Evaluation of aortic wall strength in HIV associated thoracic ascending aortic aneurysm

By

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Declaration

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Article 1: Title: ... ***Evaluation of aortic wall strength in HIV associated thoracic ascending aortic aneurysm: A pilot study***

The above article has been submitted to European journal of Cardio-Thoracic surgery for publication (outcome pending)

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Comments by primary supervisor:
The student has contributed sufficiently to the article content.

Dedication

To my mother Daphne Mokotjo and sister Botshabelo Mokotjo.

Presentations:

1. Abstract submitted and accepted by South African Heart Association Congress 2021 as a poster presentation. The abstract was published in South African heart association Journal, 2021, Nov issue.

Publication

1. Evaluation of aortic wall strength in HIV associated thoracic ascending aortic aneurysm: A pilot study submitted to Interactive Cardiovascular and Thoracic Surgery.

CHAPTER ONE: SUBMISSIBLE ARTICLE

Evaluation of aortic wall strength in HIV associated thoracic ascending aortic aneurysm: A pilot study

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LIST OF ABBREVIATIONS:

AAA= Abdominal aortic aneurysm

CHBAH= Chris Hani Baragwanath Academic Hospital

CMJAH= Charlotte Maxeke Johannesburg Academic Hospital

CT= Computed Tomography

HAART= Highly active antiretroviral therapy

HIV= Human Immunodeficiency Virus

TAAA= Thoracic ascending aortic aneurysm

Introduction

Human immunodeficiency virus (HIV) is considered to be a global pandemic which is associated with a multisystem disease [1]. The cardiovascular system is not spared. The relationship between HIV and the vascular system is unique in that it presents as aneurysms which have the propensity to dissect or rupture; and can also present as occlusive diseases [2]. It is thought to be due to an inflammatory process where leucocytoclastic vasculitis converges with the vasa vasorum [3]. Involvement of the large vessel in HIV vasculitis is uncommon and affects younger patients [2,4].

Elastin and collagen are known mechanical components of the aorta in that they aid with tensile strength. Aneurysms develop due to weakness of the segmental wall of the artery [5]. For a long time, loss of elastin and alterations of mucoid content were traditionally a trademark in the cause of aneurysms [6]. Surprisingly, collagen as a pivotal structural protein has been poorly studied as a cause of aneurysms. However, it has been shown that weakness in the aorta can be due to impaired collagen metabolism especially in rare conditions like Ehlers-Danlos [7]. The data is limited however to abdominal aortic aneurysms (AAA). No data is available on HIV thoracic ascending aortic aneurysms (TAAA).

We hypothesized that there might be a difference in collagen quantity between aneurysmal and non-aneurysmal aortic walls as a surrogate marker for aortic wall strength and therefore we aimed to investigate collagen quantity in HIV infected patients on highly active antiretroviral therapy (HAART) presenting with TAAA.

Materials and Methods

Ethics approval for the study was obtained from the Human Research Ethics Committee (Medical) at the University of the Witwatersrand, Johannesburg on the 10/08/2018, (M180368).

This was a prospective pilot study, which consecutively enrolled twelve HIV infected patients, on HAART above the age of 18 years who presented for elective TAAA surgery from the period 2019-2021 at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) Cardiothoracic department. We excluded patients with known underlying cardiovascular diseases (e.g., Myocardial infarction, pericarditis) or connective tissue diseases (e.g., Marfan syndrome) and patients presenting for emergency surgery including aortic dissection (Figure 1). This study was an extension of an already ongoing clinical study being performed at the Department of Cardiology, Chris Hani Baragwanath Academic Hospital (CHBAH) (M170389).

The patients underwent review and detailed echocardiography (Figure 2A and Figure 2B) at CHBAH by an experienced cardiologist. Aortic measurements were obtained as per standard guidelines on aorta measurements and chamber quantification [8]. Demographic and clinical findings of these patients were recorded. Once an assessment of TAAA was made the same patients underwent Computed Tomography (CT) as per standard protocols (Figure 3) [9]. Patient's demography, clinical, laboratory, echocardiography and CT angiography were captured on a Redcap data base and Microsoft Excel 2016.

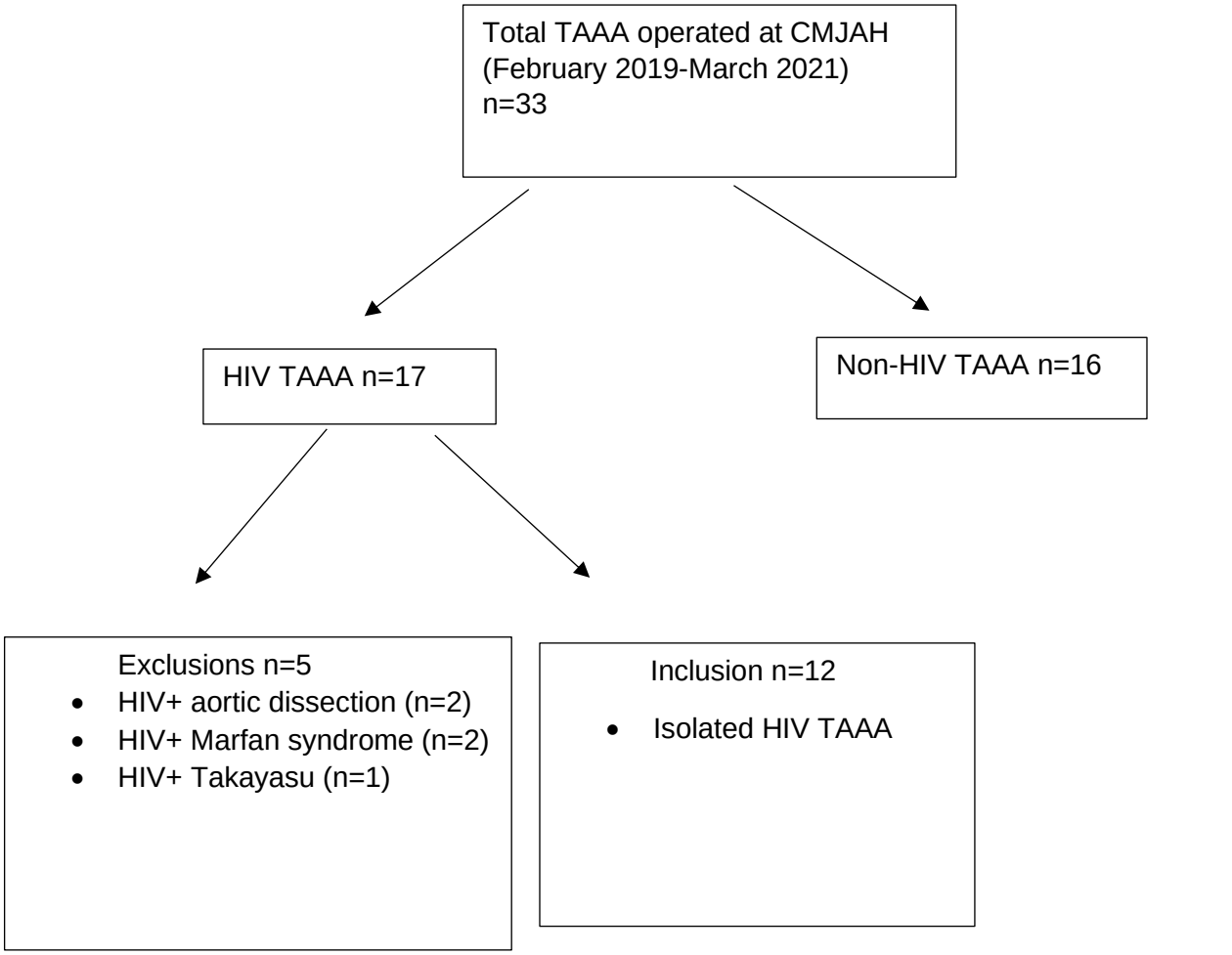

Figure 1: Flow diagram depicting inclusion and exclusion criteria for the study. Abbreviations: CMJAH- Charlotte Maxeke Johannesburg Academic Hospital, HIV- Human immunodeficiency virus, TAAA- Thoracic ascending aortic aneurysm.

Aortic sampling and analysis

The patients were operated, and the common operative procedure was a composite graft for six (50%) patients (Figure 4 and Figure 5A&B). Valve sparing aortic root replacement (Figure 5C) was done in two, sinus of Valsalva aneurysm repair and aortic valve replacement in one patient. Three patients had concomitant ascending aorta and aortic arch aneurysmal disease. Patient one received composite graft and frozen elephant trunk, patient 2 received interposition graft and frozen elephant trunk

and in patient three valve sparring aortic root replacement and frozen elephant trunk was done.

