirrors the rise in complications at this size. Energy loss correlates with imbalances in elastin and collagen composition, suggesting a measurable link between histopathology and mechanical function.

Résumé

Il n'est pas bien compris quel type d'anévrisme de l'aorte ascendant entraîne les complications. La biomécanique pourrait nous offrir des données pour prédire les risque; et de ne pas se fier seulement sur la grandeur de l'anévrisme. La perte d'énergie mécanique représente la quantité relative de l'énergie absorbée par l'aorte au cours du cycle cardiaque. Nous avons ainsi cherché à en étudier la corrélation avec l'anévrisme de l'aorte ascendante et l'histopathologie, afin de déterminer la pathophysiologie des complications liées à cette dilatation artérielle.

Les échantillons sont obtenus suite à des opérations chirurgicales sur des patients et les témoins proviennent d'autopsies ou de dons d'organes. Des essais de traction biaxiale sont réalisés au niveau des quatre quadrants de l'anneau aortique, permettant ainsi de calculer la perte d'énergie à travers l'intégrale de la courbe de contrainte-déformation, lors du chargement et déchargement de l'échantillon. Un travail de comparaison est ensuite effectué en tenant compte de la taille et de la rigidité du tissu via son module d'élasticité apparent, un élément prépondérant dans le domaine de la biomécanique. L'élastine, le collagène et la mucopolysaccharide, quant à eux, sont quantifiées par la technique de « pentachrome de Movat » sur des lames d'histologie.

Quarante-et-une aortes sont analysées (trente-quatre cas d'anévrismes et sept témoins) et des augmentations de rigidité ($p<0.0001$), ainsi que de perte d'énergie ($p<0.0001$) sont constatées. La perte d'énergie a une corrélation significative avec le diamètre de l'aorte ($p<0.0001$, $r2=0.60$). Or, lorsque ce dernier est supérieur à 5.5cm, la perte d'énergie augmente rapidement. Elle présente également une relation linéaire pour une taille de l'aorte indexée à l'air de sa surface ($p<0.0001$, $r2=0.78$). Enfin, la perte d'énergie corrèle avec l'histopathologie, en particulier avec le rapport entre le collagène et l'élastine ($p=0.0002$, $r2=0.49$).

Les variations de pertes d'énergie permettent de distinguer des patients aux histologies pathologiques à d'autres personnes saines pouvant présenter des diamètres de mêmes dimensions. Lors de la dilatation de l'aorte ascendante, la perte d'énergie est également plus importante pour des diamètres supérieurs à 5.5cm, démontrant ainsi la complexité du comportement de cette artère en fonction de ses dimensions. Pour finir, la perte d'énergie corrèle avec les répartitions uniformes d'élastine et de collagène, ce qui pourrait amener à une relation impliquant l'histopathologie et la biomécanique du tissu.

Contribution of Authors

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

Jennifer Chung: participated in experimental design, gathered data, performed mechanical experiments, gathered histology data, analysed data, wrote the manuscript, replied to reviewers.

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

Evan Wener: gathered data, performed mechanical experiments.

Raymond Cartier: assisted in coordination of tissue gathering at the Montreal Heart Institute, provided tissue from patients at the time of surgery.

Benoit De Varennes: provided tissue from patients at the time of surgery.

Richard Fraser: provided tissue from autopsy patients, reviewed histology part of manuscript.

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

1. Introduction

Patients with aneurysms of the ascending aorta are at risk of suffering catastrophic complications of dissection and rupture; elective replacement of the ascending aorta is sometimes recommended. However, the choice of whom to operate on is complex. Currently, aortic diameter is the main criteria for intervention despite known limitations. We developed a biomechanical parameter that described the relative amount of energy released by the aorta during unloading versus the amount of energy stored by the aorta during loading, and we called this parameter 'energy loss'.

In a first step towards validating energy loss as a clinically relevant biomechanical risk factor for aneurysmal aortas, we aim to characterize the energy loss of human ascending aortic tissue ex-vivo. We hypothesized the following:

1) Aneurysmal aortas demonstrate greater energy loss as compared to control.

2) Energy loss is associated with aortic size, and in fact is more closely related to size than the apparent modulus of elasticity.

3) The structural compositional changes in aneurysmal aortic walls are associated with increased energy loss.

4) Energy loss can be used to identify aortas with pathological histological changes.

2. Literature Review

2.1 The Ascending Aorta

The aorta is the main artery of the human body, acting as the conduit that transmits the left ventricle's cardiac output to peripheral vessels and the rest of the body. As blood passes the aortic valve, the first structure met is the aortic root which ends at the sinotubular junction. The aortic root comprises of three sinuses of Valsalva and provides the origin for the left and right coronary arteries. The ascending aorta follows the sinotubular junction, and under normal circumstances, it is the largest and most elastic portion of the aorta as well.[1] Its mechanical properties allow it to function in a dynamic pulsatile environment. During systole as the heart ejects, the aortic wall is able to absorb up to 50% of the cardiac output.[1] Subsequently during diastole, while the aortic valve is closed, the aortic wall recoils and delivers blood flow down the coronary arteries and maintains flow to the body. This capacitor function of the aorta is termed the Windkessel function and is performed by the aorta 60-100 times a minute for the length of a person life.

The normal aorta is structured to handle this enormous stress and strain. It is comprised of endothelial cells, elastin, collagen and smooth muscle cells. These elements are arranged in three layers: the intima, media and adventitia. Elastin and collagen are the most important contributors from a mechanical perspective, especially in the media layer. Elastic fibres are able to elongate 100% of their original length and then return to their original length.[2] Collagen has a modulus of elasticity of over 500 MPa, 1000 times greater than elastic fibre which has a modulus of elasticity of 0.6 MPa.[3] Thus, collagen is an important contributor to stiffness. By dry weight, the thoracic aorta is composed of 22-33% elastin and 18-31% collagen.[4] Smooth muscle cells only

contribute meaningfully to the tensile properties of the aorta when contracting. We have previously shown that in healthy pig ascending aortic tissue, maximal SMC activation with phenylephrine does effect the stiffness of the tissue, however the contribution is limited at physiological strains to less than 10% of stiffness increase.[5]

This complex multi-component material is well-described to have several signature characteristics. Firstly, it exhibits a highly non-linear stress-strain relationship.[2] As strain increases, significant strain-hardening occurs resulting in greater modulus of elasticity. The apparent modulus of elasticity can be used to characterize aortas, and it widely varies depending entirely on the point at which measurements are made. The apparent modulus of elasticity is the biomechanical parameter most commonly estimated by clinicians to correlate to disease.[6]

Secondly, components of aortic tissue also layer with directionality, therefore creating anisotropy.[7] In other words, any biomechanical parameter must be described in terms of whether measurements were performed in the axial or circumferential direction.

