

**The Diagnosis and Treatment of Myocardial and Arterial
Dysfunction in Marfan Syndrome.**

A Thesis submitted for the degree of Doctor of Medicine (MD)

by

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Declarations

I declare that, except where indicated by specific reference, the work submitted is the result of my own investigation and the views expressed are mine.

I declare that no portion of the work presented has been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

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Dedication

This work is dedicated to the memory of my dear wife Tamsin
and to my son Charlie XX

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Summary

Marfan Syndrome is a genetic, cardiovascular disease caused by a defect in the fibrillin 1 gene on chromosome 15. This defect causes abnormal deposition of elastin throughout the body. Elastin is found in many organs including the aorta. Marfan Syndrome is diagnosed by the Ghent criteria. The mean age at death is 44 years for men and 47 years for women, and about 70% die from acute cardiovascular complications, mainly aortic dissection.

The assessment and treatment of the aortic complications of Marfan Syndrome has not changed for many years. Serial echocardiography is performed to measure the aortic root diameter. If thought to be increasing in size, beta blockers are prescribed to delay aortic dilatation and surgery, and to prevent aortic dissection or rupture despite the paucity of good research data. I have investigated three novel diagnostic tools: Tissue Doppler Imaging, Applanation Tonometry and Wave Intensity Analysis which have potential advantages in the assessment of the left ventricle and aorta and their interaction in Marfan Syndrome. I also investigated three drugs a beta blocker, an angiotensin converting enzyme inhibitor and a calcium channel blocker to look at their impact on some of the parameters measured by these three novel tools in a double-blinded, randomised cross-over trial.

I conclude that these three novel tools would be useful adjuncts in monitoring Marfan Syndrome and their response to treatment. I also found that beta blockers may still have a role to play in delaying and preventing aortic complications when given together with an angiotensin converting enzyme inhibitor, calcium channel blocker or angiotensin receptor blocker. There are, however, other issues that need addressing to improve the management of the cardiovascular complications of Marfan Syndrome. This includes a multi-team approach to this multi-system disease and improvements in the standard of research.

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Abbreviations

A	Blood flow velocity through the mitral valve during atrial contraction
ACEi	Angiotensin converting enzyme inhibitor
AIx	Augmentation index
Am	Myocardial velocity at atrial contraction
AmBS	Myocardial velocity at atrial contraction at the basal septum
ARB	Angiotensin receptor blocker
AT	Applanation tonometry
AT1R	Angiotensin II type-1 receptor blockers
AT2R	Angiotensin II type-2 receptor blockers
BB	Beta blocker
BCW	Backward compression wave
BEW	Backward expansion wave
CAP	Central aortic pressure
CCB	Calcium channel blocker
CV	Coefficient of variation
ϵ	Myocardial strain (epsilon)
E	Blood flow velocity through the mitral valve during early diastole
EF	Ejection fraction
Em or E'	Myocardial velocity at early diastole
EmBS	Myocardial velocity at early diastole at the basal septum
FBN1	Fibrillin 1
FCW	Forward compression wave
FEW	Forward expansion wave
IVA	Isovolumic acceleration
IVC	Isovolumic contraction
IVR	Isovolumic relaxation
LMA	Lateral mitral annulus
MF	Marfan Syndrome
MMA	Medial mitral annulus
MMP	Matrix metalloproteinases
PP	Arterial pulse pressure
PWV	Pulse wave velocity
SCH	Subclinical hypothyroid
SD	Standard deviation
Sm	Peak systolic myocardial velocity

SmAS	Peak systolic myocardial velocity at the anteroseptal wall
SmBA	Peak systolic myocardial velocity at the basal anterior wall
SmBI	Peak systolic myocardial velocity at the basal inferior wall
SmBL	Peak systolic myocardial velocity at the basal lateral wall
SmBP	Peak systolic myocardial velocity at the basal posterior wall
SmBS	Peak systolic myocardial velocity at the basal septum
SmLTA	Peak systolic myocardial velocity at the lateral tricuspid annulus
SRs	Strain rate in systole
T2DM	Type 2 diabetes mellitus
TDI	Tissue Doppler imaging
TGF-beta	Transforming growth factor-beta
TIMP	Tissue inhibitor of matrix metalloproteinases
TTE	Transthoracic echocardiography
VSMC	Vascular smooth muscle cells
WIA	Wave intensity analysis

CHAPTER 1.1

A REVIEW OF MARFAN SYNDROME

History

Marfan Syndrome was first described in 1896 by Professor Antoine Bernard-Jean Marfan (1858-1942), a French paediatrician working in Paris¹. At the Medical Society of Paris meeting that year he presented the case of a 5 year old girl who had disproportionately long arms.

In 1902, Mery and Babonneix studied the same girl again, this time with the advantage of new technology in the form of radiography. They discovered her dorsal spine was malaligned and her thorax was asymmetrical. They called the condition hyperchondroplasia². In later studies further anomalies were documented, including arachnodactyly (long digits) and dislocation of the ocular lens.

In 1912, Salle³ described mitral valve abnormalities and heart dilatation in an infant with heart failure but it was not until 1943 that the typical cardiac abnormalities (aortic dilatation and dissection) were linked to the Marfan phenotype.

Cardiovascular disease accounts for more than 90% of premature deaths in patients with Marfan Syndrome⁴. In the 1950s, studies of a relatively large number of patients and their families delineated the natural history of Marfan Syndrome, particularly the cardiovascular complications. McKusick⁵, in 1955, said "What the suspensory ligament of the lens has in common with the media of the aorta is obscure. If known, the basic history of the syndrome might be understood." It was at this time that the first Marfan clinic was set up at his institution, The Johns Hopkins Hospital in Baltimore.

Before the era of open heart surgery, the majority of patients with Marfan Syndrome died prematurely of aortic rupture often by their third decade⁶. Even after open heart surgery became established, surgical management was reserved for patients who had suffered acute dissection or rupture. Results were therefore poor.

Over the last ten years there have been important advances in the understanding of the development of Marfan Syndrome and this has led to the investigation of new therapeutic targets to prevent or delay aortic dilatation. Prior to this, beta blockers have been the mainstay of medical treatment.

Incidence and aetiology

Marfan Syndrome is an autosomal dominant disorder of connective tissue that has both high penetrance and variable severity. The incidence of Marfan Syndrome is around 2-3 per 10,000 individuals⁷. In 25%, there is no family history, which suggests the condition has presented de novo. There are currently (1st September 2011) 601 identified genetic mutations of which 80% were novel⁸.

Marfan Syndrome is caused by an abnormality of fibrillin, a 350kD glycoprotein, which is the main structural component of microfibrils. Microfibrils provide a supporting scaffold for the deposition of elastin throughout the body. Fibrillin is present in many other tissues including lung, dura mater, skin, tendon, the ciliary zonules of the lens, myocardium, heart valves and periosteum. Abnormalities in these fibrillin-containing tissues are found in most patients with Marfan Syndrome.

In 1991, mutations in the fibrillin-1 gene (15q21.3) were found to cause Marfan Syndrome⁹. For many years this was thought to be the only cause of the Marfan phenotype. In 2005, however, it was reported that mutations in transforming growth factor-beta (TGF-beta) receptors 1+2 on chromosome three caused a similar but more severe vascular phenotype to that seen in Marfan Syndrome –named the Loeys-Dietz syndrome¹⁰. This is associated with aggressive aortic vascular disease and can be distinguished from Marfan Syndrome by the presence of hypertelorism, low set ears and a bifid uvula or cleft palate. In comparison to Marfan Syndrome, there is a much higher risk of dissection at a young age, at smaller vessel dimensions and in non-aortic vessels.

TGF-beta cytokines play a major role in tissue development and cellular regulation¹¹. There is a regulatory relationship between extracellular microfibrils and TGF-beta signalling so that an abnormality in either can lead to a common final pathway which causes the development of the Marfan phenotype. This will be discussed in detail in the next chapter.

Clinical features

Multiple organ systems are affected including the skeleton, eyes, heart, lungs and blood vessels. Marfan Syndrome is diagnosed in our studies using the Ghent nosology (Table 1) which combines clinical and genetic factors¹². The diagnosis is confirmed if a patient has major criteria in two or more organ systems and minor criterion in a third system or if mutation positive one major and one minor criterion.

Table 1- Ghent Diagnostic Criteria for Marfan Syndrome¹²

<u>Category</u>	<u>Major criteria</u>	<u>Minor criteria</u>
Family history	<ul style="list-style-type: none"> independent diagnosis in parent, child or sibling 	
Genetics	<ul style="list-style-type: none"> mutation FBN1 	
Cardiovascular	<ul style="list-style-type: none"> aortic root dilatation dissection of ascending aorta 	<ul style="list-style-type: none"> mitral valve prolapse calcification of the mitral annulus (<40yrs) dilatation of the pulmonary artery dilatation/dissection of descending aorta(<50yrs)
Ocular	<ul style="list-style-type: none"> ectopia lentis 	2 needed: <ul style="list-style-type: none"> flat cornea elongated globe myopia
Skeletal	4 needed: <ul style="list-style-type: none"> pectus excavatum needing surgery pectus carinatum pes planus 	2-3 major, or 1 major and 2 minor signs: <ul style="list-style-type: none"> moderate pectus excavatum high narrowly arched

	<ul style="list-style-type: none"> • wrist <i>and</i> thumb sign • scoliosis >20° or spondylolisthesis • armspan-height ratio >1.05 • protrusio acetabulae (X-ray, MRI) • diminished extension elbows <170° 	<p>palate</p> <ul style="list-style-type: none"> • typical face • joint hypermobility
Pulmonary		<ul style="list-style-type: none"> • spontaneous pneumothorax • apical bulla
Skin		<ul style="list-style-type: none"> • Striae • recurrent or incisional herniae
Central Nervous System	<ul style="list-style-type: none"> • lumbosacral dural ectasia (CT or MRI) 	

Note: lumbosacral dural ectasia and protrusion acetabulae are diagnosed using Magnetic resonance imaging or CT scan.

Diagnostic Criteria

In 1986, an international panel of experts set out the so-called Berlin nosology¹³ to diagnose Marfan Syndrome. Following the identification of the fibrillin 1 gene, the Berlin nosology was changed to the Ghent nosology due to over-diagnosis. Recently, and since I started the MD, the Ghent nosology have been revised in 2010¹⁴. Even though the original Ghent nosology confirmed Marfan Syndrome in over 95% of patients, there were concerns over the lack of validation of some of the diagnostic criteria; the application of some criteria to the paediatric population; and the availability and expense of MRI scanning for lumbosacral dural ectasia and protrusion acetabulae. The current revised diagnostic criteria rely much more heavily on the cardiovascular and ocular systems. It is thought that the new guidelines may delay a definitive diagnosis of Marfan Syndrome but will reduce the risk of premature or misdiagnosis¹⁴.

The difficulty in diagnosing Marfan Syndrome is an important one. Matching phenotype and genotype is a problem especially in a genetic disease that has over 600 genetic mutations. There can also be considerable variation in clinical features even within families with the same mutation. As with a number of genetic diseases there seems to be a spectrum of disease and people are often diagnosed as Marfanoid without meeting the full Ghent criteria for Marfan Syndrome.

Marfan Syndrome may be suspected in foetal life and can be diagnosed on antenatal ultrasound¹⁵, but the diagnosis is often not made until late childhood or adult life. In the young child it can be difficult to make a definitive diagnosis. Children often have an evolving phenotype and may need to be followed for several years before the diagnosis can be confirmed or refuted¹⁶. All these possible cases should be regularly assessed by echocardiography, optometry and skeletal survey as the child grows. A full family history and assessment of other family members also gives clues to the diagnosis. The American Academy of Paediatrics have produced detailed recommendations for the follow up of children with Marfan Syndrome which takes this difficulty into account¹⁷.

Differential Diagnosis

“Neonatal” Marfan Syndrome is a severe form of Marfan Syndrome often associated with a deletion in the exon 24-32 region of the Fibrillin 1 gene. This rare condition differs from the more usual infantile Marfan Syndrome in the severity of the cardiac and pulmonary manifestations¹⁸. Infants with the “neonatal” form often have severe mitral

and tricuspid regurgitation in addition to aortic root dilatation. Similarly, the usual arachnodactyly and tall stature may be accompanied by ectopia lentis, very loose skin “as if two sizes too big,” emphysema and joint contractures. The cardiovascular features often require surgical intervention in infancy and this may be complicated by scoliosis and pulmonary hypertension. The long term prognosis is very poor –usually due to progressive valve dysfunction or lung abnormalities^{18,19}.

Other Marfan-like syndromes do exist and there can be considerable overlap with the Sphrintzen-Goldberg syndrome, Loeys-Dietz syndrome and the vascular form of Ehlers Danlos syndrome^{14,20}. This emphasises the importance of appropriate diagnosis using the Revised Ghent criteria which takes these other syndromes into consideration.

Cardiovascular abnormalities

At 30 years of age, men with Marfan Syndrome have an annual mortality of 2%, and women 1%²¹. According to actuarial life tables, these figures represent a 20-40 fold increased risk compared with a UK population of the same age²². The mean age at death in affected individuals is 44 years for men and 47 years for women²¹, and about 70% die from acute cardiovascular complications, mainly aortic dissection²³. The in-hospital mortality of Marfan patients with dissection (21%) and the rate of complications are similar to those observed in older patients in whom the aetiology of dissection is arterial hypertension²⁴. The most important target for improving survival in patients with Marfan Syndrome, therefore, is to prevent or delay aortic dissection.

Virtually all adults with Marfan Syndrome have an abnormal cardiovascular system. The most common cardiovascular abnormalities are dilatation of the aorta and mitral regurgitation (Table 2).

Table 2: Cardiovascular Manifestations of Marfan syndrome

Lesion/ Feature	Frequency	Complications	Comments
Aortic root dilatation	60-80%	Aortic dissection	Dissection rare < 10yrs old
Pulmonary artery dilatation	76%	Dissection rare	Diagnostic feature < 40yrs old
Mitral regurgitation/ prolapse/annular calcification	52-68%	Arrhythmias Endocarditis Ventricular dysfunction	Regurgitation may be intermittent
Descending aorta dilatation		Aortic dissection	Rare in childhood
Endothelial dysfunction /abnormal aorta elasticity	80-100%	Increased vascular stiffness	May contribute to dissection risk
Tricuspid valve prolapse	4% 36% in infantile type	May progress requiring repair	Severe disease uncommon except in infantile type
Left ventricular dysfunction	Up to 100% Severity varies.	Diastolic. May be progressive to systolic dysfunction.	May occur despite normal valves
Arrhythmias	Up to 20-30%	May cause sudden death	Associated with ventricular dilatation;

Coronary artery aneurysm	< 1%	Only described in adults	
Atrial septal defect	4%	May need surgical repair	More common than in normal population

Most children with Marfan Syndrome have aortic root dilatation. The reported frequency of other valve abnormalities depends to some extent on the rigour of the method of assessment. Moreover, some abnormalities (for example, mitral regurgitation and prolapse) can be intermittent and vary from mild to severe at different times in the same patient. Patients with valvular complications are at increased risk of infective endocarditis. Recommendations for antibiotic prophylaxis have changed and rely on local policy but good dental hygiene and early treatment of skin sepsis remain vital.

Cardiac arrhythmias are an under-recognised cause of morbidity and mortality. A link between Marfan Syndrome and Wolff Parkinson White syndrome has been postulated and atrial fibrillation has been reported in children and adults^{25,26}. Minor ECG abnormalities may be present in up to 50% of children with Marfan Syndrome²⁷. In addition, ventricular arrhythmias may occur and can lead to sudden death²⁸⁻³⁰. This is not surprising given the extensive fibrillin network which extends throughout the myocardium³¹. For the same reason, paradoxical septal motion is common. There is also an important subgroup who have significant left ventricular dysfunction which is unrelated to valve regurgitation^{32,33}.

Cardiovascular Assessment of Marfan syndrome

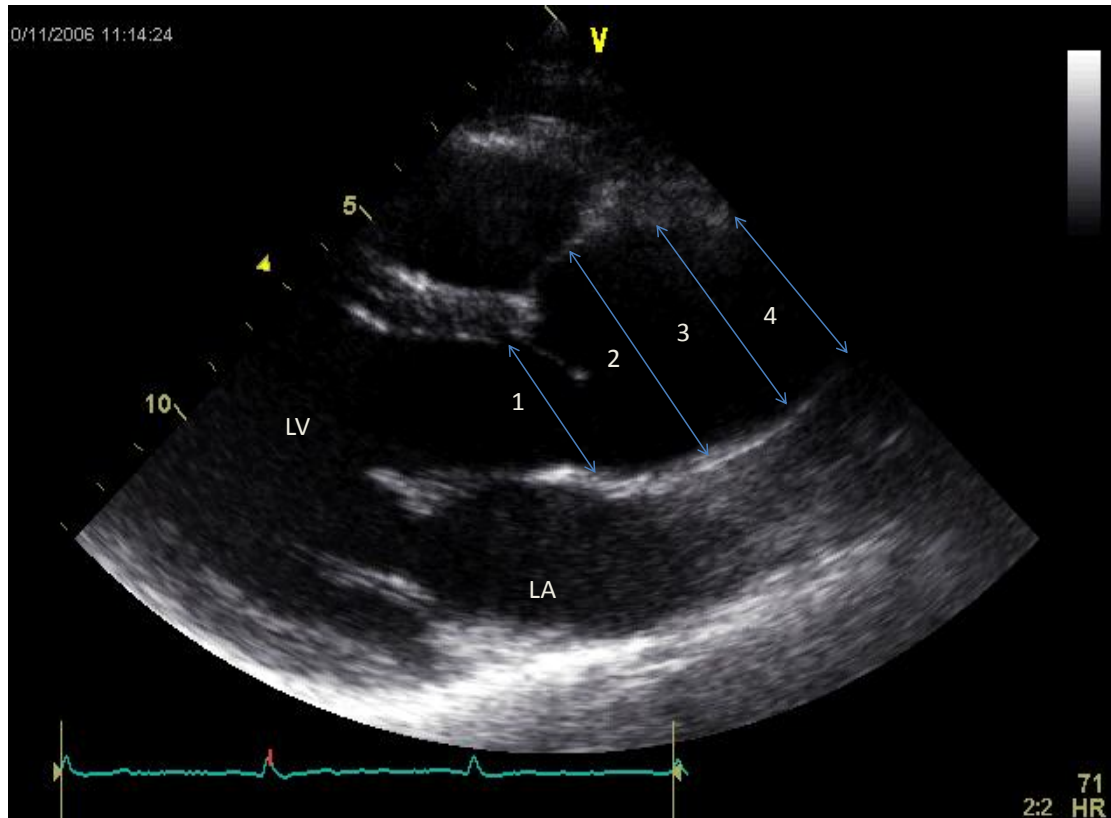
Echocardiography is the mainstay of assessment of people with Marfan Syndrome. A protocol for cardiovascular assessment is shown in Table 3.

Table 3: Protocol for cardiovascular assessment of Marfan syndrome

<i>Clinical assessment</i>	<i>Comment</i>
Weight /height	Allows calculation of body surface area for aortic root nomogram
Auscultation	Ejection click is common if aortic root dilated Midsystolic click may be present in valve prolapse. Murmurs associated with valve regurgitation.
Blood pressure	If on beta blocker, calcium antagonist or ACE inhibitor/receptor blocker
<i>Electrocardiogram</i>	12 lead ECG at each visit Consider ambulatory or event monitor if palpitations
<i>Echocardiogram</i>	Full study every 12 months. Measure LV dimensions and function, pulmonary valve diameter, aortic root diameter. Plot aortic root against body surface area nomogram.

Detailed echocardiographic assessment should include a full study of left ventricular function, aortic root dimensions and intracardiac valves. Structural lesions should be excluded – in particular, atrial septal defect. Each echocardiography department should have a standardised protocol for measurement of the aortic root to allow reproducible sequential measurements which can be plotted against body surface area³⁴ (Figure 1).

Figure 1 Echocardiographic Measurement of Aortic Root³⁴



The aortic root should be measured at the annulus (1), sinus of Valsalva (2), sinotubular junction (3) and ascending aorta (4). Measurements should be made in diastole at right angles to the aortic valve closure line using the leading edge technique. These should be plotted against normal values. Normal values are available for aortic root dimensions³⁴. These nomograms have been criticised as they do not reflect the normal aortic root dimensions in tall, slim people in whom Marfan Syndrome has been excluded. Rozendaal suggested that an adjusted nomogram derived from tall, non-Marfan people should be used to take this into account³⁵. The same group devised a discrimination score which showed that the rate of aortic root growth in children and adolescents with Marfan Syndrome differs from the normal population with a sensitivity and specificity of 84% and 73%³⁶.

Perhaps the most important factor is the need for each echocardiography unit to develop a standardised measurement technique which enables reproducible measurements to be recorded sequentially in comparison to somatic growth. This allows discrimination between normal aortic growth and progressive dilatation and enables the appropriate institution of treatment

The pattern of root dilatation should also be noted as diffuse dilatation with loss of the sinotubular junction is associated with an increased risk of dissection³⁷. In some Marfan patients it is not possible to fully assess the aorta due to a poor acoustic window. This may be exacerbated by significant scoliosis. In this situation, MRI scanning should be used. This has the benefit of allowing an assessment of the lumbar dura. Dural ectasia is present 40% of children and over 90% of adults with Marfan Syndrome^{38,39}.

The frequency of cardiovascular assessment will depend on the age of the patient, the underlying cardiovascular abnormalities and medication. In general, most patients should be assessed every 6-12 months¹⁷. This may need to be more frequent if commencing medication or if there is a rapid growth phase.

Treatment of the cardiovascular manifestations of Marfan Syndrome

General advice

Most authorities advise patients with Marfan Syndrome to avoid isometric exercise and competitive or contact sports^{7,40}. This is based on the small risk of aortic dissection on exercise^{7,41,42}. Unfortunately, this advice can occasionally lead to complete avoidance of recreational exercise. Regular exercise has many psychosocial and general health benefits⁴³. Moreover, although studies have not been performed in Marfan Syndrome, regular exercise is known to attenuate poor vascular compliance in conditions such as diabetes and hypertension^{44,45}. Consequently, patients with Marfan Syndrome should be encouraged to remain active and a specific aerobic exercise prescription may be beneficial. Similarly, adherence to a healthy "Mediterranean diet" and avoidance of obesity and cigarette smoking should be recommended as this may prevent exacerbation of the increased vascular stiffness which occurs in the Marfan aorta⁴⁶⁻⁴⁸.

Due to its autosomal dominant inheritance, relatives are also at risk from Marfan Syndrome and should be offered medical assessment. Genetic counselling for would-be parents explaining the 50% risk to their child and the potential complications during pregnancy, especially increasing aortic root dilatation, should also be discussed.

The diagnosis of Marfan Syndrome itself, with its increased mortality and morbidity also raises psychosocial issues and the early involvement of clinical psychologists and support groups such as The Marfan Association UK can help in many cases.

Risk Stratification

Risk stratification in children is difficult. In adults, excessive aortic root dilatation (>1.7mm/year), increased aortic stiffness, aortic root diameter > 55mm^{49,50} and dilatation at the aortic sinotubular junction³⁷ are significant risk factors for dissection. A family history of aortic dissection is one of the most important risk factors. The absence of lens dislocation has been reported as a risk factor for aortic dissection although this may simply reflect delay in diagnosis and treatment⁴⁹.

Cardiac Surgery

Few children with Marfan Syndrome require cardiac surgery before reaching teenage years. In the neonatal form, surgery may be necessary to repair or replace the mitral or tricuspid valves and to replace the aortic root⁵¹. Outside of infancy, tricuspid valve surgery is rarely necessary and mitral valve repair or replacement is uncommon in childhood. Tsang reported only 7 children referred over a 7 year period to a large cardiac surgical centre⁵¹. Of these, 3 had the infantile form; 2 required mitral valve replacements (aged 2 and 7 years- one of whom also underwent tricuspid valve repair) and one aortic root replacement (aged 2 years). The 4 older children all required aortic root surgery (aged 15, 17 and 18 years).

The traditional form of elective aortic root replacement is the composite (Bentall) graft⁵². This involves the resection of the aneurysmal portion of the ascending aorta and replacement with a prosthetic valve incorporated in a dacron tube. More recent alternatives include using a valve-sparing procedure or novel exostent technique.

The valve sparing procedure involves resecting the ascending aorta and replacing it with a sculpted dacron tube which sits above the native aortic valve^{53,54}. This has the major advantage of avoiding the need for anticoagulation. Although the aortic valve leaflets are abnormal in Marfan patients, the data in adults suggest that survival is similar to the composite graft and valve related complications are lower⁵⁵. Preliminary results in children suggest that the valve-sparing technique has excellent short term results but this is dependant on the precise valve-sparing method used⁵⁶.

The exostent is a new concept which involves creating a 3 dimensional model of the dilated aorta and producing a computer-designed stent which is placed on the outside of the dilated root⁵⁷. This avoids the need for bypass surgery but prevents any form of growth and therefore, could not be used in a young child. Long-term data are awaited.

The final option is the use of a human donor aorta (homograft). This has the advantage of avoiding anticoagulation but is complicated by the shortage of homografts and the poor longevity of the graft in young patients^{58,59}.

Perhaps the most important decision is the timing of aortic root surgery. The ideal time to replace the root is “one or two months before it dissects”⁶⁰. Although aortic dissection is rare in childhood, the success of elective replacement (> 95% survival) is much lower than if emergency surgery is needed. In adults, it is usually recommended that the root is replaced when the sinus of Valsalva measures 5cm although this figure is reduced if there are additional risk factors such as a strong family history of dissection^{7,61}. In older children, most authors recommend root replacement at 5cm, when enlargement is greater than 1cm per year or if there is progressive aortic regurgitation⁶².

The surgical risk is not to be underestimated and therefore, delaying the need for surgery is of primary importance to the clinician. Medical treatment in the form of beta blockade is most commonly used. Second-line therapy involves Angiotensin Converting Enzyme inhibitors (ACEi), Calcium Channel Blockers (CCB) or Angiotensin Receptor Blocker (ARB) therapy. The medical treatments that are currently used and some novel possibilities will be discussed in detail in the following chapter.

CHAPTER 1.2

CURRENT AND NOVEL MEDICAL TREATMENTS IN MARFAN SYNDROME.

Pathophysiology

The main aim of treatment in Marfan Syndrome is to delay aortic root dilatation and therefore reduce the risk of aortic dissection and delay surgery for as long as possible. As our understanding of the pathophysiology of Marfan Syndrome increases, novel areas of potential medical therapy become apparent.

Fibrillin-1 contains calcium-binding sites that are important in stabilising the microfibril against proteolytic degradation by serine proteases such as matrix metalloproteinases (MMPs) and calpains. Hence, abnormal fibrillin-1 is either not secreted or forms abnormal microfibrils which in turn lead to decreased elastin and abnormal elastic properties of the aortic wall. In 1991, Hirata⁶³ found decreased distensibility, increased stiffness index and increased pulse wave velocity in the ascending and abdominal aortas of Marfan subjects. The Marfan aorta also has abnormal endothelial function with elevated levels of the endothelial cell products factor VIII antigen and thrombomodulin⁶⁴ and impaired flow-mediated vasodilatation⁶⁵.

Over time as a consequence of central aortic pressure and waves acting on the stiff aortic wall and these abnormalities in the extracellular matrix itself, the aortic diameter enlarges eventually resulting in intramural haemorrhage (from ruptured vaso vasorum) and aortic dissection or rupture.

TGF- β

There is recent data, murine and human, on the effects of targeting TGF- β receptors and this plays an important role in the pathogenesis of Marfan Syndrome.

TGF- β 1 is a central player in the development of fibrosis in chronic inflammatory conditions. Fibroblasts and smooth muscle cells respond to TGF- β 1 by expansion of the extracellular matrix with increased collagen synthesis and deposition paralleled by downregulation of matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) and upregulation of their tissue inhibitors (TIMP). TGF- β 1 also mediates the effects of angiotensin II (All) on extracellular matrix remodelling and vascular fibrosis.

Activation of TGF- β 1 is via several signal transduction pathways. Fibrillin-1 also contributes to the regulated activation of TGF- β 1 and in Marfan Syndrome, fibrillin-1 deficiency causes enhanced signalling of TGF- β 1. In 2003, Neptune⁶⁶ reported that fibrillin-1 regulates the activation of TGF- β 1 in the developing lung and that enhanced signalling in the fibrillin-1-deficient state contributes directly to apoptosis in the lung.

In 2004, Ng⁶⁷ hypothesised that TGF- β 1 may contribute to the multisystem pathogenesis of Marfan Syndrome and his group have recently demonstrated activation of TGF- β 1 in prolapsed mitral valves in fibrillin-1-deficient mice. Moreover, they showed that TGF- β 1 antagonism in vivo caused “phenotypic rescue” of the mitral valves. In 2006, Habashi⁶⁸ demonstrated increased aortic TGF- β signalling in a mouse model of Marfan Syndrome.

The actions and interactions of abnormal fibrillin, TGF- β 1 activity, angiotensin II and the proteases MMPs and calpains, all have a role to play in the pathological process in the Marfan aorta and in the development of Marfan Syndrome. These proteins provide possible therapeutic targets for medical intervention.

Clinical studies of medical treatment for Marfan Syndrome

Until recently, only three classes of drugs had been investigated in the prevention of aortic dissection and rupture in Marfan Syndrome - beta blockers, angiotensin converting enzyme inhibitors and calcium channel blockers, and most of these studies had been of beta blockers alone. At the start of my thesis, there had been no prospective, randomised, double-blind study yet reported. Table 1 summarises all the published clinical studies of medical treatment for Marfan Syndrome in humans.