Patients underwent thoracic aneurysmal aortic surgery as detailed above and at surgery fresh aortic tissue sampling was done of the aneurysmal aorta, seemingly normal aorta 1cm downstream of diseased aorta and of the aortic valve leaflets. The specimens were taken to the School of Physiology, Witwatersrand University - stored frozen, to determine collagen content of the seemingly normal and aneurysmal aorta estimated by the hydroxyproline assay and the Department of Histopathology National Health Laboratory Service and National Institute for Occupational Health stored in formalin, for routine clinical histopathological assessment.

Hydroxyproline concentrations of tissue obtained from aneurysmal aorta, seemingly normal aorta and aortic valve leaflets were analysed. Samples of aortic tissue were stored frozen at -70°C for tissue analysis. The tissues were weighed, sealed under vacuum, and hydrolysed with hydrochloric acid at 107ºC for 18 hours. Sealed glass tubes were opened and dry hydrolysed by blowing off hydrochloric acid under nitrogen. Determination of Hydroxyproline concentration was by the method of Stalder and Stegemann (1967) [10] where the reagents chloramine T and para- Dimethyl-amino-benzaldehyde were added and the samples placed in a water bath at 60ºC for 25 min. The change in colour represented the amount of hydroxyproline present, which was measured with a spectrophotometer at 550nm against the standard curve. Standards using trans-4-hydroxyproline (µg/ml) were made. From the standard curves we determined the hydroxyproline concentration of samples in µg/mg (original weight of tissue hydrolysed) [11,12].

Statistical analyses

Statistical analysis was carried out using Microsoft excel 2016. Simple descriptive statistics was used to analyse demographic, clinical, echocardiographic, and computed tomography data. Continuous data was represented as means or median and standard deviation. The P- value was set at $P<0.05$ to indicate statistical significance. Comparison between the normal aortic tissue and abnormal TAAA wall tissue with regards to hydroxyproline concentration was analysed via an unpaired t-test. For comparisons between aneurysmal vs normal aortic tissue vs aortic valve leaflets, a one-way ANOVA was done.

Results

The clinical, demographic, echocardiographic and computed tomography findings are summarised in Table 1, Figure 2, Figure 3. The study included 12 patients (75% females, mean age of 47 ±10 years and all of African ethnicity). They were all HIV reactive on HAART, CD4 count 560 ±192 cells/uL and were virally suppressed. Hypertension was the most common comorbidity (66.6% of the patients). All patients had TAAA and 3 had concurrent aortic arch involvement. Ninety one percent (n=11) had severe aortic regurgitation with mean ascending aortic size of 60 ±8mm and preserved ejection fraction 53 ±8%.

Table 1: Demographic, clinical, echocardiography and CT findings of the study patients.

Variable	Study patients (n=12)
Age (years)	47 ± 10
Gender, n (%)	
Females	9 (75%)
Males	3 (25%)
Race, n (%)	
African	12(100%)
Body surface area (m^2)	1.7 ±0.1
Body mass index (kg/m^2)	26 ± 4
Vitals	
Heart rate (bpm),	75 ± 13
Systolic blood pressure (mmHg),	136 ± 23
Diastolic blood pressure (mmHg)	57 ± 13
Co-morbidities n (%)	
Hypertension	8 (66.6%)
Chronic Kidney Injury	1 (8.3%)
Medication n (%)	
HAART (FDC)	10 (83.3%)
HAART (second line)	2 (16.6%)

Lab results	
HB (g/dL)	13 ±2
Urea (mmol/L)	6 ±2
Creatinine (mmol/L)	80 ±24
CRP (mg/L)	<10
Cd4 count (cells/uL)	560 ±192
Viral Load (copies/mL)	Lower than detectable
Echocardiography	
LVEDD (mm)	57.9 ±9.7
LVESD (mm)	43.5 ±9.2
LVEF (%)	53 ±8
Severe aortic regurgitation n (%)	11 (91%)
Aortic annulus (mm)	23.5 ±5.2
Sinus of Valsalva (mm)	45.6 ±11.5
Sino-tubular Junction (mm)	50.3 ±12.8
Ascending Aorta (mm)	55.2 ±13.8
CT angiogram	
Sinus of Valsalva (mm)	50 ± 14
Ascending aorta (mm)	60±8
Aortic arch (mm)	46 ±17

Descending thoracic aorta (mm)	32 ±7

Normally distributed data presented as means ±SD, non-normally distributed data as medians (interquartile range). Bpm= beats per minute, CRP= C-reactive protein, FDC= fixed dose combination, HAART= highly active antiretroviral therapy, HB= hemoglobin, LVEDD= left ventricular diastolic diameter, LVEF= left ventricular ejection fraction, LVESD= left ventricular end systolic diameter.

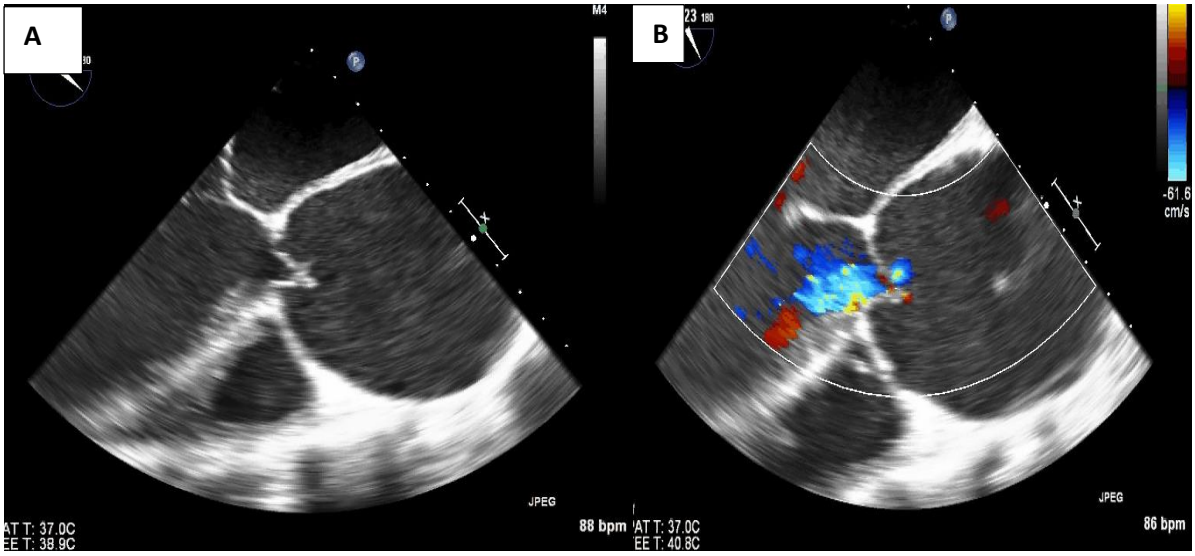

Figure 2: (A) Echocardiographic long axis views showing a large aneurysm of the ascending aorta **(B)** complicated by severe aortic regurgitation secondary to aortic root dilation.

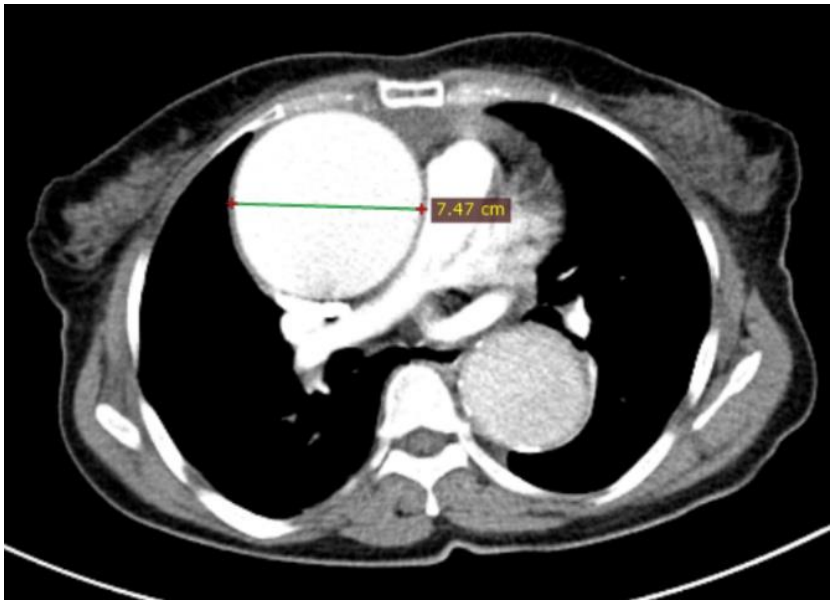

Figure 3: Computed Tomography of the ascending aorta showing a large aneurysm in a HIV patient.