An additional signature characteristic of aortic tissue is its viscoelastic nature. Aortic tissue demonstrates hysteresis, with the unloading curve following a different path from the original loading curve. The area under the curves represented energy stored during loading and released during unloading. The most comprehensive model for describing aortic biomechanics is the strain energy density function (W).[2] W can be expressed as a polynomial from which stress-strain relationships can be derived. Yet, W usually only describes either the loading or unloading curve.

Quantifying the viscoelastic nature of aortic tissue may be key to understanding how and when it fails. Fatigue describes progressive weakening of any material undergoing cyclical loading

through accumulation and expansion of microscopic cracks and defects, leading to eventual failure.[8] Aortic tissue is also a material undergoing cyclical loading and in the diseased state, forms aneurysms that fail through dissection or rupture. Creation and expansion of any defects in aortic tissue requires energy driven by the intrinsic viscous effects of the wall. Fatigue is a difficult concept to model in dynamic, healing, growing living tissue, but quantifying hysteresis is simple, and represents an underexplored concept in aortic biomechanics.

2.2 Aneurysms of the ascending aorta

Aneurysms are defined as a dilation of the vessel by at least 50%.[9] The incidence of aneurysms of the ascending aorta is estimated to be 10.4 cases per 100,000 person-years, and this number appears to be rising with improved detection techniques and the aging population.[10] The prevailing hypothesis is that this dilation is as a result of medial degeneration, a process accelerated by genetic traits, hypertension and likely many unknown factors.[11] Medial degeneration is characterized by elastic fiber fragmentation, loss of smooth muscle cells, and collagen and mucopolysaccharide deposition.[9] This leads to mechanical weakness and failure, specifically in the form of aortic dissection and rupture. Dissection and rupture is estimated to cause 30,000 to 60,000 deaths per year in the United States.[12]

The catastrophic complications associated with aneurysms of the ascending aorta are preventable with elective surgery. However, identifying appropriate patients for surgery can be challenging. Present guidelines are based predominantly on size alone and on low levels of evidence.[9] A recent study found wide variations across Canada in what surgeons believe to be appropriate size criteria for bicuspid aortopathy.[13] Studies modelling pre-dissection aortic size reveal most

patients who have suffered aortic dissection would not have been offered surgery based on guidelines.[14] Finally, the International Registry of Aortic Dissection also confirmed that size is a poor predictor of aortic dissection.[15] Given the mechanical endpoints of aortic dissection and rupture, measuring and understanding biomechanical changes associated with aneurysms is fundamental to identifying at-risk aortas. Biomechanical metrics may provide a more precise prediction model and optimize surgical selection.

The most commonly explored biomechanical metric is stiffness, quantified using the apparent modulus of elasticity or distensibility. Arterial distensibility has been linked to multiple disorders including diabetes and essential hypertension.[16, 17] In a small series, Lang et al. demonstrated the feasibility of measuring the modulus of elasticity of the descending aorta using transesophageal echocardiography.[18] An automatic border detection algorithm was used to calculate the radius, and M-mode was used to calculate aortic thickness while subclavian pulse wave tracing was used to generate instantaneous blood pressure tracing. The following equations were then used to calculate the incremental modulus of elasticity (σ = stress, P = pressure, E_{inc} = incremental modulus of elasticity, R_i = instantaneous inner radius, R_o = instantaneous outer radius, R_m = instantaneous midwall radius).

$$\sigma(t) = 2P(t) \times \left[\frac{R_i(t) \times R_o(t)}{R_m(t)}\right]^2 \times \left[{R_o}^2(t) - {R_i}^2(t)\right]^{-1}$$

$$E_{inc}(\sigma) = 0.75 \times R_m \times {d\sigma}/{dR_m}$$

Echocardiography measurements of the modulus of elasticity have evolved to use tissue doppler and velocity vector imaging.[19, 20] Other imaging modalities, specifically computed tomography

and magnetic resonance imaging have also been shown to be feasible for in vivo measurements of aortic stiffness.[21, 22]

Some investigators have used the ultimate tensile strength (UTS) as a surrogate marker for susceptibility for aortic rupture. Vorp et al., in a small series, subjected ex-vivo human ascending aortas to uniaxial tensile testing until failure.[23] The group found aneurysmal tissue to be 30% weaker than non-aneurysmal tissue. But whether extreme hypertension is the main mechanism of complications in aneurysmal aortas is debatable and this parameter requires ex-vivo measurements.

The Opening Angle (OA) is a measurement of circumferential residual stress. It is obtained by making a single radial cut on a ring of aorta. The aorta then tends to open up and the greater the angle of opening, the greater the amount of residual stress. Okamoto et al. found OA's to be higher in older patients but do not affect wall-averaged circumferential stress.[24] It is a property hindered by inherent measurement difficulties, questionable clinical relevance and in vivo translation.

After review of available literature, we identified two gaps in the available biomechanical metrics. Firstly, current metrics identify a specific point in the cardiac cycle, rather than use data derived from a full range of stresses. Secondly, current metrics do not address the pulsatility of aortic blood flow; the active diastolic, unloading phase of the cardiac cycle is disregarded. This phase is, however, paramount to the Windkessel function of the aorta as described previously. Therefore, we introduce a novel biomechanical parameter, 'energy loss', that integrates data across a full range of stresses producing a more stable metric, and one that compares both the loading and unloading phase of the cardiac cycle.

3. Article: Journal of Thoracic and Cardiovascular Surgery 2014; 148(3):1082-1089.

Preface

As described in the introduction, we identified gaps in the current metrics used for describing the biomechanics of the ascending aorta. We then developed a novel metric that does not arbitrary choose one point in the cardiac cycle, and one that compares the unloading curve to the loading curve in stress-strain relationships. Our systematic protocol to collect tissues from patients at the time of surgery has resulted in one of the largest series for this type of research. The importance of this study was recognized by a second place award in the American Association for Thoracic Surgery Resident Poster Competition 2013. This study was also a finalist for the C. Walt Lillehei Resident Forum at the American Association for Thoracic Surgery meeting in 2014. 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 field of cardiothoracic surgery. The following article has now been published and we hope that our message will reach clinicians and prompt more research.