Table 1 - Summary Table of Published Evidence in The Medical Treatment of Marfan Syndrome in Human

Subjects

<u>AUTHOR</u>	<u>DESIGN</u>	<u>INCLUSION CRITERIA</u>	<u>AGE</u>	<u>GENDER</u>	<u>DURATION</u>	<u>INTERVENTIONAL TREATMENT</u>	<u>OUTCOME</u>
BL Halpern 1971 ⁶⁹	Prospective Acute trial Non-randomised Non-blinded	N/K	N/K	N=2 M:F N/K	Acute study	Propranolol 5mg iv	Propranolol ↓dP/dT, ↓HR ↑pre-ejection period. Authors noted problems with side effects and qds regime
L Ose 1977 ⁷⁰	Retrospective Non-randomised Non-blinded	MF pts; Aortic dilatation ± AR	5-59yrs Mean 30.4± 14.8yrs	N=25 M=16, F=9	1-7yrs range, mean 3±1.8yrs	Propranolol titrated to keep HR<70 at all times. 120-160mg/day(adult); 40-80mg/day(children)	20% had acute aortic dissection and died despite Rx. Other pts had ↑ aortic root dilatation. No side effects noted
CM Reed 1992 ⁷¹	Prospective Non-randomised Non-blinded	MF assessed by geneticist	10-18yrs Mean 14.7yrs	N=9 M=5, F=4	≥6 weeks	Oral atenolol titrated to 2mg/kg/day in 2 divided doses	Significant decrease in HR, peak aortic velocity, LV ejection force and force/BSA after treatment. No change in afterload or bp
CM Reed 1993 ⁷²	Retrospective Non-randomised Non-blinded	MF assessed by geneticist	6-22yrs Mean 14±3yrs	N=22 M=14, F=8	Mean 3.9± 1.4yrs	Atenolol titrated clinically up to 2mg/kg/day	No change in pulse pressure, aortic stiffness or distensibility with atenolol in this young popn
AC Tahernia 1993 ⁷³	Prospective Non-randomised Blinded	MF by 1979 criteria	5-14yrs Mean 9.3yrs	N=6 M=4, F=2	2-5yrs	Propranolol <1mg/kg in 2 divided doses given to the 3 pts with aortic dilatation	No stats but in the 3 pts on propranolol who already had at least moderate aortic root dilatation there was no progression by echo. There was progression in the control group. No deaths/surgery occurred. No side effects noted.
J Shores 1994 ⁷⁴	Prospective Randomised Pts and investigators unblinded. Echo done blinded.	MF (Berlin); 12-50yrs; On no current treatment; mild- moderate aortic root dilatation	Mean 15.4 yrs propranolol 14.5 yrs control	N=70 M=39, F=31 32 Propranolol 38 Controls	9.3 yrs in controls, 10.7 yrs in treatment group	Propranolol titrated to keep HR<100bpm during exercise. Mean 212±68mg/day	Slower rate of aortic root dilatation in treatment group but heterogeneous response. Fewer clinical end points (AR, aortic dissection, c-v surgery, CCF, death) in treatment group (5 vs 9). Includes 2 deaths (no treatment group) but no aortic

							dissection on autopsy. 22 adverse effects in 30 pts – 1 dose reduction
MA Salim 1994 ⁷⁵	Prospective Non-randomised Non-blinded	MF (Berlin); <21 yrs old	10.4± 3.4yrs 14.1± 3.4yrs treatment 10.2± 4.6yrs controls	N=113 M=76, F=37 100 on treatment 13 control	2.1-8.2yrs	Propranolol or atenolol vs no treatment. Titrated according to protocols	The greatest rate of aortic root dilatation was in the no treatment group. 5 treated pts had asc aortic grafts despite larger doses of treatment
DI Silverman 1993 ⁷⁶	Retrospective Non-randomised Non-blinded No control group	MF (Berlin)	Mean 37±17yrs	N=417 M=213, F=204 191 (46%) received beta- blocker	5.5±2.1yrs	Propranolol, atenolol, metoprolol, nadolol or more than 1 over time	Retrospective analysis of MF pts from clinic databases. 191/417 were given a beta- blocker. There were 8 deaths (4.1%) and 58 operations(30.4%) in that group. Median cumulative survival was slightly higher (72vs70yrs) and mean age at c-v surgery was higher (33vs29yrs) in those who had taken beta blockers. No data on side effects
ME Legget 1996 ⁷⁷	Retrospective Non-randomised Non-blinded No control group	MF	Mean 21yrs Range 1-54yrs	N=83 30 (36%) received beta blockers ≥1yr	<1-16yrs Mean 4yrs	Unknown	Aortic ratios and the change in ratio between initial and final echos did not differ between groups. Number of deaths, aortic root replacement for dissection or surgery for ascending aortic aneurysm did not differ between the groups.
A Haouzi 1997 ⁷⁸	Acute trial Prospective Non-randomised Non-blinded	MF(Berlin)	32± 10yrs MF 35± 5yrs controls	13 MF pts 10 normal controls	60 mins	Pts scanned before and 60 mins after metoprolol 1mg/kg stat	100% of controls had lower stiffness indices after metoprolol cf only 62% MF pts. Non-responders had more dilated ascending aortas.
M Groenink 1998 ⁷⁹	Prospective Non-randomised Non-blinded	MF (Berlin)	21-41yrs	N=12 M=4, F=8 6 MF pts (Berlin) 6 normal controls	2 weeks- 6.7 months	200mg metoprolol once daily or 100mg atenolol once daily for at least 2 weeks	↑aortic distensibility in asc aorta and ↓pulse wave velocity in MF group after treatment diagnosed by MRI
R Rossi-Foulkes 1999 ⁸⁰	Prospective Non-randomised Non-blinded	MF (Ghent); paediatric pts; F<17yrs old M<19yrs old	Range 0.5-17.8 yrs old Mean 9.4± 5.3yrs	N=44 M=28, F=25 26 MF pts 20 on BB 6 on CCB, 5/6 on verapamil 18 MF pts on no	Mean 44± 24 months.	Calcium channel blocker (verapamil in 5/6) and beta blocker. Titrated to max tolerated ↓in HR and bp	Slower aortic root growth in medicated pts. 20% in treatment group developed major c-v complications (AR; severe MR and aortic dilatation; progressive aortic dilatation; aortic dissection;

				treatment.			descending aortic aneurysm). Too few on CCB to see any difference between BB and CCB
AS Rios 1999 ⁸¹	Prospective Non-randomised Non-blinded	MF pts; No Symptoms; 18-45 yrs	31±14.2yrs	N=23 M=11, F=12	4±2.2 yrs	Atenolol max dose to achieve HR 50-60bpm, SBP >89mmHg or side effects Mean dose 43.5± 21.6mg/day	Heterogeneous response to atenolol. Stiffness index and distensibility didn't change in 35%. Responders more likely if aortic root diameter<40mm
GJ Nollen 2004 ⁸²	Prospective Non-randomised Non-blinded	MF (Ghent) 22/32 MF patients had aortic root replacement	MF- 33yrs sd 11yrs Normals mean 29yrs sd 5yrs	25 MF pts on BB 7 MF pts no BB (M=25;F=7) 10 normal controls (M=7;F=3)	Acute study	Unknown BB	No significant difference in transition point-ie. the pressure at which the pressure-area relation deviates from its elastic (linear) to the collagen (exponential) course. This was measured at the descending aorta at the level of the pulmonary bifurcation by MRI and Finometer non-invasive bp monitor. There was also no difference in diameter of descending aorta, distensibility or mean bp between MF groups
AT Yetman 2005 ⁸³	Prospective Non-randomised Blinded echocardiographer	MF (Ghent)	Mean age 12.0 ±7.6yrs β-blocker 14.6 ±7.7yrs enalapril	N=58 M=28, F=30 32 MF pts on ACEi, 24 MF pts on atenolol 2 MF pts on propranolol	3±0.2 years	Median doses: Atenolol 25mg twice daily, propranolol 1mg/kg thrice daily, enalapril 5mg twice daily.	Enalapril improved aortic distensibility, reduced aortic stiffness, and was associated with a smaller ↑in aortic root diameter and fewer clinical end points of BB. There were 7 root replacements in BB group and 2 in ACEi group. There was 1 death -due to ventricular arrhythmia in the BB group. 8 pts were on enalapril due to ses from β-blocker
M Ladouceur 2007 ⁸⁴	Retrospective Non-randomised Non-blinded	MF (Ghent)	<12yrs Mean age 6.1±3.2yrs in BB group 7.4±5.2yrs in control group	N=155 M=82, F=73 77 MF pts on BB 78 MF pts never had BB	Mean 4.5±3.7yrs	Atenolol >70% Nadolol 17% Propranolol 6%	BB decreased the rate of aortic dilatation at the Sinuses by mean of 1.6mm/yr (p<0.05) by echo. Trend (ns) toward lower cardiac mortality, reduced need for preventive aortic surgery and less dissection in BB group.

							2/77 stopped taking BB although pts on CCBs/ACEis already excluded from study.
ESS Tierney 2007 ⁸⁵	Retrospective Non-randomised Blinded echocardiographer	MF (Ghent)	Mean 9.2±4yrs in BB group. Mean 8.8±4.8yrs in the control group	N=63 29 on BB 34 controls	Mean FU: 76 months BB; 81 months control	Atenolol mean dose 0.92mg/kg/day	No significant difference in the aortic root measurement/death/surgery between the BB and control groups. 10/29 BB group had ses.
AA Ahimastos 2007 ⁸⁶	Prospective Randomised Double-blinded Placebo-controlled	MF (Ghent)	Mean 34yrs in ACEi group Mean 31yrs in placebo group	N=17 10 on ACEi 7 on placebo	24 week FU	Perindopril 8mg/day	Patients given ACEi or placebo in addition to normal BB. ACEi group had reduced aortic stiffness, aortic root diameter, TGF-β, MMP-2 and MMP-3 levels. No ses/major events/deaths in either group
BS Brooke ⁸⁷ 2008	Retrospective Non-randomised Observational Blinded echocardiographer	MF (Ghent)	ARB median age 6.6yrs; range 1-16yrs. BB median age 12yrs; range 4months-19yrs.	N=18 on ARB N=65 on BB	Median FU 26.1 months	Losartan in 17pts Irbesartan in 1pt.	The mean rate of change in aortic diameter decreased significantly from 3.54mm during previous medical therapy to 0.46mm/yr during ARB therapy. No ses noted in ARB group.

Beta-Blockers

1950-1980

In the 1950s, it was discovered that giving reserpine to a strain of turkeys susceptible to aortic rupture prevented the birds' untimely death⁸⁸. In 1965, Wheat⁸⁹ reported increased survival of patients with dissecting aneurysm when treated with antihypertensive drugs. These studies led to the use of Propranolol and Reserpine in Marfan patients seen at The Johns Hopkins University, Baltimore. It was from this institution in 1971, that Halpern⁶⁹ described their use of Propranolol to slow aortic root dilatation and dissection in six adults with Marfan Syndrome. It was postulated that the negative inotropic and chronotropic effects of the beta blocker could be used to decrease the progression of aortic dilatation and therefore prevent acute aortic dissection. Side effects were experienced by one third of this small group of patients who were given Propranolol four times daily because of its short half-life. The authors also described the administration of intravenous Propranolol 5mg to two Marfan patients to prolong the pre-ejection period defined as the time from the Q wave on the ECG to aortic valve opening. Six years later, however, one of the original authors wrote again with Ose⁷⁰, describing 25 patients with Marfan Syndrome and aortic dilatation with or without aortic regurgitation. All had been given Propranolol (120-160mg) for one to seven years and 20% died of acute aortic rupture. In this paper published in 1977, it was suggested that Propranolol had no beneficial effect on the increasing dilatation of the aorta.

1980-2000

A reduction in vascular complications in Marfan Syndrome was not confirmed until 1994 when, in a landmark paper, Shores⁷⁴ reported a significant reduction in the rate of aortic dilatation with Propranolol compared to no treatment. Seventy adults and children with Marfan Syndrome diagnosed by the older Berlin nosology and with mild-to-moderate aortic dilatation were treated in an open-label study. Aortic diameters were measured by a blinded echocardiographer. The rate of aortic dilatation was significantly lower in the treatment group compared to the control group. On average, the rate of enlargement of the aorta in patients on treatment was less than a third the rate of patients on no treatment ($p < 0.001$). Clinical end-points were reached in 16% of patients in the treatment group and 24% of patients in the control group. Only two patients died during the study, both in the control group; post-mortem examination

revealed neither aortic dissection nor any obvious cause of death. 22 episodes of side effects were seen in the 30 treated patients, one requiring a decrease in dose. The theoretical reason suggested for the beneficial effect of beta blockers on Marfan aortas was the decrease in the rate of change in central arterial pressure (dP/dt).

Subsequently, beta blockers have been shown not to reduce central arterial pressure or stiffness⁹⁰ and other available drugs may decrease central pressure and stiffness more⁹¹⁻⁹³. The conclusions of Shores et al were supported by Salim⁷⁵ in a retrospective study of beta-blocker treatment in 100 Marfan patients compared to no treatment in 13 Marfan patients. However, in this study, 5% of the treated group went on to have ascending aortic grafts.

A trial worth reporting because of the patient size and therefore its influence in meta-analyses is that by Silverman, 1993⁷⁶. They retrospectively looked at the clinic data of 417 patients diagnosed with Marfan Syndrome by the older Berlin nosology. The trial looked at survival rates and compared them to a paper by Murdoch et al in 1972⁹⁴. On looking back over the data they found that 191 patients (102 men and 89 women-total 46% of the patients reviewed) had taken at least one beta blocker (14 took propranolol; 100 took atenolol; 5 took metoprolol; 50 took nadolol; 22 took more than one in succession). The mean duration of treatment was 5.5±2.1yrs. They remarked that the median cumulative probability of survival for the beta blocker patients was 72 yrs compared to 70 yrs for the rest (p=0.01) and mean age at surgery was 33±11yrs for the beta blocker group compared to 29±10yrs for the others (p=0.24). Aetiology of the 8 deaths in the treated group was not reported. The limitations in such a study are clear. It is, therefore, most noted for the skew it would put on a meta-analysis due to the large patient numbers.

Other mechanisms may be in action. Since conduit arteries including the aorta have adrenergic innervation⁹⁵, it has been suggested that beta blockers may also have direct effects on the elastic properties of the aorta. In 1997 Haouzi⁷⁸ showed that in 10 controls, oral Metoprolol decreased aortic stiffness acutely (beta index from 4.8 to 3.6) and increased distensibility (3.5 to 4.9 x10⁻⁶ cm².dyne⁻¹). It was also effective in eight of 13 Marfan patients (62%) (beta index 13.4 to 7.5; distensibility 1.3 to 2.5 x10⁻⁶ cm².dyne⁻¹) but in five subjects (38%) these indices deteriorated.

Other investigators also observed variable haemodynamic responses to beta blockade^{72,79}. Haouzi⁷⁸ reported that the Marfan subjects who benefited most from beta blockade were those whose aortas were less dilated (34±6 vs 38±6 mm). In 1989 Yin⁹⁶ gave intra-venous Propranolol to Marfan subjects during diagnostic cardiac catheterisation, and found that it increased the magnitude of aortic wave reflection,

reduced arterial compliance and did not decrease the maximum acceleration of blood into the ascending aorta. Other authors have reported that beta-blockade increases peripheral vascular resistance, which in turn may increase central aortic pressure and wall stress⁸¹.

GJ Nollen⁸² looked at 32 Marfan patients and 10 normal controls with MRI and a Finometer—a finger arterial non-invasive blood pressure monitor that is used to reconstruct aortic pressure. They describe the pressure-area curve derived from MRI (in the descending aorta at the site of the pulmonary bifurcation) and Finometer which changes from a linear to an exponential relationship. They suggest that this transition point corresponds to a change in load-bearing from the linear—elastin fibres, to the exponential—elastin and collagen fibres. There was no transition point in the 10 normal controls but there was in six of the 32 Marfan subjects. There was no significant difference in this relationship between the beta blocker and other Marfan group. Unfortunately, 22 of the 32 Marfan subjects had already had aortic root replacement and so their central haemodynamics and response to beta blockers will be different because of this⁹⁷.

2000- 2011

A more recent trial looked at the effect of beta blockers on ascending aortic dilatation in children less than 12 years old⁸⁴. They looked back retrospectively at their young Marfan clinic patients and analysed their echos. 77 children took a beta blocker before the age of 12 years- greater than 70% took atenolol; 17% nadolol and 6%propranolol. They were compared to a group of 78 who had never taken beta blockers. 5 children were excluded as they were taking verapamil (3) or an unnamed ACEi (2). The baseline characteristics were similar apart from the control group had a lower ratio of supra-aortic ridge to Sinus of Valsalva diameter. This is important as we know that localised aortic dilatation at the sinuses has a better prognosis and dilates slower than generalised dilatation involving loss of the sinotubular junction¹⁰⁰. This wasn't commented on in the paper. The results showed that the rate of aortic root dilatation was 1.05mm/year in the beta blocker group compared to 1.15mm/year in the control group after 4.5±3.7 years (p=0.0001). This means a total difference between groups of 4.5mm over the study period. Four children died during the period—one of respiratory distress and three sudden deaths due to atrial or ventricular arrhythmias. Three of the four were in the control group. Five of the control group had aortic surgery compared to two in the beta blocker group. Two of the beta blocker group had to stop treatment due to side effects which weren't specified.

Typical of the confusion in the data, the latest trial Tierney et al⁸⁵ tell us that beta blockers do not alter aortic root measurements in paediatric Marfan patients. They retrospectively reviewed the echo studies with blinded operators of 29 Marfan patients taking beta blockers and 34 untreated. The average dose of atenolol was 0.92mg/kg/day and the length of follow-up was 76 and 81 months in the respective groups. After this time, there was no significant difference in the aortic root diameters of the two groups. They had one death in the untreated group, a 10 year old who died perioperatively during aortic valve replacement. Two patients underwent elective aortic root replacement in the untreated group and one patient underwent surgery for aortic root dissection in the beta blocker group. Ten of the twenty-nine patients in the beta blocker group had side effects (34%) including exercise intolerance, fatigue, brochospasm and depression. Interestingly, seven of the thirty-four untreated group (21%) also reported symptoms during the study including fatigue, brochospasm, migraines or headache and depression. Seven treated patients (24%) were non-compliant at one or more clinic visit showing another difficulty faced in looking after this young population.

Meta-Analysis of Beta Blocker Trials 2007

The only meta-analysis performed, was by DR Gersony et al⁹⁸, emphasising the differences between and paucity of good trials on the efficacy of beta blockers in Marfan Syndrome. They came to the conclusion that there was no evidence that beta blockers have clinical benefit in Marfan patients. They analysed six studies⁷³⁻⁷⁷ and used the endpoints of aortic dissection or rupture, cardiovascular surgery or death. As described earlier the Silverman study⁷⁶ had a large impact due to the 417 patients in the trial. This was much larger than the next largest trial –Salim, 1994⁷⁵ whilst the smallest trial only had 6 patients -Tahernia,1993⁷³.

Variability In Trial Design and Results

It seems that there are trials confirming reduction in the rate of aortic growth with beta blockers although there are others describing varying response to treatment but no study showing a reduction in major endpoints such as aortic dissection and death.

A quick look at the table above reveals the differences between the trials in this area with regard to patient selection, age of patient, type of study, randomisation, blinding,

length of study, type and dose of beta blocker used, measures used, scanning method (MRI or echo) and the complete lack of a prospective, randomised, double-blind trial.

Concurrent vasodilators might counteract any rise in systemic vascular resistance^{71,96} but most patients receive a beta blocker such as atenolol alone, although evidence for its efficacy is limited.

Side Effects of Beta Blockers

Compliance may also be a problem in predominantly young patients who require lifelong treatment - beta blockers have a significant side-effect profile. All beta blockers are less commonly given to paediatric hypertensive patients because of their adverse events⁹⁹. This includes bronchospasm, fatigue, depression and behavioural problems impacting on school and socially. Marfan patients with the increasingly common asthma will not be given beta blockers and so will need an alternative. This all has serious implications for the young Marfan population.

Despite the varying conclusions of the trial data, the paucity of good trials and the side effect profile, beta blockers are still recommended in children with Marfan Syndrome by the American Academy of Pediatrics¹⁰¹. They remain today the first choice in the prevention of aortic dilatation, dissection and rupture in the Marfan population. I researched possible alternatives to beta blockers, firstly reviewing other medications that have any trial data and secondly, some medications that aren't used but may have potential benefits and would warrant further investigation.

Calcium channel blockers

Calcium channel blockers are sometimes prescribed for patients with Marfan Syndrome when beta blockers are contraindicated, for example in asthma, or if intolerable side effects are encountered but their use has been evaluated in only one small study. Rossi-Foulkes⁸⁰ reported a slower rate of enlargement of the aorta in 26 patients receiving treatment, compared with placebo (+0.9 vs 1.8 mm/yr, $p < 0.02$), but 20 patients received β -blockers and only six a calcium channel blocker (including Verapamil in five). No comparisons between the drugs were reported because the numbers were too small.

Since Verapamil is negatively inotropic and chronotropic and also causes generalised arterial and arteriolar dilatation, there are theoretical grounds for expecting benefit in Marfan syndrome, but they have not been tested adequately. Verapamil's slow release formulation has a half-life of 5-12 hours and so can be given as a once daily preparation to improve compliance. Calcium antagonists reduce central arterial pressure and stiffness⁹⁰. A dihydropyridine calcium antagonist such as Nifedipine or Amlodipine might have similar effects on conduit arterial function, but might be less useful because of the relative lack of effects on cardiac inotropic state. Amazingly, there are no other trials human or otherwise looking at Calcium channel blockers despite them often being used if beta blockers are contraindicated.

Angiotensin converting enzyme inhibitors

Angiotensin converting enzyme (ACE) inhibitors reduce central arterial pressure and conduit arterial stiffness⁹⁰. Preliminary evidence suggests that they may be useful in Marfan syndrome⁸³. In hypertension studies it has been suggested that Perindopril may reduce large arterial stiffness by a mechanism that is independent of its direct effect on lowering blood pressure¹⁰². Perindopril is swiftly broken down to its active metabolite perindoprilat which has a half-life of 3-10 hours and therefore, can be given once daily.

ACE inhibitors have other effects that might also be clinically useful in patients with Marfan syndrome. Cystic medial degeneration of the aorta is observed in Marfan Syndrome as well as in aortic dilatation, aneurysm and dissection related to atherosclerosis and ageing¹⁰³, perhaps caused by accelerated apoptosis of vascular smooth muscle cells (VSMC). Activation of the angiotensin II type 2 receptor (AT11R) and reduction in the expression of angiotensin II type 1 receptor (AT1R) plays an important role in promoting apoptosis of VSMCs and cystic medial degeneration in Marfan Syndrome¹⁰³. Therefore, an ACE inhibitor but not an Angiotensin II type 1 receptor (AT1R) blocker can prevent cystic medial degeneration, apoptosis of VSMCs, and aortic dissection in rats fed β -aminopropionitrile monofumarate, which induces dissection by inhibiting the cross-linking of collagen fibres¹⁰⁴. However, the authors did note that ACEIs not only block the Renin Angiotensin System pathway but also inhibit the breakdown of bradykinin and activate nitric oxide synthase which is a possible cause of their benefit.

A recent prospective, blinded, non-randomised trial⁸³ compared Enalapril and either Atenolol or Propranolol (Propranolol was given in children <12.5kg) in 57 subjects,

mean age of 14.6 and 12 years respectively. They were followed prospectively for a mean of three years. An increased aortic distensibility (3.0 ± 0.3 vs $1.9 \pm 0.4 \text{cm}^2 \text{dynes}^{-1}$; $p < 0.02$) and a reduced aortic stiffness index (8.0 ± 2.9 vs 18.4 ± 3.8 ; $p < 0.05$) were seen in the Enalapril group compared to the beta-blocker group and this resulted in a smaller increase in aortic root diameter (0.1 ± 1.0 vs $5.8 \pm 5.2 \text{mm}$; $p < 0.001$). Nine subjects underwent aortic root replacement during the study, two in the Enalapril group (6%) and seven while on beta blockers (28%). A total of twelve patients who had taken beta blockers took enalapril due to side effects whilst no-one in the enalapril group reported serious side effects. The authors gave three possible mechanisms for the beneficial effect of the ACE inhibitor. The first is inhibition of VSMC apoptosis as described above; the second is bradykinin-mediated improvement in aortic elastic tone; and the third is blocking hyperhomocysteinaemia which increases vascular stiffness. ACE inhibitors also reduce matrix metalloproteinase activity¹⁰⁵.

A recent randomised, double-blind, placebo-controlled, Australian trial by Ahimastos, 2007⁸⁶ showed that in patients already taking beta blockers there was reduced aortic stiffness, aortic root diameter, TGF- β , MMP-2 and MMP-3 levels when also given perindopril compared to placebo. 10 patients (average age 34 years) took 8mg/day perindopril compared to 7 patients (average age 31 years $p = 0.47$) taking placebo in addition to their normal beta blocker for a total of 24 weeks. There were no side effects, major events or deaths in either group. The authors suggest that the effects seen in the perindopril group likely occurred by reducing signalling through both AT1 and AT2 receptors and that the reduction in TGF- β , MMP-2 and MMP-3 are probably secondary to reduced AT1 receptor signalling. Reduction in AT2R signalling may provide additional benefit through protection from cystic medial degeneration as described previously. This suggests that ACEis should provide the Marfan aortic root more protection than AT1R antagonists alone.

Angiotensin II type-1 receptor blockers (AT1R Blockers)

Angiotensin II stimulates collagen formation, triggers matrix remodelling and vascular hypertrophy, depresses nitric oxide-dependent signalling, increases oxidant stress, and reduces elastin synthesis¹⁰⁶. It also stimulates cytokines and growth factors that contribute to an increased inflammatory response. These all contribute to arterial stiffness.

AT1R blockers are often used for conditions such as hypertension that require ACE inhibitors, when the latter cannot be used because of bradykinin-mediated side effects.

It may be helpful to decrease blood pressure in Marfan Syndrome by any mechanism but unlike the ACE inhibitors, the AT1R blockers do not inhibit VSMC apoptosis and therefore may not reduce cystic medial degeneration, the histological abnormality seen in aortic dissection. However, there is evidence that the AT1R blocker Irbesartan decreases MMP activity¹⁰⁵.

TGF- β receptor antagonism and AT1R blockers

The potential role of TGF- β receptor antagonism by AT1R blockers has caused the most excitement in this area. This antagonism has been shown to attenuate hypertension and the decline of renal function in uraemic rats¹⁰⁷. Losartan has also been shown to reverse interstitial fibrosis and the expression of collagen and TGF- β 1 in a mouse model of hypertrophic cardiomyopathy¹⁰⁸. The mechanism by which AT1R blockade antagonises TGF- β signalling is uncertain. However, activation of AT1 receptors increases the expression of TGF- β ligands and receptors and induces the activation of thrombospondin, a powerful TGF- β activator.

Habashi's Mouse Model

A paper published in April 2006⁶⁸ has caused a huge amount of excitement and provoked a flurry of new research by researchers looking at the medical treatment of Marfan Syndrome. Habashi demonstrated that TGF- β antagonism by the AT1R blocker Losartan prevented aortic aneurysm and also partially reversed impaired alveolar septation in a mouse model of Marfan Syndrome. The authors gave Losartan, Propranolol or placebo to pregnant mice which had the commonest mutation causing Marfan Syndrome. The doses were titrated to achieve comparable haemodynamic effects. The pups continued to be treated up to ten months of age. Fragmentation of aortic elastic fibres was seen in both placebo and Propranolol-treated mice but not in the Losartan group. Also, the aortic wall architecture was normal in the Losartan group relative to the placebo group and Propranolol had no effect. Aortic wall thickness and aortic diameter were similar in the placebo and Propranolol groups ($p=0.19$) but undistinguishable between the wild-type and Losartan-treated mice ($p=0.24$). The authors went on to give the same drugs to seven week-old mice to see if Losartan could have similar effects after the establishment of aortic aneurysms. They found that Losartan prevented elastic fibre fragmentation and blunted TGF- β signalling in the aortic media and that the Losartan mice had the same aortic root growth rate as the

wild-type mice ($p=0.55$). The Propranolol group had a slower rate of aortic root growth than did the placebo mice ($p<0.001$) but this was still greater than the wild-type and Losartan group ($p<0.02$). Propranolol had no effect on the aortic wall thickness ($p=0.17$) or elastic fibre architecture ($p=0.47$) compared to placebo and their effect seemed to be limited to slowing aortic root growth. Thus Losartan but not Propranolol achieved full correction of the phenotypic abnormalities in the aortic wall in the mouse model of Marfan Syndrome.

This single experimental study may revolutionise treatment in Marfan Syndrome. It has precipitated the commencement of a large-scale prospective trial comparing beta blockers to Losartan in humans with Marfan Syndrome¹⁰⁹.

Further AT1R Blocker Studies

Recently, BS Brooke 2008⁸⁷, looked at 18 Marfan children who had been followed during a median 26.1 months (range 12 to 47 months) of therapy with ARBs after other medical therapy (beta blocker alone (12pts) or in combination with an ACEi (4pts) or CCB (2pts)) had failed to prevent progressive aortic root dilatation. All patients had severe aortic root enlargement. The ARB was losartan (1.4mg/kg/day) in 17 patients and irbesartan (2mg/kg/day) in 1 patient. Treatment with the ARB significantly slowed the progress of aortic root diameter from 3.54mm per year during previous medical therapy to 0.46mm per year during ARB treatment ($p<0.001$). The degree of response, however, was variable. They compared the ARB figures with a group of similar patients who had milder aortic root disease and took beta blockers alone. The mean rates of change in aortic root diameter in the beta blocker group (1.71mm/yr) were significantly higher than the more severely affected ARB group ($p<0.001$). The more distal segments of the ascending aorta past the sinotubular junction were unaffected by the ARB therapy. No side effects were encountered.

Recently, HHC Yang et al¹¹⁰ used a mouse Marfan model to look at losartan versus doxycycline versus the combination of both with respect to aortic root diameter. At 4 months of age when the mice thoracic aortic aneurysms had established they gave the three groups ($n=15$ /group) of mice the above combination of drugs. At nine months the aortic diameter in the untreated Marfan mice was increased by 40% compared to control. Losartan or doxycycline reduced aortic diameter by 10-16% versus untreated Marfan aortas. Losartan and doxycycline combined completely prevented thoracic aortic aneurysm and improved elastic fibre organisation. They also reduced MMP-2 and 9 and TGF- β . Aortic contractile and relaxation functions were also normalised.

This result shows that the combination of the losartan to reduce TGF- β activity with the doxycycline to reduce MMP activity works synergistically in reducing the rate of aortic root dilatation in the mouse model and would therefore be interesting to trial in human Marfan syndrome.

There seems, therefore, to be two theories with contradictory beliefs of the effectiveness of general ACE inhibition compared to selective angiotensin 1 receptor antagonism. Reducing AT2R decreases VSMC apoptosis and therefore cystic medial degeneration but this receptor induces anti-inflammatory and antiproliferative effects beneficial in aortic-wall homeostasis; whereas reducing AT1R reduces TGF- β activity.

Experimental studies of alternative drugs

Aldosterone receptor antagonists

Aldosterone-responsive mineralocorticoid receptors are also present in the heart and large arteries and aldosterone is produced in the vascular wall. Aldosterone upregulates and increases the sensitivity of angiotensin type 1 receptors, and therefore mediates and exacerbates angiotensin II-induced cardiovascular damage. Thus, aldosterone receptor antagonists may be a useful adjunct to therapy, although whether their effects would be greater or even equal to those of ACE inhibitors or AT1R blockers is unknown.

Nitrates

Organic nitrates are another important class of drug which are only now being evaluated in Marfan Syndrome¹¹¹. These drugs are metabolised with the release of nitric oxide which activates guanylate cyclase and increases the formation of cGMP. cGMP activates protein kinase G and leads to a cascade of effects in smooth muscle. These effects culminate in dephosphorylation of myosin light chains and sequestration of intracellular calcium ions, causing muscle relaxation. Organic nitrates lead to venodilation and also have marked effects on large muscular arteries. This reduces pulse wave reflection from arterial branches and therefore, reduces central aortic pressure. With larger doses, resistance arteries and arterioles dilate and arterial pressure falls.

Nitric oxide also has other effects. It inhibits vascular smooth muscle cell proliferation, inhibits platelet adhesion and aggregation and inhibits monocyte adhesion and migration. Consequently, it may slow aortic dilatation and dissection. There are currently two trials in progress looking at the difference in arterial stiffness of Marfan subjects when given a nitrate or an AT1R blocker or placebo¹¹¹.

Matrix metalloproteinase (MMP) activity

MMPs are large endopeptidases that degrade matrix proteins such as collagen and elastin. MMPs and their endogenous tissue inhibitors (TIMPs) are regulated by three different mechanisms¹¹². Firstly, gene expression is tightly controlled at the transcriptional level (for MMPs and TIMPs). Secondly, plasmin and urokinase plasminogen activator (uPA) control the activation and degradation of the MMPs from inactive proenzymes. Thirdly, the endogenous TIMPs actively inhibit the proteolytic activity of MMPs.

In Marfan Syndrome, the concentration of MMPs and TIMPs may be implicated in the cardiovascular and valvular lesions. Immunohistological analysis of the aortic roots in seven patients with Marfan Syndrome revealed extensive cystic medial necrosis, loss of elastic fibres, collagen and smooth muscle cells and this was seen primarily in central regions¹¹³. Normal aortic tissue expresses modest amounts of MMP-1,-2 and –9 in endothelial cells and macrophages, weak quantities of MMPs in smooth muscle cells and virtually none in elastic fibres. Expression of TIMP-1 and –2 is weak in corresponding cell types in the normal aorta. Increased expression of MMP-2 and –9 which degrade basement membrane collagen and partly digest elastin was also demonstrated in abdominal aortic aneurysms of Marfan Syndrome patients compared with controls¹¹⁴. Affected aortic valves exhibit moderate immunoreactivity of MMPs and weakly reactive TIMPs in regions containing fibroblasts and myofibrils. Increased expression of MMP-1, -2, -3 and –9, without corresponding elevation in TIMPs –1 and –2, provides topographic evidence that abnormal remodelling might play a role in connective tissue diseases such as Marfan Syndrome. If an MMP/TIMP imbalance plays an aetiological role in tissue destruction, then future interventions may include those targeted at the extracellular matrix in Marfan syndrome.

MMP Inhibitors - Doxycycline and Tetracycline

Pharmacological agents such as Tetracycline and Doxycycline are nonselective inhibitors of MMP activity in vitro, which have been shown to suppress experimental abdominal aortic aneurysms in rodents. In small, uncontrolled clinical trials, pre-treatment with Doxycycline, twice daily doses for seven days, reduced MMP-2 and -9 expression in aortic tissues of patients with abdominal aortic aneurysms compared with untreated controls^{114,115}.