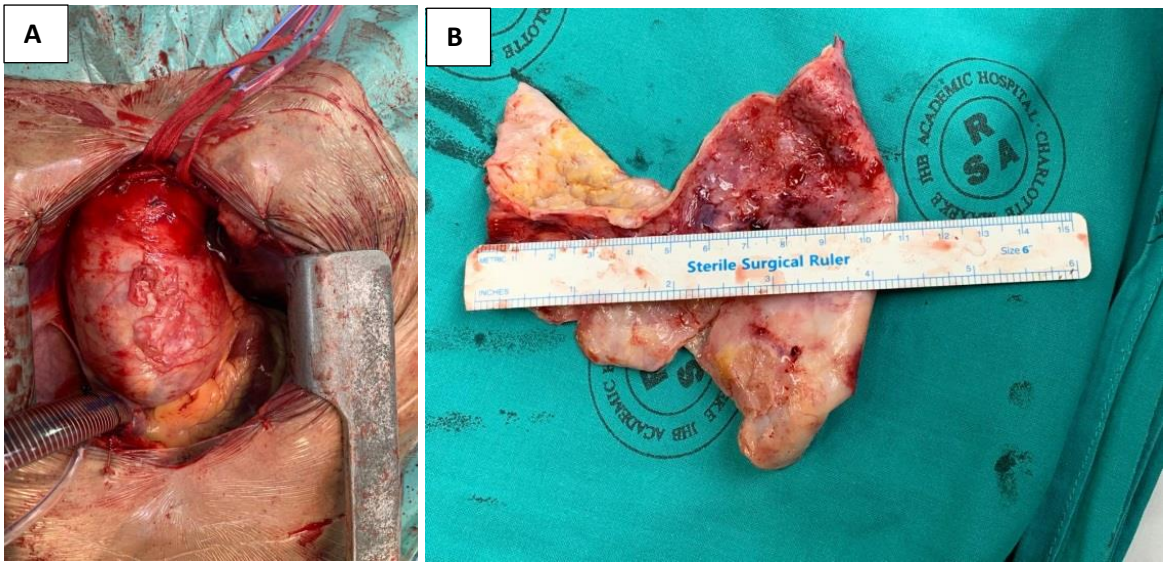

Figure 4: (A) Intraoperative thoracic aortic ascending aneurysm in HIV patient. **(B)** Post excision of a large thoracic ascending aortic aneurysm in HIV patient- diameter measuring 10 cm.

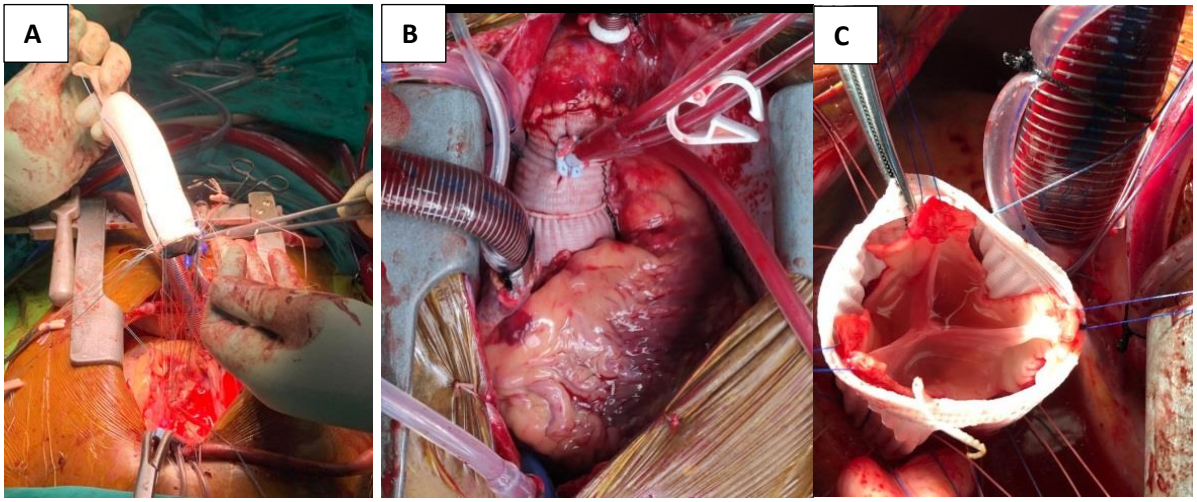

Figure 5: (A) Composite graft implantation post removal of the ascending aortic aneurysm in HIV patient. **(B)** Post implantation of the composite graft. **(C)** Competent native aortic valve post removal of the ascending aortic aneurysm, valve sparring aortic root replacement being done.

Hydroxyproline concentration

Total of 33 samples were investigated from the 12 patients (14 aneurysmal, 13 seemingly normal aortic tissue and 6 aortic valve leaflets. We took two specimens of aneurysmal aortic tissue in two patients at different sites of the TAAA where macroscopically the aortic wall tissue looked weak. Two seemingly normal tissue samples were taken from one patient whom we managed to get seemingly normal tissue proximal and distal to the TAAA). For the rest of the patients one specimen for aneurysmal and seemingly normal aortic tissue was sampled. Hydroxyproline concentration of aneurysmal aortic tissue and normal aortic tissue was 20.21 ±6.14µg/mg vs 21.20 ±8.55µg/mg (P=0.87). Hydroxyproline concentration between aneurysmal vs normal aortic tissue vs aortic valve leaflets were 20.21 ±6.14µg/mg vs 21.20 ±8.55µg/mg vs 17.79 ±8.15µg/mg (P=0.65), respectively (Figure 6).

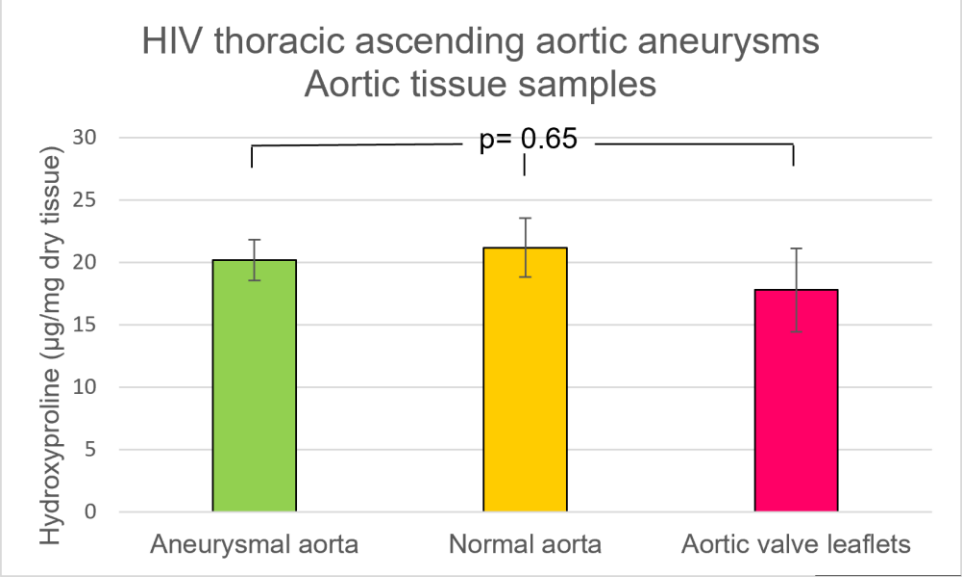

Figure 6: Hydroxyproline concentration in aneurysmal aorta vs normal aorta vs aortic valve leaflets in HIV thoracic ascending aortic aneurysms.

Histology

Eleven specimens from aneurysmal aortic tissues were sent to the National Health Laboratory Service and National Institute for Occupational Health Histopathology department (Figure 4). The 12th specimen was from the patient with the ruptured SoV aneurysm, the aortic tissue was insufficient to be sent for histopathology only aortic leaflets were sent. Fragmentation of elastin fibres was seen in 50% n=6 of the cases (Figure 7A). Infiltration of vasa vasorum by inflammatory cells was seen n=4 patients (Figure 7B), n=6 showed atherosclerosis (Figure 7C), n=3 granulomatous vasculitis (Figure 7D) (two being necrotising granulomatous, despite the Ziehl-Neelsen stain negative for acid-fast bacilli), n=2 cystic medial degeneration. From the patients that showed atherosclerosis on histology five out of six had concurrent diagnosis of hypertension and of older age 53.3±8.6 years. No mention of collagen structural abnormalities was made. Six histology aortic valve leaflet results: n=3 atheroma seen, n=1 necrotising granulomatous vasculitis, n=1 cystic myxoid degeneration, n=1 normal aortic leaflets.