Energy Loss, a Novel Biomechanical Parameter,

Correlates with Aortic Aneurysm Size and Histopathology

Jennifer Chung, MD[1]; Kevin Lachapelle, MD[1]; Evan Wener, BASc[3]; Raymond Cartier, MD[4]; Benoit De Varennes, MD[1]; Richard Fraser, MD, MSc[2]; Richard L. Leask, PhD[3,4]

[1]Department of Cardiac Surgery, [2]Department of Pathology, [3]Department of Chemical Engineering, McGill University, Montreal, QC, Canada, [4]Montreal Heart Institute, Montreal, QC, Canada

Funding: Heart and Stroke Foundation of Quebec

Corresponding Author:

Richard Leask, PhD

Wong Building, Rm 4120

3610 rue University Montreal Quebec Canada

Montreal, Quebec, H3A 0C5

Tel: 514.398.4270; Fax: 514.398.6678; Email: richard.leask@mcgill.ca

Word Count: 2606

3.1 Introduction

The catastrophic complications associated with aneurysms of the ascending aorta are preventable with elective surgery. However, identifying patients for surgery can be challenging. Present guidelines are based predominantly on size alone and on low levels of evidence.[9] A recent study found wide variations across Canada in what surgeons believe to be appropriate size criteria for bicuspid aortopathy.[13] Given the mechanical endpoints of aortic dissection and rupture, measuring and understanding biomechanical changes associated with aneurysms is fundamental to identifying at-risk aortas. Biomechanical metrics may provide a more precise prediction model and optimize surgical selection.

Currently, size is used as a surrogate biomechanical metric. Unfortunately, small aortas can experience complications while large aortas can remain stable. The IRAD database revealed aortic size to be a poor predictor of dissection.[15] Size fails to describe the quality of the aortic wall. To this end, many have quantified aortic stiffness using the apparent modulus of elasticity extracted from ex-vivo mechanical testing or estimated from in-vivo imaging.[25-27] Aneurysmal tissue is stiffer than normal aortas.[25,28] However, increased stiffness alone fails to explain why aneurysms have a propensity for complications rather than greater resilience. Moreover, the non-linear material properties of the aorta make the apparent modulus dependent on the level and rate of strain experienced by the aorta. Ultimate tensile strength usually exceeds physiological limits and is only measureable ex-vivo.[29,30]

We examined the biomechanical parameter energy loss, defined as hysteresis normalized to total stored energy, and its relationship to aortic size and histopathology. This parameter draws from the Windkessel function of the ascending aorta, which is to expand and act as a capacitor during

systole and to recoil and return the stored energy to the circulation during diastole. Greater energy loss represents greater inefficiency in performing this function and greater energy dissipated into the aortic wall. This could lead to eventual aortic remodeling and failure. Unlike the discrete nature of the apparent modulus of elasticity, which uses an arbitrary point for measurement, the energy loss parameter integrates the stress-strain relationship of the tissue over the entire cycle making it a more stable metric.

In a first step towards validating a clinically measurable biomechanical risk factor for aneurysmal aortas, we have measured the energy loss and apparent modulus of ascending aortic tissue ex-vivo. We hypothesized that energy loss is linked to aortic size, that the structural compositional changes in aneurysmal aortic walls are associated with increased energy loss, and that energy loss can be used to identify aortas with pathological histological changes.

3.2 Patients and Methods

Excised aneurysmal ascending aortic tissue was obtained at the time of surgery from July 2012 to May 2013 at McGill University Health Centre and Montreal Heart Institute. Control ascending aortic tissue was obtained from heart transplant donors and autopsy patients without heart or aortic disease. Research ethics board approval was obtained at both hospitals.

3.2.1 Biaxial tensile testing

Testing was done within 24 hours of tissue collection and specimens were kept on ice until testing was completed. All samples were collected as intact rings with orientation marked by a single clip. Four 1.5 cm by 1.5 cm squares were collected along the belly of the aneurysm. Each square underwent uni- and equibiaxial tensile testing at 37 °C in a Ringers Lactate solution to a maximum of 60% strain (EnduraTEC ELF 3200, Bose Co., Framingham, USA). We have

previously shown that in healthy pig ascending aortic tissue, SMC activation with phenylephrine does effect the stiffness of the tissue, however the contribution is limited at high strains to less than 10% of stiffness increase.[5] For consistency, smooth muscle cells were not activated in our experiments. Ten preconditioning loops were completed prior to the test loops, which were done in triplicate. Both engineering and true stress via optical tracking were measured.

Analysis was performed using MatLab R2012a (MathWorks, Massachusetts, USA). Both the engineering stress and true-stress –strain curves were modelled using polynomial functions. The apparent modulus of elasticity was calculated at 40% and 50% strain as an approximation of physiological loading conditions. We measured the percent of energy lost between the loading and unloading curves, the 'Energy Loss', to quantify the viscoelastic nature of the aorta (Figure 1). Loading and unloading of aortic tissue produces a hysteresis loop in the stress-strain curve. The area of this loop has units of energy. Once hysteresis is normalized by dividing by the total energy applied during loading (the integral of the loading stress-strain curve), this yields a unitless metric termed 'Energy Loss'. The data and figures represented here consistently use strain in the axial direction during equi-biaxial testing, and engineering stress.

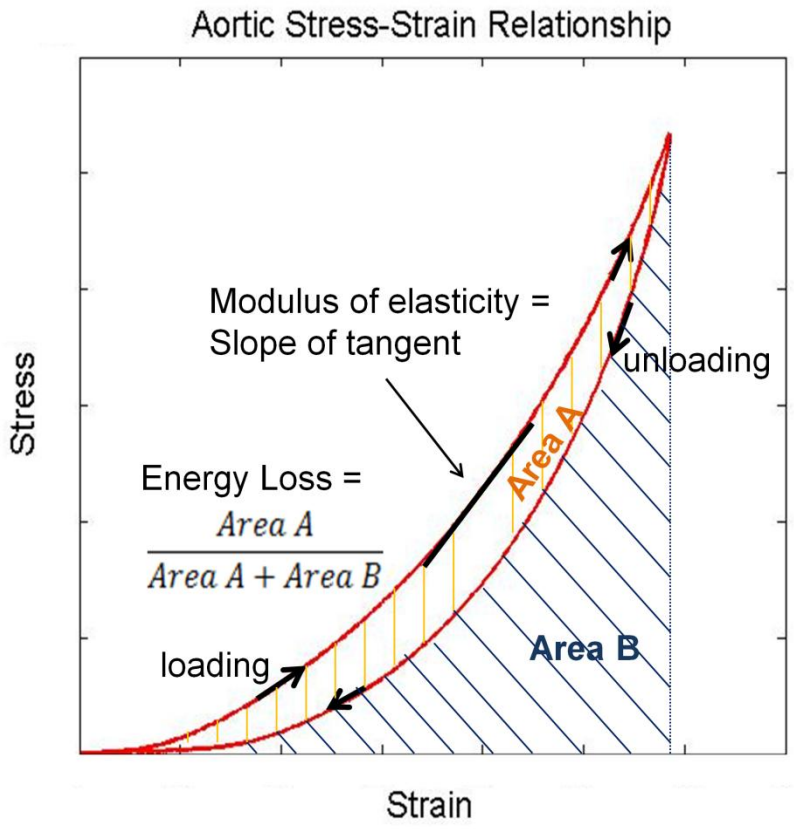

Figure 1: The stress-strain relationship of the ascending aorta, and definitions of two biomechanical parameters: apparent modulus of elasticity and percent energy loss per cycle.