Doxycycline has been shown to delay aneurysm rupture (132 vs 79 days, $p < 0.01$) in a mouse model of Marfan Syndrome, reduce aortic wall elastic fibre degradation and lower MMP-2 and -9 levels compared to untreated Marfan mice¹¹⁶. Doxycycline and derivatives seem to antagonise the activation of MMP-9 expression in tissue monocytes/macrophages and to retard proMMP-2 processing¹¹⁵.

It has also been recently shown that long-term doxycycline is more effective than atenolol in preventing thoracic aortic aneurysm in a mouse model of Marfan Syndrome¹¹⁷. This group gave a group of Marfan mice atenolol, doxycycline or left them untreated. At six and nine months the aortic root measurements of the atenolol group were significantly better than the untreated group but the doxycycline group were significantly better than the atenolol group and were no different from the normal controls. Doxycycline also improved elastic fibre integrity, normalised aortic stiffness, suppressed the upregulation of TGF- β and reduced MMP-2 and -9. Intraperitoneal injection of neutralising antibodies against MMP-2 and -9 yielded similar results to that of doxycycline.

The same group studied the additional benefit of doxycycline to losartan in preventing thoracic aortic aneurysm in the same mouse model as described earlier¹¹⁰. However, direct evidence of efficiency in man is still awaited. Enalapril and Irbesartan have both been found to decrease MMP-9 protein and MMP-9 activity in patients with coronary artery disease and arterial hypertension six to eight weeks after coronary angioplasty¹⁰⁵.

Advanced Glycation End Product Crosslink Inhibitors/Breakers/AGE receptor Blockers

Long-lived structural proteins such as collagen and elastin undergo continual non-enzymatic cross-linking with reducing sugars during life. This initial reaction is fast, reversible and dependent on the concentration of available sugars. Lowering the sugar

concentration reverses the reaction. Further rearrangement forms a more stable glycated protein. These proteins accumulate over time and can form cross-linked proteins called advanced glycation end-products or AGEs.

It is these heterogeneous AGE complexes that can irreversibly cross-link collagen and elastin increasing cardiovascular risk. This process occurs with ageing but is accelerated in diabetes, end-stage renal failure and hypertension. AGE cross-links have been implicated in age-related structural and physiological changes in the cardiovascular system, such as increased vascular and myocardial stiffness, endothelial dysfunction, altered vascular injury responses and atherosclerotic plaque formation¹¹⁸.

Many of these changes were thought to be irreversible, but studies of novel therapeutic agents that inhibit AGE cross-link formation (Aminoguanidine, Pyridoxamine, OPB-9195), break existing cross-links (DPTC, ALT-711, LR-90), or block receptors for AGEs (RAGE), support reversibility and therefore suggest potential clinical benefit.

For example, a study in aged, healthy monkeys¹¹⁹ found a significant decrease in pulse wave velocity, augmentation index, and a sustained decrease in aortic stiffness compared to pre-treatment values.

In 2001, Kass¹²⁰ carried out a phase two clinical trial in the US on 93 patients aged over 50 years with stiffened vasculature. The patients who received ALT-711 had a statistically lower arterial pulse pressure and an increase in large artery compliance compared to placebo. The drug was well tolerated and the numbers of patients reporting side-effects was the same as placebo.

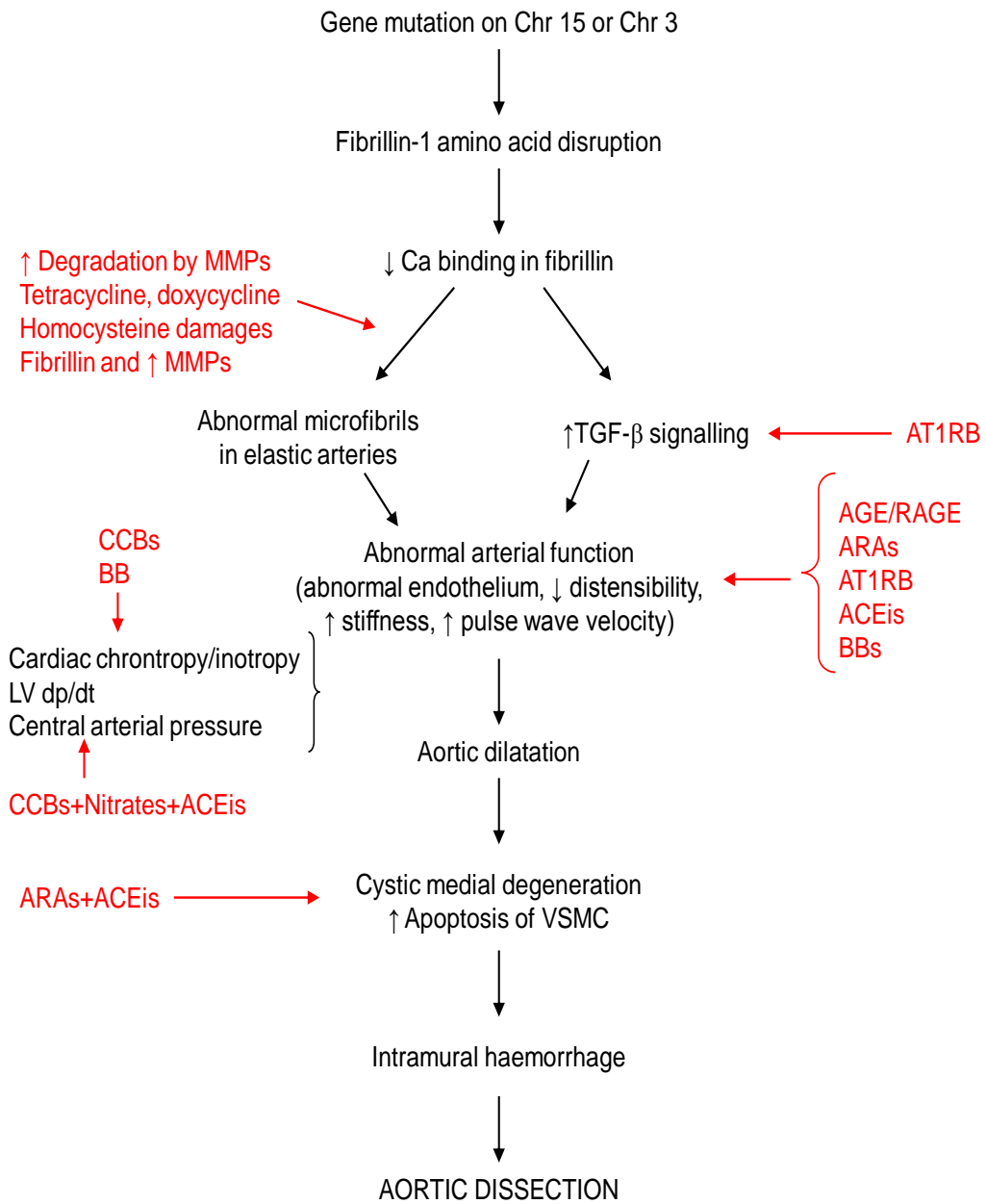
Although never tested in Marfan subjects, this new class of drugs may be of benefit in preventing or reversing aortic stiffness in Marfan Syndrome.

Drugs to decrease homocysteinaemia

In Marfan Syndrome, homocysteinaemia has been suggested to be a risk factor for aortic dissection. In 2003, Giusti¹²¹ divided 107 Marfan patients into three groups based on the severity of their cardiovascular manifestations. Total homocysteine levels were significantly higher in the group with most manifestations and the highest in those with aortic dissection. It has been postulated that hyperhomocysteinaemia induces a marked remodelling of the extracellular matrix of the arterial wall by induction of elastolysis through the activation of metalloproteinases. Moreover, homocysteine

cumulatively damages long-lived proteins and fibrillin is especially susceptible to irreversible homocysteinylation which impedes the formation of microfibrils. More research is needed to elucidate whether increased homocysteine levels are a cause or a consequence of cardiovascular manifestations in Marfan Syndrome.

Fig. 1 – Pathophysiology of Marfan Syndrome and Possible Sites for Drug Treatment



Key

- AGE = Advanced Glycation End-product crosslink breakers or crosslink inhibitors
- RAGE = Receptor for AGE blockers
- ARAs = Aldosterone receptor antagonists
- AT1RBs – Angiotensin II Type-1 Receptor Blockers
- ACEi = Angiotensin converting enzyme inhibitors
- BBs = Beta blockers
- CCBs = Calcium channel blockers
- MMPs = Matrix metalloproteinases
- TIMPs = Tissue inhibitors of matrix metalloproteinases

Conclusions

The unravelling of the complex interactions between elastin, fibrillin, aldosterone, TGF- β and the MMPs has only just started and has been benefited greatly by recent mouse models of Marfan Syndrome. The therapeutic benefits of drugs that interact with these factors (fig 1) has also just begun and needs to be investigated in the human population. In 2011, the medical treatment of Marfan patients with increased aortic diameters is given based on one non-randomised, unblinded trial published 12 years ago⁷⁴. There are now at least thirteen studies being planned to look at the medical treatment with drugs such as irbesartan, losartan, nebivolol and nitrates being investigated¹²².

When we started planning this MD in 2004, we wanted to investigate what parameters of vascular and ventricular function should be used to monitor patients with Marfan Syndrome and using what diagnostic tools. This will be discussed further in Chapter 2. We also wanted to investigate different drugs on these parameters to see if it would be appropriate to recommend the initiation of large, randomised controlled trials using alternative drug therapies. This will be discussed in Chapter 3.4.

When we started the trial, the stand-out drugs to investigate were the current normal practice beta-blocker atenolol; the calcium channel blocker verapamil; and the ACEi perindopril. The more recent studies of Losartan as a TGF- β antagonist and doxycycline as an MMP antagonist and the potential benefits of nitrates were published after our trial was commenced but have become the focus of ongoing research worldwide.

This is a syndrome that has benefitted from recent progress in mouse modelling and the discovery of potential drug benefits but due to the lack of research previously, my MD hypotheses remained unanswered.

CHAPTER 1.3

TOOLS AND PARAMETERS: TISSUE DOPPLER IMAGING

Current Transthoracic Echocardiography (TTE) Assessment of Marfan Syndrome

Transthoracic echocardiography is an easily-available, non-invasive test that has few contraindications and is well-tolerated by patients. It is able to look at the left ventricle, heart valves and aortic root in detail and is therefore the predominant investigation currently used to assess for the cardiac and aortic manifestations of Marfan Syndrome. It is also an expanding speciality with the introduction of new software packages into newer machines. In this chapter I will look firstly at the current echocardiographic parameters measured in the evaluation of Marfan Syndrome; then review the most relevant newer parameters; and throughout I will describe the general echocardiographic methodology used in our trials.

Current Assessment

As described in Chapter 1.1, the current method of investigating the myocardial and arterial abnormalities in Marfan Syndrome is by sequential scanning by transthoracic echocardiography (TTE). A routine first scan would be done and in addition to the minimum dataset (British Society of Echocardiography Education Committee¹²³) it would be used to look specifically for abnormalities more commonly seen in Marfan Syndrome.

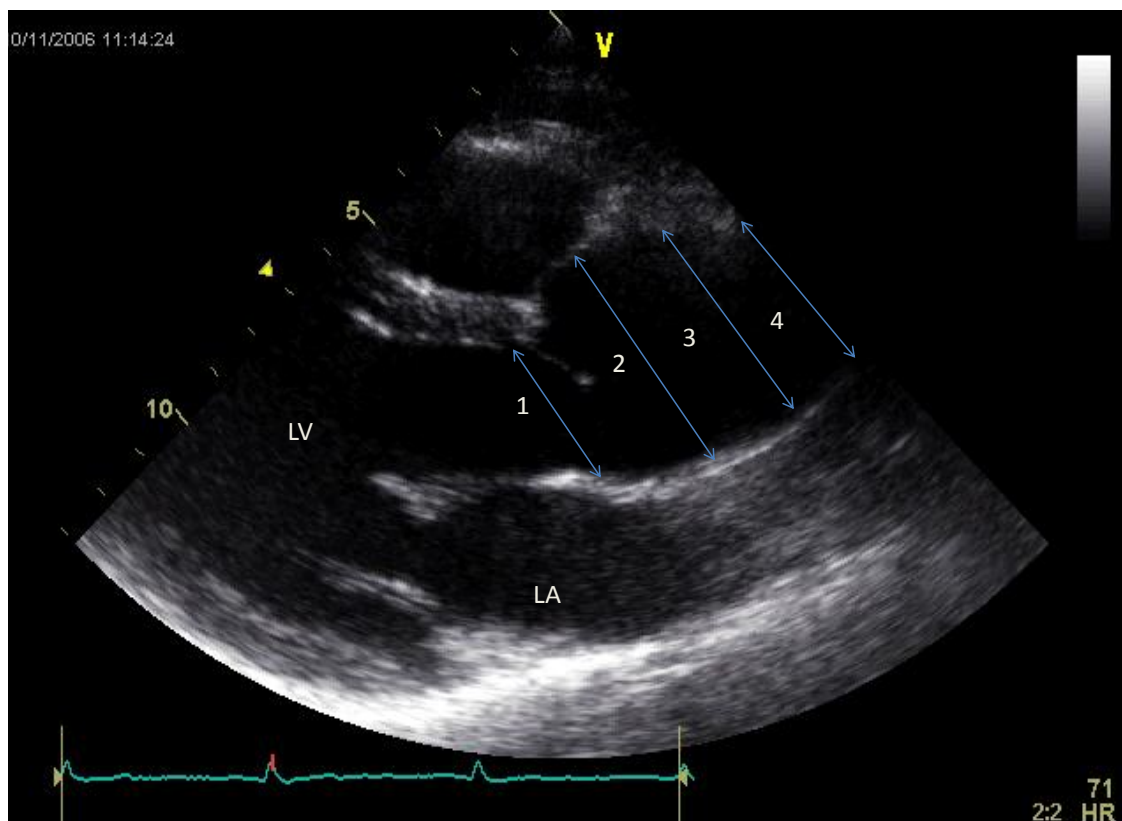
Left ventricular systolic and diastolic function can be affected and therefore should be assessed. The mitral annulus can become calcified and the mitral valve should be assessed for prolapse and regurgitation. The pulmonary artery diameter should be measured to assess for dilatation. The tricuspid valve should be assessed for prolapse. There is an increased incidence of atrial septal defects (4%) and so this should be looked for also.

Aortic root measurements are made according to set guidelines by MJ Roman¹²⁴ (figure 1). Four measurements at the aortic annulus(1), sinus of Valsalva(2), sinotubular junction(3) and ascending aorta 2cm above the sinotubular junction(4). The measurements are taken during diastole placing the calliper from leading edge to leading edge at right angles to valve closure.

The measurements gained by echo are then plotted against body surface area and inserted onto a nomogram so a comparison can be made over time and compared to the general Marfan population.

The rest of the ascending aorta, arch and descending aorta can also become dilated and dissect or rupture and so should be assessed.

Figure 1: Echocardiographic Measurement of the Aortic Root¹²⁴



TTEs are performed annually but timing varies according to the subject's risk factors for dissection:

- Aortic diameter $> 5.5\text{cm}$ ^{125,126}
- Aortic dilatation extending beyond Sinus of Valsalva¹²⁷
- Rapid rate of aortic dilatation ($> 5\%$ /year or $2\text{mm} / \text{yr}$)^{125,126}
- Family history of dissection^{125,126}
- Cigarette Smoking^{125,126}

Pregnant women are at a five times higher risk of dissection (1% increased mortality and rises with increased aortic diameter¹²⁸) due to hormonal changes on the arterial wall and an increased circulating volume. Therefore, women are scanned monthly during pregnancy.

In addition to TTE, 5 yearly MRI or CT scans are performed. Cross-sectional imaging has the advantages of looking for dural ectasia which is present in over 90% of Marfan adults¹²⁹ and is more accurate if the patient has poor echocardiography windows due to their musculoskeletal abnormalities. However, their limited availability means TTE is the workhorse of surveillance. Surgery is considered if the aortic root diameter reaches 5cm but earlier if rapid dilatation or if there is a strong family history of dissection. An aortic root diameter of 4cm is used for pregnant women or for women considering pregnancy prophylactically^{130,131}.

Problems with TTE Parameters Currently Used

There is inevitably variation in aortic diameter measurements between TTE operators and between departments. Also, an arbitrary number of 5cm will inevitably be too late for some Marfan subjects and too early for others. This risks some subjects still dissecting (the mortality rate is 5 times higher for emergency compared to elective surgery¹³²) while others will go through the trauma of open thoracotomy before the need to.

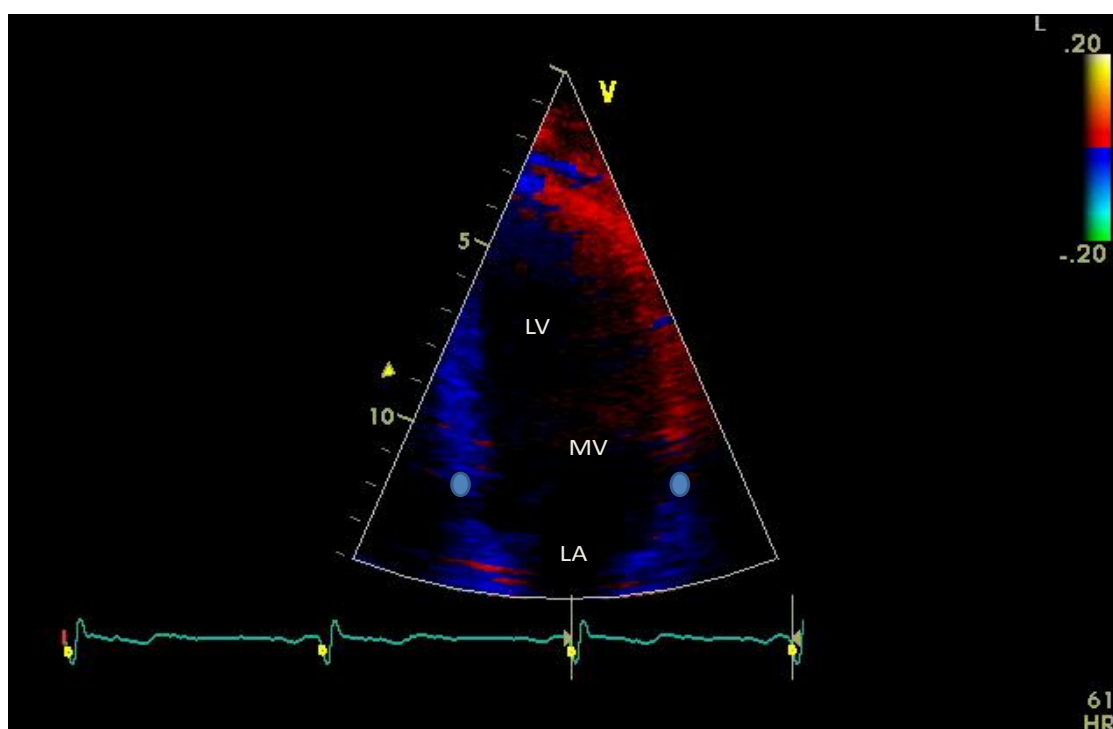
Aortic measurements can be difficult to measure even using the Roman four measurements for standardisation. The measurements can also be difficult to assess. The nomograms have been criticised as they do not reflect the normal aortic root dimensions in tall, slim people in whom Marfan Syndrome has been excluded¹³³. Not all Marfan patients are morphologically typical. Assessment becomes even more difficult in a younger population differentiating between normal somatic growth. This leads to the question whether there are other parameters that we could measure to predict dissection.

Novel Assessments in Transthoracic Echocardiography

Tissue Doppler Imaging (TDI)

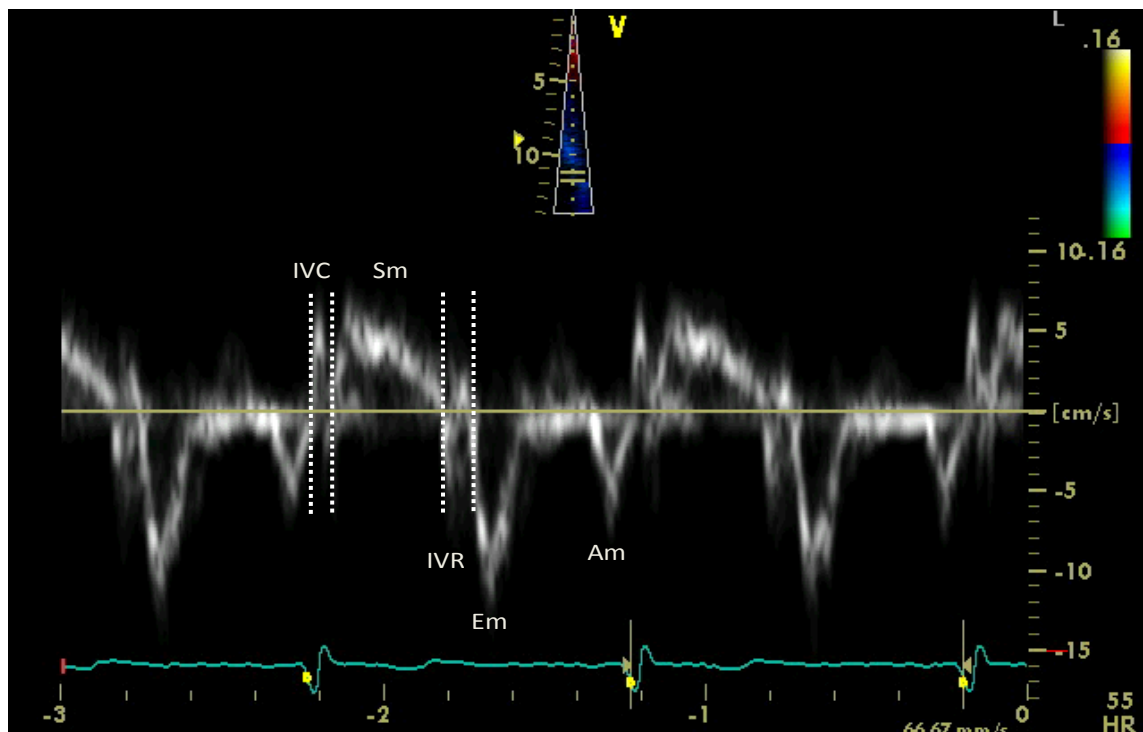
This is a modified colour Doppler technique that is used to measure myocardial wall movement. Doppler echocardiography relies on detection of the shift in frequency of ultrasound signals reflected from moving objects. Conventional Doppler techniques assess the velocity of blood flow by measuring high velocity, low amplitude signals from small, fast moving blood cells. Tissue Doppler imaging quantifies the low velocity, high amplitude signals seen with myocardial tissue motion. Pulsed TDI can be used to measure long-axis ventricular motion in all six left ventricular walls as the endocardial fibres are most parallel to the ultrasound beam in apical views. The apex of the heart remains relatively stationary throughout the cardiac cycle so that mitral annular motion is a good measure of overall longitudinal left ventricular contraction and relaxation¹³⁴. These longitudinal myocardial velocities can be measured by placing the sample volume in the ventricular myocardium adjacent to the mitral annulus (figure 2). Regional myocardial velocities can also be assessed by placing the sample volume in the mid-part of each third of each left ventricular wall and again can be applied to all six ventricular walls. A myocardial velocity trace is reproduced to give peak systolic velocities for each segment.

Figure 2: Tissue Doppler Imaging of the Left Ventricle at the Medial and Lateral Mitral Annulus



The cardiac cycle is seen in figure 3 by using Pulsed TDI at the mitral annulus and can be split into five components. Firstly, the narrow upward and downward waves immediately after the QRS complex represent movement during **isovolumic contraction (IVC)**. Secondly, the large upward wave is the peak systolic velocity, **Sm**, as the annulus moves towards the apex. Thirdly, there is a narrow downward then upward wave representing movement during **isovolumic relaxation (IVR)** (not seen in a normal right ventricle). The fourth element is **Em** (m for myocardium, also known as Ea for annulus or E¹) which represents the early diastolic myocardial relaxation as the left ventricle moves away from the apex. Lastly, **Am** (m for myocardium, also known as Aa for annulus) is the myocardial velocity associated with atrial contraction.

Figure 3: Pulsed Tissue Doppler at the Mitral Annulus



Pulsed TDI has high temporal resolution (typically 300 samples/sec) but has no spatial resolution within the sample volume and therefore, cannot be used in multiple myocardial segments simultaneously. With Colour TDI, a colour-coded representation of myocardial velocities is superimposed on gray scale images to indicate the direction and mean velocity of myocardial motion. Colour TDI has increased spatial resolution and can evaluate multiple segments in a single view.

Current Applications of TDI

Assessment of Left Ventricular Systolic Function

Sm, the peak systolic velocity when measured at the lateral mitral annulus is a measure of longitudinal systolic function. It has been correlated with LV ejection fraction¹³⁵ and peak dp/dt¹³⁶. Regional reductions in Sm are correlated with regional wall motion abnormalities and TDI is often used as part of dobutamine stress echocardiography as peak Sm increases with dobutamine and exercise and decreases with ischaemia^{137,138}. The lowest systolic velocities arise from the anterior septum (mean 7.5 cm/s) and the highest from the right ventricle (mean 15.2 cm/s). Diastolic velocities and hence E/A ratios follow a similar pattern. Additionally, the systolic and diastolic velocities decrease from the basal segment to the apex of the same myocardial wall. This is a consequence of the anatomical arrangement of the myocardial fibres: subendocardial and subepicardial fibres are mostly parallel to the long axis of the left ventricle and the midwall fibres are orientated circumferentially and hence the degree of longitudinal shortening will decrease towards the apex¹³⁹.

TDI has also given rise to new measures of regional and global cardiac function - **Strain** and **Strain rate**.

Strain (ε) is a measure of tissue deformation.

$$\epsilon = \frac{L - L_0}{L_0}$$

With L being the length of the object after deformation and L₀ its original length.

As the ventricle contracts, the muscle shortens in longitudinal and circumferential dimensions (negative strain) and thickens or lengthens in the radial direction (positive strain). Strain is measured as a percentage (%). Strain is affected by pre-load and increasing pre-load increases strain and increasing after-load decreases strain. Also, the LV cavity size matters and in small left ventricles radial strain is increased and longitudinal strain is reduced¹⁴⁰.

Strain rate (SR) is a measure of the velocity of the tissue deformation

$$SR = \frac{V^2 - V^1}{d}$$

where V¹ and V² are velocities of myocardial deformation at two points separated by a distance d. The units of SR are per sec. Unlike strain, strain rate is thought to be less

related to pre- and after-load¹³⁹. When measuring strain and strain rate by TDI, the data can be displayed by colour, with different colours representing different values, or graphically against time/cardiac cycle (Figures 4 and 5).

Figure 4: TVI Strain including Timings with sample volume at the Mid Septum of the Left Ventricle

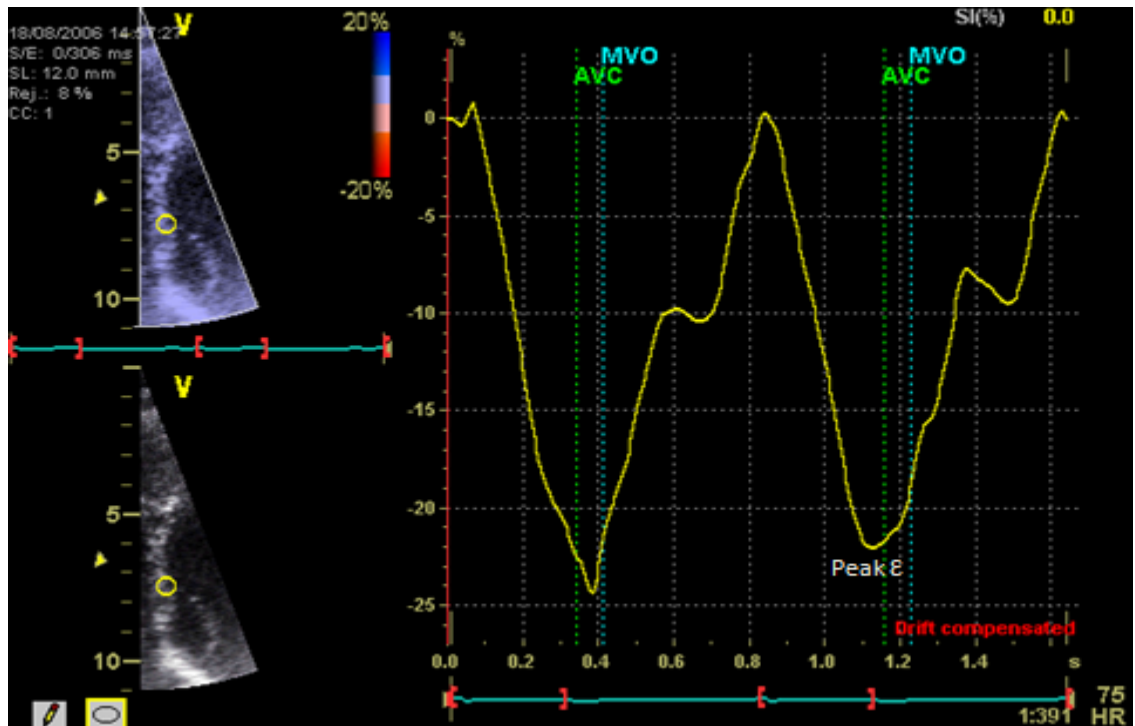
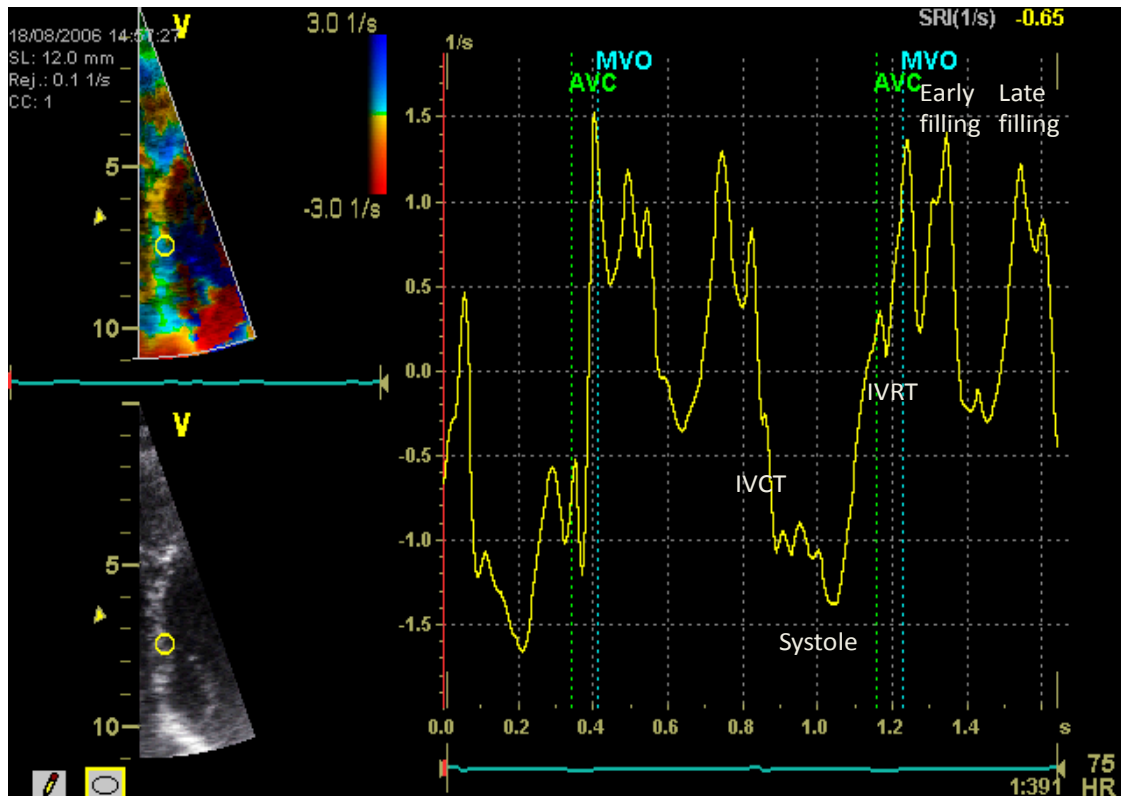


Figure 5: TVI Strain Rate including Timings with sample volume at the Mid Septum of the Left Ventricle



Correct acquisition firstly involves optimisation of the 2D image to avoid reverberation artefact. Then we narrow the sector width to improve spatial resolution and ensuring adequate frame rate (≥ 100 frames/sec). The sample volume is placed on an appropriate area of myocardium which stays on the myocardium throughout the cardiac cycle. The graphical representation of strain or strain rate can then be shown.

