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Systolic and diastolic functional parameters in Morbus Fabry patients with cardiomyopathy /

Systolische und diastolische Funktionsparameter in Morbus Fabry Patienten mit Kardiomyopathie

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to my family, for believing in me and encouraging me all the way through the development of this thesis.

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12vP	12 lead voltage duration product
2DSTE	2D speckle tracking echocardiography
α-Gal A	alpha- Galactosidase A
Angle alpha	electrical axis heart determined by the ECG
Ao	diameter of the aortic valve
ASE	American Society of Echocardiography
AV	atrioventricular
A-wave	the late- diastolic active filling of the left ventricle
A-wave Vmax	maximum velocity of A – wave
BMI	body mass index
BSA	body surface area
CCS	Canadian cardiovascular society
CH	concentric hypertrophy
CM	cardiomyopathy
CMR	cardiac magnetic resonance
CornP	Cornell Product
CP	chaperone protein
CR	concentric remodelling
CW	continuous wave
DD	diastolic dysfunction
ECG	electrocardiography

ECG-Index	Electrocardiographic-Index
EF	ejection fraction
EH	eccentric hypertrophy
ERT	enzyme replacement therapy
E-wave	early diastolic passive filling of the left ventricle
E-wave Vmax	maximum velocity of the E-wave
FOS	Fabry Outcome Survey
FS	fractional shortening
FSmid	FS applied to the middle of the ventricular wall
Gb2	Digalactosylceramide
Gb3	Globotriaosylceramide
GFR	glomerular filtration rate
GVUS	Genetic Variants of Unknown Significance
HF	heart rate
I-MIBG	123 I-meta-iodobenzylguanidine imaging
IVCT	isovolumetric contraction time
IVSd	end-diastolic thickness of the interventricular septum
IVSs	end-systolic thickness of the interventricular septum
IVRT	isovolumetric relaxation time
KG	weight
KL	height

LA	left atrium
LAd	diameter of the left atrium at atrial diastole
LAd_i	diameter of the left atrium at atrial diastole indexed by body surface area
LA_i	left atrium indexed by body surface area
LA_Vol	left atrial volume
LA_Vol_i	left atrial volume indexed by body surface area
LAX	longitudinal axis shortening
LGE	late gadolinium enhancement
LV	left ventricle
LVH	left ventricular hypertrophy
LVId	end-diastolic diameter of the left ventricle
LVIdV	left ventricular end-diastolic volume
LVIs	end-systolic diameter of the left ventricle
LVM	left ventricular mass
LVM_h	LVM indexed to body height
LVM_i	left ventricular mass indexed by body surface area
LVWT	left ventricular wall thickness
LSD	lysosomal storage disorders
MF	Morbus Fabry
MRI	magnetic resonance imaging
MVWT	mean end-diastolic wall thickness of the ventricle

NS	not significant (p > 0,3)
N	normal
n	number
NYHA	New York Heart Association
P-wave	time interval of atrial depolarisation/ contraction in the ECG
PET	positron emission tomography
PW	pulsed wave
PWd	end-diastolic thickness of the left ventricular posterior wall
PWs	end-systolic thickness of the left ventricular posterior wall
PQ	time interval from beginning of P-wave to end of Q-spike in ECG
QRS	time interval from beginning of Q-spike to end of S-spike, ventricular contraction in ECG
QT	time interval from beginning of Q-spike to end of T-wave
QTc	time interval from beginning of Q-spike to end of T-wave, corrected for heart rate
RA	right atrium
RRdias	diastolic blood pressure
RRmid	mean arterial blood pressure
RRsys	systolic blood pressure
RaVL	R- voltage in limb lead aVL of the ECG

RV	right ventricle
RV5	R- voltage in chest lead V5 of the ECG
RVAWd	right ventricular anterior wall thickness
RVH	right ventricular hypertrophy
RWT	relative wall thickness
SLP	Sokolow-Lyon Product
SSCP	single strand conformation polymorphism
SPSS	Superior Performing Software System
SV1	S- voltage in chest lead V1 of the ECG
SV3	S- voltage in chest lead V3 of the ECG
TDI	tissue Doppler imaging
TIA	transient ischemic attack

Introduction

Morbus Fabry Anderson (referred to as Morbus Fabry – abbreviated as MF – in the following thesis) is a rare X-linked lysosomal storage disorder. Due to the deficiency of the enzyme alpha Galactosidase A (α-GAL A), the substrates of this enzyme accumulate in lysosomal cells of multiple organs. As the lysosomal deposits aggregate in the kidneys, gastrointestinal tract, skin, central and peripheral nervous system and the heart, the function of these organs deteriorates. Symptoms including renal failure, cardiac insufficiency, cerebrovascular events, typical skin lesions, chronic pain and depression may occur.

One of the crucial organs affected is the heart, there within comprised, the myocardium, endocardium, endothelium and conduction cells.

In 2001 enzyme replacement therapy (ERT) was introduced, which presented the first causal therapy for this disease and has shown promising effects over the past 18 years. A new pharmacological chaperone, Migalastat HCl, has been subject to ongoing research. Migalastat binds to the misfolded but catalytically competent enzyme and traffics it into the lysosome, where α-GAL A can resume its' function. The advantage of dual therapy, Migalastat in combination with active alpha Galactosidase A versus the effects of a monotherapy are subject to current research [3].

Aim / objective of this thesis

Herewith I aspire to lay the ground work for the discussion of benefits of the Electrocardiographic-Index (ECG-Index) as an objectified diagnostic tool for the assessment and early diagnosis of diastolic dysfunction (DD) of the heart in Morbus Fabry, as early initiation of therapy is crucial in Fabry patients.

1) Comparison of systolic and diastolic echocardiographic changes between hemizygous males and heterozygous females at baseline examination before treatment.

2) Comparison of ECG changes between hemizygous males and heterozygous females at baseline examination before treatment.

3) Presentation of systolic and diastolic echocardiographic changes and electrocardiography (ECG) changes in patients with cardiomyopathy compared to patients without cardiomyopathy.

4) Presentation of systolic and diastolic echocardiographic changes and ECG changes in patients with normal diastolic function compared to patients with impaired diastolic function.

5) Performance and diagnostic value of the ECG-Index regarding diastolic dysfunction in Morbus Fabry patients

Background

Morbus Fabry is probably the most researched storage disease of at least 40 lysosomal storage diseases. The enzyme alpha galactosidase A is deficient or lacking due to a genetic mutation on the X-chromosome, which thus causes the accumulation of the substrates Globotriaosylceramide (Gb3) and Digalactosylceramide (Gb2) in multiple cell complexes, urine and blood [4]. This causes multiorgan dysfunction, a wide variety of symptoms and reduced quality of life [5-8].

Literature discussion

History

Morbus Fabry Anderson was described independently by two dermatologists in 1898, by Dr. Johannes Fabry (1860-1930) in Germany and Dr. William Anderson in England (1842-1900). Dr. Fabry described the case of a 13 year old boy who presented with albuminuria and a maculopapular rash, the purpura haemorrhagica nodularis, starting on his legs and subsequently spreading over the rest of his body in the following years [9]. Dr. Anderson in turn described a 39 year old man with finger deformities, albuminuria, varicosis, neuroma, rectal hemorrhage and angiokeratomas spread over the entire body (Angiokeratoma corporis diffusum) [10]. In 1950 the disease was classified as a lipid storage disease by Scriba [11]. 15 years later Opitz et al. discovered the condition to be an X-chromosomal recessive disease [12]. In 1967 Brady et al. proved the cause of the disease to be a deficiency of ceramidtrihexosidase (alpha Galactosidase A) [13] and in 1989 the alpha galactosidase A (A-GAL A) coding gene was sequenced [14]. The first discovery of a cardiac variant was made in 1990 [15, 16]. The first description of the clinical manifestation of a large cohort of females with Fabry disease was in 2001 [7].

Incidence, inheritance and molecular genetics of the disease

Morbus Fabry is one of approximately forty lysosomal storage disorders (LSD) which have an overall prevalence of 1:7.700 live births [17]. The lysosome is a structure that is enclosed in every cell of the body and is involved in innumerous cellular processes. When one of the enzymes which engages in these processes is deficient, a wide variety of symptoms can occur. The LSD's are classified according to the deficient enzyme [18]. All LSD's are recessively linked autosomal diseases, except for three X-chromosomally linked diseases, which include Morbus Fabry, Hunters syndrome and Danon disease. Morbus Fabry is the second most common LSD after Gaucher's disease [17-19]. Morbus Fabry has a prevalence of 1:40.000 to 1:117.000 [17, 20] for the 'classic' form. It is estimated that the unrecorded number of cases is high, especially since late-onset cardiac and renal variants have been identified, in which residual enzyme activity is preserved [15, 16, 21, 22]. A-GAL A gene sequencing in newborn screenings in Italy showed an incidence of late-onset variants

of up to 1:3.100 and a frequency of the ‘classical’ Morbus Fabry mutations of 1:37 000 [23]. In male Taiwanese newborns 1:1.250 presented with a Morbus Fabry mutation, of which 86% exhibited a mutation typical for the late-onset variants [24]. According to a recent systematic review of the prevalence of Genetic Variants of Unknown Significance (GVUS), the ratio of variants that cause the ‘classic’ Morbus Fabry compared to GVUS is approximately 1:5 [25]. Yet, as the acronym indicates the clinical importance of the various GVUS is not yet clear. The disease is prevalent in all ethnicities, but most common in the white ethnicity [26].

Morbus Fabry was believed to be transferred recessively on the X-chromosome [12], which would imply that the disease manifests only in men. However, evolving knowledge has shown that the scale of severity in females ranges from mildly affected to as severely affected as males [7, 27-30]. More recent studies suggest that Morbus Fabry is inherited in a dominant manner [28, 31]. Dobyns suggests genetically X-linked diseases may be purely X-linked, neither recessive nor dominant [32].