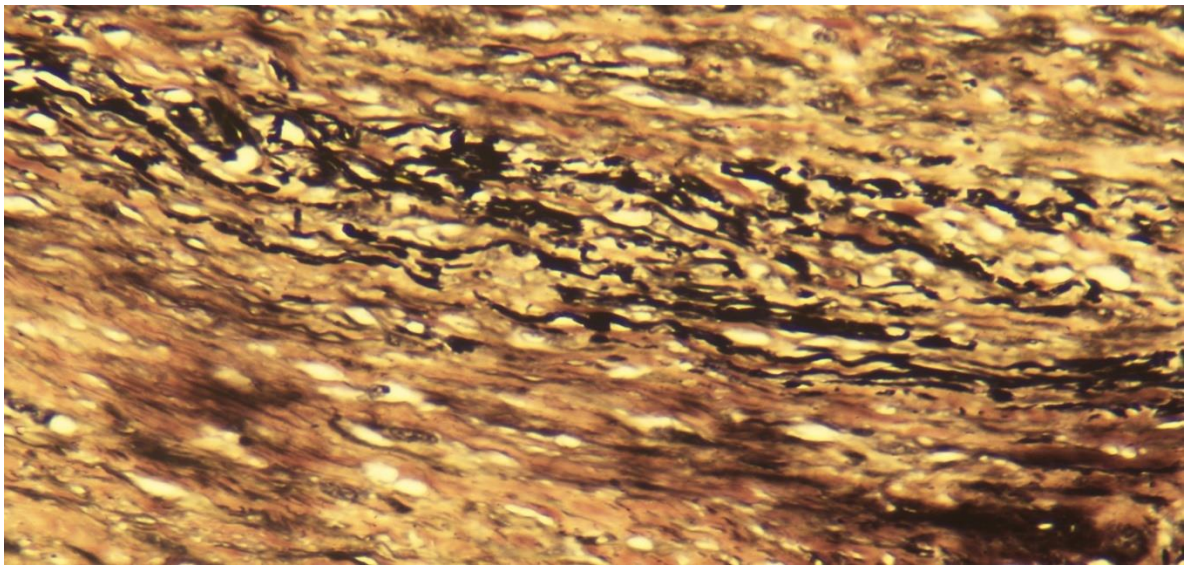

Figure 7A: Fragmentation of the elastic fibres of the aortic wall.
(EVG, original magnification 400x).

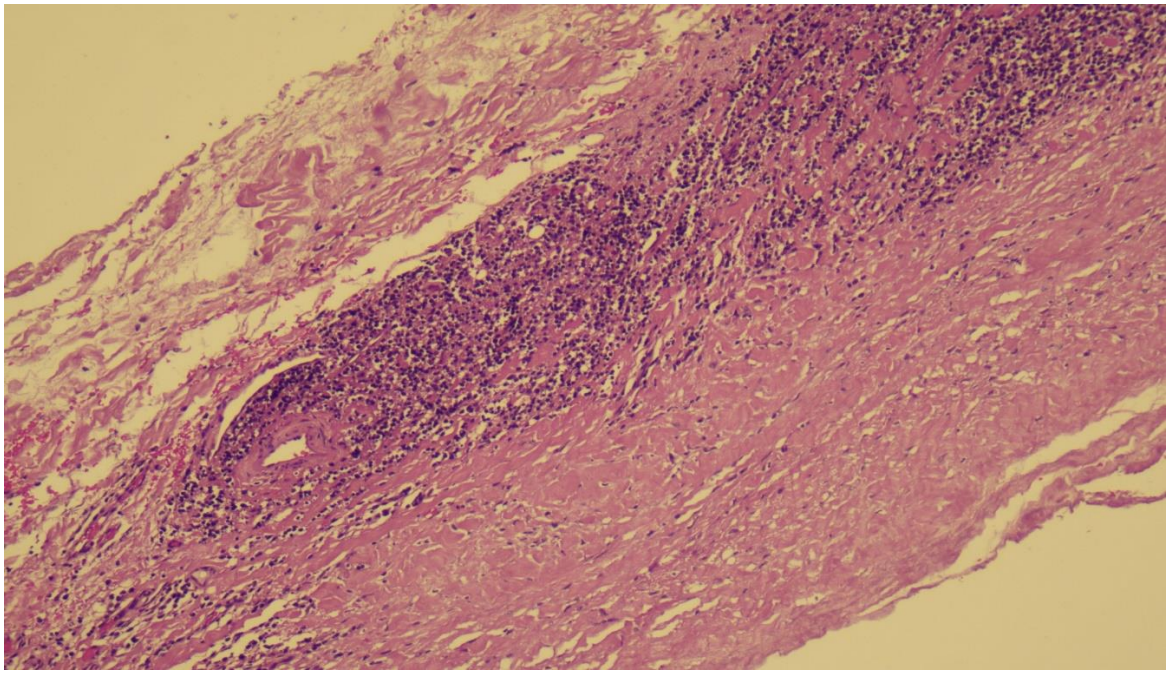

Figure 7B: Chronic inflammation surrounding the vasa vasorum.
((*H&E, original magnification 100x).*

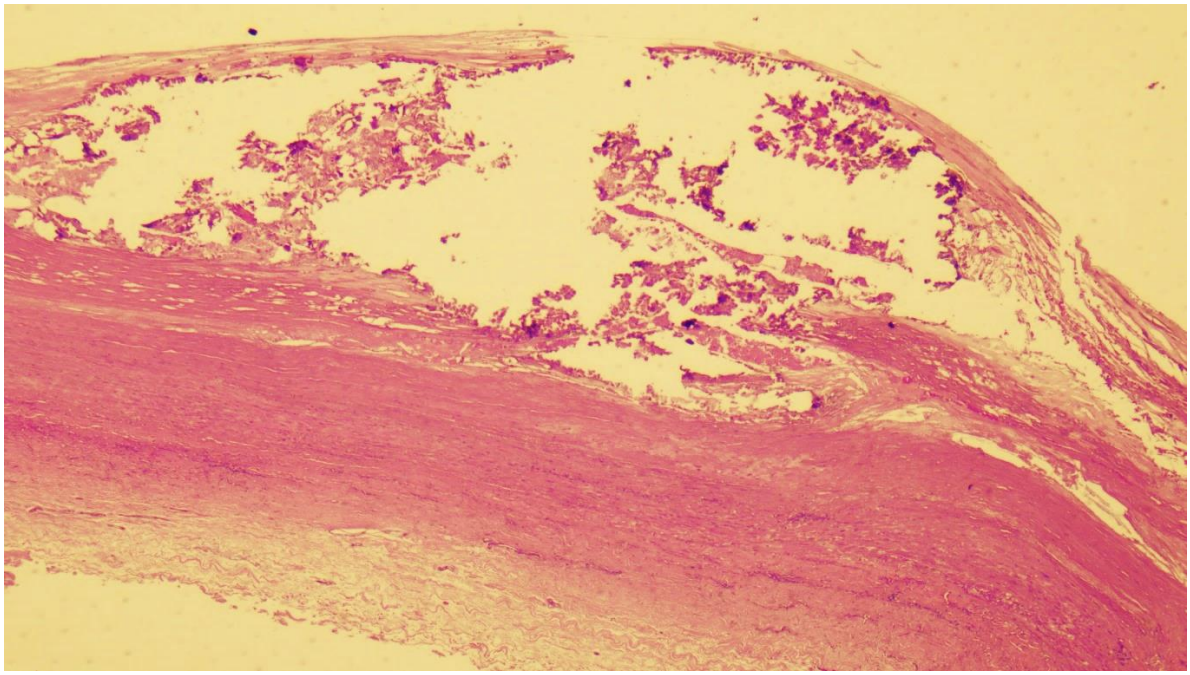

Figure 7C: Wall of aorta, with complicated atheromatous plaque.
(H&E, original magnification 40x).