The next step is to define the timing of the waveform by inserting aortic valve opening, aortic valve closing and mitral valve opening. Once the timings are in place isovolumic contraction, systole, isovolumic relaxation and diastole can be seen. The graph can then be analysed. The **normal resting values for longitudinal SR are 1.0-1.4/sec** with standard deviation of 0.5-0.6/sec. **Normal longitudinal systolic strain varies from 15-25%** with **normal radial strain ranging from 50-70%** and standard deviations of 5-7%¹⁴¹.

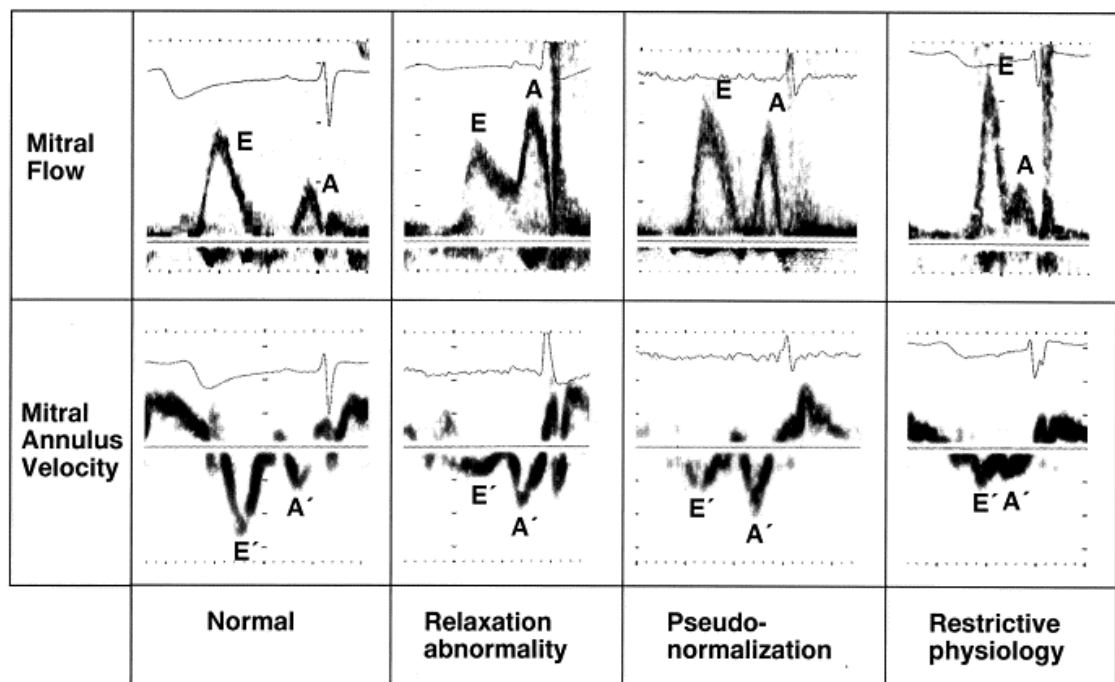
This technique has been validated firstly with sonomicrometry¹⁴² and also with MRI¹⁴³. The sensitivity of SR has made it a useful addition in the evaluation of subclinical heart disease and has been used in Amyloidosis¹⁴⁴ and Friedrich's ataxia¹⁴⁵ and in distinguishing between nonobstructive Hypertrophic cardiomyopathy and hypertensive

left ventricular hypertrophy^{146,147} and in the response to treatment in Fabry disease¹⁴⁸. This sensitivity would be an advantage in detecting early left ventricular diastolic and systolic dysfunction in the Marfan population secondary to abnormal elastin in the myocardium.

Assessment of Diastolic Function

TDI is also currently used as part of the transthoracic echocardiography examination of LV diastolic function and left atrial filling pressure (British Society of Echocardiography minimum dataset¹²³). Measurements of pulse-wave mitral inflow E and TDI lateral mitral annulus Em are used to estimate left atrial pressure. If E/Em >10 (measured at the lateral mitral annulus) or >15 (medial mitral annulus) then pulmonary capillary wedge pressure is >15mmHg with 92% sensitivity and 80% specificity¹⁴⁹. The pattern of the Em and Am waves guide assessment of LV diastolic dysfunction (figure 8).

Figure 6: Patterns of Diastolic Dysfunction¹⁴⁹



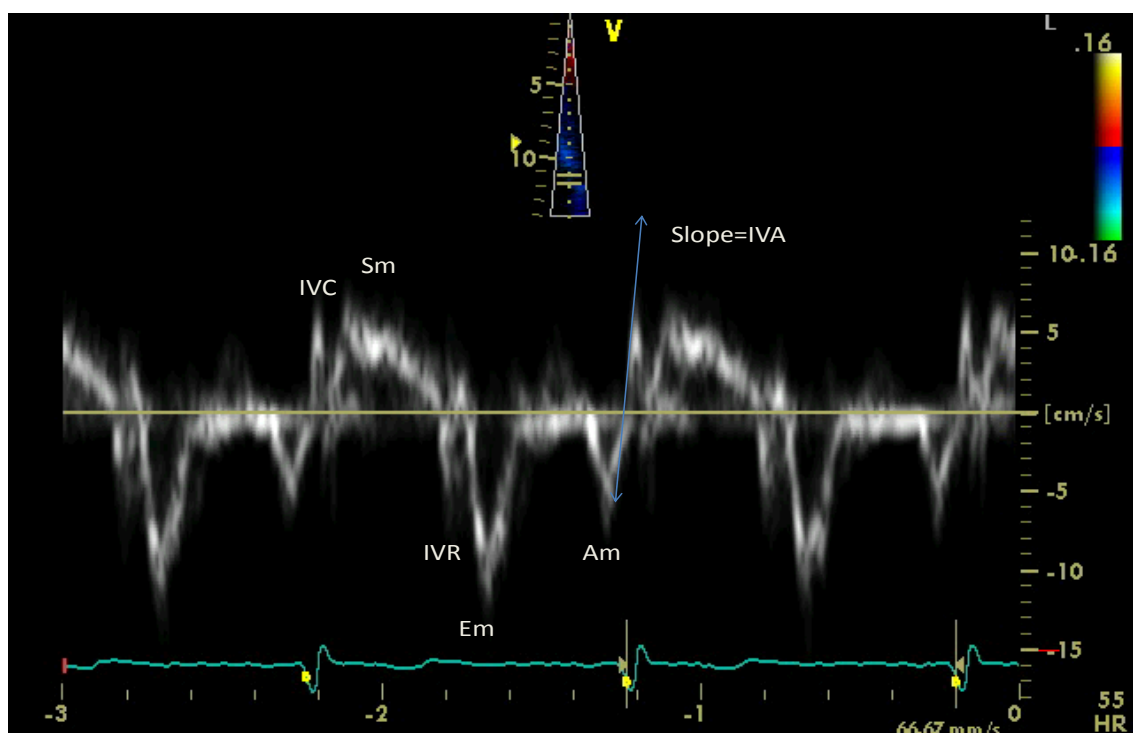
Reproduced from Sohn et al. J Am Coll Cardiol 1997;30:474-480.

Isovolumic Acceleration (IVA)

Many of the TDI indices of myocardial contractility are affected by both preload and afterload. Isovolumic acceleration (IVA), that is, myocardial acceleration during isovolumic contraction (figure 7) has been shown to be a sensitive measurement of left and right ventricular myocardial contractile function that seems unaffected by preload and afterload within a physiological range and in an animal model^{150,151}.

Figure 7 shows a TDI velocity trace at the medial mitral annulus. The trace shows isovolumic contraction (IVC) and the slope of this equates to isovolumic acceleration. Then follows peak systole (Sm), isovolumic relaxation (IVR), early diastolic filling (Em) and atrial contraction (Am) as previously described.

Figure 7: TDI Isovolumic Acceleration (IVA).



Since I started the MD, Isovolumic acceleration has been further investigated by Margulescu and colleagues¹⁵². They found that it was a useful tool to discriminate between poor and normal left ventricular function especially when measured from the medial mitral annulus. However, they reported difficulty with low reproducibility

especially in the low left ventricular function group with inter- and intraobserver variabilities of 28 and 30%.

TDI In The Aortic Wall

Harada and colleagues¹⁵³ studied wall motion velocities in the abdominal aorta using TDI in 12 Marfan patients and 30 age-matched controls. In each subject they used a transabdominal short axis view and, by M-Mode, measured aortic diastolic (onset of the ECG R wave) and systolic (point of maximal anterior motion of the aortic wall) diameters. These diameters were corrected for body surface area. They calculated the change in diameter of the abdominal aorta by $(D_s - D_d)/D_d$. They also calculated the β stiffness index by $\ln(P_s/P_d)/(D_s - D_d)/D_d$ where P_s and P_d are the systolic and diastolic blood pressures. They also measured aortic wall motion velocity by TDI pulsed Doppler with a 1mm sample volume placed on the anterior wall of the abdominal aorta. A peak systolic velocity (S) and peak diastolic velocity (D) were measured from this.

They found that the Marfan patients had significantly greater aortic stiffness index, β and significantly reduced Peak S and D ($p < 0.01$). They also had low interobserver variability between their two observers' measurements ($r = 0.94$ and 0.95).

This study interested me greatly and I wanted to reproduce its findings in our Marfan population and also to measure the same parameters but at the aortic arch where there is more elastin and where dissection and rupture is more likely to occur. Difficulty in acquiring adequate aortic root views by TTE meant that I could not test this technique more proximally.

Conclusions

Transthoracic echocardiography is the current tool of choice in the assessment of the cardiovascular abnormalities in Marfan Syndrome. It enables analysis and monitoring of both the ventricular and aortic parameters that predict aortic dissection and rupture. However, I have described some difficulties in this assessment and described some novel parameters that could potentially aid the TTE assessment. These new TTE parameters: TDI peak systolic velocity, E/E^1 , strain, strain rate, isovolumic acceleration and aortic wall velocities would be useful theoretical adjuncts in assessing left ventricular systolic and diastolic function and aortic properties in Marfan Syndrome.

CHAPTER 1.4

TOOLS AND PARAMETERS: APPLANATION TONOMETRY

In Marfan Syndrome the aim of preventing aortic dissection and rupture is by reducing the pressure in the proximal aorta. Traditionally, we have measured brachial blood pressure with a sphygmomanometer and cuff and assumed this accurately reflects the pressure “faced” by the left ventricle at each heart beat. This has been, in part, due to the need for invasive measurement of central aortic pressure and so this has been reserved for research alone. However, measuring central aortic pressure which is directly opposed to the left ventricle gives a more accurate reflection of the arterial load faced¹⁵⁵. Moreover, the shape and character of the pulse wave and not just the systolic and diastolic values adds information to the interaction between heart and arterial system¹⁵⁵. However, whereas it is simple to measure blood pressure in a limb with a cuff, it has remained, until recently, a challenge to accurately measure ascending aortic pressure.

Windkessel Effect

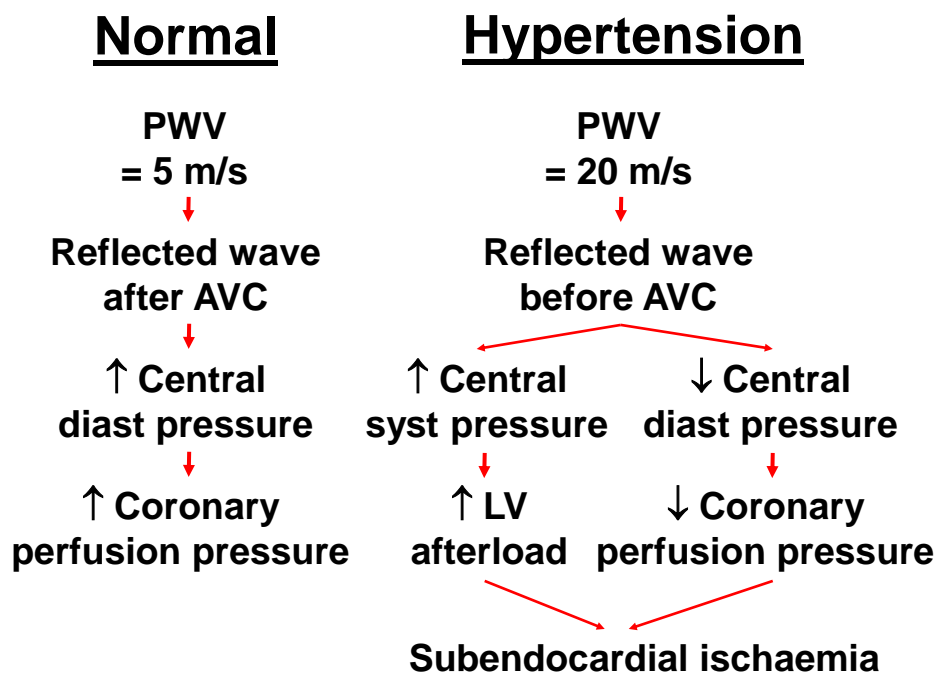
The Windkessel effect has been described as the distension of the large elastic arteries when the blood pressure rises during systole and recoil when the blood pressure falls in diastole. As the rate of blood entering the large elastic arteries is greater than the rate leaving due to peripheral vascular resistance there is a net storage of blood during systole which discharges during diastole. This effect helps in the dampening of blood pressure (pulse pressure) and aids organ perfusion during diastole when left ventricular ejection ceases. Windkessel is literally German for air chamber but implies an elastic reservoir. The concept has largely been overtaken by modern interpretations of arterial pressure and waveforms in terms of wave propagation and reflection which I will discuss both in this and the next chapter.

The Effect Of Aortic Stiffness

In studies of hypertension where, similar to Marfan Syndrome, there is also an increase in arterial stiffness, this stiffness accelerates the speed at which the left ventricular ejection pressure wave travels through the arterial system. This pressure wave acts as

a periodically oscillating wave travelling from the heart to the peripheral arteries. It is also known that throughout this journey there are changes in the arterial bed- bends, curves, arterial branches and differences in arterial properties -which cause wave reflection back to the heart. As the speed of the left ventricular pressure wave increases, the earlier the reflected wave returns to the left ventricle. When the reflected wave arrives not during diastole but in systole it augments the late systolic pressure (afterload) on the left ventricle. This increases central pulse pressure, increases left ventricular load and also because the wave is arriving less in diastole, reduces coronary artery perfusion¹⁵⁵ (figure 1). It is, therefore, vital to be able to create and understand the central aortic pressure waveform to get an accurate picture of left ventricular-arterial coupling. This has been an important factor in the latest European Society of Hypertension guidelines for the management of hypertension¹⁵⁶. They emphasise that systolic blood pressure and pulse pressure vary between the aorta and the brachial artery; that drug therapy that is successful at reducing peripheral blood pressure may not have the same beneficial effect centrally¹⁵⁶; and that central blood pressure relates to cardiovascular events¹⁵⁷.

Figure 1: The Differences In Left Ventricular-Arterial Coupling Between Hypertensive and Normal Populations.

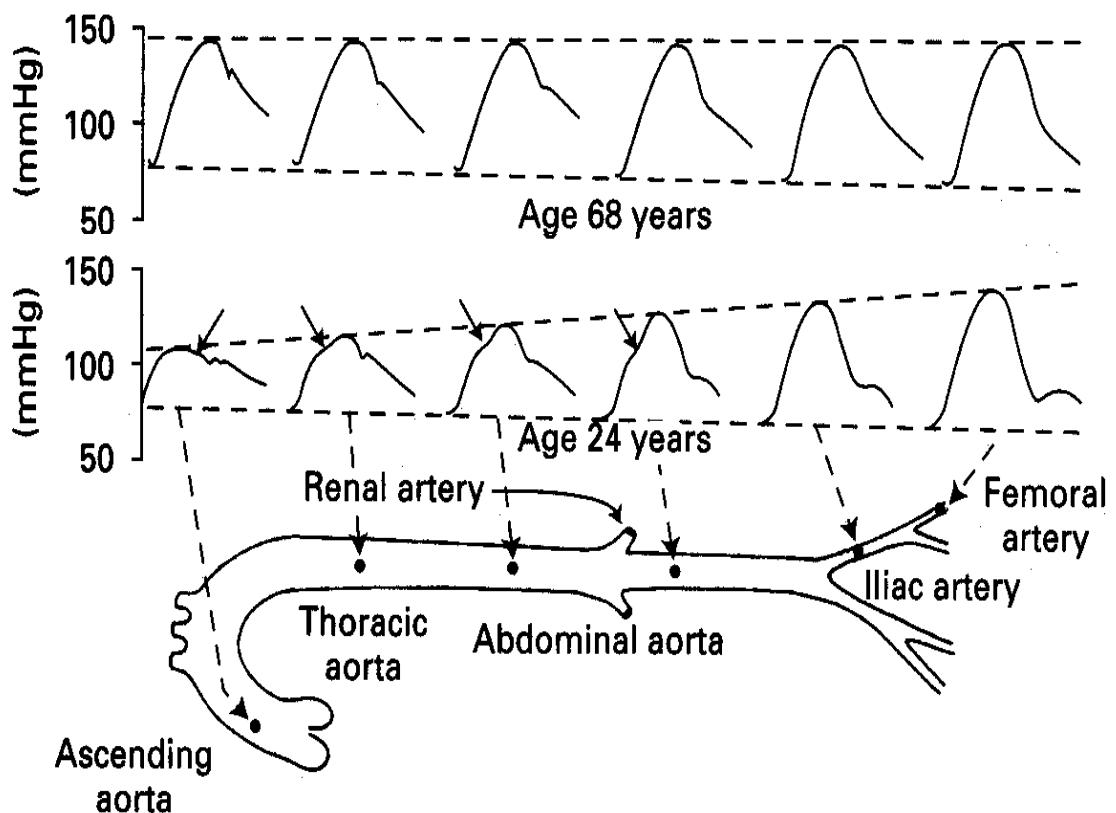


In a young normal population the pressure waveform at the ascending aorta is smaller in amplitude than at the periphery (figure 2). This is due to wave reflections encountered from all the branches on travelling through the arterial system. This is called Pulse Pressure Amplification:

$$\text{Pulse Pressure Amplification} = \frac{\text{Peripheral Pulse Pressure}}{\text{Central Pulse Pressure}}$$

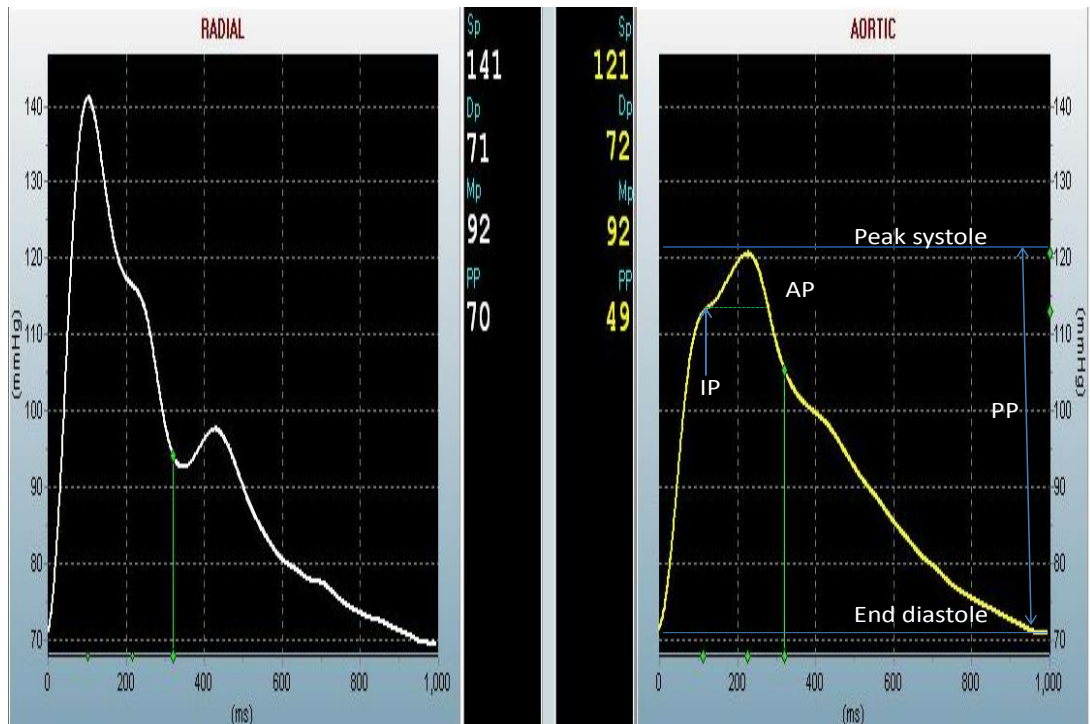
In an elderly or a hypertensive population the difference in amplitude is less. This is because wave reflections from the arterial tree return before aortic valve closure, augmenting the aortic pressure wave. The normal pulse pressure amplification if age <20 years old is 1.7; for those age >80 years old is 1.2 and there is a gender difference with amplification higher in men. Amplification is also increased in tall people and if the heart rate increases. It is decreased with age, increasing aortic stiffness, increased peripheral reflections, shorter people (due to increased reflections), hypertensives, diabetics, hypercholesterolaemia, smokers and people with cardiovascular disease.

Figure 2: The Pulse Pressure Waveform¹⁵⁸



Reproduced from McDonald's Blood Flow in Arteries, 1998, 4th Edition. Arnold. London.

Figure 3: Pulse pressure wave at the radial artery and the aorta



The amplitude of the pressure wave (figure 3) is the Pulse Pressure (PP). The augmented part of the pressure waveform is named the Augmentation pressure (AP). It begins at the site of the first inflection point (IP) which signifies the arrival of the reflected wave. Augmentation index is calculated as:

$$\text{Aix} = \frac{\text{Augmentation pressure (AP)}}{\text{Pulse Pressure (PP)}}$$

Tr is the time (seconds) from the start of the forward pulse pressure wave to the arrival of the backward reflected wave from the periphery.

Detection of Peripheral Blood Pressure Waveforms

In the past invasive measurements had to be made for detecting pulse pressure amplification, central aortic pressures and the pressure waveform by recording both peripheral and central blood pressures. However, this has obvious limitations. The peripheral pulse can now be detected by extremely sensitive pressure sensors that can be used at the tip of hand-held pencil probes. These sensors accurately measure intravascular pressure when the probe is pressed on the skin over an artery and the

artery is slightly compressed against a firm structure such as bone. This principle is known as Applanation Tonometry^{159,160}

Generalised Transfer Function (GTF)

The aortic waveform can then be reconstructed from the non-invasive radial waveform acquired by applanation tonometry by means of a “generalised transfer function.”

The characteristics of the transfer function are determined by arterial diameter, arterial wall elasticity, arterial wall thickness, number of arterial side branches and the condition of the vascular beds. Despite brachial vasculature being different between individuals it was found that the main components of the transfer function did not vary significantly in normal adults with age or in normal conditions or in conditions of vasodilatation following nitrate administration.

The use of the generalised transfer function was validated by a study by Chen¹⁵⁹ who used an invasive measurement of the aortic pressure and radial pressure in 20 patients at steady state and during haemodynamic changes incurred by the Valsalva; abdominal compression; nitrate administration and vena cava obstruction. The average of the individual transfer functions was used to determine the generalised transfer function. However, the generalised transfer function itself has never been released into the public for close scrutiny.

Reproducibility

The main element affecting reproducibility is the ability to obtain an accurate radial pulse waveform as all parameters are derived from this. This is determined by the stability of the subject’s physiological status and operator skill.

Siebenhofer et al¹⁶⁰ studied 33 healthy subjects of mean age 33 years and showed:

<u>SphygmoCor Parameter</u>	<u>Inter-operator variability</u>
Derived Systolic pressure	0.1±1.7mmHg
Derived Diastolic pressure	0.1±0.7mmHg
Augmentation Index	0.4±6.4%

Limitations

The major limitation is if the radial pulse is altered by obstruction of any part of the brachial system. If this is the case then the radial pulse cannot be used to derive the aortic pressure waveform. However, this is uncommon and further studies have shown good correlations between the derived and measured central aortic systolic pressure and Augmentation index^{158,161,162}.

Recent large trials have shown the independent role of central blood pressure as a predictor of cardiovascular events^{163,164}. The CAFE trial¹⁵⁷ was a large, prospective, randomised, open, blinded end-point study recruiting over 2000 hypertensive patients. It showed that the traditional antihypertensive therapy of atenolol ± thiazide-based regimes lower central blood pressure significantly less than the newer contemporary amlodipine ± perindopril-based regimes despite similar effects on brachial blood pressure. It also showed that central pulse pressure, augmentation and height of the aortic pulse wave up to the first inflection point were significantly associated with a composite end-point of cardiovascular and renal outcomes.

ASCOT¹⁶⁴, of which CAFE was a substudy, showed significant reductions in total coronary events, cardiovascular death and stroke with its amlodipine ± perindopril-based regime compared to the atenolol ± thiazide-based group. These results were in a hypertension population but are of course important in the context of our Marfan patients who have been predominantly taking beta blockers to reduce their central aortic pressures but are being checked in clinic by brachial blood pressure measurements.

Pulse Wave Velocity

The velocity that the pulse wave travels can also be measured between two anatomical sites and has become a widely validated and accepted measure of arterial stiffness¹⁶⁵⁻¹⁶⁹. The underlying principle is that with age and with hypertension the aorta becomes stiffer and the pulse wave therefore travels faster. Therefore, in our Marfan population we would expect an increased pulse wave velocity also. It is measured using a Doppler probe to detect the onset of flow in an artery. Doppler pulses are then recorded sequentially at two different arterial sites and compared using the R-wave of the ECG (fig 4).

Figure 4: Carotid-Femoral Pulse Wave Velocity



$$\text{Pulse Wave Velocity} = \frac{\text{Length between Measurement Sites (mm)}}{\text{Time Delay between onset of R waves (ms)}}$$

Commonly used sites are carotid-femoral and carotid-radial. In our Marfan population I will measure both but as the carotid-femoral path contains more aorta, this would be theoretically better. A 4MHz Doppler probe is used to solve the potential problem of obese or muscular necks. Carotid-femoral pulse wave velocity is the current “gold standard” measurement of large artery stiffness¹⁶⁹. It is a simple, non-invasive measurement which determines the speed of the pulse wave travelling along the aortic and aorto-iliac arterial pathway.

It has also been published that in a group of 241 patients with end-stage renal failure, increased aortic stiffness as measured by aortic pulse wave velocity, is a strong independent predictor of all-cause and cardiovascular mortality¹⁷⁰.

Applanation Tonometry, Pulse Wave Velocity and Marfan Syndrome

There have been a few studies looking at these parameters in patients with Marfan Syndrome. Yin, 1989¹⁷¹ showed an increased magnitude of wave reflection (calculated by the ratio of backward to forward components of the aortic pressure wave) measured invasively by micromanometer at the time of cardiac catheterisation in nine patients as

workup to aortic root replacement. The wave reflection was normalised by vasodilatation with intravenous nitroprusside and further increased by intravenous propranolol.

Mortensen, 2009¹⁷² has recently looked at Applanation tonometry in Marfan Syndrome. He found an independent association in 50 medically treated adults with Marfan Syndrome who hadn't previously had cardiovascular surgery, between aortic root diameter progression and Augmentation index ($p=0.02$) and lower pulse wave velocity ($p=0.03$).

Jondeau, 1999¹⁷³ measured carotid pulse pressure by applanation tonometry as a surrogate for central aortic pressure in a group of twenty patients with Marfan Syndrome. Their group found that carotid pulse pressure was a major determinant of ascending aorta diameter, whereas brachial pulse pressure was not. They also found that arterial distensibility, as measured non-invasively by ultrasound, was 38% lower in the Marfan abdominal aorta compared to the "normal" control group whereas there was no significant difference in distensibility between groups at the common femoral artery, common carotid artery or the radial artery. Interestingly, 6 of the 20 Marfan group were taking long-term beta blockers which were only stopped the day before the study.

Kiotsekoglou A, 2009¹⁷⁴ however, found that when they measured common carotid arteries in 32 Marfan subjects and 29 controls by ultrasound the intima-media thickness did not change but compliance and distensibility were significantly reduced in the Marfan group ($p<0.05$) although 13 of the 32 Marfan subjects were taking atenolol.

There have been MRI trials which have shown increased aortic pulse wave velocity in Marfan patients measured from the ascending to the abdominal aorta^{175,176} but we don't know whether the subjects were taking medication at the time of the study. This is important information missing as we know that ACEIs reduce aortic stiffness as assessed by echocardiography compared to beta blockers in Marfan Syndrome and that this is associated with a smaller increase in root diameter and fewer clinical end points.¹⁷⁷ Also in a small study looking at the addition of perindopril to normal beta blocker therapy in 17 Marfan patients, they showed reduced aortic pulse wave velocities in the perindopril group.¹⁷⁸

Thus, there have already been a small number of trials looking at the central aortic pressure wave by Applanation tonometry and aortic stiffness by pulse wave velocity and their applicability in Marfan Syndrome. These studies have demonstrated that these parameters can be measured by the Applanation tonometry tool. The 2007 European Society of Cardiology hypertension guidelines also give a warm approval for

Applanation tonometry but caveat that “The prognostic role of central as opposed to peripheral blood pressure needs to be confirmed in more large-scale observational and interventional studies,” I imagine meaning beyond ASCOT and CAFE. Nevertheless, the measurement of central aortic pressures, Augmentation index, Pulse Pressure Amplification and Carotid-Femoral Pulse Wave Velocity will become more widely used in the hypertension literature and impact on clinical end points that would also be beneficial in Marfan Syndrome, a disease so profoundly affected by ascending aortic stiffness.

CHAPTER 1.5

TOOLS AND PARAMETERS: WAVE INTENSITY ANALYSIS

Background

In transthoracic echocardiography and applanation tonometry we are investigating two distinct anatomical areas, either the left ventricle or the aorta. It is also vital to understand and investigate the interaction between the two.

A tool recently developed to address left ventricular-arterial coupling was conceptualised by Kim Parker and Chris Jones in 1990¹⁷⁹ by adapting a mathematical model used in flow mechanics. Parker described aortic blood flow as “an infinitesimal number of wavefronts that propagate along the aorta causing changes in pressure and velocity as they pass.” These wavefronts either increase the pressure in the aorta (**compression waves**) or decrease it (**expansion waves**) and either travel **forward** from the heart or **backwards** from the systemic periphery. Pressure and flow change together in forward wavefronts but in opposite ways in backward, reflected waves. This gives rise to four possibilities:

Forward compression wave (FCW) – increases pressure, increase velocity

Forward expansion wave (FEW) – decreases pressure, decreases velocity

Backward compression wave (BCW) – increases pressure, decreases velocity

Backward expansion wave (BEW) – decreases pressure, increases velocity.

Using this concept of infinitesimal discrete wavefronts as the basic elements and by using their mathematical model the aortic wave can be further analysed. The flow in elastic arteries propagates downstream and upstream with velocity:

Flow Velocity = $U \pm c$ where U is the velocity of the blood and c is the wave speed.

c , the wave speed is determined by $c=(1/\rho \cdot D)$ where D is the distensibility of the aorta and ρ is the density of the blood.