3.2.2 Histology

Samples immediately adjacent to each square were stored in 10% formalin, and underwent bulk Movat Pentachrome staining. Images were taken in three different areas per slide, giving a total of twelve images per aorta. All images were renamed and analyzed in a blinded fashion. The percent collagen, elastin and mucopolysaccharides were quantified using ImageJ 1.46r (National Institutes of Health, USA).

3.2.3 Data Analysis

All averages were represented as mean ± standard deviation. Continuous variables were compared using the Mann Whitney U test. Correlations were calculated using liner regression. Body surface area was calculated using the Dubois formula below (BSA: body surface area (m^2), H: height (m), W: weight (kg)).

$$BSA = 0.20247 \times H^{0.725} \times W^{0.425} \quad \ldots(1)$$

Statistics were performed with GraphPad Prism 5.01 (GraphPad Software, Inc, CA, USA).

3.3 Results

Forty-one ascending aortas were collected in total, including 34 aneurysmal aortas and 7 control aortas (Table 1). We found statistically significant differences in biomechanical properties between aneurysmal and control aortas. Aneurysmal tissue, with an mean energy loss of 34 ± 4%, exhibited significantly greater energy loss than control tissue, with a mean of 28 ± 2% (p=0.0005). Aneurysmal tissue also exhibited significantly greater apparent modulus of elasticity at 40% strain than control tissue (aneurysmal: 0.15 ± 0.03MPa, control: 0.10±0.03 MPa, p=0.0007). When separating the aneurysmal aorta between tricuspid and bicuspid valves, no difference was found in the apparent modulus of elasticity between the two valve types (p=0.77), while bicuspid valves exhibited less energy loss than tricuspid valves (p=0.01).

Table 1: Patient demographics.

	Control (n=7)	Aneurysm (n=34)
Age (years)	51 ± 18	64 ± 12
Male	6 (86%)	23 (68%)
Tricuspid Aortic Valves	7 (100%)	12 (35%)
Bicuspid Aortic Valves	0	20 (59%)
Mechanical Valves	0	2 (6%)
Aortic Size (cm)	2.4 ± 0.4	5.2 ± 0.7

We explored the relationship between biomechanical parameters and aortic size. There was a trend towards greater apparent modulus of elasticity, or aortic stiffness, with increasing aortic size (40% strain: $p=0.001$, $r^2=0.17$; 50% strain: $p=0.0002$, $r^2=0.30$). However, the trend disappeared once aortic size was indexed to body surface area (40% strain: $p=0.82$, $r^2=0.002$; 50% strain: $p=0.07$, $r^2=0.11$). Energy loss and aortic size significantly co-varied with greater energy loss seen in larger aortas ($p<0.0001$, $r^2=0.60$). Figure 2 demonstrates that when aortic size increased beyond 5.5 cm, energy loss escalated more rapidly with greater variability. When separating the samples between bicuspid and tricuspid aortic valve types, it appears that both valve types fall on the same curve.

The rapid rise and greater variability in energy loss beyond 5.5 cm in aortic size resulted in a group of aneurysmal aortas with wide range of energy loss indistinguishable by aortic size. Their histology was examined and advanced medial degeneration was found in aortas with elevated

energy loss whereas normal aortic wall architecture was found in aortas with lower energy loss and the same aortic size (Figure 3).

The relationship between energy loss and aortic size remained once size was indexed to body surface area (Figure 4). Indexing aortic size accounts for the concept that a 6 cm aorta is not the same in a large individual as compared to a very small individual. Interestingly, indexing resulted in a stronger more linear relationship with energy loss ($p<0.0001$, $r^2=0.78$). Notably, one can still appreciate a clear step up in energy loss beyond an indexed aortic size of 3.25 cm/m^2. Despite indexing aortic size, one sample clearly fell far from the trend-line. Histology revealed extremely disorganized medial degeneration in this aorta (Figure 4a). Another aorta of approximately the same age and indexed aortic size demonstrated uncompromised histology with orderly elastin sheets and preserved smooth muscle cells.

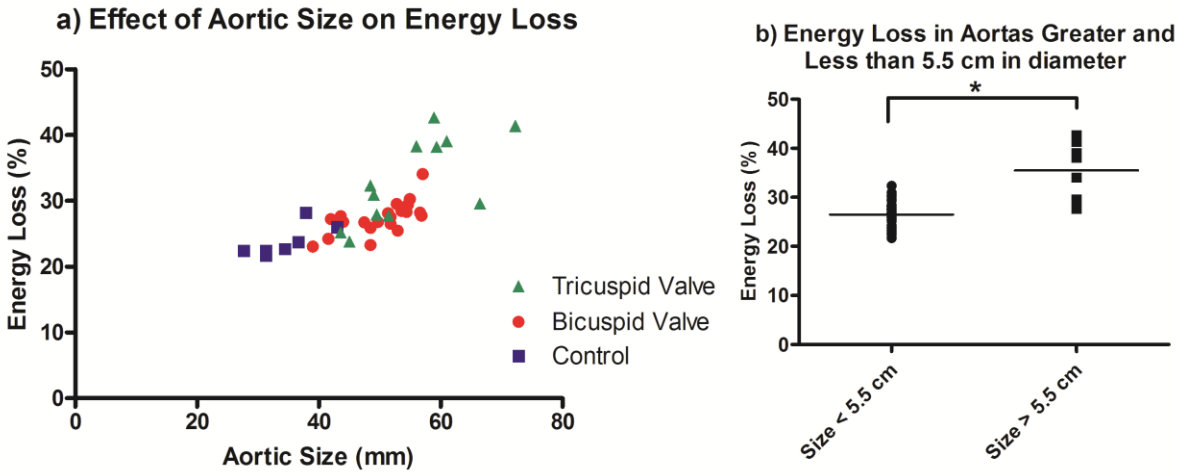

Figure 2: The effect of aortic diameter on energy loss. Energy loss rapidly increases with greater variability for aortic diameters greater than 55 mm. The average energy loss in aortas less than 55 mm is 26.5±2.7%, and 35.5±5.8% in aortas greater than 55 mm (p=0.0001).