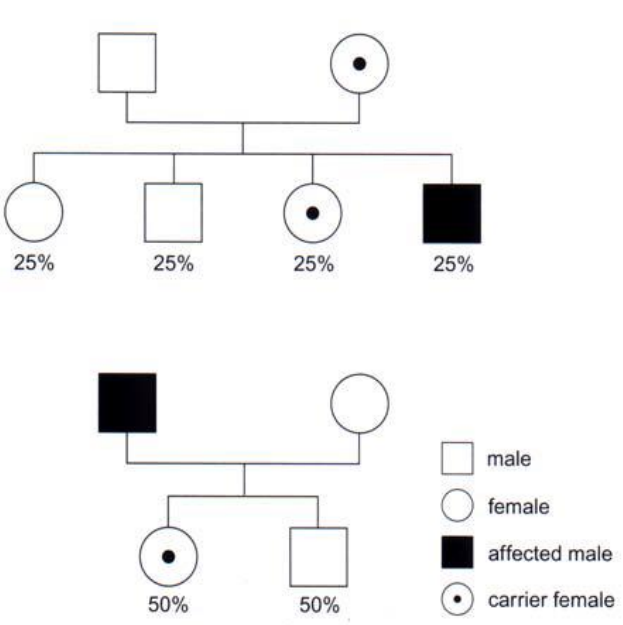

Figure 1: Classic X-linked inheritance, percentage signifies chance of inheritance respectively

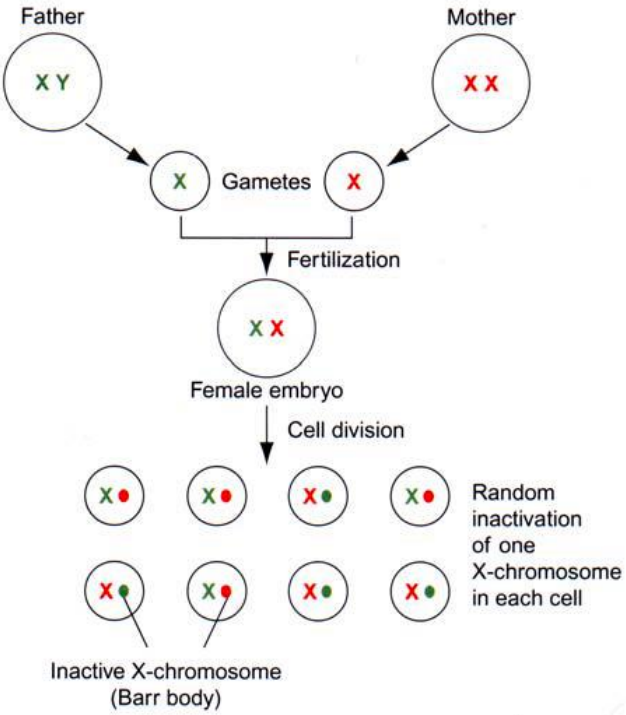

Figure 2: Graphic representation of the Lyon hypothesis

According to the X-linked inheritance when the father is affected by Morbus Fabry, the daughters are all affected whereas the male offspring is not affected at all as they receive the X chromosome from the mother. However, when the mother is affected by a X-linked disease, she has a 50% chance of passing the mutated gene to her son and 50 % of the daughters risk to be heterozygous for the disorder [33]. The expression of the mutation depends on the penetrance of the mutation [34].

The Lyon hypothesis states that a random inactivation of the mutated or non-mutated X chromosome during early embryonic stages in females takes place in every cell. The active chromosome transfers the genetic information [35, 36]. This creates a mosaic of mutated and non-mutated cells and is expressed in varying severity of symptoms [37]. On average, there is a 50:50 ratio of the mutated versus non-mutated chromosomes. As females with a mosaic of random inactivation age, the symptoms increase. This is hypothesized to be due to the lack of cross correction between healthy and affected cells, as the individual ages [38-40]. Research on the skewing of X-chromosome distribution, where either the maternal or paternal X-chromosome is predominantly expressed [38, 41], also referred to as the mosaic phenomenon, has produced conflicting results regarding the importance and influence on the onset and

progression of Morbus Fabry. However, an association between the predominantly expressed mutant X-chromosome and disease onset, severity and age has been described in multiple cases [40, 42-44]. Moreover, other studies did report evidence to support the hypothesis of X-chromosomal skewing explaining phenotypical variability [45, 46], which suggests there may be further factors influencing the genotype-phenotype relationship.

Morbus Fabry is caused by a genetically transmitted defect in the A-GAL A gene, that codes for the enzyme alpha Galactosidase A [13]. This gene is located on the q22.1 region of the X-chromosome, it has a length of 12 kilobases and is made up of 7 exons [31, 47]. The precursor glycoprotein it codes for is made up of 55 kDa (429 amino acids) and is then converted into the active enzyme which is made up of 51kDa (398 amino acids) [31, 48]. A variety of more than 900 A-GAL A gene mutation variants (Human Gene Database Professional) have been determined of which approximately 400 cause an error in the folding of the proteins [49, 50]. Approximately 75% are nonsense and the greater part are missense point mutations [51, 52]. The vast array of mutations explains the ample variety of phenotypical heterogeneity and illustrates the difficulty in finding the genotype-phenotype correlation.

Pathophysiology

The deficient activity of the lysosomal enzyme α-GAL A in leucocytes [13] causes intracellular accumulation of the lipid substrates, predominantly Gb3 and to a lesser extent Gb2. Gb3 is physiologically transformed into galactose and lactosylceramide, which can subsequently pass through the cell membrane [31]. When the substrate is not transformed due to the deficient activity of alpha galactosidase A, it progressively accumulates in the form of myelin-like, concentric lamellar inclusions with a pathogonomic periodicity of 6 to 7nm, this distinguishes Fabry's lamellar myelin-like inclusions from those commonly found in other storage diseases [31, 53-56]. The lamellar inclusions are located predominantly within the lysosomes yet also within the endoplasmic reticulum, cell membrane and nucleus [57, 58] of multiple tissues, impeding the normal function of the cell and consequently leading to multi organ pathology [31].

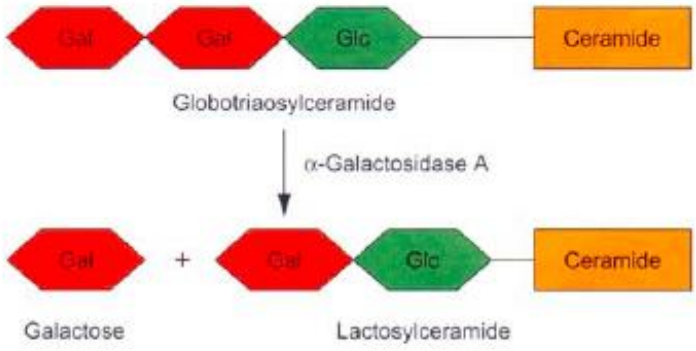

Figure 3: Enzymatic separation of galactose and lactosylceramide by alpha galactosidase A [31].

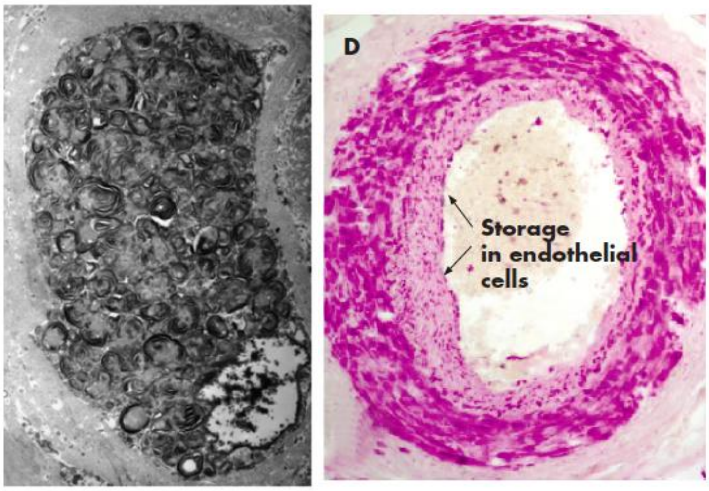

Figure 4: Electron microscopic image of a valvular fibroblast loaded with stored glycolipid (left). Transverse section of a small subepicardial branch of a coronary artery loaded with PAS + glyolipid (right) [59, 60].

Organmanifestation and complications

The affected tissue complexes are characteristically endothelial cells of the vascular intima of coronary arteries, myocytes, conduction cells and valvular fibroblasts [61-64]. The tubular epithelial cells of the renal glomeruli and the podocytes are affected as well as the endothelial and smooth muscle cells of blood vessels [65-67]. Furthermore, the corneal epithelium, the neurons of the dorsal root of nerve ganglions and the autonomic nervous system are also involved [31, 63, 64, 68-73].

	Males	Females
Cardiovascular involvement	Always	Variable
Date of onset	Earlier	Delayed (~10years)
Disease progression	Faster	Slower
Survival	Lower	Greater
Left ventricular hypertrophy (LVH)	More prevalent	Less prevalent
Myocardial fibrosis in cardiac Magnetic resonance (CMR)	Always in combination with LVH	Exists in non-hypertrophied stages of the disease
Cardiac variant	Less common	More common
Dominant cause of death	End-stage renal failure	Cardiovascular complications, heart failure

Table 1: Gender differences in Morbus Fabry [74]

Typical symptoms arise in different age groups and are summarized in Table 2.

Age	Clinical signs and symptoms
Childhood (≤ 16 years old)	Neurological symptoms: impaired hearing, neuropathic pain, acroparesthesia, dyshydrosis (hypo- and hyperhidrosis), abnormal temperature tolerance, Vestibular and auditory dysfunction Onset of cardiac and renal involvement (microalbuminuria, proteinuria, glomerular filtration rate (GFR) reduction, abnormal heart rate variability, beginning left ventricular hypertrophy (LVH) Angiokeratomas, peripheral vasospasms Ophthalmological symptoms: Eyelid oedema, cornea verticillata, tortuous retinal blood vessels Gastrointestinal problems: obstipation, diarrhoea, nausea, vomiting, abdominal pain, motility disturbances Lethargy, fatigue
Early adulthood (17-30 years old)	Aggravation of any of the above symptoms Symptoms of progressive renal insufficiency: proteinuria, lipiduria, hematuria, oedema Cardiomyopathy Transient ischemic attacks (TIA), cerebrovascular infarctions, Depression Facial dysmorphism Fever, anhydrosis, isothermia Abdominal cramps Lymphadenopathy, Extensive angiokeratomas
Late adulthood (>30 years old)	Worsening of any of the above Manifest cardiac disease (LVH, valvular dysfunction, conduction abnormalities, arrhythmia, shortness of breath, angina) Renal insufficiency Cerebrovascular events (TIA, stroke, myocardial infarction) Neurological changes comparable to multiple sclerosis Osteopenia and osteoporosis

Table 2: Age dependent symptoms of Morbus Fabry patients [6, 19, 31]

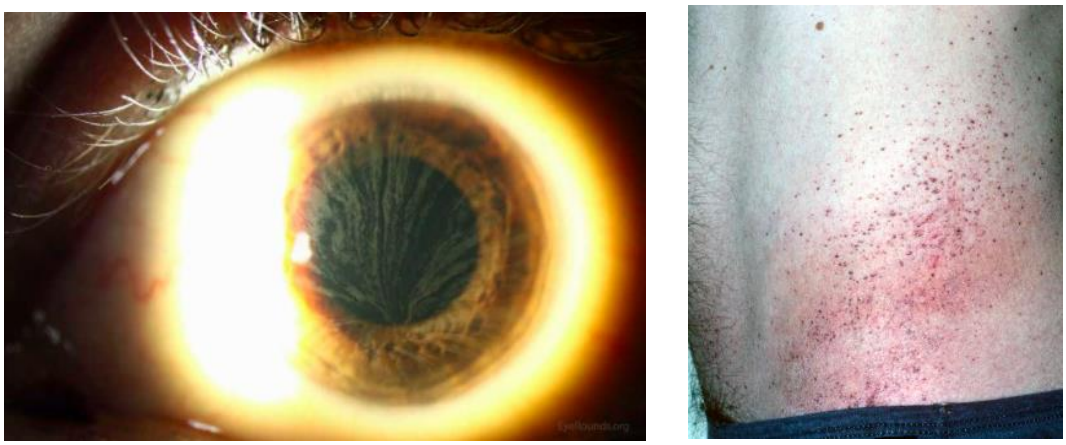

Figure 5: Cornea verticillata in a young female which led to the diagnosis Morbus Fabry (left) {Vislisel, 2019 #1077}. Angiokeratomas on the lateral side of the abdomen of a young man (right) {dermatology, 2019 #1076}.