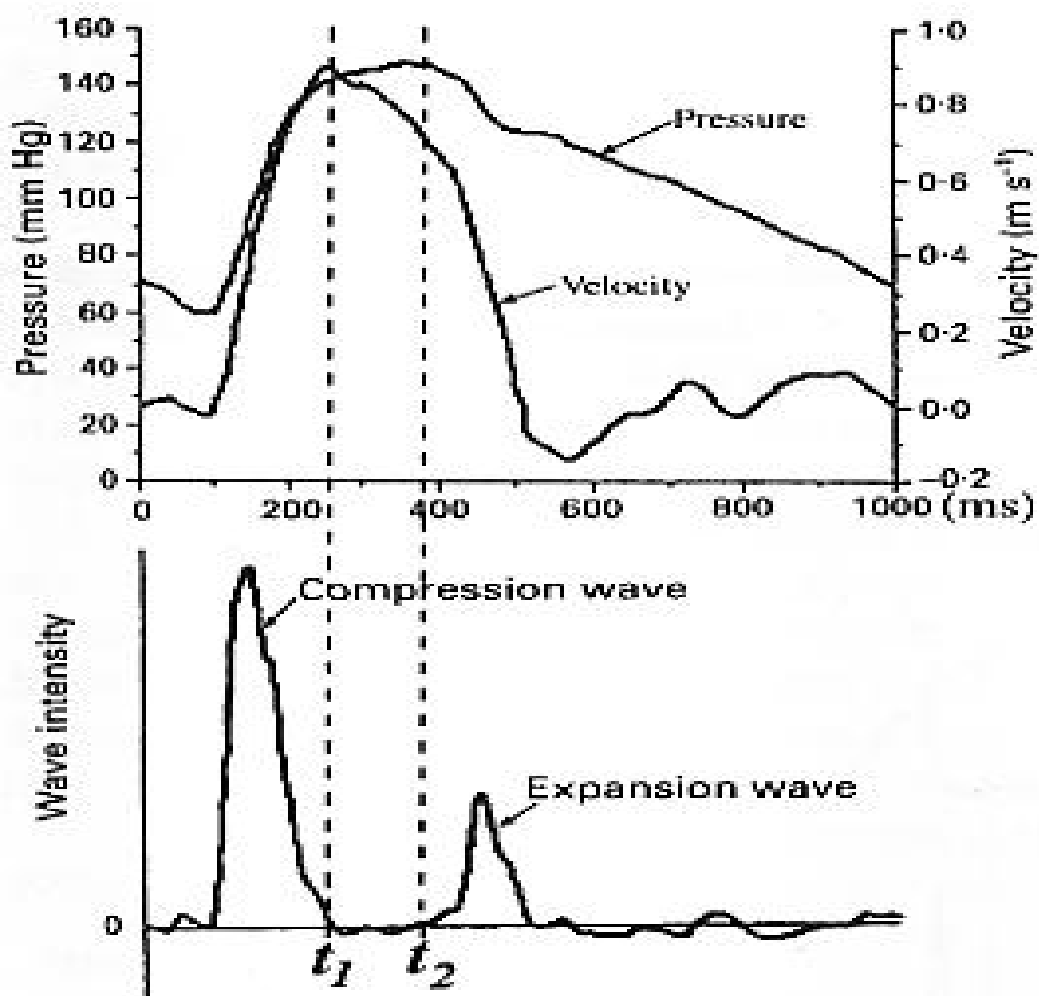
In a transient wavefront the change in pressure across the wavefront (dP) is related to the change in velocity:

$$dP = \pm \rho \cdot c \cdot dU$$

Where the + sign refers to forward direction and the – sign refers to the backward direction.

The wave intensity = $dP \cdot dU$, is the product of instantaneous changes in velocity and pressure. It is positive for forward waves and negative for backward waves (figure 1). This approach is now generally known as **Wave Intensity Analysis (WIA)**.¹⁸⁰

Figure 1: Aortic Pressure, Velocity and Net Wave Intensity



Measuring Pressure and Velocity

WIA has an advantage in that it can be used in the time domain allowing us interpretation of temporal events in the cardiac cycle. In this way, WIA can be calculated from simultaneous measurements of pressure by measuring the diameter of

the blood vessel (common carotid artery is used as the most proximal, easily accessible artery) and velocity by measuring flow¹⁸¹.

Diameter has been shown to relate linearly to pressure over the physiological range ($r^2 > 0.97$) and can be calibrated by measuring the brachial blood pressure. Flow can be measured using pulse wave doppler positioned in the lumen of the common carotid artery.

Separated Waves

Initially, it was only possible to calculate the net Wave Intensity (fig 1). Parker, Fraser, Rakebrandt and colleagues worked closely with the Japanese company Aloka (Hitachi-Aloka, Tokyo, Japan) who adapted their ultrasound machine to enable the acquisition of the patient data required. Additionally, new software¹⁸² was developed which enabled separation of the net WI into the four individual forward and backward-moving waves. The timing, duration, peak amplitude and net energy of the separated waves can now be measured.

Local wave speed can also be measured from the slope of the pressure-velocity loop in early systole when forward wave travel is predominant¹⁸³. Augmentation index, Peterson's pressure-strain elastic modulus (epsilon) and the β stiffness index (beta) can also be calculated¹⁸⁴.

Implications of Measuring Separated Waves

The amplitude of the forward compression wave is an index for left ventricular contractile function ($+dp/dt$).¹⁸⁵ The net negative wave intensity in midsystole arises from reflections (backward compression waves) and increases central aortic pressure and decreases forward flow. The expansion wave that starts in late systole before aortic valve closure marks the onset of left ventricular relaxation and is equivalent to a forwards-travelling suction wave that decelerates flow and reduces pressure, leading to aortic valve closure.¹⁸⁶

By measuring the forward compression wave and the backward compression wave it would give us an insight into the interaction between left ventricle, aorta and peripheral vasculature in our Marfan population that no other tool can do. Additionally, it allows the measurement of Augmentation index and indices of vascular stiffness – local

wavespeed, Peterson's pressure-strain elastic modulus (epsilon) and the β stiffness index (beta).

Evidence for WIA

WIA has been confirmed in experimental and clinical studies¹⁸⁷⁻¹⁸⁹ and has been used to differentiate between myocardial and vascular effects of antihypertensive treatment.¹⁹⁰ Swampillai, 2006¹⁹¹, investigated a group of healthy volunteers and found caffeine consumption was associated with an increase in the forward compression wave and local wave speed without changes in pressure-strain elastic modulus (ϵ) and (β) stiffness index; whereas cigarette smoking resulted in only an acute increase in local arterial stiffness indices. This suggests a prevalent myocardial inotropic effect of caffeine as opposed to the mostly vascular action of smoking and WIA was useful in distinguishing these factors.

Disadvantages of WIA

Wave Intensity signals are intrinsically noisy and there is considerable biological variability in net wave intensity.

The common carotid artery is used to measure the flow and diameter, where reflections are influenced by cerebral vasomotor tone as well as local arterial properties¹⁹². In our Marfan population the common carotid artery has less elastin compared to the Aorta and this may influence results. In the wider population, the clinical utility of WIA has not yet been established.¹⁹³ The use of WIA has never been reported in Marfan Syndrome.

CHAPTER 1.6

HYPOTHESES:

My thesis has been designed to investigate firstly, the **parameters** that could most usefully be measured in monitoring the myocardial and arterial dysfunction in patients with Marfan Syndrome. Secondly, I wanted to investigate the **tools** that could be best utilised to measure these parameters. Lastly, I wanted to investigate the effects of three different **medical treatments** on these parameters using the different tools.

Hypothesis 1: New parameters of myocardial and arterial function would be useful adjuncts in monitoring the vascular and myocardial manifestations of Marfan Syndrome

Hypothesis 2: Tissue Doppler Imaging, Applanation Tonometry and Wave Intensity Analysis would be useful tools in monitoring the vascular and myocardial manifestations of Marfan Syndrome

Hypothesis 3: ACEI and CCB reduce central aortic haemodynamics more than Beta Blockers in Marfan Syndrome

CHAPTER 2.1

PATIENTS, TRIAL APPROVAL PAPERWORK AND METHODS

Marfan Patients

We approached 55 Marfan patients from the South Wales and South West regions via databases from the two main tertiary congenital heart centres: The Congenital Heart Disease Centre, University Hospital of Wales, Cardiff; and the Bristol Congenital Heart Centre, Bristol. Local expertise was gained and aided recruitment – Dr Dirk Wilson in Cardiff and Dr Graham Stuart in Bristol. We also sought help from the local genetics department in Cardiff – Dr Sally Davies and all Cardiology Consultants in South Wales were written to in order to aid recruitment of Marfan patients who were being followed-up in their clinics. The patients were written to and asked to contact me if they were interested in participating in our trial. They then attended the Wales Heart Research Institute, Cardiff or the Bristol Congenital Heart Centre, Bristol for confirmation of their diagnosis by the Ghent criteria and initial assessment to see if they met the inclusion or exclusion criteria. They were all given verbal and written information about the trial. We recruited patients from Cornwall to West Wales.

Inclusion criteria: were subjects aged 16-60 years, who were either on no treatment, or were taking a β -blocker or other monotherapy only. Patients with previous aortic dissection or aortic surgery, severe heart valve regurgitation or aortic diameter at the sinotubular junction ≥ 5.0 cm were excluded. In addition, for the medical treatment study, patients who had contraindications to specific drug treatment (such as asthma), and women who were pregnant, at risk of pregnancy or breast-feeding, were excluded.

22 patients attended the Wales Heart research Institute for assessment. One subject did not have Marfan Syndrome. 2 Marfan patients were breast-feeding or trying to get pregnant. 1 patient attended this session but did not attend consequently. The latter 3 all had initial studies only.

In the medical treatment study, 18 patients started the trial and 14 completed it. 1 subject thought they were having side effects from the medication but when we broke their drug code they had in fact been taking the blinded version of their normal medication. 1 patient got a University place away from home and could not attend due to distance. 1 patient attended for 2 sessions only, because her lesbian partner was undergoing a renal transplant. 1 patient got into trouble with the law and could only attend 3 sessions.

Trial Approval Paperwork

Full written consent was obtained from all subjects. We had full MREC (Multiple Research Ethics Committee) approval after completing the paperwork and attending an interview at the Royal Free Hospital in London.¹⁹⁴

I received honorary contracts from the two hospitals in Cardiff and Bristol. We completed R and D (Research and Development) paperwork and were given local permission from both Cardiff and Bristol to perform a trial on their sites.

Further paperwork was sent to the MHRA (Medicines and Healthcare products Regulatory Agency) and we got their approval to conduct a trial involving medication. We purchased the trial medication from a local supplier in Cardiff but the drugs had to be firstly manufactured and then packaged in exactly the same capsules for blinding. We contacted both pharmacies and Biochemistry laboratories at Cardiff and Bristol to enable correct dispensing of the drugs and to check renal function after commencement of the ACEI, perindopril.

I attended a Good Clinical Practice in Research Training day in Cardiff and filled in the paperwork to access medical records for research. A sponsorship form was completed and Cardiff University agreed to sponsor our study.

During this time new Standard Operating Procedures for Research Ethics Committees came into place (March 2004)¹⁹⁴.

R+D: 05/CAD/3320

EudraCT 2005-000749-13

Cardiff University Sponsorship SPON CU101

Data protection number RD 05198

MREC 05/Q0501/41

Heart Research UK Ref: RG2535/07/09

We eventually, achieved full approval and could commence the trial.

Baseline Studies

At baseline, essential anthropometric data such as height, weight, age, and disease severity were recorded. Beta blockade or other monotherapy was withdrawn over a period of two weeks in subjects already on treatment, and then all subjects had a two-week period off all treatment, during which they received a placebo single-blind.

Blood pressure was measured from the brachial artery with an appropriately sized cuff in the supine position following quiet rest for 10 minutes using a validated semi-automated oscillometric device (Dinamap, General Electric, Connecticut, US).

Transthoracic Echocardiography Methods

Transthoracic echocardiography was performed using commercially available equipment (Vivid 7, General Electric, Connecticut, US). A minimum dataset was initially recorded in each patient, according to the recommendations of the British Society of Echocardiography. At each acquisition, the image was optimised to obtain the highest frame rates and best quality tissue doppler data.

Aortic root diameter was measured according to Roman at end-diastole in the parasternal long-axis view, at 4 levels: annulus, sinuses of Valsalva, sino-tubular junction, and ascending aorta 2 cm above the sino-tubular junction.

Left ventricular global, longitudinal and radial function were measured using conventional (Simpson's biplane method and annular plane systolic excursion) and tissue Doppler indices at each left ventricular wall in parasternal long axis, parasternal short axis, apical 4 chamber, apical 2 chamber and apical long-axis views. Left-ventricular stroke volume was calculated by both Doppler and Simpson techniques. Left ventricular contractile function was estimated from isovolumic acceleration and peak systolic mitral annular velocities as described in Chapter 1.3. Diastolic filling pressure was estimated using the E/e' ratio.

The amplitude and timing of expansion in the aortic arch and abdominal aorta were measured using pulsed tissue Doppler, as previously published by Harada. When we deviated from the above methods, for example in Chapter 3.1.1, it is fully explained in the text.

Applanation Tonometry Methods

In our Applanation Tonometry studies, we initially invited the subjects to rest supine for 10 mins in a quiet room. Blood pressure in the brachial artery was measured as described above.

Pressure waveforms in the radial artery were recorded by applanation tonometry with a Millar tonometer (SPC-301) and calibrated to the brachial blood pressure. The waveforms were analysed with commercial software (SphygmoCor version 7, AtCor, New South Wales, Australia) to obtain an averaged radial arterial waveform, and to derive a corresponding central aortic pressure waveform using the generalised transfer function. Augmentation index, defined as the ratio of augmentation pressure to central pulse pressure expressed as a percentage, was calculated from three or more consecutive radial recordings, and an average of the two readings with the lowest standard deviations was used in the analysis. Pulse pressure amplification was measured as brachial pulse pressure/central aortic pulse pressure.

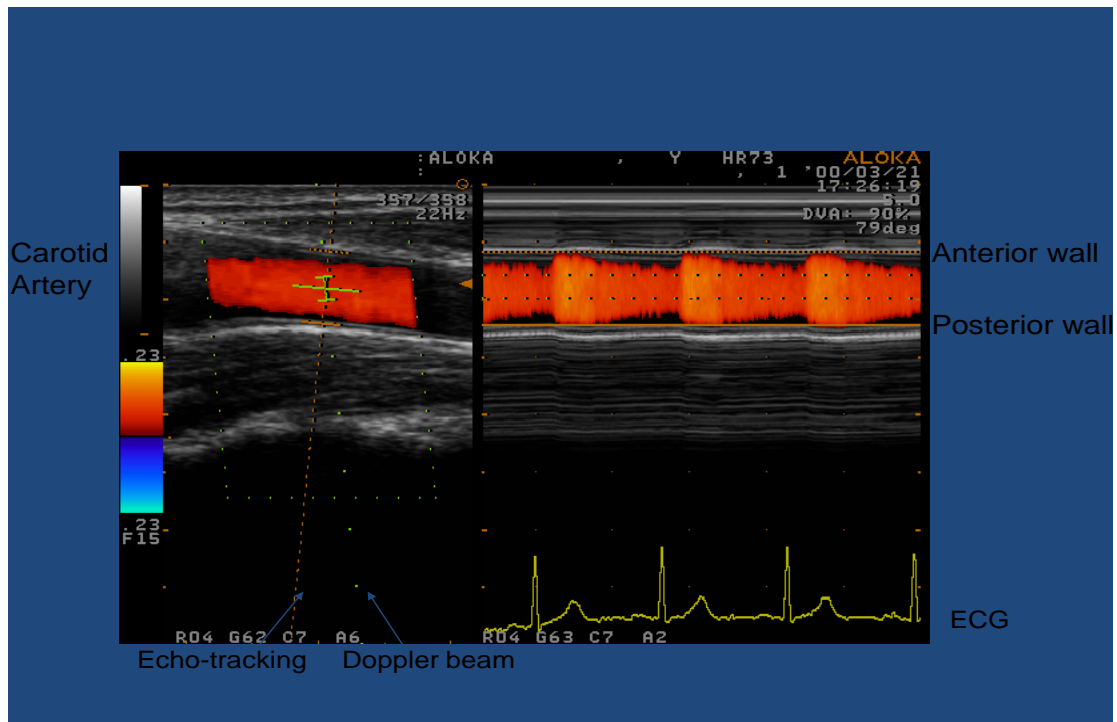
Carotid-to-radial and carotid-to-femoral pulse wave velocities were obtained, as an index of aortic stiffness. The carotid, radial and femoral arteries were applanated using the Millar tonometer, and sequential recordings of pressure waveforms were compared using pulse wave analysis. The surface distances between the sampling points were measured and the transit time was calculated using the SphygmoCor software. Data were collected by two trained researchers (myself and fellow researcher Dr Damien Kenny, Bristol) and the means of the two measurements with the lowest standard deviations were used in the analysis.

Wave Intensity Methods

Our subjects were studied reclined on a couch following a 10 minute rest in a dark, quiet room. Left brachial blood pressure was measured before each scan as described above. A 3 lead electrocardiogram was monitored throughout.

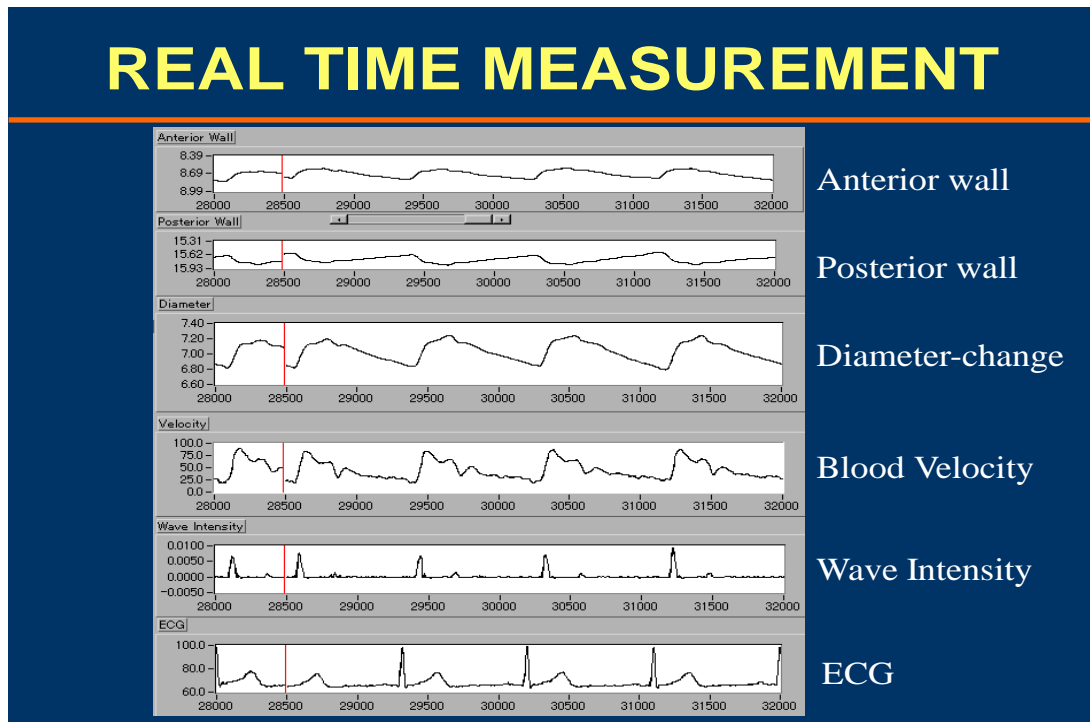
The right common carotid artery was exposed and scanned using a 7.5MHz linear array probe incorporating a 5MHz Doppler transducer. This was connected to an Aloka SSD-5500 ultrasound machine (Hitachi-Aloka, Tokyo, Japan)¹⁹⁵ (figure 1).

Figure 1: Ultrasound Measurement of Common Carotid Artery Diameter and Flow to Calculate Net Wave Intensity



As seen in fig 1. A longitudinal view of the right common carotid artery was acquired and the probe positioned so the anterior and posterior wall intima were clearly seen. A single scan line was aligned perpendicularly to the vessel walls at a site 2cm proximal to the carotid bulb. The anterior and posterior intima-media borders were tracked using high-resolution online wall tracking with a sampling rate of 1kHz¹⁹⁶. Arterial pressure waveforms were obtained automatically in real time by calibrating peak and trough values with systolic and diastolic blood pressure measured by sphygmomanometry.¹⁹⁶ A pulse wave Doppler beam was aligned to the artery walls to measure velocity from a colour flow Doppler box covering the lumen. Velocity was calculated from the mean of the colour Doppler data. Arterial diameter and velocity were recorded continuously for 20 secs. After acquisition, 20 beats were selected with noisy waveforms rejected. These beats were signal-averaged to give single waveforms of diameter and velocity (fig 2).

Figure 2: Wave Intensity Analysis Real Time Measurement



The MATLAB program was used to calculate net Wave Intensity and then to separate the waves into their four components and to calculate the local wavespeed, Peterson's pressure-strain elastic modulus (epsilon) and the β stiffness index (beta).

CHAPTER 3.1.1 **REPRODUCIBILITY OF TISSUE DOPPLER IMAGING – THE M4 STUDY**

Introduction

Regional left ventricular function can now be quantified precisely by tissue Doppler (myocardial velocity imaging), giving numerous specific measurements of motion and deformation that can be used to diagnose disease even at a preclinical stage. Many echocardiography departments use more than one brand of echocardiography machine. Unfortunately, however, manufacturers process the signals in different ways and so it is uncertain if measurements can be compared between machines. In addition, if the technique is to be standardised, the same patient having studies on different machines by different operators, should give comparable results.

Studies so far have been extremely variable. In one previous study¹⁹⁷ they showed that two commercially available speckle-tracking software appear to be comparable when quantifying left ventricular function in a healthy population of 28 people. However, a multicentre study¹⁹⁸ also looking at strain by speckle-tracking showed considerable variation that could only partly (16% of variance) be explained by patient factors. It is important to note that both these two studies were of a healthy population. A study in 2006¹⁹⁹, reported 20 healthy participants having a coefficient of variation of up to 19% in the measurement of tissue velocity, strain and strain rate but no significant difference between the two systems trialled. A further study in the US also shows variation²⁰⁰. They tested three common commercial US systems with a Doppler string phantom. In measuring pulsed and continuous-wave Doppler velocities one of the systems consistently overestimated velocity by 5% whereas the other two were similar and accurate.

Variation between systems has also been looked at briefly in magnetic resonance imaging.²⁰¹ In this study, Kornaat and colleagues looked at two surrogate markers for osteoarthritis between two 3.0T MRI systems from different manufacturers. In this small trial of five healthy volunteers they found no differences in these markers between the two scanners.

With such variation in the literature, I, therefore, wanted to look at these potentially important differences in a more real-life situation. We arranged four of the most-used types of echocardiography machines in a healthy and unhealthy population using several echocardiographers from our department. We took a wide range of measurements including tissue Doppler, real-time and off-line measurements.

I, therefore, set out a list of objectives:

- To compare reproducibility of tissue Doppler measurements obtained on the same patients, between different machines and operators
- To compare real-time and processed measurements
- To compare the reproducibility of tissue Doppler measurements with the reproducibility of standard echocardiographic measurements (M-mode, 2D, blood pool doppler)

Methods

4 of the latest model, high-tech machines were obtained for 2 weeks from GE, Toshiba, Acuson and Aloka. 60 patients each attended for one morning or one afternoon with a wide range of diagnoses and ages varying from normal to severe heart failure. Each patient had 4 consecutive echocardiographic studies performed on each machine independently by a separate operator, in random order (each starting with a different system but then consecutively all the others, in a circuit). Each operator made measurements for that system following a common protocol.

I specifically looked at:

- What was the intermachine/operator reproducibility of myocardial velocity?
- What was the intermachine/operator reproducibility of myocardial strain?
- What was the intermachine/operator reproducibility of myocardial strain rate?
- Was there a difference in the reproducibility of systolic, early diastolic (E), and late diastolic (A) indices?
- Was reproducibility affected by frame rate?
- Was reproducibility affected by image quality?
- Was there a systematic difference between machines/operators or is it random?
- Were velocities consistently more reproducible between systems, than strain and strain rate?
- What were the implications for drawing conclusions from repeated measurements?

Protocol

Anthropometric data were collected: age, gender, height, weight, body mass index, diagnosis. The times for analyses were recorded and image quality was also graded by the operators on a scale of 1 to 5. The image was optimised to get the best frame rate and therefore best quality tissue Doppler data.

In the Parasternal long-axis (**PLAX**) view, the measurements obtained were for radial (*short-axis*) function of the left ventricle in the basal posterior (BP) segment:

Sm velocity in systole

Em velocity in early diastole

Am velocity during atrial contraction

and the derived parameters

SRs strain rate in systole

S strain in systole

In the Apical imaging planes we used: Apical 4-chamber view (**A4C**) – for septal and lateral walls; Apical 2-chamber view (**A2C**) – for inferior and anterior walls; Apical long-axis view (**APLAX**) – for posterior and anteroseptal walls.

The three apical views were acquired at 60 degree intervals by rotating the transducer positioned over the apex of the heart. All these views were used to measure *long-axis* function of the left ventricle (movement/contraction along the plane from the base of the heart to the apex).

5 parameters were measured in the basal segment of each wall (same parameters as for the short-axis measurements from the parasternal window) and the frame rate was documented for each imaging plane/pair of walls.

Myocardial segments

The study protocol included a focussed image just of the septum, to give higher frame rates in order to optimise signal quality (**Septum with high FPS**). From these images, the same 5 parameters were measured at three levels corresponding to the **basal**, **mid**, and **apical** segments.

Right ventricle

We included only one set of measurements from the right ventricle, in order to simplify acquisition and analysis and because the right ventricle is rarely affected in Marfan Syndrome. These parameters were velocities of the lateral tricuspid annulus (**LTA**) at the base of the right ventricular free wall as this is used clinically. Deformation indices are never measured at the annulus and so they were not recorded.

Real-time vs off-line measurements

Deformation indices (i.e. strain and strain rate) can only be measured “off-line”, that is by processing of digitally stored velocity data.

Velocity, however, can be measured in 2 ways – either in “real-time” from a pulsed Doppler measurement, or by processing digitally stored data to obtain “off-line”

measurements at the same site. There is an important systematic difference between these measurements since the pulsed, real-time technique gives peak velocities and the processed off-line technique gives mean velocities; thus the “off-line” measurements would be expected to be about 25% lower.

To assess this, velocities were measured at the medial mitral annulus (**MMA**) and lateral mitral annulus (**LMA**) by both techniques.

Timing of myocardial motion

Accurate measurements of the timing of heart muscle movement is now important for studying dyssynchrony in the normal and diseased heart (for example, to identify patients who might benefit from resynchronisation therapy with a biventricular pacemaker). There have been no reported comparisons of timings between machines. We measured timings of only one parameter – that is, time from the onset of the QRS complex on the ECG to the time of peak systolic velocity of mitral annular motion (**Time to peak**). This was done at two sites (**medial and lateral mitral annulus**) and using 2 methods (**real-time, off-line**).

Estimated left atrial filling pressure

A combination of one blood-pool Doppler measurement (velocity of flow across the mitral valve in early diastole) and one tissue Doppler measurement (mitral annular velocity during early diastole, E_m , using real-time measurements) is now widely used to estimate left atrial pressure non-invasively. We therefore made recordings to allow us to assess the intermachine variability of this derived haemodynamic index.

We recorded velocities of blood flow through the mitral valve in early diastole (E) and during atrial filling (A); in clinical practice these are often combined in the E/A ratio which is an indicator of myocardial relaxation.

I was involved in the setting up of this study. The echocardiographic data were recorded independently by four other clinical research fellows. I then compiled the data base summarising all the results. I calculated the derived measurements and undertook the initial analyses. I prepared the database for further statistical analysis which was performed by Tomas Andersson, Karolinska University, Stockholm.

Results

The baseline characteristics of the study group are seen in Table 1 with a wide range of age and size with a 62% male preponderance. 20% are a normal population and the remaining subjects had a range of cardiac and non-cardiac disease mirroring a normal hospital outpatient echocardiography list. The GE machine was given the highest

image quality average (4.3 out of 5) and the Toshiba system the lowest image quality average (3.3 out of 5) by the echocardiographers although it must be noted the GE system is the machine the scanners were more experienced in using and this is a very subjective measure.

Table 1: Baseline Characteristics of the Study Group (n=60 patients)

Characteristic	Value
Age (yrs)	
-mean	52.6
-range	19-88
Height (cms)	
-average	168
-range	146-190
Weight (kgs)	
-average	77
-range	51-122
BMI	
-average	27.4
-range	20.6-39.7
Male gender n° (%)	37/60 (62%)
Image quality average (rated 1-5 by echocardiographer)	
-Machine A (GE)	4.3
-Machine B (Acuson)	3.4
-Machine C (Toshiba)	3.3
-Machine D (Aloka)	3.8
Diagnosis n° (%)	
-Normal	12 (20%)
-Heart Valve disease	11 (18%)
-Heart Failure	9 (15%)
-Coronary artery disease	9 (15%)
-Cardiomyopathy	8 (13%)
-Non-cardiac disease	8 (13%)
-Hypertension	5 (8%)
-Atrial fibrillation	4 (7%)

Table 2 shows the correlations of tissue Doppler measurements at the basal septum of the left ventricle in an apical 4 chamber view. This shows good correlations between machines for peak systolic ($r=0.64$), early ($r=0.73$) and late (0.54) diastolic velocities but a disappointing correlation for strain rate (0.18) and strain (0.19). It is also noted that there wasn't a specific machine combination where the correlations were particularly good or poor. For example, when measuring peak systolic velocity, early and late diastolic filling the worst correlations were with machine A vs B; for strain rate it was machine C vs D; and for strain it was machine A vs D.

Table 2: Correlations and R^2 Of Tissue Doppler Measurements at The Basal Septum on Apical 4 Chamber View

	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D	Mean R of all 6 combinations
SmBS	0.54 0.29	0.68 0.46	0.63 0.4	0.69 0.48	0.59 0.35	0.70 0.49	0.64
EmBS	0.58 0.34	0.69 0.48	0.65 0.43	0.81 0.66	0.81 0.66	0.81 0.66	0.73
AmBS	0.47 0.22	0.59 0.35	0.54 0.29	0.56 0.31	0.52 0.27	0.57 0.32	0.54
SR	0.23 0.05	0.28 0.08	0.05 0.00	0.23 0.05	0.25 0.06	0.01 0.00	0.18
Ss	0.15 0.02	0.35 0.12	0.02 0.00	0.14 0.02	0.23 0.05	0.22 0.05	0.19

Table 3 shows the correlations for all 6 left ventricular walls, the lateral tricuspid annulus and the basal septum at high frame rate. These figures show a mean correlation between machines of $r=0.56$ for all 8 walls and a range of correlations of $r=0.44$ at the left ventricular basal posterior wall to $r=0.64$ at the left ventricular basal septum at normal frame rate. Interestingly, the high frame rate seemed to have no impact on the peak systolic velocities with a correlation of $r=0.62$ compared to a correlation of $r=0.64$ when a lower frame rate was used at the same site –left ventricular basal septum.

Table 3: Correlations and R² Of Peak Systolic Velocities at the Six LV Walls, at High Frame Rate and The RV FreeWall

	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D	Mean R of all 6 combination
SmBP	0.39 0.15	0.49 0.24	0.49 0.24	0.50 0.25	0.34 0.12	0.44 0.19	0.44
SmBS	0.54 0.29	0.68 0.46	0.63 0.4	0.69 0.48	0.59 0.35	0.70 0.49	0.64
SmBS high frame rate	0.52 0.27	0.74 0.55	0.58 0.34	0.65 0.42	0.55 0.30	0.69 0.48	0.62
SmBL	0.49 0.24	0.58 0.34	0.43 0.18	0.56 0.31	0.33 0.11	0.80 0.64	0.53
SmBI	0.45 0.20	0.66 0.44	0.61 0.37	0.63 0.40	0.71 0.50	0.74 0.55	0.63
SmBA	0.33 0.11	0.51 0.26	0.45 0.20	0.51 0.26	0.60 0.36	0.71 0.50	0.52
SmAS	0.52 0.27	0.56 0.31	0.61 0.37	0.26 0.07	0.61 0.37	0.70 0.49	0.54
Sm LTA	0.46 0.21	0.67 0.45	0.53 0.28	0.70 0.49	0.47 0.22	0.53 0.28	0.56

We also looked at real-time and processed measurements. Table 4 compares early diastolic filling at LMA and MMA showing a large difference in reproducibility between machines at the LMA (r=0.80) compared to the MMA (r=0.52). Time to peak systolic velocities were measured in real-time and processed and the correlations were lower when processed times were used (r=0.41 and 0.49) compared to real-time (r=0.52 and 0.55) and this was true whether it was measured at the lateral or medial mitral annulus. However, the correlations were again lower for MMA (r=0.41 and 0.52) compared to LMA (r=0.49 and 0.55).