Next, we quantified changes in the underlying histology among a subset of the aneurysmal aortas by colourimetrically separating components of the aortic wall into elastin, collagen, smooth muscle cells and mucopolysaccharides. Individual components as well as the elastin to collagen ratio were compared to aortic size, energy loss and modulus of elasticity. Increasing levels of energy loss and increasing aortic size correlated significantly with increasing proportions of collagen (energy loss: $p=0.004$, $r^2=0.33$; size: $p=0.009$, $r^2=0.27$) and decreasing proportions of elastin (energy loss: $p=0.003$, $r^2=0.34$; size: $p=0.009$, $r^2=0.27$). The best correlations were found with increasing collagen to elastin ratio (energy loss: $p=0.0002$, $r^2=0.49$; size: $p=0.0007$, $r^2=0.42$). There was no statistically significant relationship between the modulus of elasticity and aortic wall composition. Mucopolysaccharide content did not correlate with aortic size or either of the biomechanical parameters.

3.4 Discussion

The exact mechanisms of non-traumatic aortic complications are not well delineated. Generally, larger aortic sizes are associated with higher risks of dissection and rupture. Thus clinicians have used aortic size almost exclusively to gauge the need for prophylactic surgery. This is despite great heterogeneity in growth rates and lack of clarity in the natural history of ascending aortic aneurysms. Ascending aortic dissection and rupture occur when the mechanical integrity of the aorta is overcome by stresses on the wall. Aortic size is not a material property and is an inadequate surrogate for wall stresses. Therefore, biomechanics can provide mechanistic insight into aortic aneurysm complications and more sophisticated patient selection, reducing exposure to the real risks of even elective surgery.

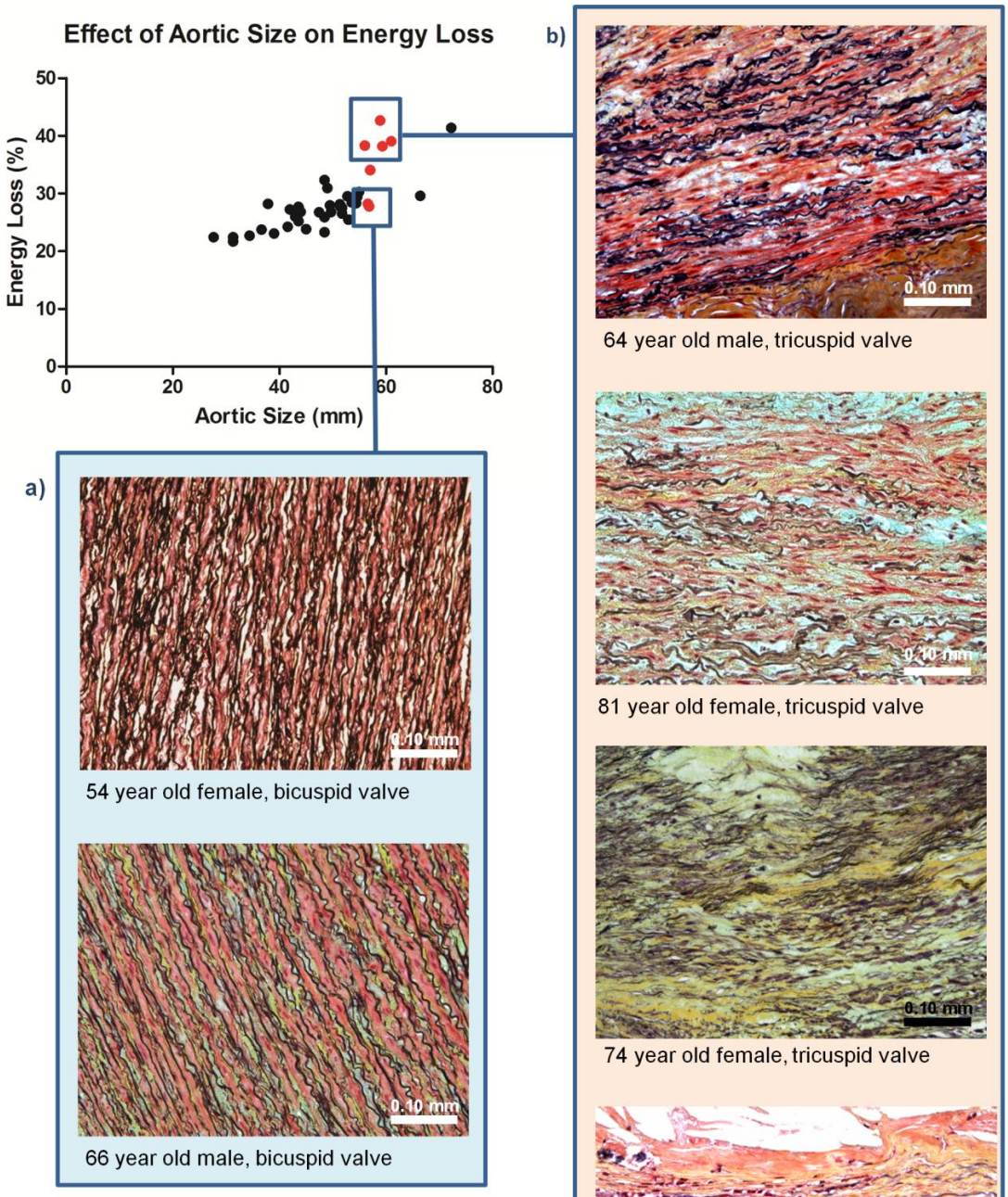

Figure 3: Above 5.5 cm in size, energy loss rapidly rises with greater variability. The red dots represent aortas indistinguishable by aortic size, yet with a wide range in energy loss. Aortas with low energy loss, box a), display normal histology with preserved elastic laminae (brown to black) and intact smooth muscle cells (red to purple). On the other hand, aortas with elevated energy loss, box b), display fragmentation of elastin, loss of smooth muscle cells, and replacement with collagen (yellow).