One of the earliest manifestations and most common of the disease is the development of chronic neuropathic pain [7, 27]. Other symptoms include hypohydrosis, gastro-intestinal symptoms, corneal changes and angiokeratomas [7, 8]. Another onerous consequence is depression, most likely as a result of reduced quality of life [5, 26]. As the disease progresses multiorgan dysfunction occurs with renal failure, cardiac insufficiency and cerebrovascular incidents such as strokes and heart attacks [31, 75]. End-stage renal disease generally affects males around the age of 40 years [75, 76]. In women it is less severe and progresses more slowly [7]. In the end stages of the renal disease, dialysis or kidney transplants are often necessary [8, 76]. In 2001 renal failure was the most common cause of death reported by the Fabry outcome survey (FOS). Currently cerebrovascular incidents are the leading cause of death in Morbus Fabry patients [39, 77, 78], probably due to the increased number of kidney transplants performed since 2001 [77].

Other cardiac involvement such as valvular dysfunction and conduction abnormalities respectively also determines the rate of morbidity and mortality to a great extent [7, 8, 79, 80].

1.1.1. Cardiac manifestation

The accumulation of Gb3 is found in multiple cardiac tissue complexes [61-63]. This leads to arrhythmia, dyspnoea (shortness of breath) and angina pectoris (sudden chest pain), which occurs in up to 65% of female and 69% of male Fabry patients

[27]. Further symptoms include syncope, fatigue and palpitations [64]. Cardiac dysfunction is caused by arrhythmia, conduction abnormalities, valvular alterations and insufficiencies [39, 81]. Yet the most prominent finding is left ventricular non-dilative hypertrophy.

1.1.1.1. Cardiac hypertrophy

Morbus Fabry is characterized by a progressive, infiltrative, mostly concentric left ventricular hypertrophy (LVH) of the heart, which occurs in 18-33% of women and 53-61% of men [39, 59, 81, 82]. Heterozygous women develop LVH 10 to 15 years later and the left ventricular mass increases at a lower rate than in hemizygous males [29, 59, 81-84]. Right ventricular hypertrophy (RVH) is also common and occurs in up to 56% of females and 84% of males [84, 85].

The most common form of LVH in Fabry patients is concentric hypertrophy [31, 59, 82, 86], it is preceded by concentric remodeling, which is frequently found in patients with normal left ventricular mass (LVM) [59, 60]. Morbus Fabry's ventricular hypertrophy is known to be symmetric, yet an asymmetrical variation may occur in 5 – 66% of patients [59, 73, 86, 87].

The increase of left ventricular wall thickness in Morbus Fabry has been confirmed by imaging techniques for example echocardiography and magnetic resonance imaging (MRI), scintigraphy and patho-anatomical analysis [31, 59, 83, 88, 89]. Histologically the highest quantity of lamellar Gb3 deposits occurs in the heart compared to other organs, yet the glycosphingolipid deposits merely make up approximately 1% of the total mass of the heart (11mg/g heart wet weight) [15]. It is therefore likely that other neurohormonal, inflammatory or vasoreactive mechanisms play a role in tissue ischemia, hypertrophy and fibrosis [90].

Aerts et al. identified high concentrations of Globotriaosylsphingosin, a deacylated Gb3 metabolite, in the blood, which is likely to act as an inhibiting factor to α-GAL A activity and a proliferating factor to smooth muscle cells [91]. Brakch et al. hypothesized that Sphingosin-1-Phosphat, which can be found in increased concentrations in plasma of Fabry patients, functions as a proliferating factor and is furthermore associated with cardiovascular remodeling [92]. Moreover, Barbey et al. showed a strong positive correlation between the intima media thickness of the

common carotid artery and increased LVM and suggest a growth promoting factor, confirmed in vitro, may be the underlying cause [93]. Another hypothesis indicates that Gb3 deposits trigger the production of reactive oxygen species in cultured endothelial cells, which may influence the development of hypertrophy [94]. Yet another approach to explain the mechanism leading to cardiac hypertrophy is mitochondrial dysfunction and thus inefficient energy utilization caused by the Gb3 accumulation [95-97]. Thus, further research is needed to evaluate the exact mechanisms leading to LVH.

An increased LVM has been found to correlate with age, increased occurrence of valvular disease, conduction abnormalities, arrhythmia and severity of typical cardiac symptoms such as angina [29, 59, 82, 86]. Furthermore, LVH is associated with mostly preserved systolic function, mild to moderate diastolic impairment and increased LV filling pressures [98, 99]. However, the end stages of cardiomyopathy are characterized by fibrosis, marked by late enhancement during cardiac magnetic resonance (CMR), and regionally severely reduced left ventricular (LV) function. This comprises the LV longitudinal function, which deteriorates first and the radial function [100, 101]. Consequently, LVH is an important risk factor for cardiovascular disease progression and cardiac events [80, 102].

1.1.1.2. Cardiac fibrosis:

Myocardial fibrosis is found in 25-83 % of Fabry patients [84, 103-106] and occurs in both ventricles [17, 61, 86, 100, 103, 105-110]. Cardiac fibrosis mostly seems to occur in conjunction with hypertrophy [4, 100, 101, 105, 106]. Yet signs of fibrosis have been found in women without the development of LVH, which suggests fibrosis and hypertrophy are not necessarily associated [101, 108, 111, 112]. Fibrosis can be detected using late gadolinium enhancement (LGE) during CMR. Intravenously applied gadolinium contrast diffuses freely between the cells and does not transcend cell membranes. When the extracellular space (intercellular space) is widened due to fibrosis, combined with slower wash out kinetics, the contrast can be detected in the late washout phase, as is the case in Fabry patients [101, 113].

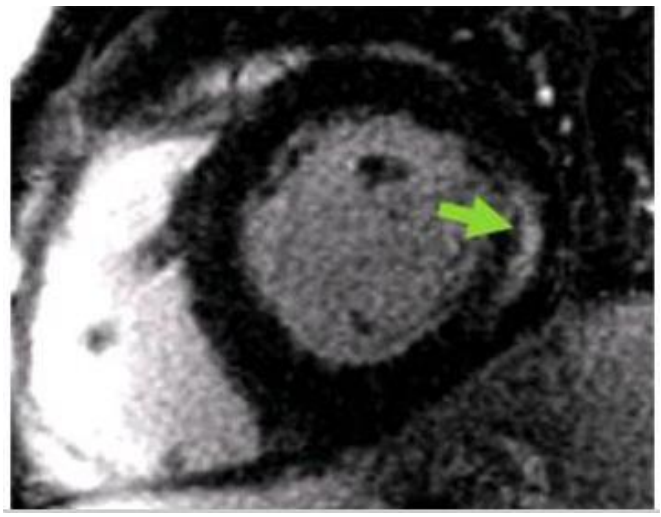

Figure 6: Cardiac MRI showing marked thickening of the left ventricle with late gadolinium enhancement in the basal posterolateral myocardial segments {Yousef, 2013 #310}.

In patients with pacemakers or cardiac defibrillators, that cannot be examined by MRI and patients with end-stage renal disease, in which case contrast agents are contraindicated, the only method to examine this group of patients would be by echocardiography. There are two methods to detect replacement fibrosis indirectly: strain rate imaging and two-dimensional speckle tracking.

The fibrotic process initially starts with midmyocardial (intramural) involvement and advances by means of transmural progression. It is predominantly localized in the basal inferolateral myocardial segments [22, 90]. The mechanism behind the specific pattern of distribution is not entirely clear, however this has also been found in other metabolic cardiomyopathies, such as Duchenne's dystrophia and Friedreich's ataxia [114-116]. Presumably fibrosis is associated with cardiac kinetics, work load and structural wall differences, which subsequently lead to pressure overload and increased wall stress stimulating fibrosis [22, 100]. Furthermore, chronic inflammation may play a role in the pathogenesis of myocardial fibrosis in Fabry patients [111, 117]. Moreover, recent CMR/positron emission tomography (PET) studies, that combined metabolic and molecular imaging with morphological and functional imaging, suggest chronic inflammation to be the histological correlate of LGE, rather than fibrosis [101]. Further research is needed to establish the exact process of fibrosis and its' association with LVH in Fabry patients.

1.1.1.3. Conduction tissue disease

Lipid deposits (Gb3) in the conduction system dispose the heart to atrioventricular (AV) conduction delay, complete AV blocks [118], bundle branch blocks, progressive sinus node dysfunction [119] and premature supraventricular beats [31, 119-126].

Tachy- and bradyarrhythmia have also been found to be common in Fabry patients. Furthermore, impaired autonomous regulation can cause an irregular heart rate and decreased heart rate variability [64, 68, 127-129]. Longitudinal studies found 15-42% of males and 21- 27% of females to suffer from arrhythmia [39, 75]. The most common types of arrhythmia are supraventricular tachycardia, atrial fibrillation and atrial flutter [119]. The occurrence of malignant arrhythmia has been found to correlate with the extent of cardiomyopathy and burden of fibrosis [130].

1.1.1.4. Electrophysiological abnormalities in Morbus Fabry

The most common electrocardiographic abnormalities in Fabry patients with cardiac manifestation are repolarization irregularities and high amplitudes in chest leads reflecting left ventricular hypertrophy [59, 131-133]. A recent study found ECG abnormalities in general to significantly correspond with the progression of cardiac disease, irrespective of age, left ventricular mass and gender. Patients with ECG abnormalities showed an eight fold higher relative risk for cardiac disease progression than patients with a normal ECG[134]. The following ECG abnormalities in Fabry patients have been recorded in the different phases of the heart action.

1.1.1.4.1. Atrial depolarization

A study comparing 30 newly diagnosed Fabry patients to heart rate and age–matched controls found the PQ interval to be shortened [135]. This was attributed to a shortened P-wave, due to accelerated intra-atrial conduction as a result of an increased diameter of the conduction cells by Gb3 accumulation [120, 135, 136]. A larger study with 150 genetically confirmed Fabry patients and varying levels of cardiomyopathy did not find a significant difference in the duration of the P-wave or PQ segment in Fabry patients compared to the norm [133].

1.1.1.4.2. Atrioventricular conduction

A shortened PR interval was observed in early reports [121]. Since then multiple studies including two large studies of 207 and 150 cases found a shortened PR interval in 14% and 16% of patients respectively [118, 133, 137]. PR prolongation correlates with increasing age and disease progression [122, 138]. This suggests that LVH and increasing Gb3 burden may slow down atrioventricular conduction due to cellular dysfunction[122, 138].

1.1.1.4.3. Ventricular depolarization

Namdar et al. found the shortening of the QRS segment in a cohort of 30 Fabry patients compared to heart rate and age matched healthy persons [135]. Contrarily a prolonged QRS segment was found to correlate strongly with age [138] in Fabry patients and with LVM in echocardiography [119, 131, 138] and CMR [133]. This suggests the shortening of the QRS segment is a characteristic of early stage cardiomyopathy in Fabry patients and QRS prolongation correlates with the progression of the disease, which is in agreement with the findings above (PR segment).