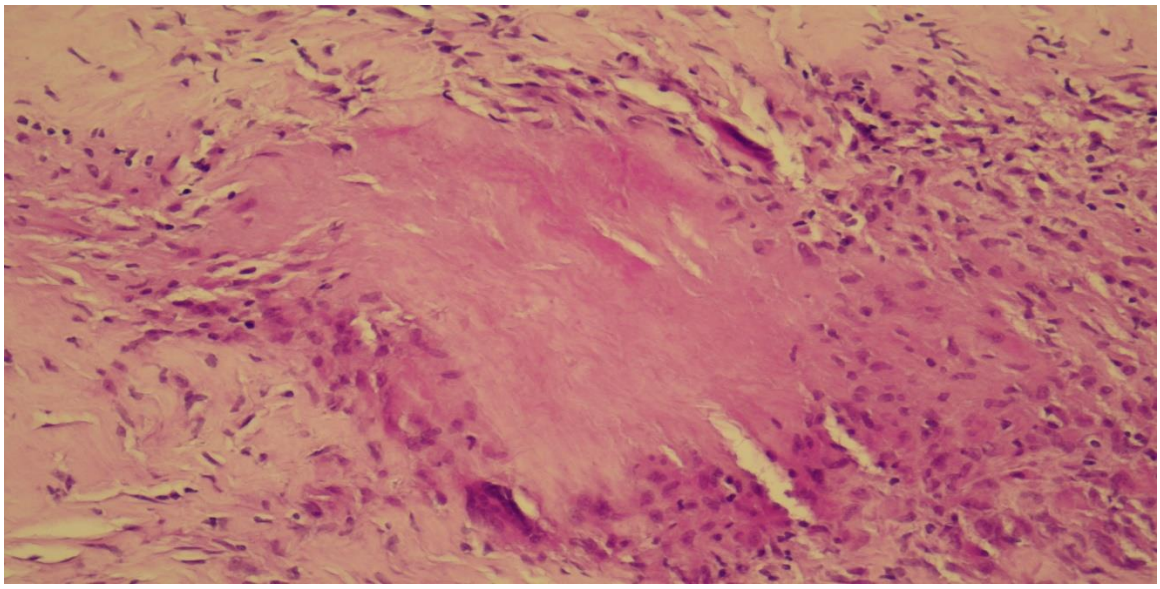

Figure 7D: Granulomatous inflammation within the wall of the aorta.
(H&E, original magnification 200x).

Discussion

The extracellular matrix of the aorta is a very important element of the tensile strength of the aorta. Any changes in its composition - mainly the collagen and elastin components - can lead to mechanical failure of the aortic wall which can represent as aneurysms that progress to aortic dissection or rupture [5]. Our study is the first study to assess collagen quantity in terms of hydroxyproline concentration in TAAA patients with HIV.

Our study population was young patients with mean age of 47 ±10 years, this was comparable to a study of HIV-associated extracranial arterial aneurysms [13]. However, we had predominantly women (75%), which contrasts with studies in some first world countries, where the males predominate [14]. A possible explanation could be that in South Africa women are more likely to seek medical assistance and be diagnosed with HIV and take highly active antiretroviral therapy than men [1]. Also women are more likely to contract HIV due to biological differences [15].

Surprisingly there was no significant difference in hydroxyproline concentration of aneurysmal aortic tissue and normal aortic tissue. This is contrary to a study that observed 50% decrease in hydroxyproline content in abdominal aortic aneurysms (AAA). They compared AAA with control group of autopsies of non-aneurysmal abdominal aortas not specific to HIV related groups [16].

Borges and colleagues [6] evaluated collagen in thoracic aneurysms compared with control of normal aortic tissue. They excluded connective tissue diseases as in our case because there are known reasons for aneurysms (i.e., Marfan syndrome=mutation in fibrilinin-1 gene). Sirius red stain was used for evaluation of collagen quantity which is comparable to hydroxyproline content. They found that there

was decrease in collagen content in the thoracic aneurysms compared to the controls and this could be associated to the weakness of the aortic wall [6].

The insignificant difference in hydroxyproline concentration in our study between the two specimen groups is comparable to hydroxyproline concentration in a control group of normal aortic tissues in a study that looked at AAA (21±7µg/mg) [16]. This could support that collagen content has no bearing on the aortic tensile strength in HIV TAAA, but this finding needs to be interpreted with slight caution as our study lacked a normal control group specific to this population. Yet in another study by Pichamuthu et al that compared hydroxyproline concentration in TAAA between patients with bicuspid aortic valve and tricuspid aortic valve,18.9 ±1.9µg/mg vs 19.0±1.5µg/mg p=0.12, the hydroxyproline concentrations were comparable to our results of aneurysmal and normal aortic tissue in patients with HIV TAAA [5].

There have been reports that in the elderly (age 50 years or greater) HIV infected patients there is an increase in collagen and elastic fibres in the abdominal aorta compared to non-HIV infected patients. As we know HIV is a systemic disease, so the vascular remodelling is commenced by inflammation from the HIV itself and therefore there is activation of endothelial cells and expression of collagen [18]. The aforementioned finding contrasts with the current study, whereby, in addition to a lack of change in collagen content we noted fragmentation of elastin fibres, this difference could be accounted for by the fact that we studied the ascending aorta which differs from the abdominal aorta in its collagen and elastin content, which would result in different reaction to injury by the two areas of the aorta [19] as well as different methodologies utilised in the two studies to measure the collagen and elastin content. Additionally, our sample size was too small to allow for meaningful subgroup comparisons between the young and the elderly patients with HIV.

The reason of no change in collagen content between the two groups (aneurysmal vs non-aneurysmal aortic tissue) in our study could lend support to the hypothesis that that it is not perhaps the quantity of collagen but the quality of the collagen that may be responsible for weakening of the aortic wall and resultant aneurysm formation in patients with HIV aortopathy. This may be analogous to alteration in the microstructure of the collagen in ageing [17,20].

Aortitis as evidenced by infiltration of the vasa vasorum by inflammatory cells was also observed in some of our cases, which is the classic sign of HIV aortopathy and is suggested to cause transmural ischemic necrosis. There have been reports of leucocytoclastic vasculitis converging with the vasa vasorum [3,19].

In addition to elastin fragmentation, atherosclerosis of the aneurysmal aorta was a common finding in the current study. It is suggested that the premature acceleration of atherosclerosis in HIV patients is from a sequela of 1. Inflammation, 2. Migration of monocyte and transformation to macrophages and foam cells, and 3. Development of plaques with focal thickening of the intima through calcium dependent endoplasmic reticulum after apoptosis of the foam cells [21]. Another contributing factor in accelerated atherosclerosis may also be from the second line protease inhibitors [22].

There is a known strong association of atherosclerosis with hypertension and age. In our study, we found out that 83% of the patients with atherosclerosis on histology had concurrent hypertension diagnosis and they were of older age. This could mean that the findings may be due to the comorbidities instead of HIV itself or a combination of all mentioned disease entities [23].

Out of the 6 aortic valve leaflets that were sent for histology, only one was normal, and the rest displayed atherosclerotic, necrotising granulomatous vasculitis and cystic

myxoid degeneration disease process akin to the ascending aorta. Based on the aforementioned finding, together with a lack of difference in collagen content between the aortic leaflets and the ascending aorta one may conclude that aortic leaflets are a continuum of the adjacent aorta [24].

Study limitations

The limitation to the study is that of small sample size because the number of cases with this particular disease entity is quite low, however this study is the largest published so far. Another limitation is that there was no control group consisting of aortic tissue from normal living participants and this would nevertheless have been difficult to obtain due to obvious ethical considerations. Hence, all the specimens were taken from the HIV patient with TAAA delineating aneurysmal tissue from non-aneurysmal tissue.

Conclusion

Surprisingly, we found no difference in collagen concentration in aneurysmal vs non-aneurysmal aortic tissue in HIV related TAAA. Therefore, the alteration in tensile strength in such patients with HIV associated TAAA is not attributed solely to the amount of collagen concentration. In this preliminary analysis, the contributors to weakening of the aortic vessel wall are likely multifactorial as evidenced by the mixed findings of inflammation, degeneration, elastic tissue fragmentation and atherosclerosis. Further studies are needed to confirm this finding and unravel the pathophysiology of HIV related aortopathy.

Conflict of interest

None declared.

Data Availability Statement

The data used and/or analysed during the current study is available from the corresponding author on reasonable request.

Authors contributions

Moleboheng Mokotjo: Protocol definition and approval, data collection, laboratory work, data handling, data interpretation, preparation of the original manuscript, and review of manuscript, approval of last version of manuscript.

Angela J Woodiwiss: Ideation and study design**,** Protocol definition and approval, Data collection, statistical analysis, data interpretation, reviewed the manuscript approval of last version of manuscript.

Shungu Mogaladi: Ideation and study design, Protocol definition and approval, Data collection, reviewed the manuscript.

J. Michael Hasenkam: Ideation and study design**,** Protocol definition and approval data interpretation, preparation of manuscript, reviewed and provided academic input to the manuscript, approval of last version of manuscript.