The best correlations between the machines were seen with normal pulsed Doppler early and late diastolic filling at mitral inflow. The correlation for early diastolic filling was r=0.89 and late diastolic filling was r=0.81.

Table 4: Correlations and R² At Mitral Inflow and the Mitral Annulus

	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D	Mean R of all 6
E	0.92	0.89	0.93	0.85	0.86	0.87	0.89
A	0.81	0.85	0.79	0.86	0.75	0.79	0.81
Em at LMA	0.81 0.66	0.84 0.71	0.85 0.72	0.79 0.62	0.76 0.58	0.76 0.58	0.80
Em at MMA	0.85 0.72	0.73 0.53	0.21 0.04	0.76 0.58	0.32 0.10	0.25 0.06	0.52 0.27
Time to peak Sm real-time LMA	0.71 0.50	0.64 0.41	0.65 0.42	0.60 0.36	0.37 0.14	0.35 0.12	0.55
Time to peak Sm processed LMA	0.37 0.14	0.60 0.36	0.63 0.40	0.44 0.19	0.35 0.12	0.57 0.32	0.49
Time to peak Sm real-time MMA	0.52 0.27	0.71 0.50	0.62 0.38	0.54 0.29	0.31 0.10	0.40 0.16	0.52
Time to peak Sm processed MMA	0.30 0.09	0.63 0.40	0.47 0.22	0.32 0.10	0.32 0.10	0.40 0.16	0.41

Table 5: Bland-Altman Mean differences between the Four Machines for the six main parameters.

	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D	Mean of all 6
E (m/s)	0.0±0.1	0.1±0.1	0.0±0.2	0.1±0.1	0.0±0.2	-0.1±0.2	0.0
SmBS (cm/s)	0.6±1.5	1.6±1.2	1.7±1.4	0.8±1.4	1.1±1.7	0.1±1.4	1.0
SmBS HFR (cm/s)	0.1±1.6	1.6±1.1	2.4±1.6	1.5±1.4	2.0±1.8	0.6±1.4	1.4
SsBS (%)	-5±25	-1±19	0±29	5±21	6±26	0±22	1
SRsBS (.s⁻¹)	-0.8±1.8	-0.1±2.4	-0.3±2.6	1.0±2.5	0.9±2.4	0.1±2.8	0.1
Time to peak Sm processed LMA (ms)	5±66	-12±49	-3±46	-15±64	0.2±62	11±47	-2

Table 5 illustrates the mean differences in measurements between the different machine combinations for six specific parameters. It shows very little difference between machines with respect to early diastolic mitral blood flow Doppler (E). However, there are large mean differences and standard deviations in the other parameters especially systolic strain (SsBS) and strain rate (SRsBS). The timing parameter shows larger mean differences for the combinations A vs C, B vs C and C vs D compared to A vs B, A vs D and B vs D. However, the standard deviations are about the same for all six combinations.

Figure 1 shows a good correlation $r=0.92$ between the GE and Acuson machines for early diastolic filling (E).

Figure 1: Scatterplot of Early diastolic filling (E) as measured by the GE Vivid 7 and the Acuson (r=0.92).

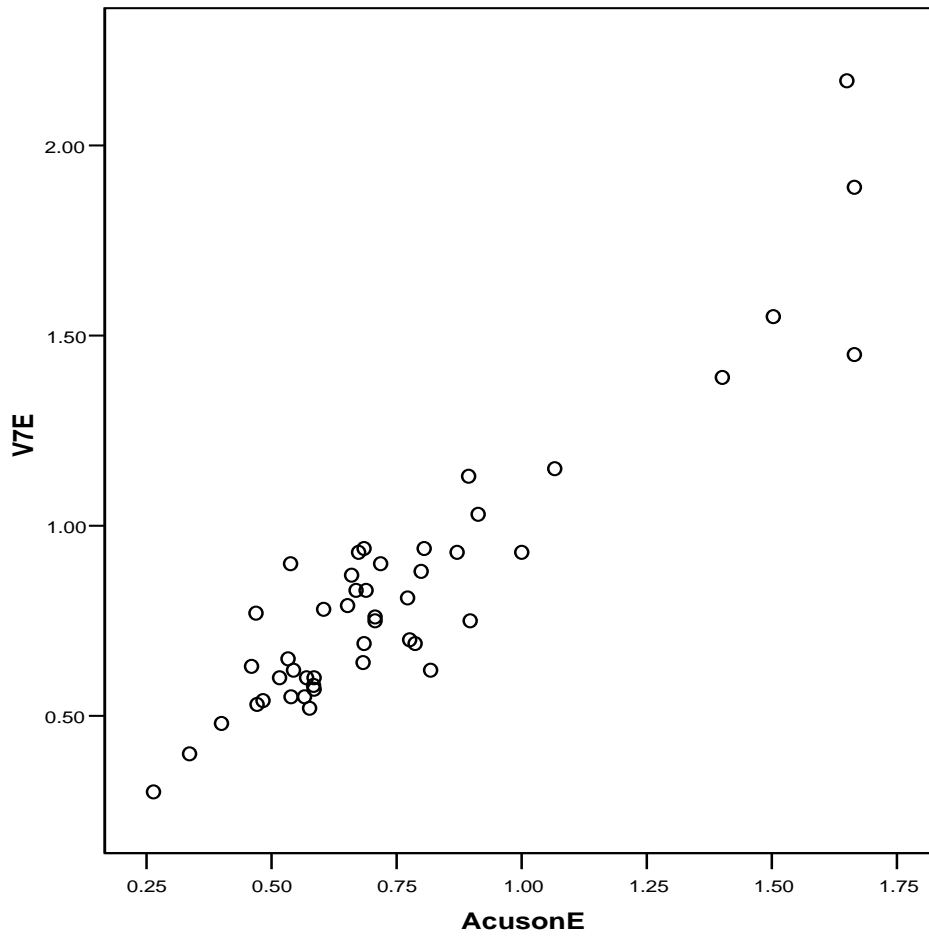


Figure 2 illustrates the layout for the next 2 figures. Figures 3 and 4 highlight the variability in correlations and mean differences between two parameters – the first in figure 3 shows little intermachine variation, the second in figure 4 showing huge intermachine variation.

Figure 2:

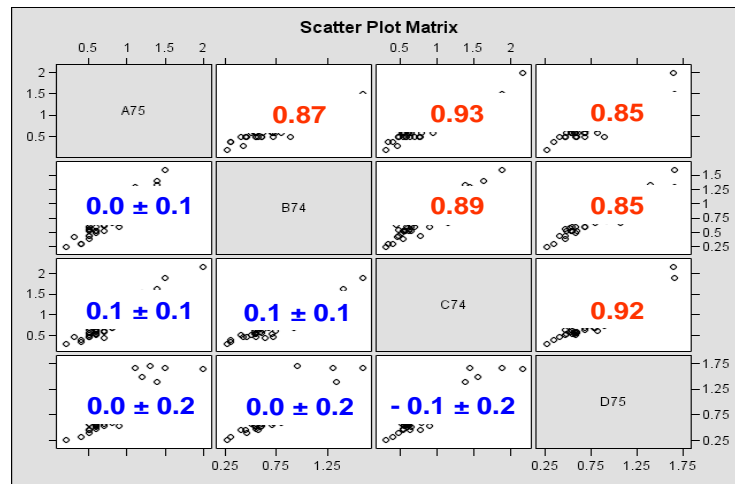
	A	B	C	D
A				
B				
C				
D				

Aloka "A" / Toshiba "B" / GE V7 "C" / Acuson "D"

Figure 3: Correlations and Mean Differences for early diastolic mitral blood flow doppler (E) between Machines

Mitral E velocity, pulsed Doppler

Correlations 0.89



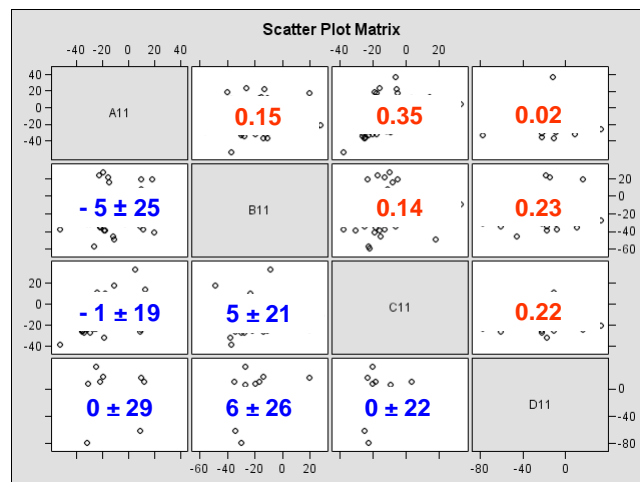
Mean differences (m/s)

0.0

Figure 4: Correlations and Mean Differences for systolic strain at the basal septum (SsBS) between Machines

Ss basal septum A4C

Correlations 0.19



Mean differences (%)

1

Discussion

Firstly, it must be noted that having different fellows performing the echocardiography is a confounding variable in this study and may alter the impact of the machines themselves on the measured variables. This would benefit from a more complex statistical review. However, in this study, there were considerable and potentially clinically significant variations in myocardial velocities and deformation recorded on different echocardiographic machines by different operators, in the same patients under similar physiological conditions.

It is worth explaining that this study is not looking at the accuracy of the four individual machines per se but emphasises the differences between them. However, I have shown a large variation in correlations amongst machines in the tissue Doppler parameters especially pronounced with strain and strain rate. The correlations for strain ($r=0.19$) and strain rate ($r=0.18$) were so low that it must be raised whether these parameters are reliable enough to be used in echocardiography labs with different manufactured machines. Correlations of peak systolic velocities of between 0.44 and 0.64 are however better but the lack of difference with a higher frame rate was a surprise ($r=0.62$ HFR compared to 0.64). This may be because our echocardiographers were very experienced in a research setting and optimized the echo parameters as routine and therefore, the high frame rate had less of an impact. Tissue Doppler measurement of early diastolic filling at the LMA ($r=0.80$) was much better than at the MMA ($r=0.52$) and maybe adds weight to measuring this parameter, to calculate left atrial filling pressure, routinely at the lateral mitral annulus or by averaging the two. Current British Society Of Echocardiography guidelines suggest using either sites.

There was also a notable difference between specific machines especially when generally the correlations were lower. However, we did not find evidence to show that 2 particular machines were always very different compared to other combinations. It was reassuring to see high correlations between machines for pulsed Doppler E and A measurements of early and late diastolic filling at mitral inflow. These parameters are measurements of left ventricular relaxation and are found in all guidelines and are taken at every basic echocardiography exam.

The causes of these differences need to be understood and overcome by standard acquisition, tracking and signal averaging if the diagnostic potential of Tissue Doppler is to be optimised. At present, it seems sensible to at least sequentially scan the same Marfan patient with the same machine and ideally by the same operator. It also seems sensible to purchase one type of echocardiography machine for each department although cost, new software updates and different personal preferences amongst Consultants makes this practically difficult.

CHAPTER 3.1.2

Tissue Doppler Imaging In A Normal, A Subclinical Hypothyroid, A Type 2 Diabetic And A Marfan Population

Introduction

In Chapter 1.3, we identified two novel parameters of left ventricular myocardial function that had good theoretical reasons for being useful adjuncts in our assessment of the cardiovascular abnormalities seen in Marfan Syndrome, - isovolumic acceleration (IVA) and peak systolic velocity (Sm).

IVA, that is, myocardial acceleration during isovolumic contraction at the base of each of the six left ventricular walls has been described in chapter 1.3 as a sensitive measurement of left²⁰²⁻²⁰⁴ and right²⁰⁵ ventricular myocardial contractile function that seems unaffected by preload and afterload at least within physiological range. IVA correlates closely with invasively derived haemodynamic indices of contractility such as dp/dt and end-systolic elastance.²⁰² IVA can also be measured in each left ventricular wall and regionally.

Peak systolic velocity when measured at the lateral mitral annulus is a measure of longitudinal systolic function. It has been correlated with LV ejection fraction²⁰⁶ and peak dp/dt²⁰⁷. Regional reductions in Sm are correlated with regional wall motion abnormalities and TDI is often used as part of dobutamine stress echocardiography as peak Sm increases with dobutamine and exercise and decreases with ischaemia.^{208,209}

The most commonly used index of left ventricular function, the Ejection Fraction (EF), is affected by volume and is a marker of global left ventricular function.

I, therefore, wanted to investigate these two new parameters in terms of their reproducibility and the variation at the six different left ventricular walls and their ability to expose subclinical deterioration in left ventricular function. Members of our research group had already performed echocardiography on a subclinical hypothyroid and type 2 diabetic population. As both populations would be expected to have subclinical left ventricular systolic dysfunction I analysed the data acquired and compared it to a normal population group and my Marfan population.

Hypothesis 1: IVA and Peak systolic mitral annular velocity will detect subclinical left ventricular impairment in our 3 abnormal populations.

Hypothesis 2: IVA and Peak systolic mitral annular velocity will detect regional differences in left ventricular impairment.

Hypothesis 3: Peak systolic mitral annular velocity will have lower variability compared to IVA.

Methods

Subjects

A total of 85 volunteers were examined at our institution. They consisted of 20 normal volunteers, 21 female subclinical hypothyroid patients (SCH), 23 type 2 diabetic patients (T2DM) and 21 Marfan patients (MF). All volunteers were given written information and gave informed consent. The baseline characteristics are recorded in Table 1 and the thyroid function of the subclinical hypothyroid group in Table 2.

Transthoracic Echocardiography Acquisition

All volunteers underwent transthoracic echocardiography using a GE Vivid 7 machine (General Electric, Connecticut, US) equipped with a 2.5MHz probe. Optimised Tissue Doppler images were attained using a frame rate of $>100\text{s}^{-1}$ and the loops were stored for post-processing. Two different echocardiographers were used for the diabetic, subclinical hypothyroid and normal groups and I was the echocardiographer for the Marfan group.

Longitudinal IVA and peak systolic velocity (Sm) were measured at the septal, lateral, inferior, anterior, basal-posterior and anteroseptal walls from the apical 4-chamber, apical 2-chamber and apical long-axis views. Ejection fraction (as the most commonly used index of left ventricular function) was measured by the Simpson's biplane method. IVA was measured by placing the cursor at the base of the left ventricular wall immediately (0.5cm) below the insertion of the mitral valve leaflet as described by Vogel and colleagues.²⁰² A three beat loop was recorded but a single beat loop was selected to increase the size of the image for accuracy and the slope was measured at isovolumic acceleration. This was done in 2 of the 3 beat loops recorded and an average taken. IVA was measured as the slope from the first positive deflection after

the onset of systole, as ascertained from the surface ECG, during isovolumic acceleration from zero to peak velocity.

The peak systolic velocities (S_m) were also measured using Tissue Doppler Imaging and recorded using maximal frame rates at the base of each of the six left ventricular walls

Statistics

I analysed all the echo data from all four groups to calculate the peak systolic velocities and the IVA. I produced the database and performed the statistical analysis using SPSS Version 18. The data was checked for Normality by inspecting histograms and Q-Q Plots. The means of IVA and S_m at each wall were calculated with the standard deviations. The differences between the four populations were calculated using a One Way Anova with Tamhane's T2 posthoc calculation. This gave a mean difference, standard error and a significance level. A p value <0.05 was considered statistically significant. The correlation between the independent predictors of the key cardiac variables and the variables themselves was performed using Pearson's Correlation Coefficient. Reproducibility is expressed as the Coefficients of Variation (CV). Each variable was acquired once by the operator but measured twice by me in all subjects after a time period of 24 hours and compared to the same measurement calculated by another fellow. CV was measured by Standard Deviation (SD)/mean \times 100. The SD is the standard deviation of the measurement error associated with a single measurement calculated as the SD of residuals (measurement 1 - measurement 2) divided by $\sqrt{2}$.

Table 1: Baseline Characteristics of the Four Different Populations

	Normal Population	Subclinical Hypothyroid Patients	Type 2 Diabetic patients	Marfan Patients
Number	20	21	23	21
Age (yrs)	25.7 ±2.9	49.2 ±3.8	64.1 ±8.5	30.4 ±12
Male/Female	50%/50%	0%/100%	70%/30%	43%/57%
Smokers	0%	5.3%	20%	7%
BMI (kg/m ²)	24.1 ±5.4	29.9 ±6.7	31.6 ±8.9	22.9 ±4

Data are mean ±SD.

Table 2: Baseline Thyroid function In the Subclinical Hypothyroid Patients (SCH)

	Subclinical Hypothyroid Patients
TSH (mIU/litre) 0.5-5.0 normal	8.8 (range 5.7-21.6)
Free T4 (ng/dl) 0.8-1.8 normal	1.0 ±0.1
Free T3 (ng/dl) 0.2-0.5 normal	0.38 ±0.03
Thyroid Peroxidase Antibody +ve	89%

Data are mean ±SD.

Results (Tables 3+4)

Ejection Fraction:

Although both the SCH and Marfan population had mean ejection fractions measured that are below the cut-off of normal, that is 55%, there was no significant difference between the groups. However, the standard deviation for the three abnormal groups was much higher than the normal group indicating the abnormal groups did contain

subjects with below normal ejection fractions. The heart rates were the same for all groups. It is noted that the Marfan group stopped taking their medication, mostly beta blockers, two weeks prior to testing.

Peak Systolic Mitral Annular Velocities (Sm):

The peak systolic mitral annular velocities were significantly lower in both the T2DM group and the SCH group compared to the normal group in all six left ventricular walls ($P>0.05$). The velocities were also significantly lower in the MF group at the anterior and anteroseptal walls. The velocities were also lower in the MF group at the remaining septal (18%), lateral (16%), inferior (10%) and basal-posterior walls (12%) but did not reach statistical significance.

Intraobserver variability for peak systolic mitral annular velocity was 15% and interobserver variability was 20%.

Isovolumic Acceleration:

There was no statistically significant variation in longitudinal IVA between the six left ventricular walls. IVA was significantly different between the normal population and the SCH and the normal population and the T2DM group in both the septum (by 38% for both comparisons) and anterior walls (31% and 44% respectively, $p<0.05$). There was no statistical difference in IVA between the normal and Marfan population. When we calculated the average of IVA in the six left ventricular walls, there was no statistical difference between the three groups.

Intraobserver variability for IVA was 28% and interobserver variability was 30%.

Table 3: Comparison of Regional Peak Systolic Mitral Annular Velocities Between the Four Groups

	Mean Sm ± Standard Deviation (cm/s)				Mean Differences ± Standard Error			Significance Level		
	Normal (A)	SCH (B)	T2DM (C)	MF (D)	A vs B	A vs C	A vs D	A vs B	A vs C	A vs D
Septum	6.6 ± 1.1	5.2 ± 0.7	4.9 ± 1.4	5.4 ± 1.8	1.3 ± 0.3	1.7 ± 0.4	1.2 ± 0.5	P<0.001*	P<0.001*	P=0.1
Lateral	8.0 ± 1.3	5.7 ± 1.5	4.8 ± 2.1	6.6 ± 2.8	2.3 ± 0.4	3.2 ± 0.5	1.3 ± 0.7	P<0.001*	P<0.001*	P=0.32
Inferior	6.9 ± 1.1	6.0 ± 1.0	5.7 ± 1.7	6.2 ± 1.2	1.0 ± 0.3	1.3 ± 0.4	0.7 ± 0.4	P<0.05*	P<0.05*	P=0.28
Anterior	7.3 ± 1.5	5.3 ± 1.5	4.4 ± 1.4	5.4 ± 2.0	2.0 ± 0.5	3.0 ± 0.5	1.9 ± 0.6	P<0.001*	P<0.001*	P<0.01*
Basal-posterior	7.4 ± 1.6	6.1 ± 1.0	5.3 ± 1.6	6.5 ± 1.8	1.3 ± 0.4	2.2 ± 0.5	0.9 ± 0.5	P<0.05*	P<0.001*	P=0.4
Anteroseptal	6.6 ± 1.3	4.4 ± 1.1	3.9 ± 1.6	4.3 ± 1.3	2.2 ± 0.4	2.7 ± 0.4	2.2 ± 0.4	P<0.001*	P<0.001*	P<0.001*
HR (bpm)	65 ± 14	67 ± 10	66 ± 12	65 ± 11	-2 ± 4	-1 ± 4	0.2 ± 4	P=1.0	P=1.0	P=1.0
EF (%)	60 ± 5	54 ± 9	56 ± 11	53 ± 13	5 ± 2	4 ± 3	6 ± 3	P=0.2	P=0.6	P=0.2
Frame Rate	175 ± 20	165 ± 25	161 ± 26	170 ± 22	9 ± 6	10 ± 5	5 ± 3	P=0.5	P=0.3	P=0.9

Table 4: Comparison of Regional Isovolumic Acceleration Between the Four Groups

	Mean IVA \pm Standard Deviation (m/s ²)				Mean Differences \pm Standard Error			Significance Level		
	Normal (A)	SCH (B)	T2DM (C)	MF (D)	A vs B	A vs C	A vs D	A vs B	A vs C	A vs D
Septum	1.6 \pm 0.7	1.0 \pm 0.5	1.0 \pm 0.5	1.2 \pm 0.7	0.5 \pm 0.2	0.6 \pm 0.2	0.4 \pm 0.2	P<0.05*	P<0.05*	P=0.3
Lateral	1.3 \pm 0.6	1.2 \pm 0.5	0.9 \pm 0.8	1.1 \pm 0.6	0.2 \pm 0.2	0.4 \pm 0.2	0.3 \pm 0.2	P=0.93	P=0.39	P=0.54
Inferior	1.3 \pm 0.5	1.1 \pm 0.3	1.0 \pm 0.6	1.1 \pm 0.4	0.2 \pm 0.1	0.3 \pm 1.8	0.2 \pm 0.2	P=0.65	P=0.51	P=0.68
Anterior	1.6 \pm 0.6	1.1 \pm 0.6	0.9 \pm 0.6	1.3 \pm 1.0	0.6 \pm 0.2	0.8 \pm 0.2	0.4 \pm 0.3	P<0.05*	P<0.005*	P=0.6
Basal-posterior	0.9 \pm 0.6	1.2 \pm 0.5	1.1 \pm 0.9	1.2 \pm 0.6	-0.3 \pm 0.2	-0.2 \pm 0.2	-0.3 \pm 0.2	P=0.66	P=0.97	P=0.56
Anteroseptal	1.1 \pm 0.5	0.9 \pm 0.4	0.8 \pm 0.4	0.8 \pm 0.9	0.1 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.2	P=0.97	P=0.37	P=0.77
6 Wall Av.	1.4 \pm 0.5	1.1 \pm 0.4	1.0 \pm 0.5	1.1 \pm 0.4	0.3 \pm 0.1	0.4 \pm 0.2	0.2 \pm 0.1	P=0.2	P=0.09	P=0.7

Table 5: Correlation between Age and BMI and the Regional Peak Systolic Mitral Annular Velocities For all Groups

	Age	BMI	Septum	Lateral	Inferior	Anterior	Baso-posterior	Antero-septal
Age	1.0	0.8	-0.31	-0.50	-0.32	-0.43	-0.43	-0.24
BMI	0.8	1.0	0.11	0.24	0.12	0.25	0.28	0.11

Table 6: Correlation between Age and BMI and the Regional Isovolumic Acceleration For all Groups

	Septum	Lateral	Inferior	Anterior	Baso-posterior	Antero-septal	6 wall average
Age	-0.02	-0.15	-0.07	-0.28	-0.08	-0.08	-0.17
BMI	0.11	<0.01	<0.01	0.16	0.05	0.11	0.13

Table 5 shows the correlations between the two key independent predictors age and BMI was 0.8. Therefore, as expected, a strong correlation that as age increases so does BMI. Tables 5 and 6 show there were much lower correlation values for both regional peak systolic mitral annular velocities and regional isovolumic acceleration with age and BMI. The highest correlation was -0.5 between age and lateral wall peak systolic mitral annular velocity. The six wall IVA average had very low correlations with age (-0.17) and BMI (0.13).

Discussion

During tissue Doppler imaging, high frame rates were achieved for each population and therefore, the results should be accurate. Peak systolic mitral annular velocities detected abnormalities in all walls in the T2DM and SCH groups compared to the normal population. Abnormalities were also detected in the anterior and anteroseptal walls in the MF group. The ejection fractions measured suggest that all three abnormal groups had subjects with below normal left ventricular systolic function. The reason for their reduction in left ventricular function is difficult to speculate on due to the different ages in the four groups which was confounded with their underlying diagnosis. The mean ages were 26 years in the normal group; 49 years in the SCH; 64 years in the T2DM; and 30 years in the MF groups. The reason the MF group only had significantly different peak systolic mitral annular velocities in only two of the six walls may be due to their relative youth. Also, there was a difference in the sex of the groups due to the medical disorders they had with 100% female sex in the subclinical hypothyroid group and significantly more men in the diabetic group. It must be noted that performing correlations with age and BMI against the key cardiac variables in the four groups combined (tables 5 and 6) did not reveal any significant correlations. However, the patient characteristics revealed important differences and if repeated the groups should be better matched.

The only statistical differences in IVA measurements between the normal control group and the other three abnormal groups were seen at the anterior wall in the T2DM and SCH groups and at the septum in the T2DM group. This is a marked difference from the peak systolic mitral annular velocities. It may be partly explained by difficulties encountered during measurement. Acquisition of the trace required for IVA at each site was not difficult but the actual measurement itself was difficult and prone to error. Choosing the correct slope was subject to considerable intraobserver (28%) and interobserver (30%) variability and scores poorly compared to the variability with the peak systolic velocity (15 and 20%).

My results are similar to that found in a recent paper²¹⁰ looking at IVA in three groups - a normal population, a type 2 diabetic population and a heart failure population. The mean LVEFs in these three groups were 60%, 56% and 32% respectively. They concluded that IVA may be used as a research tool but the clinical applicability is hampered by low

reproducibility, especially in patients with impaired left ventricular function in whom it would be most useful. We did not look at heart failure subjects in our study but at three groups where abnormal left ventricular function would be more subtle which would be more applicable to our Marfan population. However, my IVA variability was similar if not worse than this recent paper. Their mean intraobserver variability was 12%(normal group), 18%(T2DM group) and 30%(HF group) compared to our mean intraobserver variability of 15%(normal group) and 28%(abnormal groups). Their mean interobserver variability was 23%(normal group), 21%(T2DM group) and 28%(HF group) compared to our mean interobserver variability of 25% (normal group) and 30% (abnormal groups).

Peak systolic velocity at the mitral annulus however did exhibit significant differences between the normal control group and the three abnormal populations in all six ventricular walls. The velocities were markedly lower in the SCH and T2DM groups and to a lesser degree in the MF group perhaps reflecting less left ventricular pathology in this group. The peak systolic velocities were far easier to acquire and measure and the intraobserver (5% for the normal group and 8% for the abnormal group) and interobserver (10% for both groups) variabilities were much lower compared to that seen with IVA.

This study suggests that Tissue Doppler Imaging in the form of peak systolic mitral annular velocity may play a real role in evaluating Marfan patients' left ventricular function and indeed pick up subtle and subclinical changes not yet reflected in symptoms or even in measured or visually interpreted ejection fraction. IVA cannot be recommended as such and remains a research tool only at the present time.

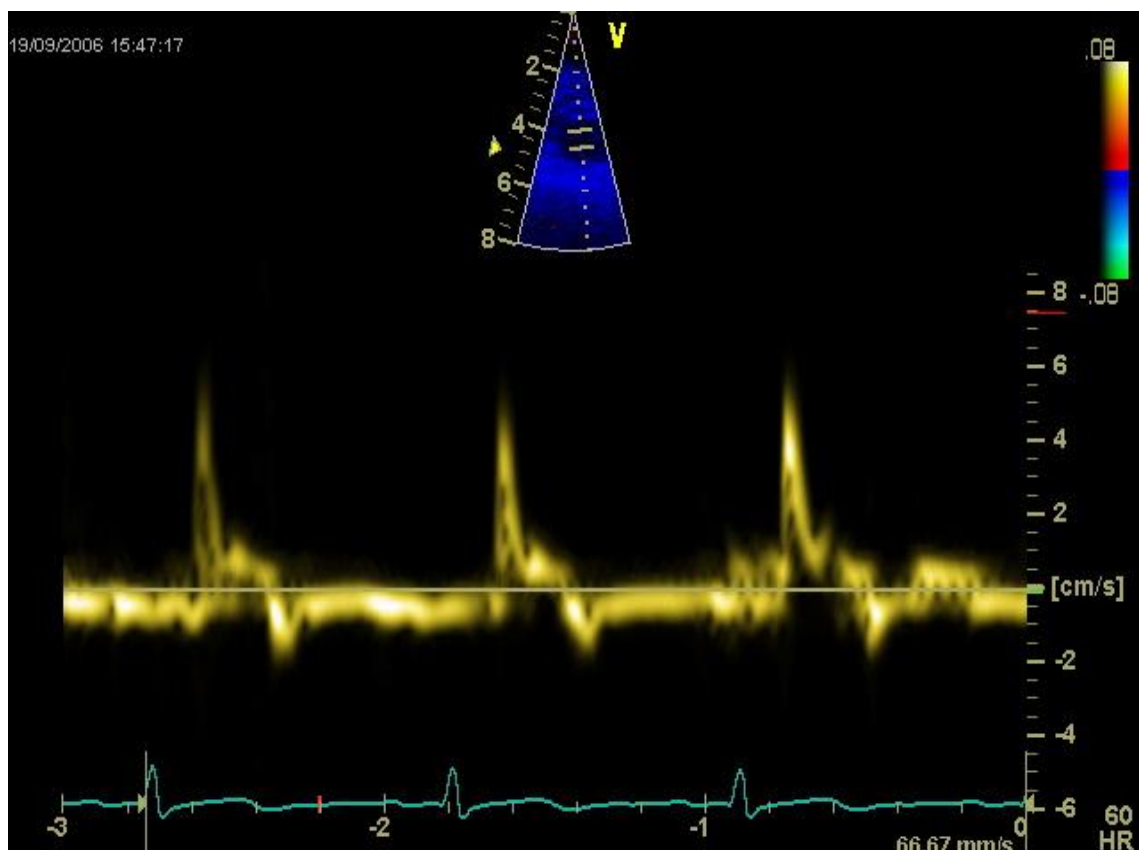
CHAPTER 3.1.3

TDI IN THE MARFAN AORTA STUDY

Introduction

As described in Chapter 1.3 and in the paper by Harada 2004²¹¹, Tissue Doppler Imaging can also be applied to the abdominal aorta in Marfan patients to assess aortic wall stiffness (fig.1).

Figure 1: Pulsed-Wave TDI of the Abdominal Aorta



It is not certain that the abdominal aorta is always involved in the Marfan Syndrome phenotype reflected by the fact that dilatation or dissection of the descending aorta under the age of 50 years is only included as a minor criterion in the Ghent Diagnostic nosology²¹² and not included at all in the scoring of systemic features in the Revised Ghent criteria.²¹³ Utilising TDI to investigate not only the abdominal aorta but also the ascending aorta and arch in Marfan Syndrome may add further information to the clinician beyond the normal practice of serially measuring aortic diameters.

Methods

I investigated 21 Marfan patients diagnosed by the Ghent criteria²¹² and recruited from two tertiary congenital heart centres (Bristol Congenital Heart Centre, Bristol and Congenital Heart Disease Centre, University Hospital of Wales, Cardiff). Full written consent was obtained from the subjects and I had full MREC and local R and D approval as described in Chapter 2.1. Subjects with previous aortic dissection or surgery, severe valvular regurgitation or an aortic diameter ≥ 5.0 cm were excluded. The subjects were told to abstain from their usual medication over a period of two weeks prior to the trial.