1.1.1.4.4. Ventricular Repolarisation

The QT, QTc and QT dispersion was normal in a cohort of 150 untreated Morbus Fabry patients [133]. Namdar et al. found the QTc interval to be prolonged and the repolarization dispersion to be more distinct than in age and heart rate matched controls and suggests this may predispose to ventricular arrhythmia [135].

1.1.1.4.5. Electrocardiographic detection of fibrosis

ST segment and T-wave abnormalities commonly occur in the precordial leads V4-V6 [131, 132, 139]. This correlates well with the typical localization (basal postereolateral wall) of fibrosis determined by LGE during CMR. Niemann et al. found normal values for ST or T-waves to correlate with the absence of late enhancement in the CMR and in reverse conclusion he suggests that abnormalities of the ST segment or T-wave may reflect the development of fibrosis and may be an indication to proceed with an echocardiography if CMR is not available to assess the presence of cardiac fibrosis

[133]. Linhart et al. describe an association between ST segment/ T-wave abnormalities and LVH and cardiac remodeling [59].

1.1.1.5. Valvular disease

Gb3 accumulation and fibrosis of valvular tissue is responsible for valvular disease in Morbus Fabry patients [140, 141]. Valvular involvement is found in up to 55% of Fabry patients [59, 83, 142, 143]. Minor abnormalities of the mitral and aortic valve are common and only seldomely associated with a severe degree of valvular regurgitation. Specifically, mild aortic valve regurgitation is reported in 10-15%, tricuspid valve regurgitation in 34% and mitral valve regurgitation in 25-51% of Morbus Fabry patients [143]. Moderate valve regurgitation is reported in less than 4% of Fabry patients [144].

Aortic root and aortic valve abnormalities are more frequent in older patients, as well as in patients with cardiac disease progression defined by the presence of LVH, whereas mitral valve thickening and prolapse is typical in younger patients [59, 83, 145]. Nevertheless, valvular dysfunction rarely progresses to a degree which requires an intervention [146].

The pulmonary valve is only very rarely affected, this is presumably due to lower hemodynamic stress compared to the left heart valves, which also explains the relatively low occurrence of tricuspid valve involvement [137].

1.1.1.6. Vascular disease

Up to every second Fabry patient has experienced chest pain [7, 8, 75]. Yet less than 1 % of Fabry patients require coronary revascularization reported by the FOS database in 2005. The pain usually presents itself despite the lack of coronary artery stenosis [81, 146]. Nevertheless, Fabry patients with relevant symptoms should undergo coronary catheterization to exclude the stenosis as they seem to accumulate cardiovascular risk factors i.e. hyperlipidemia, hypertension and renal disease [39, 81]. Factors contributing to chest angina may be increased oxygen requirement by hypertrophied cells, decreased coronary reserve and raised diastolic filling pressures, resulting in decreased blood flow in subendocardial capillaries, which may cause anginal chest pain [59, 81, 146]. Moreover, microscopically, endothelial dysfunction caused by Gb3 accumulation may be associated with

coronary vasospasm, inducing anginal pain [146]. Furthermore, endothelial dysfunction can cause thromboembolic incidents [73].

1.1.1.7. Diastolic function

Diastolic function is made up of four phases: the relaxation period of the ventricle and the passive filling period, which requires good compliance. The diastasis phase, occurs when the left atrial (LA) and LV pressures are almost equal and the filling of the left ventricle is determined by the flow in the pulmonary veins and lastly the active phase, which involves the atrial contraction [147]. Diastolic dysfunction occurs when the active or passive phase of the filling of the ventricle is shortened, lengthened or incomplete [148]. This is commonly due to increased stiffness of the left ventricle which equates to decreased elasticity and compliance during the filling phase and therefore a restricted amount of blood flow into the ventricle. This leads to more blood accumulation and higher pressures in the left atrium and subsequently atrial enlargement develops [73, 149, 150]. This was confirmed by Saccheri et al. who recorded an enlargement of the indexed left atrial volume in 20 Fabry patients with LVH [151]. Another study comprised of 33 Fabry patients (14 with LVH), found increased LA volume and reduced left atrial compliance in non-ERT Fabry patients compared to age- and gender-matched controls [152]. However, this is in disagreement with Putko et al, who did not find a significant enlargement of the left atrium in 31 Fabry patients compared to healthy controls, yet 58% of their patients were under ERT, which may have influenced the absence of atrial remodelling [153]. Furthermore, left atrial function and size has been described as an important marker for the severity and chronicity of diastolic dysfunction [151, 154].

Mild to moderate DD is common in Fabry patients. Severe global diastolic dysfunction is rare, yet when it occurs it results in decreased cardiac output [31, 59, 61, 81, 98, 146, 155, 156]. Clinically, DD may be asymptomatic, however in Fabry patients it is the main cause of dyspnoea [81, 98, 147].

The classical diastolic functional parameters, early (E)- and late (A)-wave were recorded by means of pulsed wave Doppler and the isovolumetric relaxation time (IVRT) was recorded by continuous wave Doppler. The E-wave, corresponds to the maximum velocity of the early diastolic passive filling of the left ventricle and the A-wave, is equivalent to the maximum velocity of the late diastolic active filling (atrial

contraction) of the left ventricle. IVRT is the time between the closing of the aortic valve and the opening of the mitral valve, during which the ventricle loses its capacity to shorten and generate power and recoils into a state of unstressed length and force [157]. Another parameter to measure diastolic function is the E/A ratio, which determines the ratio of the early (E) and late (A) maximum mitral inflow velocities during diastolic filling of the LV. It is sensitive to change when more than half of the LV segments are affected by impaired relaxation [155]. The E/A ratio is an important factor in the estimation of LV filling and hereby has shown a high value in the diagnosis of diastolic dysfunction [147, 156]. It was therefore used as a criteria of diastolic grading in this study [158].

IVRT prolongation, a sign of abnormal relaxation of the ventricle, is more common in Fabry patients [31, 155, 159]. Fabry patients with LVH or increased relative wall thickness show longer IVRT, than patients without LVH or relative wall thickness [31, 73]. One study found IVRT to be within the normal range in Fabry patients with and without LVH [82]. In contrast, IVRT may also be shortened, which indicates a restrictive filling pattern, yet this is extremely rare in Fabry patients [31, 59, 81, 85, 98, 160]. The E/A ratio correlates inversely with LV- and LA- wall thickness and LVM in Fabry patients. In patients with LVH the E/A ratio decreases and the A-wave Vmax significantly increases compared to patients without LVH and controls [73].

Diastolic dysfunction is not only relevant in the left side of the heart, it also commonly occurs in the right ventricle. Palecek et al. found DD in 47% of 45 Fabry patients. Furthermore, DD is associated with RVH and correlates significantly to increased right ventricular wall thickness, age and indexed LVM [156, 161].

1.1.1.8. Systolic function

Systole is made up of the simultaneous contraction of both ventricles, with the left ventricle moving its volume into the aorta and the right ventricle moving its content into the pulmonary artery. Echocardiographic parameters commonly used for the evaluation of the global systolic function are the ejection fraction (EF) and fractional shortening (FS) of the diameter of the ventricle. FSmid corresponds to FS applied to the middle of the ventricular wall. In cases where FS and EF may not adequately represent reduced systolic function due to altered left ventricular geometry, FSmid is a reliable parameter. FS and FSmid represent the radial function, whereas the

longitudinal axis shortening (LAX) reflects the longitudinal function. Differences in EF and FS between genders have not been established and the global systolic function is typically normal in Morbus Fabry patients [73, 82-84, 100, 145, 159, 162]. Yet with the progression of the disease and increasing LVM, FS and FSmid decrease [29, 31, 59, 81, 100, 159]. One study was not able to confirm these results [82]. Similarly, LAX is impaired as LVM increases [29, 31, 81, 100]. The longitudinal function of the left ventricle is impaired before the radial function is reduced [29, 81, 84, 100].

Early dysfunction of the LV and moreover of the RV and LA, such as decreased contractility [100] and reduced myocardial velocities can be detected in asymptomatic carriers without LVH [73] and when conventional echocardiography parameters have not detected change. The following methods are used for early detection; tissue Doppler imaging (TDI), ^{123}I-meta-iodobenzylguanidine imaging (I-MIBG imaging) and 2D speckle tracking echocardiography (2DSTE) [73, 84, 107, 155, 159, 163-166]. A possible explanation for the dysfunction may be a mechanical and functional interference of the Gb3-deposits with the contraction/relaxation cycle of the sarcomeres, leading to cellular hypertrophy and decreased tissue velocities for the contraction and relaxation cycles [73]. This is underlined by the fact that histological evidence of Fabry's cardiomyopathy and myocyte abnormalities can be found at early stages of the disease, when LVH has not yet developed [73, 155]. Isovolumetric contraction time (IVCT) has also been described as a very sensitive tool to determine preclinical cardiomyopathy in Fabry patients. The prolongation of IVCT may be due to the onset of compensating mechanisms caused by reduced myocardial function [155].

Systolic dysfunction of the ventricle primarily seems to occur regionally. The basolateral segment is most commonly the initial area involved, which is concurrent with the area of initial development of fibrosis. This raises the question whether the determining factor in the development of systolic dysfunction may be fibrosis rather than an increase in LVM. Histologically this may be explained by increased interstitial volume, not macroscopically visible, and a decrease in contractile fibers impeding the ventricles function [61, 100, 105, 106, 108, 113, 162, 167]. Furthermore, regarding the right ventricle, Weidemann et al. found a significantly reduced RV longitudinal function in patients with fibrosis, and a normal RV longitudinal function in the patient group with hypertrophy and without fibrosis. The radial function of the right ventricle

was not significantly different to controls. A second study found a normal regional myocardial function of the right ventricle [84, 100]. The discrepancy between these two studies may be explained by the absence of patients with fibrosis in the study of Weidemann et.al.

On the contrary, a recent study demonstrated a stronger link between global LV myocardial dysfunction and left ventricular wall thickness (LVWT) than between global LV myocardial function and fibrosis [107]. Conform to these findings, another study found basolateral segments with impaired longitudinal function in the early stages of the disease, in the absence of cardiac fibrosis [100, 162]. Either way, left ventricular hypertrophy, myocardial fibrosis and left ventricular dysfunction characterize the end stages of Fabry's cardiomyopathy and ultimately lead to progressive heart failure [100, 163, 164] .

Diagnosis and prognosis

The first symptoms arise at a mean age of 11-14 years in males and 16-23 years in females. The time between the onset of the first symptoms and the diagnosis ranges between 14 years in men and 16 years in females [26, 27]. One of the reasons for this is that the disease is rare, another is that awareness of the disease is not widespread amongst clinicians and the public. Furthermore, a contributing factor might be the unspecific symptoms and the fact that the disease manifests in different organs and creates multiple symptom complexes, which differ in degrees of severity.

The median life expectancy is 50 years in men and 70 years in women, which is a reduction of 20 and 10 years respectively compared to the United States general population's average life expectancy [7, 8, 75, 168]. This is mostly due to cerebrovascular, renal and cardiac complications [31, 75].