Ruchika Meel: Ideation and study design**,** Protocol approval**,** data collection, data interpretation, preparation of manuscript, and substantially reviewed and provided academic input to the manuscript, approval of last version of manuscript.

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CHAPTER TWO: LIST OF APPENDICES

Ethics Clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr M Molopa et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M180368

NAME: (Principal Investigator)	Dr M Molopa et al
DEPARTMENT:	School of Clinical Medicine Department of Surgery Division of Cardio-Thoracic Surgery Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Evaluation of aortic wall strength in human immunodeficiency virus-associated thoracic aortic aneurysm
DATE CONSIDERED:	06/04/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor JM Hasenkam
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	10/08/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **March** and will therefore be due in the month of **March** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date

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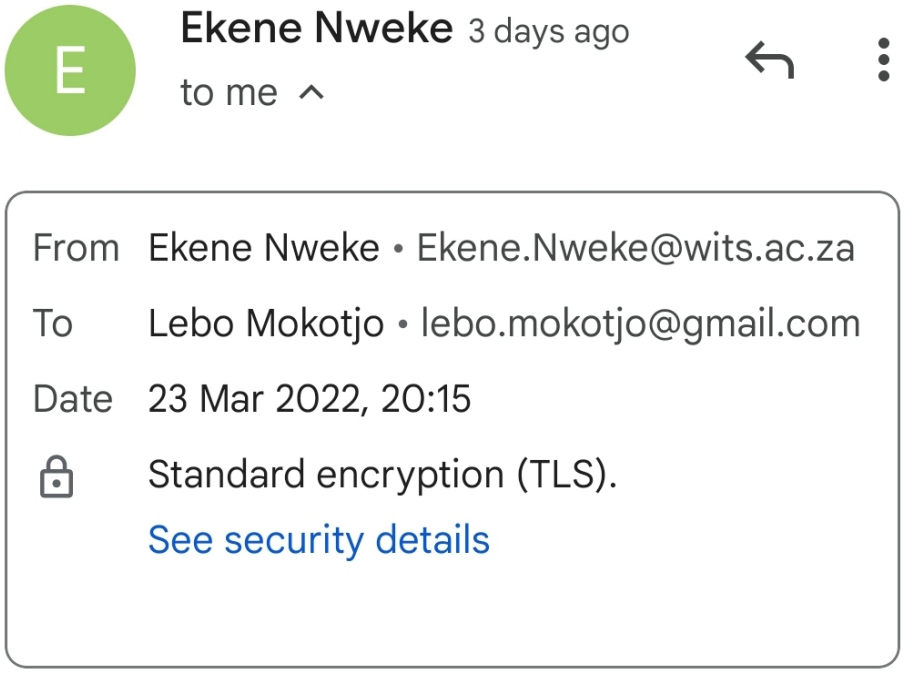

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	"Differential Tensile Strength and Collagen Composition in Ascending Aortic Aneurysms by Aortic Valve Phenotype", The Annals of Thoracic Surgery, 2013 Publication	
17	Yu Wang, Jing Jing, Yuesong Pan, Mengxing Wang, Xia Meng, Yongjun Wang. "Clinical characteristics and prognosis in oldest old patients with ischaemic stroke or transient ischaemic attack in China", Annals of Palliative Medicine, 2021 Publication	<1 %
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Approved protocol with extended literature review

Evaluation of aortic wall strength in HIV associated thoracic ascending aortic aneurysm

Researcher

Dr Moleboheng Mokotjo MBChB, FC Cardio (SA)

Student number: 1778980

Supervisors

Prof Ruchika Meel, MBChB, MMED (Cum Laude), Cert Cardio (CMSA), PhD, FEACVI and Post-Doctoral Carnegie Fellow. Consultant in the Division of Cardiology Chris Hani Baragwanath Academic Hospital.

Dr Shungu Mogaladi, BSc PreMed (UL), MBChB, MMED (Thorax Chir), FC Cardio(associate), CSTMP (Unisa). Chief Specialist, Head of Department Cardiothoracic Surgery, University of Witwatersrand, Charlotte Maxeke Johannesburg Academic Hospital.

Prof J. Michael Hasenkam MD DMSc, Professor of Experimental Cardiac Surgery Aarhus University Hospital Denmark.

Executive Summary

Patients with HIV are known to develop an inflammatory process of the arterial wall. The exact mechanism of this entity has not been fully understood, but intuitively is thought to develop because of the pro-inflammatory process, which alters the biomechanical characteristics in the arterial wall. These changes lead to weakness of the vessel wall and subsequently arterial aneurysms, which although rare, have a propensity to rupture even in younger patients and are mostly fatal when this complication is encountered. HIV associated aortopathy is a rare but a lethal disease entity. In greater South Africa and Gauteng, we have a high volume of patients with HIV and a high throughput of patients at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) from large volume referral centre such as Chris Hani Baragwanath academic hospital (CHBAH). It provides us with a unique opportunity to closely study this disease entity. We have a unique possibility to investigate the aortic wall strength in this population with thoracic ascending aortic aneurysms (TAAA). This study will provide longitudinal sections of aneurysmal and seemingly normal aortic tissues removed as per routine during elective surgery for TAAA in HIV infected patients at the CMJAH. These samples will then be subjected to biomechanical analyses of the vessel walls. Comparative analysis between seemingly normal and diseased aorta will then be employed.

This pilot study will help to provide insight into the underlying pathophysiology of this entity and form a basis for future studies looking at screening for patients at risk and possible therapeutic or preventative measures that can be undertaken.

Background

The aorta is composed of three layers. The luminal intima layer, middle elastic media layer and the peripheral collagenous adventitia layer (figure 1) [1]. It is important to understand the biomechanics of the aorta in TAAA. Evidently the cause of dissection and, or rupture is due to mechanical failure of the vessel wall whereby the local stresses in the tissue wall exceeds the mechanical quality of the vessel. The mechanical components are elastin and collagen [2]. Both are important to determine the tensile strength and the durability of the aorta [3].

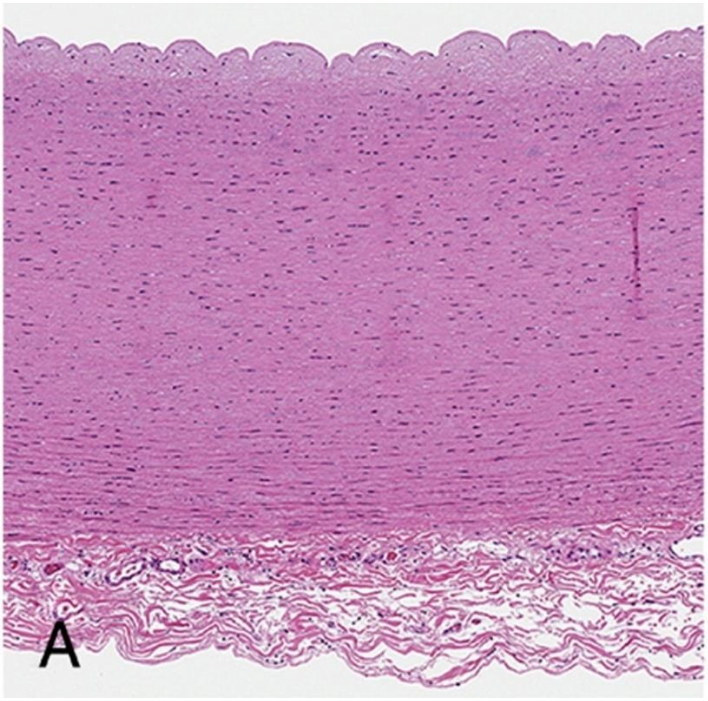

Figure 1: Normal aorta demonstrating all three aortic layers, transverse section: intima (top), media and adventitia (H&E, original magnification 50x) by Halushka MK et al [1].

Documented risk factors of developing TAAA are advanced age, hypertension, atherosclerosis, developmental defects such a bicuspid aortic valve (BAV), connective tissue disorders like Marfan syndrome and Ehlers-Danlos where the flaw is the stiffness of the aorta and the weakness in structural support of the aorta [2].

A study was done to determine the effect of age and diseases on collagen content in aortic tissue. It was reported that there was an increase in collagen content with age. The study was done using hydroxyproline assay which is proportional to the collagen content. Specific to the thoracic aorta the collagen content was constant for patients under 45 years of age then started to increase slowly thereafter. The structure of the collagen was changed as well with increasing age by show of irregular collagen fibres in thoracic aorta media layer [4].