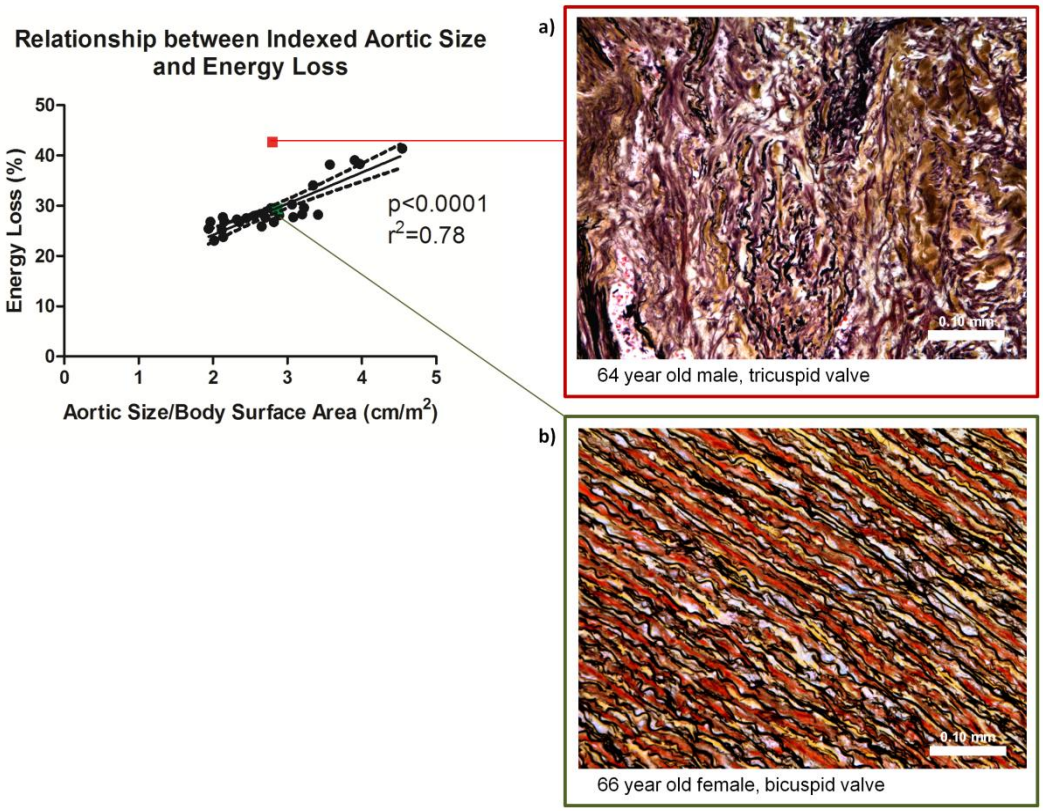

Figure 4: Once aortic size is indexed to BSA, the relationship between size and energy loss linearizes, although a step up is still appreciated after 3.25 cm/m^2. Energy loss pulled one sample off the trendline conspicuously as an outlier (red square). Box a) shows the abnormal histology of the outlying sample: fragmented elastin (black), disorganized smooth muscle cells (purple), and large amounts of disorganized collagen (yellow/brown). The green square represents a sample with a similar indexed aortic size, and box b) shows the corresponding histology with orderly elastic lamellae, orderly smooth muscle cells and only moderate amounts of collagen.

Ex vivo biaxial tensile testing as performed here is the ideal experimental setting. The stress-strain characteristics of the tissue can be obtained under controlled loading or displacement over a wide range of amplitudes and frequencies without interference of surrounding tissue. Currently available medical imaging provides estimates of strain over the limited uncontrolled displacement of the cardiac cycle. Stresses are rough estimates based on blood pressure and relies on many assumptions of the vessel material properties. Although in vivo measures have shown good co-variance with patient age and aortic diameter,[25-27,31,32] they have never been validated against the tissue material properties of the ascending aorta.

Aortic stiffness, characterized by apparent modulus of elasticity, is an intuitive parameter thought to be one of the earliest markers of vessel disease,[33] previously described in association with essential hypertension and aneurysms.[29,16,23] However, how it is related mechanistically to complications is unclear. Also, its non-linear variation with strain presents significant difficulties in comparing between studies and between patients, and in providing meaningful in vivo measurements. In our study, apparent modulus of elasticity demonstrated no correlation with the underlying histopathology.

Energy loss is by its definition a more stable robust variable less susceptible to noise. Potentially, its simplicity can make in vivo measurements more feasible and comparisons between studies and patients easier as well. As a normalized measurement of hysteresis per cycle of loading and unloading, it represents the viscous component of the aortic wall. We found by analyzing histology that unlike apparent modulus of elasticity, energy loss did reflect the structural makeup of the aortic tissue, especially the collagen to elastin ratio. Poor efficiency in returning energy when cycled was associated with more advanced medial degeneration and higher collagen to elastin ratios. Whether it acts as a stimulus for vessel wall remodeling or is a by-product of

remodeling is unknown. However, if the amount of energy absorbed by the aortic wall exceeds the aorta's ability for repair, it would ultimately render the material more prone to failure.

The calculation of energy loss is completely independent of aortic size, and yet a highly significant association was found between the two properties ($p<0.0001$). We demonstrated that at the high end of aortic size, aortas exhibit a rapid rise in levels of energy loss. Further examination showed that aortas with greater energy loss had much more medial degeneration than their counterparts of the same size.

By indexing aortic size to body surface area, we achieved greater linearization of the relationship between energy loss and aortic size ($r^2=0.78$). These results provide biomechanical support for the concept that indexed size may be more important than size itself.[34] This is in contrast to the traditional parameter of apparent modulus of elasticity, which did not have any relationship to indexed aortic size. The step-up in energy loss seen at 3.25 cm/m^2, or its rapid rise beyond 5.5 cm, may point to the mechanism behind the hinge-point in complication rates with increasing aortic size.[35]

An important finding was that energy loss was able to clearly separate an aortic sample with very pathological changes seen on histology. The energy loss was well off the curve expected given either size or indexed size, and the histological changes were not seen in aortas of the same size. The severity of disease is unlikely to have been identified based on the age, gender and valve type of this 64 year-old male patient with a tricuspid valve. These results suggest that energy loss may provide additive value to examining aortic size alone. Upon examination of Figure 2, one may hypothesize a level of 30% in energy loss to represent a cut-off other than size to recommend intervention. If such a cut-off was used, 18% of the aneurysmal aortas in our cohort

would not have been removed. Clearly, it is unknown what the clinical consequences of that would be at this time. It is not certain if and how the pathological changes we observed would translate into aortic dissection. However, a contemporary case series of aortic dissection note the importance of subadventitial collagen hyperplasia, offering some merit to the importance of increased collagen to elastin ratio.[36]