Therapy

1.1.2. Enzyme replacement therapy

Morbus Fabry disease is the second lysosomal storage disease following Gaucher's disease that has enzyme replacement therapy available. Two different products (Algasidase alfa and beta) were granted marketing authorization in 2001 and multiple

studies have since determined the effect of the products on different organs and their functions.

Quality of life and average pain improved over 4 – 5 years [169-171]. The stabilisation or slower deterioration of the kidney function, has been noted [66, 170-178]. Furthermore, the effects of treatment on left ventricular mass and function of the heart seem to be inconsistent or generally diminish over time [102, 163, 179-181]. In patients without LVH, LVM was found to stabilize in multiple studies [99, 163, 169-171, 182, 183]. Yet in patients with LVH (LVM>50g/$m^{2.7}$) and severe LVH (LVM>85g/$m^{2.7}$), the LVM decreased constantly over a period of 10 and 4 years respectively [99, 171]. Moreover, the benefit of ERT in terms of myocardial function, exercise capacity and the reduction of the left ventricular mass, was increased in patients without fibrosis compared to patients, who had already developed mild to severe fibrosis. These patients showed no improvement of cardiac function and merely a mild reduction in LVM [105, 163, 182].

Schuller et al. criticizes that many studies used biomarkers that were insufficiently studied as clinical endpoints specifically in regard to morbidity and mortality in the evaluation of ERT and therefore found the effectiveness of ERT to be unclear [184]. However, a recent review in an overview of 77 cohort studies with over 15.000 patients, revealed that agalsidase beta is associated to a significantly lower incidence of renal, cardiovascular and cerebrovascular events compared to no ERT and to a significantly lower incidence of cerebrovascular events than algasidase alfa [185].

Patients responses to ERT are variable and may be explained by the serum mediated inhibition of ERT (in 40-88% in male Fabry patients), which may increase risk for the development of Fabry specific symptoms such as decreased kidney function and increased LV mass [186-189]. In the cases of ERT inhibition positive patients, immune modulation therapies are subject to future studies, although a recent study by Sato et al. using anti B- lymphocyte antibodies has shown promising results in terms of inducing immune tolerance in 50% of Fabry mice models during ERT. The effect in humans remains to be investigated [190]. Another reason for variable effects of ERT may be different cellular pathomechanisms influencing variable activity, insufficient distribution and concentration of the infused enzyme in different cell types [70, 187, 191].

1.1.3. Migalastat HCl

A new product was granted marketing authorization in April 2016. The product Galafold, which consists of migalastat as the active substance, is a chaperone protein (CP). A chaperone protein binds to the misfolded but catalytically competent enzyme alpha Galactosidase A, which is generally destined to stay in the endoplasmic reticulum due to its mutation. However, the chaperone-linked enzyme is trafficked into the lysosome where it can resume its function. Simultaneously Migalastat (1-deoxygalactonojirimycin) is a competitive inhibitor of α-GAL A and in subinhibitory doses induces the effective clearance of lysosomal Gb3 storage in multiple organs in transgenic mice [192], in fibroblasts of cell cultures [193, 194] and furthermore enhances the activity of α-GAL A in cultured lymphoblasts [194-197].

The co-administration of ERT and Migalastat HCl has been found to have a superior effect compared to exclusive ERT therapy in plasma of Morbus Fabry patients and in cultured Morbus Fabry fibroblasts. It resulted in increased activity of α-GAL A in plasma and within the cells. Additionally, the amount of intralysosomal α-GAL A increased and the clearance of Gb3 was improved [3, 198].

Positive effects have been recorded in patients with and without a history of ERT treatment [3, 199, 200]. Hughes et al. showed similar effects of ERT and Migalastat on renal function, yet a significant decrease in left ventricular mass indexed to body surface area (LVM_i) after 18 months of Migalastat treatment was documented, whereas under ERT treatment no significant change was observed [200]. However, Migalastat is only administered in patients with amenable A-GAL A gene mutations (responsive to migalastat) [3]. The advantage of this product is the method of administration, as Migalastat is administered as a capsule in contrast to ERT treatment which is an infusion administered intravenously biweekly, which is more invasive and may lead to the delay of the initiation of therapy. Furthermore, in transgenic mice the chaperones have been shown to reach different types of tissues for example brain tissue [192]. Whereas in ERT the enzyme insignificantly reduces the accumulation of Gb3 in some cell types [201] or the administered α-GAL A is not detectable in some tissue types, potentially due to inefficient delivery and uptake [70, 202]. Additionally the PC could avoid the risk of immunogenicity reactions and complications related to ERT [186, 200].

Importance of early Diagnosis:

The Gb3 accumulation begins early in life, yet left ventricular hypertrophy most commonly manifests itself only decades later; at a median age of 43.5 years 51.5% of men and at 54.9 years 38% of women presented with LVH [39, 82, 139]. Consequently, the detection of left ventricular hypertrophy may not be useful for the recognition of early stages of the disease. Furthermore, evidence has shown that a determining factor in cardiac outcome under ERT is the severity of baseline left ventricular hypertrophy and fibrosis [105, 139, 163, 182]. It is therefore suggested that early treatment is most effective [203-205] . Yet adversely Rombach et al. shows progression in adolescents with early disease manifestation does occur and suggests that long-term ERT does not prevent this, but rather decreases the risk of developing additional complications [180]. Nevertheless, increased awareness and subsequent earlier diagnosis of the disease at least enables earlier symptomatic treatment by means of administration of diuretic, antiarrhythmic, antiplatelet and anti-heart failure drugs decreasing the rate of complications.

ECG-Index

This thesis serves as ground work for the further evaluation of the hypothesis that ECG parameters and specifically the ECG-Index, can serve as an early diagnostic marker for diastolic dysfunction before abnormalities are recorded in echocardiography. The index was developed using the parameters that correlated best to diastolic dysfunction [206].

$$ECG - Index = \frac{Tend - P}{PQ\ x\ Age}$$

Equation 1: ECG-Index: Electrocardiographic index with Tend-P [ms], PQ [ms], Age [years]

It was tested on a group of 172 non-Fabry individuals that suffered from diastolic dysfunction diagnosed by means of echocardiography. The index proved a high accuracy for the diagnosis of DD and showed an even higher diagnostic value in combination with left atrial volume [206].

Methods

Patient acquisition

The pediatric department of the university hospital in Mainz is specialized and performs extensive research on storage disorders such as Morbus Fabry. The research center therefore has a large collective of patients that can be tracked and observed for research benefit. Using family trees, the group of patients was enlarged by recruiting all potentially affected male and female family members of patients, that were already registered in Mainz.

The group of patients that qualified for this research project was derived from this large pool of patients. Patients were excluded if they showed signs of atrial fibrillation, higher than grade 1 AV block, had atrial or ventricular pacing or had already started ERT. This was necessary to prevent wrong measurement of the different ECG durations and furthermore atrial fibrillation for example may influence diastolic function and therefore may be a confounding variable.

Diagnosis

The potential candidates were thoroughly clinically examined for variable symptoms corresponding with clinical signs of Morbus Fabry. In correspondence, the patients were genetically tested for the various mutations and the level of the alpha-galactosidase in leucocytes was determined to confirm the diagnosis.

1.1.4. Alpha Galactosidase activity

The level of enzyme activity was determined in the laboratories of the pediatric department of the university hospital of Mainz (then director: Prof Dr. Beck). The assay was conducted with the substrate alpha Galactosidase A- 4-methylbelliferyll-alpha-D-galactopyranosid (sigma chemical, ST. Louis, USA) and N-acetyl-D-galactosamine was added to inhibit alpha-N-acetylgalactosaminidase [31, 207, 208].

1.1.5. Proof of the genetic mutation

The genetic sequencing was performed by the institute of human genetics at the university hospital of Hamburg-Eppendorf (then director: Prof. Dr. med A. Gal). The

seven exons of the gene that are responsible for the coding of the α-GAL A enzyme were sequenced and analysed by means of single strand conformation polymorphism (SSCP) [31].

Patient collective description

The following demographic data was recorded:

- Gender [male/female]
- Age [years]
- Height [cm]
- Weight [kg]

For the comparability and standardization of the parameters, the body surface area (cm^2) is an appropriate parameter. It is calculated by the formula of Du Bois and Du Bois [31, 209]

$$BSA = KL^{0.722} * KG^{0.425} * 71.84$$

Equation 2: BSA: Body Surface Area [cm^2], KL: height [cm], KG: body weight [kg]

The body weight also has an influence on the extent of the left ventricular mass, therefore the BMI is taken into account.

$$BMI = \frac{KG}{KL^2}$$

Equation 3: BMI: Body Mass Index [kg/m^2]: KG [kg]; KL [cm]

1.1.6. Cardiological examination

All patients that were diagnosed with Morbus Fabry were subsequently cardiologically examined. A clinical examination including the measurement of blood pressures was performed and cardiac function was assessed by means of an electrocardiographic and echocardiographic examination. Only patients with a complete set of data were admitted to the study.

1.1.6.1. Conduct of examination

Due to the reduced physical capacity of the patients, they were examined after a 15 minute rest on the bed without any disturbing factors. No sedation was necessary during any of the examinations [31].

1.1.6.2. Determination of the arterial blood pressure

The blood pressure of the right arm was measured every 5 minutes during the echocardiographical examination. The cuff used for the measurements was adapted to the body weight and age of the patient. The measurements were performed with a Dinamap (Criticon, USA). The mean of the measured systolic and diastolic blood pressures and mean blood pressure calculated by the Dinamap were used to calculate the final mean blood pressure [31]. Arterial hypertension was classified according to the current guidelines at the time of data acquisition [210, 211].

1.1.6.3. Electrocardiographic examination

All electrocardiographic examinations were conducted under standardized conditions with a Megacard ECG System (Siemens Elema, Sweden). They were recorded at a speed of 50mm/s and had an amplitude of 1mV/10mm with an alternating current filter of 50 Hz. The electrocardiographic derivations were made up of the bipolar limb leads according to Goldberger and Einthoven and the precordial derivations V1 to V6 (according to Wilson). It is presumed that the leads V1 and V2 represent the area to the right side of the septum, V3 and V4 the septum itself and V5 and V6 the electrical activity to the left of the septum. The leads consequently represent the structures in the above mentioned areas.

After the recording, the ECGs were scanned and digitally evaluated using the “Image Tool” Software Package (developed at: the University of Texas Health Science Centre, San Antonio, Texas, USA) [31].

1.1.6.3.1. Measured ECG parameters

- P-wave: time interval of atrial depolarisation in the ECG

- PQ: time interval from beginning of P-wave to end of Q-spike in ECG
- QRS: time interval from beginning of Q-spike to end of S-spike, ventricular contraction in ECG
- QT: time interval from beginning of Q-spike to end of T-wave
- RaVL: the voltage of the R-wave in limb lead aVL
- RV5: the voltage of the R-wave in chest lead V5
- SV1: the voltage of the S-wave in chest lead V1
- SV3: the voltage of the S-wave in chest lead V3
- Angle alpha: electrical axis of the heart in ECG

1.1.6.3.2. Calculated Parameters

Tend-P

Tend-P is the duration between the end of the T-wave to the beginning of the P-wave. It was calculated by the measurement of one complete cycle (RR) minus PQ minus QT.