With regards to the effect of collagen on ascending thoracic aortic aneurysm, there was an increase in collagen content in the adventitia layer and decrease collagen content in the media and intimal layer. There was overall decrease in collagen concentration in the aneurysmal tissue [4].

TAAA has been correlated with decreased strength of the aortic wall by means of elastin destruction and collagen disruption that leads to change in collagen structure. As a result, weakness in the vessel wall predisposes to aneurysm formation which has a propensity to dissect and or rupture with catastrophic consequences [3].

Vorp and co- workers conducted a study to assess the effect of aneurysm on tensile strength and biochemical behaviour of the ascending thoracic aorta. Longitudinal and circumferential strips of aortic aneurysmal and normal tissue were compared and tested. The conclusion was that thoracic aortic aneurysm (TAA) formation was associated with 30% weakening and stiffening of the aortic wall compared to normal aortic tissue [3].

Another study was done to compare tricuspid aortic valves (TAV) and BAV wall strength in TAA, as well as to measure the content of collagen in the wall of TAA. It is known that BAV is a congenital heart malformation, one of the notable associations in

these patients is the presence of TAA. In this study however, tensile strength was higher in BAV compared to TAV. There was no difference in the content of collagen between the two suggesting that there might have been microstructural changes in the collagen framework rather than the decreased collagen content [5].

The current consensus on standard surgical care for patients with TAAA should undergo prophylactic surgery when the aneurysmal diameter is above 55mm and patients with Marfan syndrome with additional risk factors should undergo surgery above diameter 45mm. Marfan syndrome with no additional risk factors and BAV should undergo surgery >50mm. Patients with indication for aortic valve surgery with ascending aortic diameter >45mm should undergo surgery addressing the aortic valve and ascending aorta to avoid the aforementioned consequences [6]. Resection of the TAAA and replacement with the synthetic graft is recommended [2].

The link between HIV and large vessel aneurysms was first described in case reports in 1989 [8]. Subsequently HIV has been associated with vasculitis of all different sized arteries [9].

It has been postulated that it is this viral induced inflammatory process of the arterial wall that eventually leads to the alteration in the biomechanical characteristics of the arterial walls, which again leads to aneurysm formation in the affected vessel wall [8]. A study found that with arterial aneurysm associated with HIV, young patients were at higher risk and the aneurysms occurred at unusual sites [10]. Histopathology results of these patients reported acute and chronic inflammatory changes of the aneurysmal walls. The authors implied that the association between HIV and aneurysms may be caused by direct viral action or by bacterial infections from immunosuppression [10].

Brand and co-workers identified conclusively that histopathological evidence existed in large vessel wall inflammation in about 90% of patients who were HIV positive. No HIV negative patients had evidence of adventitial leukocyte vasculitis of the vasa vasorum. Seventy percent of HIV positive patients had evidence of adventitial slit-like vessels compared to HIV negative patients who had none. In addition, HIV positive patients demonstrated T-Lymphocytes in the intima in 80% and 30% in the media and in HIV negative patients T-Lymphocytes were noted only in the intima in 50% of patients. No significant differences were noted regarding CD4 counts [11].

There are various methods of evaluating the biomechanical properties on vessels and their influence on the vessels wall weakness and the resultant consequences. Ex vivo mechanical testing is seen as a gold standard where the aortic tissue is stretched, and the tension is measured in the wall. It is divided into uniaxial tensile testing system and biaxial tensile testing. The aortic tissue is stretched, and the corresponding tension is measured in the wall [1]. Hydroxyproline has also been used as a biomarker of biomechanical tensile strength testing [12,13].

Hydroxyproline is an amino acid that helps to stabilize the collagen structure [14]. The Hydroxyproline assay is a relatively cheap and less labour-intensive method than other biomechanical tests. It greatly reduces the time required for the analysis of the excised tissue [15]. It measures vessel wall strength as it is a direct measure of collagen content, we therefore use tissue concentration of hydroxyproline as a surrogate measure of aortic tissue strength [12,13].

Most of the data on mechanical properties of aortic walls in aneurysmal diseases are limited to abdominal aortic aneurysms. Currently there are no studies of the mechanical properties of TAAA in HIV infected individuals.

Based on the clinical findings in these HIV-infected patients on highly active antiretroviral therapy (HAART) we hypothesize that there will be a difference in collagen quantity between the aneurysmal and seemingly normal aortic tissue as a surrogate measure for aortic wall strength.

Aim

To conduct a prospective study in patients who are HIV infected on HAART presenting with TAAA at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) Department of Cardiothoracic Surgery to determine whether there are differences in collagen quantity in seemingly normal and aneurysmal aortic wall segments as a surrogate measure for aortic wall strength.

Objectives

1. To obtain longitudinal sections of aneurysmal and seemingly normal aorta resected during elective surgery of TAAA in HIV infected patients on HAART as to quantify aortic wall strength of seemingly normal aortic wall and diseased aortic wall tissue using hydroxyproline assay and to send aneurysmal specimen for Histopathological assessment.

2. To document demographic, clinical, laboratory, echocardiographic and Computed Tomography (CT) angiography findings in HIV infected patients with TAAA.

Methodology

This is a prospective consecutive, within patient-controlled cohort study which will enrol HIV–infected patients on HAART who present for elective TAAA surgery comparing their diseased and seemingly normal aorta.

Patient Subjects

This study is an extension of an already ongoing clinical study being performed at the Department of Cardiology, Chris Hani Baragwanath Hospital (M170389).

The study population will be HIV infected patients on HAART with TAAA who present for elective surgery at our department of Cardiothoracic Surgery at CMJAH during the period of February 2019 - March 2021. These patients will be counselled for surgery as per our normal hospital protocol. Once patients have consented for the elective surgery, informed consent for participation into the study will then be obtained by one of the investigators.

Inclusion Criteria

- HIV-infected patients with confirmed ELISA tests on HAART presenting for TAAA surgery at the CMJAH Cardiothoracic Surgery Department.
- Age >18 years of age
- Provision of informed written consent.

Exclusion Criteria

- Patient with known underlying cardiovascular disease (e.g., Myocardial infarction, pericarditis) or connective tissue disease (e.g., Marfan syndrome) or other aetiologies of ascending aortic aneurysms.
- Patients presenting for emergency surgery including aortic dissection.

Sample size:

There are no existing data from similar studies. Due to the explorative Pilot nature of this study, we have defined the sample size to be at least 10 consecutive patients – depending on patient inflow in the study period. It is within a patient-controlled study design.

Data collection:

The following data will be collected from the patients:

- Demographics: name, age, sex and race
- HIV status, CD4 count and viral load.
- Echocardiogram, CT angiogram diameter and morphology of TAAA
- Tissue sampling: fresh TAAA sampling of seemingly normal aorta 1cm distal and proximal from diseased aorta harvested as intact tubular structure during elective surgery. It will then be divided into two longitudinal strips. 1. Physiology- stored frozen, to determine collagen content of the seemingly normal and abnormal aorta estimated by hydroxyproline assay. 2. Histopathology stored in formalin, to examine vasculitis.
- Hydroxyproline assays between seemingly normal and abnormal aorta will be analysed. Samples of aortic tissue will be weighed and stored frozen at -70°C for

tissue analysis. Determination of Hydroxyproline concentration ([HPRO]) will be by the method of Stalder and Stegemann (1967) after acid (HCl) hydrolysis. To determine the extent of collagen cross-linking, aortic collagen will be extracted and digested with cyanogen bromide (CNBr) [16]. The CNBr- collagen digested sample will be subjected to acid hydrolysis to determine [HPRO]. The amounts of cross-linked and non-cross-linked collagen in the aortic tissue will be determined from the product of the percentage of collagen soluble to CNBr digestion and the total collagen concentration and the difference between the total collagen concentration and soluble collagen concentration, respectively. The relationship between soluble and insoluble collagen will be used as an indicator of the degree of collagen cross-linking [17,18].

Data Analysis

Patient data will be stored on the REDCap electronic data capture tool affiliated to the University of the Witwatersrand, Johannesburg. Research data will be represented as tables and figures using Microsoft excel 2010. Statistical analysis will be carried out using Statistica software. Continuous data will be represented as means or median and standard deviation. The P value will be set at $P<0.05$ to indicate statistical significance. Comparative statistics between the normal aortic tissue and abnormal TAAA wall strength with regards to hydroxyproline concentration and percentage of cross-linking will be analysed.

Simple descriptive statistics will be used from the HIV-infected patients' data presenting for TAAA repair at CMJAH Cardiothoracic Department. (Demographics, clinical, echocardiography and CT angiography). This data will be analysed to identify

potential relations with aortic aneurysm formation and the mechanical properties of the aortic wall.