Our results suggest that aneurysms associated with bicuspid aortic valves have similar energy loss to tricuspid valves at any given aortic size**.** Other studies on surgical specimens have found biomechanical differences between the two valve types in terms of stiffness, delamination strength and tensile strength.[37,38] We also found a difference in energy loss between bicuspid and tricuspid valves ($p=0.02$, data not shown), but when once plotted against aortic size, the two valve types fell on the same curve. Certainly there is a high incidence of aneurysms among patients with bicuspid aortic valves,[39] and compelling hemodynamic data exists on how wall stress is different in bicuspid aortopathy.[40] Still, whether a smaller aortic size is more significant in this population is debatable,[41,42] as reflected by changes in the 2014 American Heart Association/American College of Cardiology valve guidelines which raised the threshold for intervention for bicuspid aortopathy from 5.0 cm to 5.5 cm.[43] Aortopathy among patients with bicuspid aortic valves exhibit heterogeneity,[44] and not all patients may merit early surgery. Energy loss may tease out those who fall off the normal curve.

Our future objectives include establishing a non-invasive method of measuring metrics such as energy loss in vivo. We are currently validating both pre-operative MRI and intra-operative TEE data with our ex-vivo data using previously described method.[26,45] Also, the genetic and biochemical factors that contribute to elevated energy loss need further exploration. We envision

refining risk stratification and surgical selection of candidates for ascending aortic aneurysm repair through the addition of simple biomechanical parameters to aortic size.

3.5 Conclusions

A highly significant association was found between energy loss and aortic diameter, with aortas exhibiting a rapid rise in energy loss levels at the high end of aortic size. Aortas with greater energy loss had much more medial degeneration than their counterparts of the same size. In fact, energy loss had a significant association with the underlying histological make-up of the aorta. By demonstrating a measurable link between aortic function and structure, energy loss may provide an additional tool in distinguishing aortas at risk for complications.

4. Summary and Conclusions

We defined a novel biomechanical parameter, "energy loss", based on the ascending aorta's physiological role as a capacitor and hypothesized that decreased efficiency in performing this role would be linked to structural changes associated with disease (specifically aneurysmal disease). Experiments on ex-vivo human ascending aortic tissue confirm the difference in biomechanical properties between aneurysmal and normal aorta, i.e. aneurysmal aortas demonstrate greater apparent modulus of elasticity and energy loss. Energy loss demonstrated good correlation with histology, especially the collagen to elastin ratio. Most significantly, elevated levels of energy loss appear to be able to identify aortas with highly pathological changes in histology.

5. Future Directions

Our study suggests that energy loss provides incremental information about the pathological state of ascending aortas. In order to determine whether use of energy loss would result in superior risk stratification, the new metric needs to be clinically applicable. The first step would be to provide a reproducible algorithm for determining energy loss using available medical imaging. Thus we plan to incorporate magnetic resonance imaging and transesophageal echocardiography into our data collection protocol. Validation of a non-invasive imaging modality for calculating energy loss enables more wide spread use of the metric and population level based studies. We will also continue data collection and mechanical testing as in the current study, as a larger sample size would make it possible to analyze patient and biochemical factors related energy loss.

6. References

1. Belz GG. Elastic properties and Windkessel function of the human aorta. *Cardiovascular drugs and therapy / sponsored by the International Society of Cardiovascular Pharmacotherapy.* 1995;9:73-83.
2. Humphrey JD. *Cardiovascular solid mechanics: cells, tissues, and organs*. Springer; 2002.
3. Lasheras JC. The Biomechanics of Arterial Aneurysms. *Annual Review of Fluid Mechanics.* 2007;39:293-319.
4. Humphrey JD, Holzapfel GA. Mechanics, mechanobiology, and modeling of human abdominal aorta and aneurysms. *Journal of biomechanics.* 2012;45:805-14.
5. Tremblay D, Cartier R, Mongrain R, Leask RL. Regional dependency of the vascular smooth muscle cell contribution to the mechanical properties of the pig ascending aortic tissue. *Journal of biomechanics.* 2010;43:2448-51.
6. Lehmann ED. Terminology for the definition of arterial elastic properties. *Pathologie-biologie.* 1999;47:656-64.
7. Haskett D, Johnson G, Zhou A, Utzinger U, Vande Geest J. Microstructural and biomechanical alterations of the human aorta as a function of age and location. *Biomech Model Mechanobiol.* 2010;9:725-36.
8. Lemaitre J, Lippmann H. *A course on damage mechanics*. Springer Berlin; 1996.
9. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE, Jr., 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:e266-369.

10. Clouse WD, Hallett JW, Jr., Schaff HV, Gayari MM, Ilstrup DM, Melton LJ, 3rd. Improved prognosis of thoracic aortic aneurysms: a population-based study. *JAMA : the journal of the American Medical Association.* 1998;280:1926-9.
11. Isselbacher EM. Thoracic and abdominal aortic aneurysms. *Circulation.* 2005;111:816-28.
12. Elefteriades JA, Farkas EA. Thoracic aortic aneurysm clinically pertinent controversies and uncertainties. *Journal of the American College of Cardiology.* 2010;55:841-57.
13. Verma S, Yanagawa B, Kalra S, Ruel M, Peterson MD, Yamashita MH, et al. Knowledge, attitudes, and practice patterns in surgical management of bicuspid aortopathy: A survey of 100 cardiac surgeons. *The Journal of thoracic and cardiovascular surgery.* 2013;146:1033-40 e4.
14. Rylski B, Branchetti E, Bavaria JE, Vallabhajosyula P, Szeto WY, Milewski RK, et al. Modeling of predissection aortic size in acute type A dissection: More than 90% fail to meet the guidelines for elective ascending replacement. *The Journal of thoracic and cardiovascular surgery.* 2014;148:944-8 e1.
15. Pape LA, Tsai TT, Isselbacher EM, Oh JK, O'Gara P T, Evangelista 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;116:1120-7.

16. Isnard RN, Pannier BM, Laurent S, London GM, Diebold B, Safar ME. Pulsatile diameter and elastic modulus of the aortic arch in essential hypertension: a noninvasive study. *Journal of the American College of Cardiology.* 1989;13:399-405.

17. Giannattasio C, Mancia G. Arterial distensibility in humans. Modulating mechanisms, alterations in diseases and effects of treatment. *Journal of hypertension.* 2002;20:1889-99.