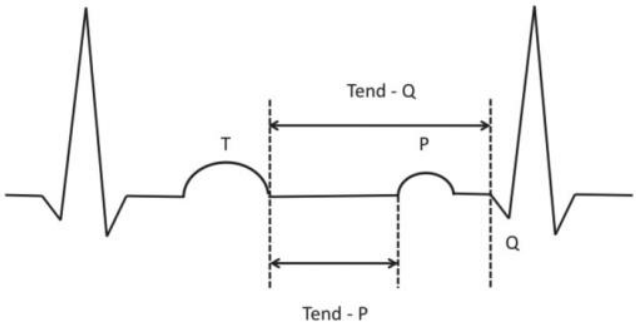

Figure 7: Schematic illustration of Trend-P and Trend-Q measurements reflect the electrical and mechanical diastole respectively [206].

ECG-Index

The ECG-Index is a parameter highly associated with diastolic dysfunction [206]. It is based on the PQ interval, which is significantly longer in patients with DD, the Tend-P reflects the electrical diastole, which is shortened in DD [206, 212]. Furthermore, the index takes age into account, which is associated with an increasing incidence of DD [206].

$$ECG - Index = \frac{Tend - P}{(PQ \times Age)}$$

Equation 4: Electrocardiographic index: Tend-P [ms], PQ [ms], Age [years] [206]

1.1.6.3.3. Myocardial Hypertrophy

Since patients suffering from Morbus Fabry commonly develop left ventricular hypertrophy, the above measured ECG parameters were implemented in formulas that describe left ventricular hypertrophy. However, these formulas are typically used to detect left ventricular hypertrophy in patients suffering from arterial hypertension or other conditions that result from excessive pressure in the left ventricle.

Sokolow- Lyon Index- LVH

This index is calculated by creating the sum of the amplitudes of S in V1 and R in V5 [213].

Cornell Index

The Cornell index is calculated by creating the sum of the amplitudes of R in aVL and S in V3. To correct the gender dependence, 8mm (0.8mV) are added to the Cornell index, when a female is concerned [214].

12 Lead voltage

The total amplitude of all derivations are added and result in the 12 lead voltage [215].

123 Lead voltage

The amplitude of the I, II and III derivations are added and result in the 123 lead voltage.

Voltage duration products

Considering that the QRS duration is also an indicator for myocardial hypertrophy, voltage duration products can be calculated by the multiplication of the QRS duration with the Sokolow-Lyon Index, the corrected Cornell Index and the 12 Lead Voltage and respectively creates the Sokolow-Lyon Product (SLP), the Cornell Product (CornP) and the 12 lead voltage duration product (12VP) [214] The different products have shown higher correlations to the different types of hypertrophy compared to the necroptic weight of the heart, than purely the indices or the duration of QRS [31].

1.1.6.4. Echocardiographic examination

Echocardiography is a non-invasive reliable tool to determine the systolic function, diastolic function and dimensions of the heart [216]. The echocardiographic examination followed the electrocardiographic examination. Patients were positioned on their left side slightly tilted backwards towards the stretcher which they were lying on during the examination. To rule out machine-dependent variances, a single digital Ultrasound machine (Power-Vision, SSA380, Toshiba, Japan) was used for all echocardiographic examinations. Depending on the age of the patient either a 2.5 MHz, 3.5 MHz or 5 MHz sector scanner was used.

The pre- and postprocessing signal was set and the same mode was used for all patients. The depth sensitivity is very variable in every echocardiographic examination. Therefore, manual adjustments were made according to set criteria of optimal optical configuration. Only echocardiograms with sufficient quality were analysed. The signal was transferred onto a MD (Sony, USA) as a video loop or as a single image. The videos were copied onto SVHS Video, which resulted in the loss of image quality. They were therefore only used as a control. Consequently, only Cine-Loops and images of MD Digital were used to evaluate the echocardiograms.

Due to the variability caused by breathing and the change in pre- and afterload of the heart, the measurements were recorded a minimum of three times. ‘Beat to beat’ variability was minimized using the mean of multiple measurements. A ‘day-to-day’ variability was not considered due to the model of the study.

Simultaneously ECG leads were attached to the patients’ limbs and synchronized to the 2D, Doppler and M-mode on the monitor. The recording speed was set at

50mm/s or 100mm/s to record and analyse one full cycle of information on the monitor and on a single picture. All measurement were performed in end-expiration [31].

1.1.6.4.1. M- Mode echocardiographic parameters

The standardized direct measurements of specific cardiac structures were conducted based on the guidelines of the American society of echocardiography [31, 217]. The M-Mode allows measurements of only a very limited area of the heart, therefore, to determine the function of the heart it is supposed that the measured diastolic and systolic function applies to the rest of the left ventricle's function. The data gathered by M-mode was verified by 2D visualization. The end-systolic and end-diastolic function was determined in the parasternal axis of the M-mode's sonographic wave [31, 218].

The following echocardiographic parameters were obtained and calculated

- End-diastolic thickness of the interventricular septum IVSd
- End-systolic thickness of the interventricular septum IVSs
- End-diastolic diameter of the left ventricle LVId
- End-systolic diameter of the left ventricle LVIs
- End-diastolic thickness of the left ventricular posterior wall PWd
- End-systolic thickness of the left ventricular posterior wall PWs
- Diameter of the aortic valve Ao
- Diameter of the left atrium at atrial diastole LA

All measurements were obtained using the synchronized 2D picture, after the position of the M-mode was verified.

1.1.6.4.2. Left ventricular muscle mass

The left ventricular muscle mass was calculated with the above parameters according to the Guidelines of the American Society of Echocardiography (ASE) and its modifications by Reichek et al. [31, 219].

$$LVM_{ase} = 1.04 * ((LVId + PWd + IVSd)^3 - LVId^3)$$

Equation 5: LVMase: left ventricular muscle mass according to the ASE norm (1.04: specific weight of the heart muscle) [g]

$$LVM = 0.8 * LVM_{ase} + 0.6g$$

Equation 6: LVM [g]: echocardiographically determined left ventricular muscle mass that correlates to the muscle mass of a necroptic heart specimen

For the comparability of the left ventricular mass, the LVM is indexed to body height.

$$LVM_{\_h} = \frac{LVM}{KL^{2.7}}$$

Equation 7: LVM_h [g/m$^{2.7}$], LVM [g] und KL [m]

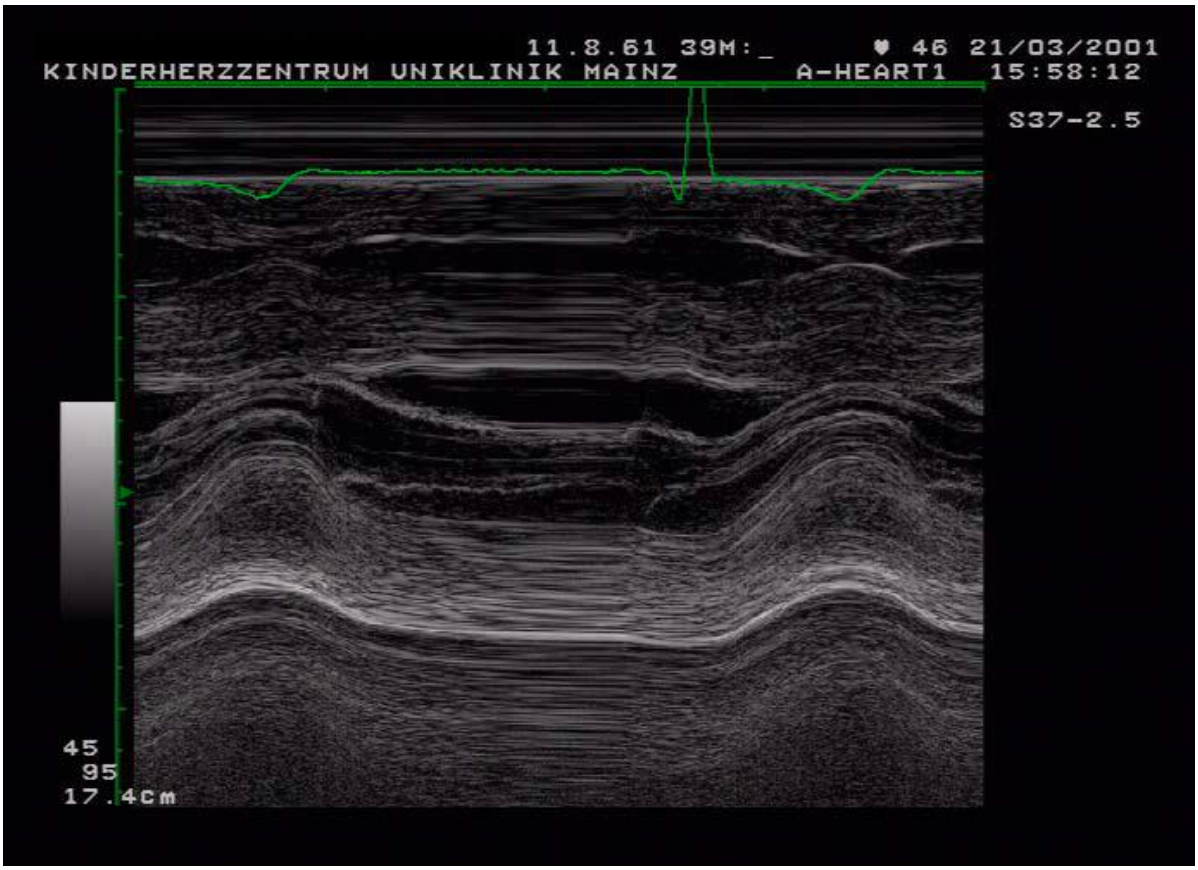

Figure 8: Digital M-mode image of patient with severe left and right ventricular hypertrophy.