Ethical consideration

Approval to conduct this study will be obtained from the Faculty of Health Sciences' Graduate Studies Committee and the Human Research Ethics Committee (Medical) of the University of the Witwatersrand. Permission to conduct the study will be requested from the CEO of CMJAH.

The surgery will be performed only on patients who have clear indications according to standard clinical practice. The patients will only have diseased tissue removed and 1cm of normal tissue proximal and distal to TAAA which is performed according to normal clinical practice, as part of their treatment. Only tissue which is routinely removed as part of the treatment (except for the extra amount of blood taken in conjunction with routine blood sampling) will be harvested for the above-described investigations.

To avoid any bias or coercion the informed consent for surgery will be obtained by members of the unit who are not involved in the study. The information sheet and consent of the study (see appendix) will be explained to the patient in the language they understand. If the patient wishes to withdraw from the study, he/she can do so at any time – also after surgery before the study has begun.

The study will be conducted in accordance with the South African good clinical practice guidelines and with the principles of the declaration of Helsinki.

Budget

Consumables	Mainly Stationary- R400
Hydroxyproline assay	R50/ sample
Tubes for storage	R550
Formalin	R650/ 5L

The cost of the above will be at the investigators expense.

Prof A Woodiwiss will cover costs of hydroxyproline assays.

Timing

	Feb 2018	Mar 2018	Apr 2018	Feb-Dec 2019	Jan-Dec 2020	Jan-Mar 2021	April 2021	May 2021	June 2021	Sep- Dec 2021
Literature Review										
Preparing Protocol										
Protocol assessment										
Ethics application										
Collecting of data										
Data analysis										
Writing up-paper										

References:

1. Halushka MK, Angelini A, Bartoloni G, Basso C, Batoroeva L, Bruneval P, et al. Consensus statement on surgical pathology of the aorta from the Society for Cardiovascular Pathology and the Association for European Cardiovascular Pathology: II. Noninflammatory degenerative diseases - Nomenclature and diagnostic criteria. Cardiovasc Pathol. 2016;25(3):247-57.
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Patient Information Sheet

Study title: Evaluation of aortic wall strength in HIV associated thoracic ascending aortic aneurysm.

Good Day

We (Dr M Molopa, Dr B Ngutshane, Dr D Scott) are clinicians at the Charlotte Maxeke Johannesburg academic hospital. We are currently conducting a research study titled “Evaluation of aortic wall strength in HIV associated thoracic aneurysm” at the University of the Witwatersrand.

The nature and purpose of the study

Research is a process used in seeking new knowledge. In this study the aim is to determine how strong the enlarged part (aneurysm) of the blood vessel that comes from the heart and compare it to the normal part of the same vessel. This study will collect information compiled by your cardiologist (clinical data, investigations- heart sonar and CT chest scan) and evaluate the strength of the diseased enlarged and normal blood vessel that comes from the heart taken out during your operation.

Explanation of the procedures to be followed

As you are aware in a few days’ time you will be having surgery to repair the enlarged part of your blood vessel that comes from the heart. From the blood vessel that will be taken out during your operation (diseased part and 1cm of normal tissue on either part of the diseased vessel) we would like to have your permission to perform some tests to assess its strength.
Clinical data, which includes hospital file with medical records, laboratory results, imaging will be accessed from various databases including TrakCare (National Health Laboratory), Hospital records and RedCap hosted by the University of the Witwatersrand, Johannesburg

Risks and discomfort involved

There will be no additional risk towards you taking the study. Some discomfort will result from drawing up blood.

Possible benefits

This study will help us understand the cause of your disease better and how it progresses over years. This in turn will aid in understanding timing of operation and may help in development of future medical therapy for this problem.

Participation is voluntary

Participation in this study is voluntary. If you choose to withdraw from the study at any stage, there will be no negative impact on your management in the hospital. There will also be no costs for your participation in this study as well as no financial compensation towards your participation in this study.

Ethical approval

This clinical trial protocol was submitted to the Faculty of Health Sciences Research Ethics Committee at the University of the Witwatersrand and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki which deals with the recommendations guiding doctors in biomedical research involving human subjects. A copy of the declaration may be obtained from the investigator should you wish to review it.

Confidentiality

All records obtained during this study will be kept confidential. Results will be published or presented in such a fashion that you remain unidentifiable. The results of the study will be made available for publications in medical scientific journals.

We are inviting you to take part in our research study.

If you agree to participate in this study, you may sign this document. Please note that you may withdraw your name from this study any time, for whatever reason, without any question being asked and this will not affect your treatment.

If you have any queries regarding this project please contact the research team, alternatively you may contact Human Research Ethics Committee (Medical) (HREC).

Kind Regards
Research team

Contact details of research team.
Dr M Molopa 0733840505
Dr B Ngutshane 0722281421
Dr D Scott 0732011598

Contact details of HREC (Medical)

Prof Clement Penny Tel: 011 717 2301 email: Clement.Penny@wits.ac.za
Ms Zanele Ndlovu- Tel: 011 717 2700 email: zanele.ndlovu@wits.ac.za
Mr Rhulani Mkansi Tel: 011 717 2656 email: Rhulani.mkansi@wits.ac.za

Thank you for reading this study information sheet.

Consent Form –current testing

Evaluation of aortic wall strength in HIV associated thoracic ascending aortic aneurysm

---

I

_____

_____, Hospital number _____, hereby grant permission to take part in the above-mentioned study.

I have read and it has been verbally explained to me the aim and procedures of the study.

1. That participation is purely voluntary.
2. That I may withdraw my consent at any time without providing any reasons and without any disadvantage to my treatment as a result.
3. Participation or non-participation will not influence any aspect of my treatment
4. Results obtained in the study will be reported anonymously without mention of any identifying information

_____

Participant's Signature Date

_____

Witness Signature Date

_____ _____

Patient data sheet

Evaluation of aortic wall strength in HIV associated thoracic ascending aortic aneurysm

Demographic Information

Record ID	_____ ___
Patient Details	
Age	_____
Gender	○ Male ○ Female
Race	○ African ○ Caucasian ○ Coloured ○ Asian ○ Indian
Clinical Information	
Referring Cardiologist	_____
Referring Hospital	○ CMJAH ○ CHBAH ○ HJ ○ Klerksdorp ○ Sebokeng ○ Leratong ○ Other
Date of Referral	_____

Operation Related Data

Pre-operative assessment	
Admission Date	_____
Pre-op Diagnosis	_____
Comorbid Conditions	☐ Hypertension ☐ COPD/Asthma ☐ Diabetes ☐ Renal Disease ☐ HIV ☐ Infective Endocarditis ☐ Rheumatic Heart Disease ☐ Other
CD4 count	_____
Viral Load	_____
BMI/BSA	_____

_____

Extremities	☐ Clubbed ☐ Splinter Haemorrhage ☐ Pedal oedema
Pedal Oedema Grade	☐ 1 ☐ 2 ☐ 3 ☐ 4

EXAMINATION	
General	☐ Pale ☐ Cyanosed ☐ Jaundice ☐ Dehydrated ☐ Clubbing
Pulse Rate	_____
Systemic BP	
Chest	☐ Clear ☐ Crepitations ☐ Pleural effusion ☐ Consolidation ☐ Wheezing
Abdomen	☐ Hepatomegaly ☐ Pulsatile ☐ Splenomegaly ☐ Ascites

INVESTIGATION

Lab results

HB g/dL
Urea mmol/L mean
Creatinine mmol/L
Cd4 count cells/uL
Viral Load copies/mL
CRP (mg/L)

Echocardiography findings

LVEDD mm
LVEDS mm
LVEF %
Aortic regurgitation
Aortic annulus mm
Sinus of Valsalva mm
Sinotubular Junction mm
Ascending Aorta mm

CT angiogram

Sinus of Valsalva mm
Ascending thoracic aorta mm
Aortic thoracic arch mm
Descending thoracic aorta mm

Details of Procedure

Date of Procedure

_____
____

Description of Procedure

_____
_____

Operation Notes

Photographic representation of specimen

Outcome of Procedure

☐ Alive and Well
☐ Alive with Minor Morbidity
☐ Alive with Major Morbidity
☐ Dead

Study Data

Histopathology

Report

_____
_____

Attach Report

Physiology

Hydroxyproline Assay of Normal Aorta

_____
_____

Study Completion Data

Consent	
Did patient consent for study?	○ Yes ○ No
State date which consent was obtained	_____ ___
Did Patient Complete the Study?	○ Yes ○ No
if no, please state reason	☐ Patient withdrew consent ☐ Missing information ☐ Other