18. Lang RM, Cholley BP, Korcarz C, Marcus RH, Shroff SG. 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;90:1875-82.

19. Vitarelli A, Giordano M, Germano G, Pergolini M, Cicconetti P, Tomei F, et al. Assessment of ascending aorta wall stiffness in hypertensive patients by tissue Doppler imaging and strain Doppler echocardiography. *Heart.* 2010;96:1469-74.

20. Kim KH, Park JC, Yoon HJ, Yoon NS, Hong YJ, Park HW, et al. Usefulness of aortic strain analysis by velocity vector imaging as a new echocardiographic measure of arterial stiffness. *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography.* 2009;22:1382-8.

21. Metafratzi ZM, Efremidis SC, Skopelitou AS, De Roos A. The clinical significance of aortic compliance and its assessment with magnetic resonance imaging. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance.* 2002;4:481-91.

22. Ahmadi N, Nabavi V, Hajsadeghi F, Flores F, Azmoon S, Ismaeel H, et al. Impaired aortic distensibility measured by computed tomography is associated with the severity of coronary artery disease. *The international journal of cardiovascular imaging.* 2011;27:459-69.

23. Vorp DA, Schiro BJ, Ehrlich MP, Juvonen TS, Ergin MA, Griffith BP. Effect of aneurysm on the tensile strength and biomechanical behavior of the ascending thoracic aorta. *The Annals of thoracic surgery.* 2003;75:1210-4.

24. Okamoto RJ, Xu H, Kouchoukos NT, Moon MR, Sundt TM, 3rd. The influence of mechanical properties on wall stress and distensibility of the dilated ascending aorta. *The Journal of thoracic and cardiovascular surgery.* 2003;126:842-50.

25. Koullias G, Modak R, Tranquilli M, Korkolis DP, Barash P, Elefteriades JA. Mechanical deterioration underlies malignant behavior of aneurysmal human ascending aorta. *J Thorac Cardiovasc Surg.* 2005;130:677-83.

26. Oishi Y, Miyoshi H, Mizuguchi Y, Iuchi A, Nagase N, Oki T. 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;57:354-9.

27. Petrini J, Jenner J, Rickenlund A, Eriksson P, Franco-Cereceda A, Caidahl K, et al. Elastic Properties of the Descending Aorta in Patients with a Bicuspid or Tricuspid Aortic Valve and Aortic Valvular Disease. *J Am Soc Echocardiogr.* 2014.

28 Choudhury N, Bouchot O, Rouleau L, Tremblay D, Cartier R, Butany J, et al. Local mechanical and structural properties of healthy and diseased human ascending aorta tissue. *Cardiovasc Pathol.* 2009;18:83-91.

29. Khanafer K, Duprey A, Zainal M, Schlicht M, Williams D, Berguer R. Determination of the elastic modulus of ascending thoracic aortic aneurysm at different ranges of pressure using uniaxial tensile testing. *J Thorac Cardiovasc Surg.* 2011;142:682-6.

30. Martin C, Sun W, Pham T, Elefteriades J. Predictive biomechanical analysis of ascending aortic aneurysm rupture potential. *Acta Biomater.* 2013;9:9392-400.

31. Oishi Y, Miyoshi H, Iuchi A, Nagase N, Ara N, Oki T. Negative impact of cardiovascular risk factors on left atrial and left ventricular function related to aortic stiffness--new application of 2-dimensional speckle-tracking echocardiography. *Circ J.* 2013;77:1490-8.

32. Petrini J, Yousry M, Rickenlund A, Liska J, Hamsten A, Eriksson P, et al. The feasibility of velocity vector imaging by transesophageal echocardiography for assessment of elastic properties of the descending aorta in aortic valve disease. *J Am Soc Echocardiogr.* 2010;23:985-92.

33. Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. *J Am Coll Cardiol.* 2011;57:1511-22.

34. Davies RR, Gallo A, Coady MA, Tellides G, Botta DM, Burke B, et al. Novel measurement of relative aortic size predicts rupture of thoracic aortic aneurysms. *Ann Thorac Surg.* 2006;81:169-77.

35. Elefteriades JA. Natural history of thoracic aortic aneurysms: indications for surgery, and surgical versus nonsurgical risks. *Ann Thorac Surg.* 2002;74:S1877-80; discussion S92-8.

36. Schmitto JD, Popov AF, Coskun KO, Friedrich M, Sossalla S, Didilis V, et al. Morphological investigations of type A aortic dissection. *Ann Thorac Cardiovasc Surg.* 2010;16:331-4.

37. Pasta S, Phillippi JA, Gleason TG, Vorp DA. Effect of aneurysm on the mechanical dissection properties of the human ascending thoracic aorta. *J Thorac Cardiovasc Surg.* 2012;143:460-7.

38. Pichamuthu JE, Phillippi JA, Cleary DA, Chew DW, Hempel J, Vorp DA, et al. Differential tensile strength and collagen composition in ascending aortic aneurysms by aortic valve phenotype. *Ann Thorac Surg.* 2013;96:2147-54.

39. Siu SC, Silversides CK. Bicuspid aortic valve disease. *J Am Coll Cardiol.* 2010;55:2789-800.

40. Pasta S, Rinaudo A, Luca A, Pilato M, Scardulla C, Gleason TG, et al. Difference in hemodynamic and wall stress of ascending thoracic aortic aneurysms with bicuspid and tricuspid aortic valve. *J Biomech.* 2013;46:1729-38.

41. Tzemos N, Therrien J, Yip J, Thanassoulis G, Tremblay S, Jamorski MT, et al. Outcomes in adults with bicuspid aortic valves. *JAMA.* 2008;300:1317-25.

42. Michelena HI, Desjardins VA, Avierinos JF, Russo A, Nkomo VT, Sundt TM, et al. Natural history of asymptomatic patients with normally functioning or minimally dysfunctional bicuspid aortic valve in the community. *Circulation.* 2008;117:2776-84.

43. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP, 3rd, Guyton RA, et al. 2014 AHA/ACC Guideline for the Management of Patients With Valvular Heart Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol.* 2014.

44 Fedak PW. Bicuspid aortic valve syndrome: heterogeneous but predictable? *Eur Heart J.* 2008;29:432-3.

45. Fattori R, Bacchi Reggiani L, Pepe G, Napoli G, Bna C, Celletti F, et al. Magnetic resonance imaging evaluation of aortic elastic properties as early expression of Marfan syndrome. *J Cardiovasc Magn Reson.* 2000;2:251-6.