On arrival, I measured their weight and height. They then reclined on a comfortable couch and rested for 10 minutes. The blood pressure was recorded from the right arm using an electric cuff device (Dinamap). I used a GE Vivid 7 echo machine and measurements taken. I measured Peak S and D and β -index as described above by Harada et al and in Chapter 1.3. I also measured the time to peak systole (from the R wave on the ECG to the Peak S on Pulsed-wave TDI) with the sample volume placed over 3 sites: the aortic arch wall, the abdominal aorta wall and in the blood flow itself. The data acquisition, post-processing, database set-up and statistical analyses were performed by myself.

Hypothesis: Time to peak systole in the aortic arch will be shorter as the stiffness index increases in a Marfan population.

Statistics

The data was analysed using SPSS version 18.0. Correlations between the stiffness index and the timings were calculated using the Pearson and Spearman correlation coefficients. Intraobserver Reproducibility was measured by Coefficient of Variation. I acquired each variable twice per subject on the same date one measurement straight after the other. The CV was then measured by calculating the Standard Deviation (SD)/mean x 100. The SD was calculated by (measurement 1 – measurement 2) divided by $\sqrt{2}$.

Results

Feasibility: in most subjects I was able to complete the measurements. Finding the aortic arch in a suprasternal window is a difficult skill developed with practice. When scanning

the abdominal aorta I used a set anatomical landmark, 2cm above the umbilicus, for probe placement. This resulted in 100% success rate. Analysis of the time to peak systole in the arch and abdominal flow was more difficult reflected in the low numbers in whom it was possible to get a reading (5 and 9 subjects respectively).

The results are shown in Table 1. The aortic arch and abdominal aorta were of normal dimensions in our group. The mean abdominal aorta stiffness index (β) was 6.7. Peak S and D in this adult Marfan population were 3.0 and 2.0. Peak systole (S) in the abdominal aorta correlated with the time to peak systole in the abdominal aortic flow ($r=-0.7$, $p<0.05$) and with the peak diastole (D) in the abdominal aorta ($r=-0.5$, $p<0.05$). There was no correlation between time to peak systole in the aortic arch and the stiffness index, β ($r=0.3$, $p=0.15$).

Intraobserver Reproducibility was low for beta index, Peaks S and D (CV 19%) but higher for the timings reflecting the large choice of places to select as your area of sampling (CV 35%).

Table1: Pulsed-Wave TDI Measurements at the Aortic Arch and Abdominal Aorta In A Marfan Population.

	Marfan Group Mean \pm Standard Deviation
Age (years)	29.4 \pm 10.8
Sex	M=7, F=14
BMI (kg/m ²)	22.8 \pm 3.7
Body Surface Area (m ²)	2.0 \pm 0.3
Arch diameter (cm)	2.1 \pm 0.8
Beta Abdominal Aorta	6.7 \pm 4.3
Peak S (cm/s)	3.0 \pm 0.01
Peak D (cm/s)	2.0 \pm 0.01
Time to peak systole in Arch Wall (ms)	129 \pm 34
Time to peak systole in Arch Flow (ms)	173 \pm 39
Time to peak systole in abdo aorta wall (ms)	182 \pm 26
Time to peak systole in abdo aortic flow (ms)	221 \pm 51

Discussion

This is the first study to look at timings of wave travel through the Marfan Aorta by TDI. It is difficult to compare my results as the only other published data on this is the Harada

paper which was in a paediatric Marfan population (mean age 11 ± 4 years) compared to our adult Marfan population (mean age 29 ± 11 years). This study would have benefited from a normal age-matched control group. However, this was a sub-study of my main pharmacological intervention study (Chapter 3.4.1) which was a crossover trial using the Marfan subjects themselves as their own controls. If time had permitted, I would have recruited a further age-matched normal control population for these sub-studies.

The results show that the mean beta stiffness index, measured in my population, of 6.7 had a high standard deviation of 4.3 and may explain the lack of significant correlations. Harada's group's beta index was 3.6 ± 0.5 . It must be remembered that this is the first study of these parameters and as experience of acquiring and measuring them increases I would expect the variability and standard deviations to reduce.

Despite this, it is a relatively simple procedure to acquire the echocardiography windows. However, the large choice of where your sample volume should be placed, the size of the sample volume compared to the small diameter of the aortic wall and the noise associated also increases the reproducibility for the timings significantly compared to the beta index and Peaks S and D.

The location of the abdominal aorta away from musculoskeletal pathology in the thorax is an advantage when scanning but the disease process does not always involve the abdominal aorta as described in the introduction. Measurements at the aortic arch are more attractive as pathology here makes the diagnosis of Marfan Syndrome. It is also the site closest to the aortic root and ascending aorta where the high cardiovascular risk of Marfan Syndrome derives from and of course, the timings would reflect wave travel through these conduits.

Theoretically, you should see lower times from the Electrocardiogram R wave to peak systole as the aorta stiffens and wave travel becomes faster. Subjects with Marfan Syndrome have at least annual transthoracic echocardiography and including these parameters should not add much to the acquisition time and only a little extra time to the post-processing. The intraobserver reproducibility of 35% is high and so until further studies look at this and/or more experience of the technique is gained, these measurements must remain a research tool only for the present time.

CHAPTER 3.2.1

APPLANATION TONOMETRY IN MARFAN SYNDROME

Introduction

The aim in Marfan Syndrome is to lower central aortic pressure, heart rate, dp/dt and central aortic stiffness. Beta blockers, such as atenolol, lower central blood pressure less and peripheral pressure more compared to other newer antihypertensives such as ACEIs and CCBs. Investigating parameters of central and peripheral blood pressure and comparing the two has obvious attractions in the assessment of Marfan patients as described in Chapter 1.4 and would be a useful method of assessing drug treatment effects. Measurements made non-invasively from the wrist, neck and groin also provide an additional benefit if the tool is to be used regularly. A recent paper from St George's Hospital²¹⁴ described central and peripheral haemodynamics in a Marfan population measured by Applanation Tonometry. We wanted to measure the same parameters as this group did and compare the results to our Marfan population.

Hypothesis: Applanation Tonometry can be used as a tool in measuring central and peripheral haemodynamics in our Marfan population

Methods

I invited 21 patients from my recruited Marfan population (as described in Chapter 2.1) for investigation at the Wales Heart Research Institute and at the Congenital Heart Centre, Bristol. They underwent baseline assessment and Applanation Tonometry as described in the methods section 2.1.

Statistics

Data were collected by a single trained observer (me). I acquired at least 3 measurements and the mean of the two measurements with the lowest standard deviations were used in the data analysis. The 2 measurements themselves were used for reproducibility as described below. Statistical analysis was done using SPSS version 18.0. The data were checked for Normality by inspecting histograms and Q-Q Plots. The means and standard deviations were calculated and a p value <0.05 was considered statistically significant.

Intraobserver Reproducibility is expressed as the Coefficients of Variation (CV). CV was measured by Standard Deviation (SD)/mean x 100. The SD is the standard deviation of the measurement error associated with a single measurement calculated as the SD of residuals (measurement 1-measurement 2) divided by $\sqrt{2}$. The two measurements used for each variable were the two highlighted by the Sphygmocor software as those with the lowest standard deviations. All acquisition, post-processing and statistics was performed by myself.

Results

The results are shown in Tables 1 and 2.

Table 1: Peripheral Haemodynamics in a Marfan Population as Measured by Applanation Tonometry.

	UHW MARFAN PATIENTS Mean ± standard deviation
Age (years)	27.6 ±7.5
Sex (Male/Female)	43%/57%
BMI (kg/m ²)	22.3 ±3.6
Body Surface Area (m ²)	2.0 ±0.3
Heart Rate (bpm)	65.3 ±9.6
Peripheral Systolic blood pressure (mmHg)	118.7 ±15.7
Peripheral Diastolic blood pressure (mmHg)	74.6 ±7.6
Mean arterial pressure (mmHg)	88.9 ±9.5
Peripheral Pulse Pressure (mmHg)	44.1 ±13.0

Table 2: Central Haemodynamics in a Marfan Population as Measured by Applanation Tonometry.

Central Systolic blood pressure (mmHg)	105.9 ±12.4
Central Diastolic blood pressure (mmHg)	75.6 ±7.9
Mean Arterial pressure (mmHg)	85.7 ±8.6
Central Pulse Pressure (mmHg)	30.3 ±9.6
Pulse Pressure Amplification	1.5 ±0.2
Augmentation index (%)	9.0 ±8.3
Carotid-Femoral Pulse Wave Velocity (m/s)	6.4 ±1.4
Carotid-Radial Pulse Wave Velocity (m/s)	7.6 ±1.1

Our intraobserver reproducibility using the Sphygmocor machine was excellent. For central systolic blood pressure the CV was 1%, for Augmentation index it was 18% and for Pulse Wave Velocity it was 6%.

Discussion

The ease of use was apparent using the Sphygmocor system and Applanation Tonometry. Locating the peripheral pulses was actually easier than normal due to the tall, thin body habitus exhibited by most of our Marfan patients. The learning time for acquiring the skills needed is short and the Sphygmocor programme which can be downloaded to your own laptop is simple and easy to understand. It has an in-built quality control which advises you if you have poor arterial tracings and the programme includes an easy to manage

database. It is also quick and a patient can have a whole study completed in 5-10 minutes.

Table 3: Peripheral Haemodynamics in the St George's Hospital Marfan Population as Measured by Applanation Tonometry.

	ST GEORGE'S HOSPITAL MARFAN PATIENTS Mean \pm standard deviation
Age (years)	30 \pm 11
Sex (Male/Female)	65%/35%
BMI (kg/m ²)	21.2
Body Surface Area (m ²)	1.96 \pm 0.23
Heart Rate (bpm)	52 \pm 12
Peripheral Systolic blood pressure (mmHg)	112.9 \pm 11.6
Peripheral Diastolic blood pressure (mmHg)	75.2 \pm 8.2
Mean arterial pressure (mmHg)	87.7 \pm 8.7
Peripheral Pulse Pressure (mmHg)	37.7 \pm 7.9

Table 4: Central Haemodynamics in the St George’s Hospital Marfan Population as Measured by Applanation Tonometry.

	ST GEORGE’S HOSPITAL MARFAN PATIENTS Mean ± standard deviation
Central Systolic blood pressure (mmHg)	100.9 ±11.2
Central Diastolic blood pressure (mmHg)	75.6 ±8.6
Mean arterial pressure (mmHg)	86.4 ±8.6
Central pulse pressure (mmHg)	25.4 ±8.7
Pulse Pressure Amplification	1.48
Augmentation Index (%)	12.4 ±13.3
Carotid-Femoral Pulse Wave Velocity (m/s)	7.0 ±1.7
Carotid-Radial Pulse Wave Velocity (m/s)	7.7 ±1.8

As in the last chapter, this sub-study would have benefited from a normal, age-matched control group. Again, due to the main study (Chapter 3.4.1) using the Marfan subjects as their own controls and lack of time, this was not possible. However, it compares well with published data from a similar Marfan group at St George’s Hospital. The University Hospital of Wales (UHW) and the St George’s Hospital (SGH) Marfan groups are similar. They are age-matched, with similar body mass indices and both groups were off medication for the purposes of the studies. The St George’s published data are in tables 3 and 4 for comparison.

The heart rate in the SGH group was 20% lower than our group despite neither groups reported to be on medication. Both peripheral and central systolic blood pressures were also lower by 5% in the SGH group. The SGH's group have higher Augmentation index (by 27%) although the standard deviations are wide. Their group also had higher pulse wave velocity (by 4%) with lower amplification (by 1%).

The SGH's group had lower heart rates and peripheral blood pressures but were of similar ages. This suggests that if this was not a true effect then maybe their drug washout time was too short. The SGH group had higher Augmentation index and pulse wave velocity with lower amplification suggesting stiffer aortas and therefore slightly more advanced aortic disease. In fact, compared to a recently published normal reference range²¹⁵ for carotid-femoral PWV, our subjects were not different from a normal population (mean PWV of 6.2m/s in their normal under 30 years old cohort). However, the two groups were comparable and this is reassuring firstly that our population are typical of other Marfan patients and secondly, that our application of Applanation Tonometry and Pulse Wave Velocity is accurate and similar to published data.

The reproducibility we calculated was also excellent – the figures I have used were taken the first time I used the system and would be even lower in experienced hands.

In conclusion, Applanation Tonometry is an attractive additional method of evaluating and monitoring Marfan aortic properties. It highlights differences in central and peripheral blood pressures and as such could be used to monitor change over time or evaluate effectiveness of drug treatment. Additionally, my patients' results are comparable to a Marfan cohort and their data published from South London.

CHAPTER 3.3.1

WAVE INTENSITY ANALYSIS- A Comparative Study Between a Normal and Marfan Population.

Introduction

Wave Intensity Analysis is a research tool that has been used and investigated by our institution for the last ten years. It has potential attractions in our Marfan population as it looks specifically at aortic wave travel, aortic stiffness indices and the interaction between the left ventricle and the aorta as described in Chapter 1.5. We therefore, wanted to investigate this tool further. We performed WIA on a normal population and our Marfan population and compared the results.

Methods

60 healthy volunteers had been recruited for another study at our institution and I had helped with the data acquisition. In addition, I recruited our 21 Marfan subjects as described in Chapter 2.1. All were invited to attend the Wales Heart Research Institute to participate in a WIA study. The Marfan population consisted of 7 men and 14 women of average age 29.4 ± 10.8 years (see Table 1). The healthy volunteers had no history of cardiovascular disease, hypertension, diabetes or major illness and none was taking any medication. They consisted of 37 men and 23 women of average age 47.7 ± 13.7 years.

On arrival they underwent baseline assessment as described in Chapter 2.1. After resting for ten minutes WIA was performed on them as described in Chapter 2.1 by me.

The data was analysed using SPSS version 18.0 (SPSS Inc, Chicago, IL, USA). The data were checked for Normality by inspecting histograms and Q-Q Plots. The means and standard deviations were calculated and the differences between the populations analysed by a Mann Whitney U-test as the indices were not normally distributed. Statistical significance was taken as a p value ≤ 0.05 .

Intraobserver Reproducibility was expressed as the Coefficients of Variation (CV). CV was measured by Standard Deviation (SD)/mean x 100. The SD is the standard deviation of the measurement error associated with a single measurement calculated as the SD of

residuals (measurement 1-measurement 2) divided by $\sqrt{2}$. I acquired two measurements for each key variable for each subject one straight after the other to calculate this.

The data acquisition, set-up of the database and all statistical calculations were performed by me. The separation of the net WI into the four individual waves was done by a single operator using software he had designed specifically for this purpose as described in Chapter 1.5.

Hypothesis: Marfan patients will have higher stiffness indices and higher wave reflections as evidenced by the backward compression wave.

Results

The arterial haemodynamics in each population and the differences between the two are shown in Tables 1 and 2.

Table1: Arterial Haemodynamics and Local Stiffness Indices In a Normal and Marfan Population

Parameter	Units	Normal Population Mean \pm sd	Marfan Population Mean \pm sd	Mann Whitney U-Test
Number		60	21	
Sex (M/F%)		62%/38%	33%/67%	P<0.05*
Age	years	47.7 \pm 13.7	29.4 \pm 10.8	p<0.001*
Heart Rate	bpm	60.8 \pm 9.3	57.0 \pm 7.3	P=0.10
Systolic blood pressure	mmHg	121.0 \pm 15.0	117.9 \pm 17.4	P=0.37
Diastolic blood pressure	mmHg	75.1 \pm 9.8	71.2 \pm 9.3	P=0.09
Mean arterial blood pressure	mmHg	90.4 \pm 10.9	86.8 \pm 10.6	P=0.12
Local wavespeed	m/s	6.7 \pm 3.8	10.7 \pm 21.6	P=0.49
Beta stiffness index		7.6 \pm 2.8	5.9 \pm 1.8	P<0.05*
Epsilon (Elastic Modulus)	kPa	99.4 \pm 43.5	73.5 \pm 28.0	P<0.05*
Augmentation index	%	9.2 \pm 13.7	8.9 \pm 15.2	P=0.67

Table 2: Separated Wave Amplitudes In a Normal and Marfan Population

Parameter	Units	Normal Population Mean \pm sd	Marfan Population Mean \pm sd	Mann-Whitney U-test
Forward Compression Wave Peak	W/m ²	8.6 \pm 4.9	8.0 \pm 6.1	P=0.67
Forward Compression Wave Integral	W/m ²	375.0 \pm 193.2	306.7 \pm 294.5	P=0.13
Backward Compression Wave Peak	W/m ²	-2.2 \pm 1.6	-3.5 \pm 3.0	P=0.07
Backward Compression Wave Integral	W/m ²	-111.5 \pm 72.4	-148.5 \pm 117.3	P=0.17
Forward Expansion Wave Peak	W/m ²	2.0 \pm 1.0	1.6 \pm 1.2	P=0.07
Forward Expansion Wave Integral	W/m ²	59.5 \pm 27.9	49.9 \pm 42.1	P=0.38
Backward Expansion Wave Peak	W/m ²	-0.4 \pm 0.3	-0.6 \pm 0.5	P=0.30
Backward Expansion Wave Integral	W/m ²	-13.8 \pm 7.4	-17.5 \pm 13.4	P=0.48

The Marfan population were significantly younger than our normal population (29.4 ± 10.8 years vs 47.7 ± 13.7 years, $p < 0.001$) and the proportion of females higher (67% vs 38%, $p < 0.05$).

The heart rates, systolic, diastolic and mean arterial blood pressures were the same between groups and were normal. The two local stiffness indices, beta and epsilon were significantly lower in the Marfan group ($p < 0.05$) which was unexpected. Local wave speed was higher in the Marfan group (10.7 ± 21.6 m/s vs 6.7 ± 3.8 m/s, $p = 0.49$) but not statistically significant. This was probably in part due to the high standard deviation in the Marfan group which was double the mean.

Augmentation index, expected to be higher in the Marfan group, was similar between the two groups. The amplitude of the forward compression wave (FCW), an index of left ventricular function, was lower in the Marfan group (8.9 ± 15.2 W/m² vs 9.2 ± 13.7 W/m², $p = 0.67$) but was not statistically significant. The backward compression wave (BCW), an index of reflections from the peripheral vascular bed, displayed a trend of being higher in the Marfan group (-3.5 ± 3.0 W/m² vs -2.2 ± 1.6 W/m², $p = 0.07$) than the normal group but again did not reach statistical significance. The forward expansion wave (FEW) displayed a trend of being lower in the Marfan group. The ratio of the amplitudes of the BCW to the FCW was 26% (normals) and 44% (Marfan group). The differences between the two groups with regard the backward expansion wave (BEW) did not reach significance.

The overall Intraobserver variability was 20%.

Discussion

Firstly, it must be highlighted that the two groups were significantly different with regard age and sex. The Marfan group were significantly younger with a higher female preponderance. This is due to the difficulty in getting normal age-matched controls for the Marfan group as described in earlier chapters. The normal population used in this study were originally from another study. It was difficult to use the younger members of the normal group because even they were significantly older than the Marfan population. This is a flaw in the study.

The most significant finding in this study was that the stiffness indices were higher in the normal group than the Marfan group. This reflects local common carotid artery stiffness

and was an unexpected finding. These results probably reflect the difference in age and sex between the groups. The haemodynamics on a whole were more reflective of a healthy population and the measurements in both groups reflected this. For example, the mean Augmentation indices in our groups of 9.2% (normals) and 8.9% (Marfans) were far lower than the normal controls (13.1%) in a paper looking at arterial wave reflection in a hypertensive group before and after treatment with barnidipine²¹⁶ where the control group's mean age was 58 years.

There was a trend for the backward compression wave to be higher in the Marfan group which is a marker of wave reflections from the peripheral arterial bed back to the heart. The amplitude of BCW/FCW was also higher in the Marfan group. Higher reflections may be expected in the Marfan group but the timings of the reflections would have given more information. If the reflections return to the heart before aortic valve closure this increases left ventricular afterload and can impact on coronary perfusion.

As described, the major limitation in this study is the difference in ages and sexes of the two groups. The mean age of the Marfan group (29.4 years) was 18 years younger than our normal controls (47.7 years). The normal group also contained more men: increasing age and male sex are positively correlated to impaired arterial haemodynamics and so the two groups may have been artificially similar due to these two important baseline characteristics. Alternatively, the Marfan group had similar haemodynamics to a normal population group almost 20 years older.

However, the results do lead to important questions concerning the validity of arterial measurements at the common carotid artery in a Marfan population where the pathological effects are seen maximally in the largest elastic arteries, namely the aorta. Can we extrapolate data from the carotid and assume it accurately reflects the aorta in this group? We also know from previous studies that the carotid measurements are affected by cerebral vasomotor tone.²¹⁷

The acquisition of the data was not particularly difficult once trained and a little experience gained. However, the software used is only available through one company (Aloka) at present and the post-processing is time-consuming. The separation of the waves was done by a single person who wrote a programme specifically for this purpose - this again takes time. The wave intensity signals were intrinsically noisy and affected by patient factors such as depth of the carotid artery and suboptimal windows. Variation in respiration, movement and physiological changes also impede the signals. Once filtering

and averaging procedures are introduced to decrease the noise you can lose some integrity of the data. Another factor for discussion is the intraobserver and interobserver variabilities. My own intraobserver variability was 20% and variability has been a large problem with this technique in the past.²¹⁸

Whilst theoretically Wave Intensity Analysis would be a very interesting tool to aid evaluation of our Marfan population, at present there are too many unanswered questions. These include the validity of the carotid artery to assess the aorta - although assessment of the proximal aorta itself is currently technically impossible.

The variability is too high to be used routinely and the acquisition and post-processing of the data needs to be made more available, efficient and accurate. Overall, this technique cannot be used in the routine assessment of a Marfan patient but may be in the future if the problems highlighted are remedied.

CHAPTER 3.4.1

The Effects of Atenolol, Perindopril and Verapamil on Haemodynamic and Vascular Function in Marfan Syndrome – A Randomised Double-Blind Crossover Trial

Introduction

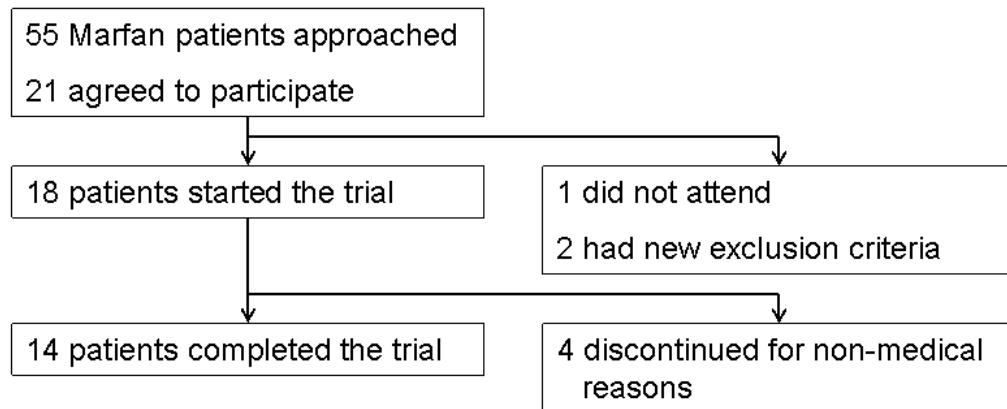
Almost exclusively, medical therapy to date in Marfan Syndrome has been with β -blockers, based on limited reports suggesting a reduced rate of aortic dilatation compared with no treatment.²¹⁹ More recent data suggest that β -blockers have less effect than other antihypertensives on central aortic pulse pressure²²⁰ which is one of the main determinants of ascending aortic dilatation²²¹. Angiotensin converting enzyme inhibitors (ACEI) and calcium channel blockers (CCB) reduce central systolic pressure and conduit arterial stiffness when compared to β -blockers, in adults with hypertension.²²² There are some recent reports of beneficial effects of ACEI on central aortic stiffness in patients with Marfan syndrome^{223,224} but no studies have been reported that compare the effects of these three classes of drugs on estimated central aortic systolic pressure and pulse pressure in Marfan syndrome.

The objectives of my study were to compare the effects of an angiotensin converting enzyme inhibitor (perindopril), a non-dihydropyridine calcium channel blocker (verapamil) and a β -blocker (atenolol) on central aortic pressure, augmentation of central pressure, conduit arterial stiffness, and left ventricular function, in patients with Marfan syndrome.

Methods

My twenty-one Marfan patients described in Chapter 2.1 agreed to participate in the trial, which was conducted at the Bristol Congenital Heart Centre and the Congenital Heart Disease Centre, University Hospital of Wales. 18 patients started the trial (figure 1). All patients received detailed written information and gave written informed consent. The protocol was approved as reported in Chapter 2.1.

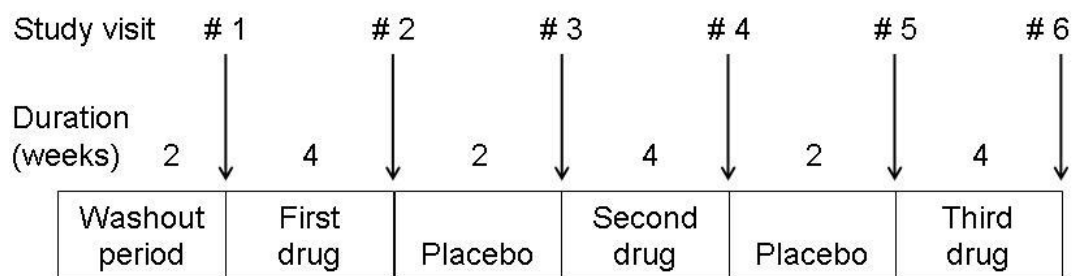
Figure 1: Recruitment



Inclusion criteria were subjects aged 16-60 years, who were either on no treatment, or were taking a β -blocker or other monotherapy only. Patients with previous aortic dissection or aortic surgery, severe heart valve regurgitation, aortic diameter at the sinotubular junction ≥ 5.0 cm, contraindications to specific drug treatment (such as asthma), and women who were pregnant or at risk of pregnancy, were excluded. At baseline, essential anthropometric data such as height, weight, age, and disease severity were recorded. Beta blockade or other monotherapy was withdrawn over a period of two weeks in subjects already on treatment, and then all subjects had a two-week period off all treatment, during which they received a placebo single-blind. Subjects were then randomised double-blind to atenolol 75 mg, or perindopril 4 mg, or sustained release verapamil 240 mg, in a cross-over design. These drugs and doses were selected because of their long half-life, to improve compliance and efficacy, and each patient was instructed to take the medication once daily as a single dose, at 8 a.m. An established computer-generated randomisation process specified the drug allocation sequence. There was a two-week washout period after each treatment before a new baseline recording, during

which patients again received matched placebo tablets in an attempt to ensure compliance. Clinical investigations were performed in the same order at each visit, starting 2 hours after the last dose of treatment (i.e. at peak drug concentration). Each treatment was administered for 4 weeks with a total study duration for each patient of 18 weeks (figure 2). Each patient was requested to attend for non-invasive investigations on 6 occasions (as a baseline before each treatment, and to assess drug effects after 4 weeks of each treatment), and for simple blood tests alone on 3 occasions (2 weeks after starting treatment, to check renal function).

Figure 2: Summary of the design of the trial



Echocardiographic Measurements

At each visit, transthoracic echocardiography was performed as described in Chapter 2.1 according to the recommendations of the European Association of Echocardiography. Aortic root diameter, left ventricular function (by conventional and tissue Doppler parameters), left-ventricular stroke volume (by both Doppler and Simpson techniques), isovolumic acceleration, peak systolic velocities and E/e' ratio were all measured. The

amplitude and timing of expansion in the aortic arch and abdominal aorta were measured using pulsed tissue Doppler, as previously described in chapter 1.3.

Blood pressure, pulse wave analysis and pulse wave velocity

Blood pressure in the brachial artery was measured after the subject had rested supine for 10 minutes. We used the Millar tonometer, Applanation Tonometry and SphygmoCor software to derive central aortic pressure, Augmentation index and pulse pressure amplification as described in Chapter 1.4. Carotid-to-radial and carotid-to-femoral pulse wave velocities were also obtained, as an index of aortic stiffness.

Conduit arterial stiffness (beta and epsilon indices) was estimated in the right common carotid artery by Wave Intensity Analysis (Chapter 1.5).

Data were acquired and collected by two trained personnel (myself and Dr Damien Kenny in Bristol). The statistics was performed by Prof Frank Dunstan (Professor of statistics at Cardiff University).

Statistical analysis

Data were checked for outlying values. Results are expressed as mean values with 95% confidence intervals, unless otherwise indicated.

Within a single period, the effect of each drug on each parameter was assessed by carrying out a paired t-test and calculating a confidence interval for the mean difference. The comparison of the three drugs was performed using a crossover analysis, with drug, period and subject effects, and using the baseline value as a covariate. Residuals were checked for normality. Probability values <0.05 were considered significant. Using the data from published literature Ahimastos et al²²³, in order to detect a 10% change in arterial stiffness parameters and a 5% change in aortic diameters with an $\alpha=0.05$, the study had 80% power.

Results

Fourteen of the 18 patients who were recruited, completed the trial; 4 discontinued because of social reasons (figure 1). No significant adverse effects were observed. The baseline characteristics of the study population are summarised in table 1.

Table 1: Baseline characteristics of the study population

Gender	Female 8, Male 6
Age (yrs)	30.4 (11.7)
Weight (kg)	77.7 (14.8)
Height (m)	1.86 (0.13)
Body Mass Index (kg/m ²)	22.7 (3.8)
Smokers	1
Family history of dissection	6
Urea (mmol/l)	4.60 (0.92)
Creatinine (μmol/l)	77.3 (14.6)

Results are expressed as mean (s.d.)

Hemodynamic effects and measurements of arterial function are shown in table 2.

Atenolol reduced resting heart rate by 16% ($p=0.006$) while perindopril and verapamil had no effect ($p<0.001$ for between-drug comparison). Mean cardiac output fell by 17% after atenolol but there were no significant changes ($p=0.1$).

Although baseline blood pressure measurements were within the normal range, atenolol, perindopril, and verapamil all lowered both peripheral (brachial arterial) blood pressure and calculated central arterial pressure (all, $p<0.05$ for within-drug comparisons). The differences between the drugs were not significant – atenolol lowered central pressure by a mean of 7% compared with 10% after perindopril and 9% after verapamil. There were similar non-significant trends in augmentation index which was reduced by 44% after atenolol, 52% after perindopril, and 69% after verapamil.

Aortic function as assessed by pulse wave velocity was not altered by atenolol, perindopril, or verapamil. Carotid arterial stiffness was also unaffected by treatment – no significant between-drug effects were observed in the beta index or in Peterson's elastic modulus (epsilon), although a reduction in epsilon was documented after atenolol ($p=0.005$).

The time interval from the onset of systole to the peak velocity of aortic systolic expansion was delayed by atenolol, by an average of 8% in the aortic arch and by 11% in the upper abdominal aorta (table III), while it was unaltered by perindopril and verapamil ($p<0.01$ and $p<0.05$ respectively, for between-drug comparisons).