1.1.6.4.3. Myocardial hypertrophic changes

Myocardial hypertrophy is defined as an increase in myocardial mass exceeding 118g/m^2 in hemizygous individuals and 104g/m^2 in heterozygous individuals [220, 221]. By analogy to Devereux this was indexed to the body length and BMI [222]:

Males:	BMI ≤ 26kg/m^2 =>	LVM > 51g/m$^{2.7}$
	BMI >26kg/m^2 =>	LVM > 53g/m$^{2.7}$
Females:	BMI ≤ 26kg/m^2 =>	LVM > 48g/m$^{2.7}$
	BMI >26kg/m^2 =>	LVM > 60g/m$^{2.7}$

Types of myocardial hypertrophy

The relative wall thickness is calculated as [31]:

$$RWT = \frac{(\mathrm{IVSd} + \mathrm{PWd})}{\mathrm{LVId}}$$

Equation 8: RWT [ ]: IVSd [mm], PWd [mm], LVId [mm]

The following types of left ventricular hypertrophy are classified according to the relative wall thickness and left ventricular mass [1, 59]:

Normal (N): normal LVM and relative wall thickness with regards to the end-diastolic diameter of the left ventricle < 0.45

Concentric remodeling (CR): normal LVM and relative wall thickness with regards to the end-diastolic diameter of the left ventricle ≥ 0.45

Concentric hypertrophy (CH): increased LVM and the relation between end-diastolic septum wall thickness and end-diastolic posterior wall thickness or respectively the relation between posterior wall thickness and septum wall thickness < 1.25

Eccentric hypertrophy (EH): increased LVM and the relation between end-diastolic septum wall thickness and end-diastolic posterior wall thickness or

respectively the relation between the posterior wall thickness and the septum wall thickness ≥ 1.25

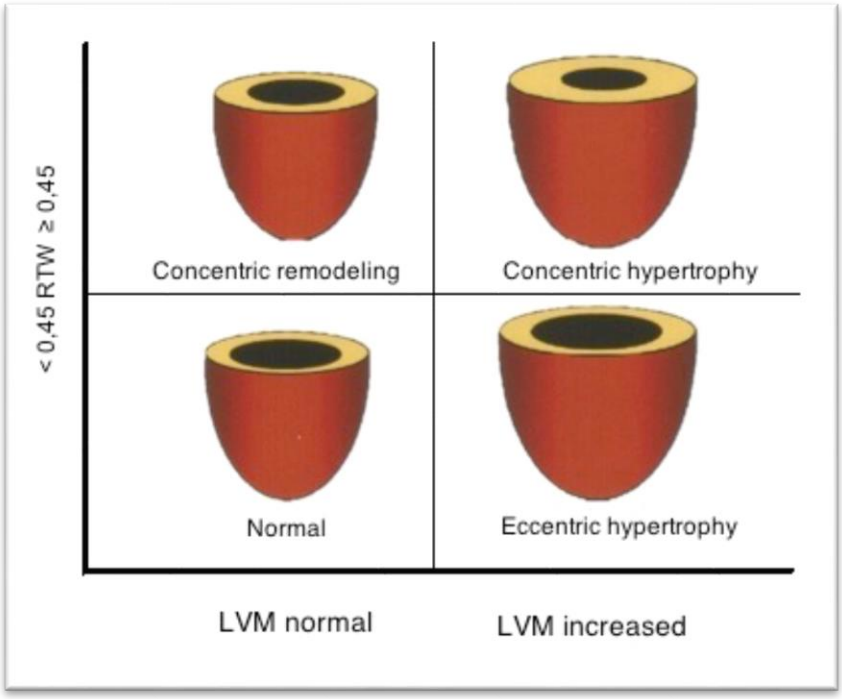

Figure 9: Schematic classification of diffferent types of left ventricular geometry by Ganau et al. [1] edited by Wiethoff 2008 [2].

The left ventricle is defined as dilated, when the end-diastolic diameter indexed to the body surface area exceeds 32 mm/m^2. The enlargement of the aorta or the left atrium is defined as the diameter of the structures exceeding 22mm/m^2 [31, 223]

$$MVWT = \frac{(\mathrm{IVSd} + \mathrm{PWd})}{2}$$

Equation 9: MVWT [mm]: mean end-diastolic wall thickness of the ventricle

Severe myocardial hypertrophy is defined as MVWT ≥15mm [224].

1.1.6.4.4. Doppler sonographic data

All sonographic doppler data was recorded under standardized conditions [225, 226]. The measurements of maximum flow velocity below 1.5m/s were recorded using the Pulsed Wave (PW) Doppler technique and when the maximum flow velocity

exceeded 1.8m/s it was measured with the Continuous Wave (CW) Doppler technique [31].

The following parameters of the left ventricular inflow (over the mitral valve) were recorded:

- Maximum velocity of the E-wave — E-wave Vmax [m/s]
- Maximum velocity of the A-wave — A-wave Vmax [m/s]

The above parameters were adapted according to heart rate [31, 227]:

$$Parameter_{cor} = \frac{Parameter}{\sqrt{R-R-Distance}}$$

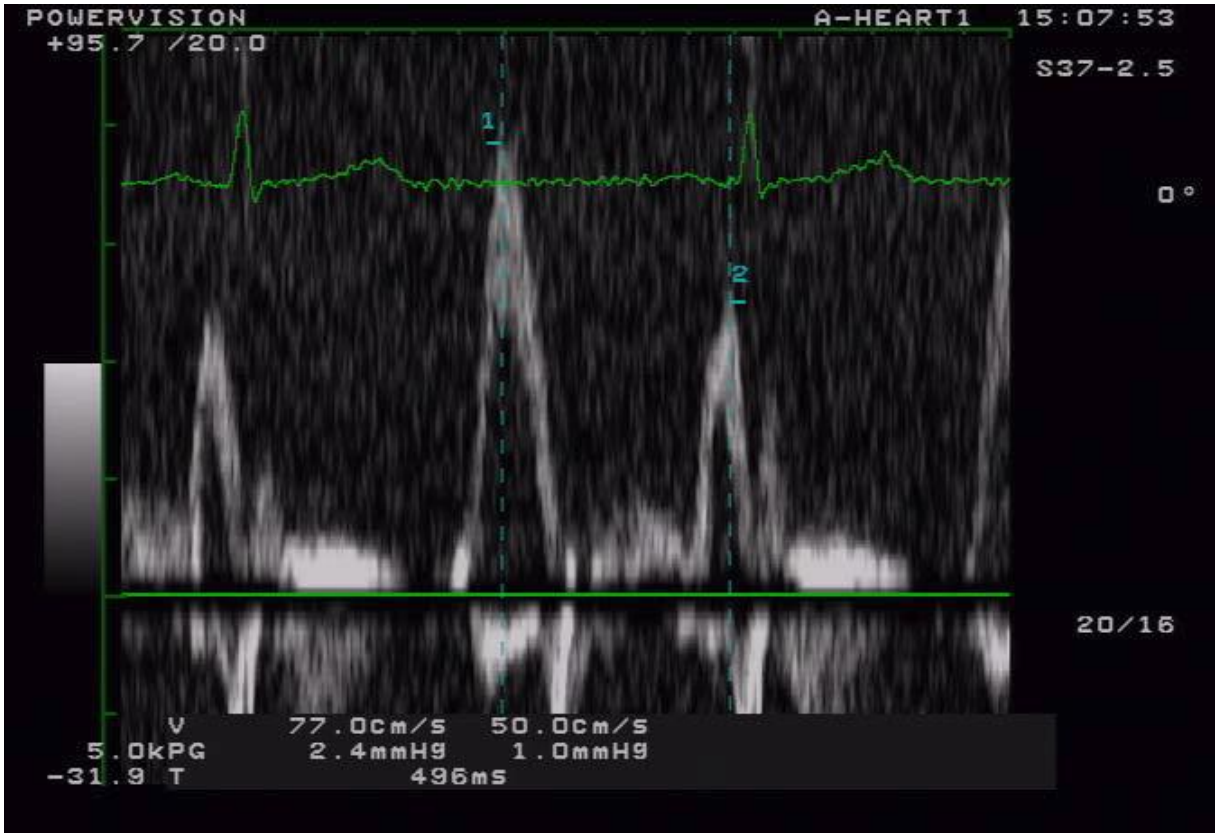

Figure 10: PW Doppler flow over the mitral valve, measurement of the E-wave Vmax (77.0 cm/s) and A-wave Vmax (50.0 cm/s) [31], E/A ratio= 1,54, which would correspond to moderate diastolic dysfunction if the LA_Vol_i ≥34ml/m², see Figure 11.

1.1.6.4.5. Systolic Parameters

Fractional shortening (FS)

This describes the diameter reduction of the left ventricle in systole and is independent of the heart rate [31]:

$$FS = \frac{LVId - LVIs}{LVId} * 100$$

Equation 10: FS [%]: shortening fraction of the left ventricle

The ASE advises the use of 25% as the lower limit of FS [228, 229]. In the cohort described below Kampmann et al. used 28% as the minimum reference value [31].

Midwall related fractional shortening (FSmid)

Due to hypertrophic changes of the heart, the value for FS is generally falsely increased. Consequently, the calculation of FS is related to the midwall, whereby the thickness of the septum and posterior wall in the end-diastole and end-systole are considered, which produces a more accurate value [31, 230, 231].

$$FS_{mid} = \frac{\pi * \left(LVId + \frac{1}{2}IVSd + \frac{1}{2}PWd\right) - \pi * (LVIs + \frac{1}{2}IVSs + \frac{1}{2}PWs)}{\pi * (LVId + \frac{1}{2}IVSd + \frac{1}{2}PWd)} * 100$$

Equation 11: FSmid [%]: fractional shortening of the left ventricle related to the wall thickness

Systolic long axis shortening (LAX)

The global left ventricular systolic long axis shortening is estimated by taking the fractional thickening of the posterior wall into account [232]. Furthermore, the perturbed function of the long axis shortening may be an indication for diastolic dysfunction [31, 233, 234].

$$LAX = \left(\frac{1}{\left(1 + \frac{\frac{LVId\ +\ PWd}{2} - \frac{LVIs\ +\ PWs}{2}}{\frac{LVId\ +\ PWd}{2}}\right) * \left(1 + \frac{PWs\ -\ PWd}{PWd}\right)} - 1 \right) * 100$$

Equation 12: LAX [%]: calculated long axis shortening of the left ventricle

Ejection Fraction (EF)

Analogous to FS, EF is calculated using the difference in the end-systolic and end-diastolic volume in relation to the end-diastolic volume. The volumes are calculated by applying the Teichholz formula: [235]

$$LVIdV = \frac{7}{2.4 + LVId} * LVId^3$$

Equation 13: LVIdV [ml]: left ventricular end-diastolic volume

The left ventricular end-systolic volume is calculated with the same formula, yet LVId is replaced by LVIs.

$$EF = \frac{LVIdV - LVIsV}{LVIdV} * 100$$

Equation 14: EF [%]: Ejection fraction

1.1.6.4.6. Parameters of diastolic function

Diastolic function is analysed by parameters which record the blood flow over the mitral valve into the left ventricle. The following parameters illustrate an accurate representation of diastolic function using the PW Doppler mode [31, 224, 236-239].

E/A Ratio

The E/A ratio is calculated by dividing the maximum velocity of the E-wave by the maximum velocity of the A-wave. For this calculation, the heart must be in sinus rhythm, otherwise there is no A-wave.

$$E/A\ Ratio = \frac{E - Vmax}{A - Vmax}$$

Equation 15: E/A Ratio [ ], the relation of the maximum flow over the mitral valve of the E-wave and the A-wave.

Isovolumetric relaxation time

The IVRT is generally recorded in [ms] and reflects the time between the closure of the aortic valve and the opening of the mitral valve [236, 238, 240].