Table 2: Arterial effects of atenolol, perindopril and verapamil

	Atenolol			Perindopril			Verapamil			P value
	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change	
Heart rate (bpm)	61.1	51.6	-9.5* (-15.7, -3.3)	64.5	63.9	-0.62 (-8.3, 7.1)	61.4	61.4	0.0(-5.2, 5.2)	<0.001
Brachial systolic BP (mmHg)	122.4	110.6	-11.8* (-19.0, -4.6)	118.9	108.6	-10.3* (-17.0, -3.6)	120.1	112.8	-7.3* (-13.5, -1.1)	0.685
Brachial diastolic BP (mmHg)	72.7	66.5	-6.2* (-11.6, -0.9)	72.1	67.0	-5.1* (-9.8, -0.5)	73.9	69.1	-4.8* (-8.8, -0.8)	0.967
Mean arterial pressure (mmHg)	89.3	81.2	-8.1 (-13.4, -2.7)	87.7	80.9	-6.8 (-11.5, -2.2)	89.3	83.7	-5.6 (-9.3, -1.9)	0.306
Peripheral pulse pressure (mmHg)	49.7	44.1	-5.6 (-11.4, 0.3)	46.8	41.6	-5.2 (-10.9, 0.6)	46.2	43.7	-2.5 (-9.1, 4.1)	0.630
Pulse wave velocity (carotid-radial) (m/s)	7.9	7.6	-0.3 (-0.9, 0.2)	7.7	7.9	0.2 (-0.5, 0.8)	7.7	7.9	0.2 (-0.5, 1.0)	0.582
Pulse wave velocity (carotid-femoral) (m/s)	7.2	6.6	-0.6 (-1.3, 0.2)	6.8	6.6	-0.2 (-0.7, 0.3)	6.8	6.8	0 (-0.4, 0.5)	0.724
Augmentation index (%)	7.3	4.0	-3.2 (-7.0, 0.6)	12.2	5.9	-6.3 (-12.8, 0.2)	8.0	2.5	-5.5 (-11.6, 0.7)	0.780
Central systolic BP (mmHg)	103.9	96.8	-7.1* (-12.3, -1.9)	105.8	95.4	-10.3* (-16.1, -4.6)	104.9	95.8	-9.2* (-14.6, -3.7)	0.776
Central pulse pressure (mmHg)	32	30.3	-1.7 (-6.9, 3.6)	30.6	27.8	-2.8 (-7, 1.3)	29.8	26.7	-3.2 (-8.1, 1.8)	0.180
Pulse pressure amplification (mmHg)	1.48	1.39	-0.09 (-0.27, 0.09)	1.51	1.45	-0.06 (-0.31, 0.19)	1.52	1.47	-0.09 (-0.29, 0.20)	0.084
Beta (units)	6.81	5.79	-1.02 (-2.04, 0.00)	6.20	5.65	-0.54 (-1.56, 0.47)	6.76	6.13	-0.64 (-2.34, 1.06)	0.487
Epsilon (kPa)	86.9	66.9	-20.0* (-32.1, -7.9)	78.0	71.5	-6.6 (-19.0, 5.9)	84.7	75.4	-9.3 (-24.8, 6.3)	0.198

The Values expressed as means (95% confidence intervals).

* p<0.05 for changes from baseline for each drug. P value (in last column) = summary for comparison *between* drugs.

Table 3: Echocardiographic comparisons of atenolol, perindopril and verapamil

	Atenolol			Perindopril			Verapamil			P value
	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change	
Aortic root diameter (at sinuses of Valsalva) (cm)	3.77	3.78	0.01 (-0.11, 0.12)	3.77	3.75	-0.02 (-0.13, 0.09)	3.83	3.80	-0.03 (-0.17, 0.10)	0.848
Aortic root diameter (at sinotubular junction) (cm)	3.49	3.59	0.1 (-.08, 0.27)	3.63	3.39	-0.23* (-0.43, -0.04)	3.48	3.50	-0.02 (-0.09, 0.13)	0.059
Doppler LV stroke volume (ml)	71.9	63.5	-8.5 (-22.8, 5.9)	69.8	68.0	-1.8 (-9.6, 5.9)	69.6	71.0	1.4 (-8.8, 11.5)	0.210
Doppler cardiac output (l/min)	4.6	3.8	-0.8 (-2.2, 0.6)	4.4	4.5	0.1 (-0.6, 0.8)	4.6	4.5	-0.1 (-0.9, 0.7)	0.110
LV ejection fraction (%)	53.2	57.5	4.2 (-4.9, 13.2)	55.3	54.2	-1.1 (-9.0, 6.9)	57.9	52.4	-5.5 (-11.9, 0.8)	0.052
Vs mean (cm/s)	5.5	5.5	0.0 (-0.5, 0.5)	5.9	6.1	0.2 (-0.3, 0.7)	5.9	5.8	-0.0 (-0.6, 0.5)	0.180
Isovolumic acceleration (m/s ²)	3.0	2.3	-0.8 (-1.8, 0.3)	2.6	2.8	0.3 (-0.8, 1.3)	2.8	2.8	-0.04 (-1.1, 1.0)	0.211
E/e' ratio (units)	6.1	6.4	0.3 (-0.7, 1.3)	6.2	6.3	0.1 (-0.7, 0.9)	6.0	5.7	-0.3 (-1.2, 0.7)	0.450
Time to peak systolic velocity (aortic arch) (ms)	119	129	10* (0, 20)	120	123	3 (-12, 19)	115	112	-3 (-12, 6)	0.003
Time to peak systolic velocity (abdominal aorta) (ms)	180	200	20* (11, 28)	184	189	5 (-2, 12)	179	190	11*(1, 21)	0.043

Values expressed as means (95% confidence intervals). * p<0.05 for changes from baseline for each drug; P value (in last column) = summary for comparison *between* drugs. Vs mean: peak systolic velocity of longitudinal function, averaged at 6 sites around the mitral annulus. E mitral inflow velocity, e' lateral mitral annular velocity, both measured in early diastole.

Echocardiographic studies

The results of the detailed echocardiographic studies are shown in table 3.

Global left ventricular function (ejection fraction), longitudinal left ventricular function (mean velocity of long-axis systolic shortening), and estimated mean filling pressure (E/e') as an index of diastolic function, were not altered by atenolol, perindopril, or verapamil. Isovolumic acceleration was reduced by an average of 27% after atenolol while it was unchanged or increased with the other drugs, but there were no significant differences.

After four weeks of treatment with perindopril, a small reduction (-6%) was observed in the mean diameter of the aorta at the level of the sino-tubular junction ($p=0.024$), but no change was observed after perindopril in the diameter at the sinuses of Valsalva and there were no significant differences between drugs.

Discussion

The results of this small study must be interpreted with caution but the detailed non-invasive comparisons that were performed raise interesting questions. Unexpectedly, we demonstrated that in young patients with Marfan syndrome, central arterial pressure was reduced by atenolol as well as by perindopril and verapamil, although to a lesser extent.

A strength of our trial was its cross-over, double-blind design, but the protocol was demanding of patients since they were asked to attend for 6 visits within 18 weeks; its weakness was that of 55 eligible patients who were asked to participate, only 21 initially agreed to do so and fewer completed the study. For ethical considerations, subjects were restricted to those whose aortic root dimensions were <5 cm. Many previous pharmacologic studies in Marfan syndrome have had similar numbers of subjects²²⁵, however, and it would be impractical to undertake similarly detailed investigations in a large clinical trial.

Rationale of the study design

The primary goal of medical treatment in patients with Marfan syndrome is to reduce the rate of dilatation of the aortic root. Since a major determinant is central pulse pressure²²¹ we selected classes of drugs that have been demonstrated to lower central pulse pressure in subjects with hypertension^{220,226} – an ACEI (perindopril) and a CCB (verapamil) – and preparations with a long half-life so that they could be given once daily in order to optimise compliance.

After our trial was started, experimental studies in an animal model demonstrated that the angiotensin receptor blocker (ARB), losartan, may prevent or delay the phenotypic expression of Marfan syndrome²²⁷ by antagonising TGF- β and slowing or even reversing defragmentation of the elastic fibres of the aorta. ACE inhibitors have similar effects on TGF- β signalling.^{223,228} They also reduce angiotensin II levels which are associated with cystic medial degeneration contributing to aortic rupture in Marfan syndrome²²⁹. The scientific rationale for testing an ARB in children with Marfan syndrome is strong²³⁰ and initial clinical experience is promising²³¹, but the benefit is unproven, and in older subjects ACEI may be an effective alternative.

CCBs reduce central aortic pressures in adult hypertensive patients²²⁶ but similar effects in patients with Marfan syndrome have not been described. One small study of combined treatment demonstrated a slower rate of aortic enlargement compared with placebo²³². We chose a CCB with negative chronotropic and dromotropic properties. Atenolol was included as standard treatment of patients with Marfan syndrome, yet this practice is based on few reports²²⁵. The strongest evidence comes from a prospective randomised trial comparing propranolol with no treatment, by Shores et al²¹⁹. They concluded that the benefit of β -blockade in Marfan patients was caused by a reduced rate of rise of central arterial pressure (+dp/dt). Further reports have demonstrated reduced aortic root dilatation^{233,234} but the effects of β -blockade on aortic compliance have been varied^{235,236} and one study reported an increase in central aortic pressure and wall stress²³⁷. In young patients, side-effects such as excessive tiredness may reduce compliance. Since atenolol is not the most effective drug at lowering central pressure in patients with hypertension, its routine use in Marfan syndrome has been questioned.

Our study is the first formal comparison of an ACEI, CCB and β -blocker in Marfan syndrome. Our hypothesis was that the ACEI and CCB would both lower central aortic pressure, whereas the β -blocker would not, but this was not confirmed.

Central hemodynamic effects

In healthy young people, blood pressure measured in the arm by sphygmomanometry is an uninformative indicator of pressure in the ascending aorta, because of the phenomenon of peripheral pulse pressure amplification,²³⁸ in older subjects it may also be inaccurate, due to augmentation of central pressures caused by earlier wave reflections in stiffer aortas. We therefore used applanation tonometry to estimate central arterial pressure. We demonstrated that all three drugs lowered both peripheral and central pressures.

Perindopril and verapamil reduced systolic pressure in the ascending aorta, as expected. They reduced augmentation index more than atenolol did, but the changes

were not significant, perhaps because the within-subject variability of some measurements in our study was quite high, as reflected in their confidence intervals, and this reduced the power for identifying differences between the drugs. Ahimastos et al compared perindopril with placebo over 24 weeks in a double-blind trial in 17 patients with Marfan syndrome; they observed only small reductions (-1 mmHg) in mean brachial blood pressure and in carotid arterial pulse pressure, and they did not report central aortic pressure²²³. In patients with hypertension, changes in carotid pressure with ACEI have correlated with reductions in peripheral blood pressure.²³⁹ ACEIs may alter reverse remodelling in small arteries²³⁸, and a distal shift in the origin of peripheral reflections would delay wave travel and reduce augmentation of central pressures, without any change in conduit arterial function.

None of the drugs significantly reduced central pulse wave velocity, which is an index of aortic stiffness. In the other recent study, perindopril did reduce pulse wave velocity and increase aortic compliance in patients with Marfan syndrome, but after 24 weeks²²³, and so it is possible that our study was too short to demonstrate these effects. The measurements of wave velocity and augmentation index that we obtained were similar to those reported in Marfan patients by Kiotsekoglou et al²⁴⁰ and higher than those observed in normal age-matched controls.²⁴¹ Pulse wave velocity was also unaltered in hypertensive subjects who were given atenolol, perindopril, lercanidipine and bendroflumethiazide.²⁴²

This study was not designed to examine the longer term impact of treatment on the rate of aortic root dilatation, but we noted a small but significant reduction in the diameter of the aorta at the level of the sino-tubular junction after perindopril had been given for only 4 weeks. Ahimastos et al reported a greater effect after 24 weeks, with reductions in diameter of 3-7 mm²²³ that correlated with reduced circulating levels of TGF- β and matrix metalloproteinase-3.²⁴³ Similar changes have not been reported and would not be expected with verapamil, and since it reduced central pressures in our study but did not slow heart rate, it would not have any advantage over an ACEI in the treatment of patients with Marfan syndrome.

Indices of local arterial stiffness in the common carotid artery (beta and epsilon) were not altered. We observed a reduction in epsilon after treatment with atenolol (-23%; compared with -8% after perindopril, and -11% with verapamil) but there were no significant differences between drugs. It is now uncertain if the carotid arteries are significantly affected by Marfan syndrome, and it is possible that stiffness is increased especially in the thicker-walled aorta where there is more elastic tissue. More studies are needed to investigate regional variations in conduit arterial function in Marfan syndrome²⁴⁴, in order to identify indices that can best serve as surrogate targets for monitoring treatment.

Beta-blockade in Marfan syndrome

This study tested the hypothesis that β -blockade would not lower central pressure, because it causes vasoconstriction that shifts arterial reflection sites proximally, compared with ACEI and/or CCB,²⁴⁵ and because it is not as effective at lowering central pressure in arterial hypertension.²²⁰ Atenolol did reduce central arterial pressure, however, to a lesser degree than was observed with the ACEI and CCB. This might be explained by a reduction in cardiac output (which fell by a mean of 17%, $p=0.24$) related to the reduction in heart rate (by a mean of 16%, $p=0.006$) more than to any change in stroke volume (-12%, $p=0.22$). Alternatively, a negative inotropic effect would be expected to reduce the amplitude of aortic wave reflections during systole. It has been demonstrated using non-invasive measurements of wave intensity that the amplitude of the forwards-travelling compression wave during ejection correlates well with LV $+dp/dt$ ²⁴⁶, and also that the energy of mid-systolic wave reflections (the backwards-travelling compression wave, which augments central aortic systolic pressure) can largely be accounted for by the amplitude of the forward compression wave.²⁴⁷ Baumgartner et al observed improved aortic elastic properties in 70% of 30 patients treated with atenolol for an average of 39 months, particularly in those with smaller aortic root diameters.²⁴⁴

Isovolumic acceleration (IVA) is a non-invasive indicator of LV contractility²⁴⁸ but it has high variability.²⁴⁹ In our study, there was a trend for IVA to be reduced with atenolol whereas it was unchanged with perindopril and verapamil. The mean velocity of long-axis shortening of the left ventricle, which is inversely related to arterial stiffness, was lower than normal values for age-matched subjects, but was unaltered by treatment. The timing of the onset of systolic expansion in the aorta can be measured accurately with tissue Doppler, as first reported in children with Marfan syndrome by Harada et al.²⁵⁰ We report the first use of this measurement in adults with Marfan syndrome. Atenolol but not perindopril or verapamil delayed wave travel in the arch and abdominal aorta. The clinical significance of this finding is uncertain but it suggests that atenolol reduced $+dp/dt$.

In engineering, the fatiguing effect of cyclic stressors depends on the number of cycles as well as the amplitude of stress. Beneficial effects on aortic dilatation with β -blockers may be due to a reduction in heart rate rather than in the amplitude of central distending forces. Lowering brachial systolic pressure and pulse pressure does not appear to affect aortic dilatation.²⁵¹

It may be premature to abandon β -blockers in Marfan patients, and nebivolol, a beta-1 receptor blocker with nitric oxide potentiating vasodilatory effects, may be a more appropriate choice than atenolol. In patients with hypertension, it reduces central pulse

pressure and augmentation index more than atenolol²⁵², and it reduces central arterial pressure and left ventricular hypertrophy more than metoprolol.²⁵³

Conclusions

Recent experimental studies have prompted several large prospective clinical trials of ARBs in children and adults with well-characterised Marfan syndrome. The results are eagerly awaited and may change clinical practice. This study adds weight to the argument that ACEI may also be effective, but more importantly, perhaps, it suggests that a combination of a β -blocker with an ARB or an ACEI may be the most effective. This strategy is also being tested.²⁵⁴ While an ARB or ACEI may lower central pressures by reducing or delaying peripheral reflections, a β -blocker may reduce reflections by an effect on the left ventricle.

CHAPTER 4

DISCUSSION

What The Results Show

We have investigated several new parameters and three new tools –Tissue Doppler Imaging; Applanation Tonometry; and Wave Intensity Analysis- to assess Marfan patients in order to extract more information about the pathology of the aorta and the left ventricle and the interaction between the two. This has the aim of better quantitative assessment of a Marfan aorta's properties to improve the timing of surgery; aiding a clinician's evaluation of risk in Marfan patients; and assessing the effects of medical therapy. The ultimate aim is to improve the mortality and morbidity associated with aortic dissection and rupture in Marfan Syndrome.

Tissue Doppler Imaging

Chapter 3.1.1 showed that there is considerable variability in myocardial velocities and deformation between different echocardiography machines, used by different operators, in the same patients. There appears to be no consensus amongst Industry to standardise their machines. The poor intermachine correlations were especially marked with strain and strain rate ($r=0.19$ and 0.18) but improved with peak systolic velocities (up to $r=0.64$ with high frame rates).

With such low correlations, it must be considered whether the use of strain and strain rate is reliable enough to be used in echocardiography labs that have more than one type of machine or if a patient changes hospital. Certainly, the lack of equivalence between machines must be highlighted and remembered clinically. The causes of these differences need to be understood and overcome by standard acquisition, tracking and signal averaging if the diagnostic potential of Tissue Doppler is to be optimised.

Chapter 3.1.2 looked specifically at two parameters of left ventricular function by tissue Doppler imaging. IVA, which is unaffected by loading and peak systolic mitral annular velocities which are. These two parameters were investigated because of their ability to detect subclinical left ventricular impairment and also because they can assess regionality, two important aspects which the currently used Ejection fraction cannot assess. IVA had considerable problems with variability. We calculated an intraobserver variability of 28% and interobserver variability of 30% and these figures are consistent with the Margulescu paper.²⁵⁵ Acquisition of the trace was not difficult but there were problems in calculating the slope needed and this has been shown to worsen as left ventricular function reduces (Margulescu). I conclude that IVA may be used as a research tool but the clinical applicability is hampered by low reproducibility.

Peak systolic velocity at the mitral annulus however did exhibit significant differences between the normal control group and the three abnormal populations in all six ventricular walls. The measurements were far easier to acquire and measure and the intraobserver (5% for the normal group and 8% for the abnormal group) and interobserver (10% for both groups) variabilities were much lower compared to that seen with IVA. Therefore, peak systolic velocity may have a role in the future assessment of Marfan Syndrome.

The last study looking specifically at tissue Doppler imaging was reported in Chapter 3.1.3. I was excited by the Harada paper investigating tissue Doppler measurements in the abdominal aorta in Marfan Syndrome and wanted to reproduce their findings.²⁵⁶ However, as the abdominal aorta is less affected than the ascending aorta and arch in Marfan Syndrome, we designed a study to investigate the more proximal part of the aorta and to calculate tissue doppler-derived timings as a marker of stiffness. In practice, there was a high intraobserver variability (CV of 35%) when measuring the timings. This was caused by poor echo windows in a population known to have thoracic musculoskeletal abnormalities and difficulty in knowing exactly where you place your sample volume. If these aspects can be addressed this may be an interesting parameter to revisit.

Applanation Tonometry

Chapter 3.2 investigated the tool of Applanation Tonometry. It can give measurements of central aortic pressures and stiffness that usually would have to be made invasively. There seems to be a change in thinking in the Hypertension world when it comes to accurately assessing blood pressure away from peripheral measurements and there are similarities between the hypertensive and Marfan populations. I found the Sphygmocor system easy to use and did not take a lot of training which is important in a new tool like this. My intraobserver reproducibility for central systolic blood pressure was 1%, for Augmentation index it was 18% and for Pulse Wave Velocity it was 6%. This remains an exciting new tool used increasingly frequently in hypertensive research and starting to be used clinically. It would certainly add to the assessment in Marfan Syndrome.

Wave Intensity Analysis

Wave Intensity was studied in Chapter 3.3.1 because it can measure the interaction between the left ventricle and the aorta giving measurements equating to left ventricular function, peripheral reflections and aortic stiffness indices. However, we found that the signals were very noisy and as such the intraobserver variability was 20%. The validity of the carotid artery to assess the aorta is a problem with WIA but

currently it cannot be used at the aortic root. A further problem is the acquisition and post-processing of the data, which needs to be made more available, efficient and accurate. Overall, this technique cannot be used in the routine assessment of a Marfan patient but may be in the future especially if the aorta itself can be scanned and if the variability is reduced.

Study Limitations

Chapter 3.1.1 showed intermachine variation in some important echocardiography parameters used in clinical practice including those that would be theoretically useful in Marfan Syndrome. I have described the problems with statistical help in the chapter and the lack of statistics is an issue. Four different echocardiographers were employed and each used the same echo machine. This is an important confounder and requires more advanced statistical help.

A major drawback to the sub-studies undertaken (Chapters 3.1.3, 3.2.1, 3.3.1) was the lack of a normal, age-matched control group. As described before, this was due to the main study Chapter 3.4.1 using a cross-over design and therefore, the Marfan subjects were their own controls. This showed a lack of foresight on my part at the start of the MD. Chapter 3.1.2 studied TDI in patients with medical disorders chosen due to their similarity to Marfan Syndrome in producing subclinical left ventricular dysfunction. However, these groups were dissimilar due to the nature of the disorders themselves and by the age and sex of patients who suffer from these disorders. Ideally, I should have studied groups or a single group of similar age and sex to our Marfan population.

The main limitation of the medical intervention study, Chapter 3.4.1, is the low number of Marfan subjects. However, this was much more difficult to amend. I approached everyone that met the inclusion criteria known to the UHW Genetics team in Cardiff and wrote to all Cardiology Consultants in South Wales as part of the recruitment drive. I also involved a second regional centre in Bristol to increase the numbers using their South West England cohort which went as far as Cornwall. I was also involved in discussions with the Institute of Child Health, London but the logistics of me travelling to London became too much and we couldn't find a local fellow to help. It therefore, must be accepted that there will not be enough Marfan patients who meet the inclusion criteria in one or even two regional centres.

Regional Aortic Variation

The characteristics of Marfan Syndrome are caused by abnormal fibrillin in elastic tissues and therefore, tissues that contain a lot of elastin are affected (for example, the

eyes, aorta). We also know that the arterial system contains progressively less elastin the more distally you go. What is uncertain, however, is how these different vessels are affected. We investigated three tools, two of which looked at other arteries as surrogates for central aortic effects. Using the radial, carotid and femoral arteries may be misleading if the lack of pathology in these more distal arteries belies pathology proximally. I feel there is a need for a post-mortem study looking at the regionality of arterial involvement in Marfan Syndrome and also robust testing of Applanation Tonometry and Wave Intensity Analysis in the Marfan population.

What Drugs To Use

Chapter 3.4 investigated three drugs used in Marfan Syndrome and their effects on some of the parameters from the three different tools of Tissue Doppler Imaging, Applanation Tonometry and Wave Intensity Analysis. Atenolol was investigated as the current treatment of choice in many practices but also because I believed it would perform badly in comparison with the other two drugs due to its relatively smaller effect on central aortic pressure and increased peripheral reflections. I chose perindopril because it does reduce central aortic pressure and also reduces vascular smooth muscle cell apoptosis. The third drug chosen was verapamil because it reduces central aortic pressure and it is negatively chronotropic.

Table 1: Advantages and Disadvantages of the Medical Therapies in Marfan Syndrome

Drug	Advantages	Disadvantages
Beta Blockers	-Negatively chronotropic -Antiarrhythmic	-Less effective in reducing central arterial pressure (CAP). -Increase peripheral reflections -Side effect profile -No effect on TGF- β
ACEIs	- \downarrow CAP - \downarrow VSMC apoptosis -low side effect profile -benefits poor LV function	-no effect on TGF- β -no effect on HR
Calcium Channel Blockers	- \downarrow CAP - \downarrow heart rate -low side effect profile	-no effect on TGF- β
ARBs	- \downarrow CAP - \downarrow TGF- β -low side effect profile -benefits poor LV function	-no effect on HR -no anti-arrhythmic effects
Nitrates	- \downarrow CAP	-no effect on TGF- β -no effect on LV -no effect on HR -moderate side effect profile

In the medical treatment study I found that the beta blocker, Atenolol, did reduce CAP, although less than verapamil and perindopril, and that this may be due to its negatively chronotropic and inotropic effect. Basically, if the heart beats less often and less hard then there will be less of an impact on the abnormal aorta, especially over the course of many years.

As shown in table 1, there are a number of currently available drugs that may have benefits in retarding aortic dilatation in Marfan Syndrome. A caveat is that none of the drugs above have been proven to slow dilatation, reduce dissection or improve mortality in a blinded, randomised control trial in humans. ARBs have only proven to be of benefit if you are a mouse with Marfan Syndrome. The beta blockers have less

effect in reducing central aortic pressure; have had mixed results in the literature; and the landmark Shores paper²⁵⁷, which is the evidence given for taking beta blockers, has no mortality data.²⁵⁸ ACEIs may be of benefit but do not antagonise TGF- β which seems an important property as it had such an impact on the mouse model of Marfan Syndrome.

The answer to the drug question would appear to be in 2 parts. Firstly, design and run a large, multicentre, multinational blinded, randomised control trial in order to get the patient numbers needed to see the effects. We managed to investigate only 14 patients recruiting from Camarthen to Cornwall and using the resources of two large tertiary referral centres. Investigators need to measure the correct parameters using the most appropriate tools: aortic dilatation and subtle left ventricular impairment using TTE including TDI; central aortic pressure and aortic stiffness using Applanation Tonometry and run it over many years to get morbidity (aortic surgery, dissection) and mortality data. Secondly, use drugs that may theoretically benefit. We now know that there are certain factors to target –Heart rate; CAP and stiffness; TGF- β ; and VSMC apoptosis. There is added benefit if a drug is anti-arrhythmic or has a beneficial effect on left ventricular function. These factors may only be fully targeted by using combination therapy and so this also should be further investigated. For example, the beta blocker Nebivolol, which has arterial vasodilatory properties, may be a good choice plus an ARB/ACEI; or if asthmatic Verapamil plus an ARB/ACEI.

Further Problems

There are other issues in addition to the lack of randomised control trial data in humans; not knowing what drug(s) to prescribe; and only using the older, more traditional assessments of left ventricular and aortic pathology.

Diagnostic Problems

The third version of diagnostic criteria since 1986 have recently been published—firstly the Berlin criteria were used, then the Ghent criteria and now the Revised Ghent criteria. The authors describe in their paper that the latest criteria “may delay a definitive diagnosis of Marfan Syndrome but will facilitate worldwide discussion of risk and follow-up/management guidelines.”

The diagnosis is, of course, very important. It has all sorts of implications from stigmatisation (especially in exercise-restricted children) to insurance and mortgage issues. It can lead to restriction in career aspirations and an increased financial burden from frequent outpatient appointments. The anticipated reduction in lifespan can lead

to anxiety and depression. There are also issues around marital and reproductive decisions in this autosomal dominant condition.

However, the diagnosis can also be comforting. These patients are often in the medical system for years pre-diagnosis not knowing what is wrong with them and living in uncertainty often visiting a number of different medical specialities. Just having a concrete diagnosis often reduces anxiety and stress and enables the patient to contact helpful organisations such as the Marfan Association UK.

The diagnosis, however, is difficult and in our own Marfan population, one subject was taken out of the trial because he did not have Marfan Syndrome despite being followed-up for some time with serial echocardiography in the local Cardiology clinic.

Follow-Up Problems

Other patients in our group were followed up in a variety of clinics including Cardiology, Rheumatology, Genetics, under the care of the General Practitioner or Opticians or under no follow-up at all. This reveals another problem in this syndrome, that is, the lack of a multi-team approach to a multisystem problem. This seems to become more of an issue when the Marfan children reach adulthood and have to leave the more generalist paediatric clinic.

I believe there should be regional centres for patients with Marfan Syndrome with a multi-team approach to clinics led by an expert in Marfan Syndrome, with input from ophthalmology, rheumatology, cardiology and genetics. Ideally, this would be in a tertiary centre with inpatient access to cardiothoracic surgery where the surgeons perform high volume aortic work.

There should also be a national registry so we know who has the diagnosis which would make research easier, especially as the process of research is being made more difficult with increasing amounts of form-filling (Chapter 2.1).

The Future

The future assessment of people with a possible diagnosis of Marfan Syndrome should involve:

The right patient – with a firm diagnosis of Marfan Syndrome by the Revised Ghent Nosology or being followed-up appropriately due to a possible diagnosis but not yet fulfilling the full diagnostic criteria;

being monitored by the right Consultants – ophthalmology, rheumatology, cardiology and genetics in a specific Marfan multi-disciplinary clinic in a tertiary centre with cardiothoracic surgeons experiencing high volumes of aortic and chest wall surgery with a research ethos;

having the right assessment – TTE looking at aortic dimensions serially using the 4 Roman aortic measurements indexed for BSA. Using the same echo machines in a department which monitors/audits the measurements it's echocardiographers make. Using TDI to monitor for subclinical left ventricular systolic dysfunction. Using Applanation tonometry to evaluate central aortic pressure, augmentation, amplification and stiffness indices by Pulse Wave Velocity. Researching some of the other parameters I have looked at such as WIA and IVA;

taking the right medications – I suggest beta blockers at present or ACEIs or ARBs if they cannot take beta blockers. Ongoing trials will hopefully tell us whether BBs or ARBs or combination therapy reduces aortic dilatation, aortic dissection, cardiovascular surgery and death;

all based on the right trials – Multicentre, multinational, randomised, prospective, blinded controlled trials investigating BBs, ACEIs, ARBs, CCBs and combinations looking at left ventricular and aortic parameters, TGF- β , MMP, VSMC apoptosis in genotyped subjects over a long enough time period so we see the hard end-points of aortic dilatation, aortic dissection, cardiovascular surgery and death. If there are deaths then I feel it would be important for a thorough post-mortem to determine causality.

With the number and quality of ongoing and recently started trials looking into the medical therapy in Marfan Syndrome (Table 1) I feel the future is as bright as it has been since that famous Medical Society Meeting in Paris in 1896.

Table 1: Ongoing or Future Marfan Syndrome Trials March 2012: www.clinicaltrials.gov and www.nhs.uk

	<u>Title</u>	<u>Investigators</u>	<u>Medication</u>	<u>Patient number</u>	<u>Start Date</u>	<u>End Date</u>	<u>Age years</u>	<u>Trial Type</u>
1	Atenolol vs Losartan in the Prevention of Progressive Dilatation of the Aorta in MFS (LO-AT-ARFAN01).	Dr Albert Forteza, Spain	Atenolol vs Losartan	140	Oct 2008	Feb 2013	5-60	Echo: Aortic diameter.
2	Effects of Losartan vs Atenolol on Aortic and Cardiac Muscle Stiffness in Adults with MFS.	Mark Creager, Boston, Mass, USA	Atenolol vs Losartan	50	Oct 2007	Jan 2012	>25	Echo, AT: Aortic stiffness, LV diastolic function.
3	Study of the Efficacy of Losartan on Aortic Dilatation in Pts with MFS (MARFANSARTAN).	Guillaume Jondeau, Paris, France	Losartan vs Placebo	300	Sept 2008	March 2014	≥10	Echo: Aortic diameter.
4	Losartan vs Atenolol for the Treatment of MFS.	George Sandor, Vancouver, BC, Canada	Losartan vs Atenolol	17	Jan 2008	Dec 2011	12-25	PWV Echo.
5	Comparison of 2 Medications Aimed at Slowing Aortic Root Enlargement in Individuals with MFS – Pediatric Heart Network.	Lynn Sleeper, New England Research Institutes (NHLBI, NMF)	Losartan vs Atenolol	604	Jan 2007	March 2014	6 month to 25	Echo <i>Single-blind.</i>
6	A Randomised, Open-Label, Losartan therapy on the Progression of Aortic Root Dilatation in Pts with MFS.	Mei-Hwan Wu, Taipei, Taiwan	Losartan plus atenolol or propranolol vs Atenolol or propranolol	44	Feb 2007	Jun 2011	≥1yr	Echo <i>Open-label.</i>
7	Nebivolol vs Losartan vs Nebivolol+Losartan Against Aortic root Dilatation in Genotyped MFS Pts (MaNeLo).	Eloisa Arbustini, Pavia, Italy	Nebivolol vs Losartan vs Nebivolol+Losartan	291	Jul 2008	Jul 2013	1-55	Echo, TGF-β, Gene expression, Carotid arterial stiffness.
8	Randomised, Double-Blind study for the Evaluation of the effect of Losartan vs Placebo on Aortic Root Dilatation in Pts with MFS under Treatment with Beta-blockers.	Julie De Backer, Ghent, Belgium	Losartan or Placebo added to β-blocker	174	Jun 2009	Dec 2014	≥10	Echo, Aortic stiffness by MRI, Genetic polymorphisms (losartan).
9	The Effects of Irbesartan on Aortic Dilatation in MFS.	Michael Mullen, Royal Brompton Hospital, UK	Irbesartan vs Placebo	490	Sept 2010		>6<40	Echo.
10	Losartan and MFS –the COMPARE Study.	M Groenink, Amsterdam, Netherlands	Losartan + normal meds vs no treatment + normal meds	330	Feb 2008		Adult	MRI, Echo, Skin biopsy-for gene + protein expression. <i>Open-label.</i>

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Stuart AG, **Williams A**. Marfan syndrome and the heart. Arch Dis Child 2007;92:351-356.

Williams A, Davies S, Stuart AG, Wilson DG, Fraser AG. Medical treatment of Marfan syndrome: a time for change. Heart 2008;94:414-421.

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