Left atrial volume

The left atrial volume was calculated according to the cubic formula with the left atrial diameter (which one) measured in the parasternal long-axis view and indexed by BSA [241].

$$LA_{Vol_i} = \frac{LA^3}{BSA}$$

Equation 16: LA_Vol_i[mm/cm^2]: Left atrial volume indexed by body surface area

1.1.6.4.7. Grading of the diastolic function

Diastolic function is classified in the following grades [156, 158]:

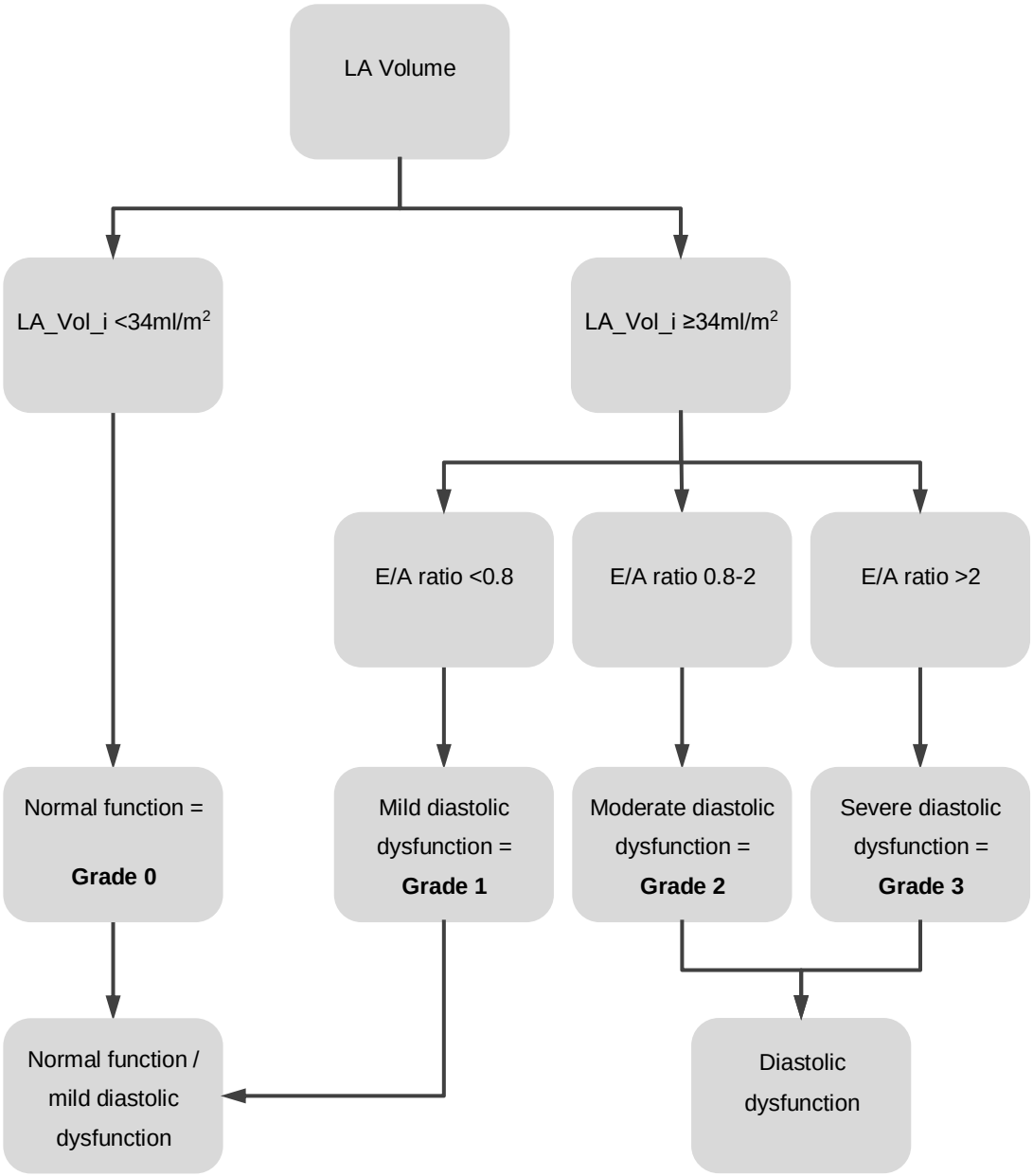

Figure 11: Grading of diastolic function

In view of the small group of patients, the four grades of diastolic function were grouped into two larger groups to facilitate improved comparability.

Control group

To compare Fabry patients from this study with healthy individuals, reference values from current literature were utilized.

Analysis Process

All data was recorded and entered in a spreadsheet program (Excel, Fa. Microsoft). The calculations were performed with routine procedures of Excel. The data was transferred from Excel to SPSS version 10.07 for windows. This program was used for the analysis.

1.1.7. Statistical methods

The data was broken down for comparison and analysis by looking at the mean ± standard deviation, mostly supplemented by the 95% confidence interval and the minimum and maximum values. Furthermore, when comparing a continuous and standardly distributed variable between two patient groups the T-test for independent samples was conducted. The Mann-Whitney-U test was conducted when the data was unequally distributed between the two patient groups. The X^2 Test (chi-square) was used in multiple field tables.

As this study is an explorative data analysis the p-values (value of probability) are exclusively descriptive. Adjustment for the p-values for multiple testing was not conducted. Statistical significance of the results was established when $p \leq 0.05$. When this value was exceeded a considerable difference could be noted, yet non-significant. In the tables, non-significance was marked as NS, when the p-value exceeded > 0,3. The number of patients included in the analysis are indicated in every table. Drop-outs result through missing data.

1.1.8. Graphic presentation

The graphs and tables were produced by the routines of the graphic package of the SPSS – program, the tables were then transformed in the Excel program (Excel, Fa. Microsoft) and subsequently inserted into this Word document (Word, Fa. Microsoft), the graphs were directly imported from SPSS into Word. Solely the arrow diagrams were directly generated in Word.

Results

Patient collective

This study includes 118 patients, data was collected from August 1999 – December 2012. The Morbus Fabry – Anderson disease was proven enzymatically and/or genetically in each of the subjects. The cardiologic examination was conducted according to the methods explained above. Only the base line examination was considered.

As Morbus Fabry is inherited X-chromosomally, all parameters are initially analysed according to gender. In part 2 this is no longer considered, due to the relatively small sample of patients included in this study.

Inclusion and exclusion criteria

The patients were selected according to a number of criteria:

1) Only patients over the age of 18 years, who had not yet started ERT were included in this study.

2) All patients with pacemakers or any form of cardiac arrhythmia (i.e. atrial fibrillation or atrioventricular blocks) were excluded.

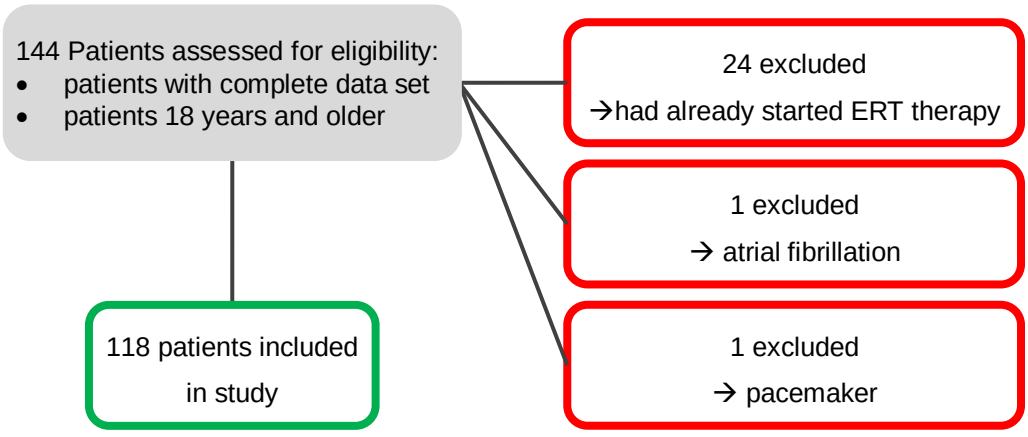

Figure 12: Patient flow chart

Comparing males and females

1.1.9. Basic clinical data and cardiac parameters

The following table (Table 3) displays the basic biometric and clinical cardiac data of the studied group of patients. Out of 118 patients which were analysed 41 were men and 77 were female. The mean age of males was 35.0 years and the mean age of females was 39.8 years. Furthermore, as recorded in previous studies, the data shows men to have a significantly larger body surface area [59] and a lower body mass index compared to women [31, 167].

	All	Males	Females	p
n	118	41	77	
Age [years]	38.2 ± 13.8	35.0 ± 12.1	39.8 ± 14.5	0.074
	(18.5 – 66.3)	(18.3 – 63.5)	(18.5 – 66.6)	
	18.1 – 70.8	18.1 – 68.0	18.0 – 70.8	
Weight [kg]	67.2 ± 13.2	69.8 ± 14.8	65.9 ± 12.2	0.131
	(49.9 – 94.1)	(46.6 – 100.9)	(50.0 – 91.3)	
	46.0 – 107.5	46.0 – 107.5	47.0 – 96.0	
Height [cm]	168.0 ± 9.0	176.0 ± 8.2	164.8 ± 6.8	<0.0001
	(154 – 186)	(163.1 – 188.9)	(153.9 – 177.0)	
	148.5 – 194.5	161.5 – 194.5	148.5 – 180	
BSA [m^2]	1.76 ± 0.18	1.82 ± 0.20	1.70 ± 0.15	<0.0001
	(1.48 – 2.06)	(1.48 – 2.2)	(1.47 – 1.99)	
	1.43 – 2.36	1.47 – 2.36	1.43 – 2.06	
BMI [kg/m^2]	23.7 ± 4.5	22.5 ± 4.15	24.3 ± 4.5	0.032
	(17.0 – 31.9)	(16.88 – 28.4)	(18.4 – 34.3)	
	15.9 – 37.4	15.9 – 35.0	16.6 – 37.4	

Table 3: Basic clinical data in male and female MF patients

The diastolic, systolic and mean arterial blood pressure did not show significant differences between the sexes (Table 4). However, the systolic blood pressure of men at 130.6 ± 16.7 mmHg tended to be higher than in women at 126.1 ± 13.7. The heart rate in men tended to be lower than in woman, with a mean heart rate of 65.8 bpm in men and 70.4 bpm in woman.

	All	Males	Females	p
n	118	41	77	
RRsys [mmHg]	127.7 ± 14.9 (107.9 –155.2) 84 – 167	130.6 ± 16.7 (100.9 – 158.0) 84 – 159	126.1 ± 13.7 (107.8 – 155) 97 – 167	0.121
RRdias [mmHg]	73.3 ± 9.5 (56 – 89.1) 50 – 98	73.6 ± 11.0 (55.1 – 89.9) 50 – 98	73.1 ± 8.6 (58.7 – 85.9) 55 – 97	NS
RRmid [mmHg]	91.4 ± 9.6 (76.0 – 107.7) 64.7 – 118	92.6 ± 11.6 (72.6 – 112.3) 65 – 115	90.8 ± 8.4 (78.4 – 105.1) 70 – 118	NS
n	114	40	74	
HF [s^{-1}]	68.8 ± 14.1 (51.8 – 95.5) 44 – 134	65.8 ± 15.1 (49.1 – 85.7) 44 – 134	70.4 ± 13.3 (52.8 – 100) 50 – 106	0.1

Table 4: Basic cardiac parameters in male and female MF patients

Hypertension is more than twice as common in men compared to women in this cohort ($p<0.01$ – chi square). 22.9% of the 118 Fabry patients showed hypertensive values defined according to the WHO by systolic blood pressure >140mmHg or a diastolic blood pressure of >90mmHg (Table 5).

		Males	Females	Total
Hypertension	No	26 63.4%	65 84.4%	91 77.1
	Yes	15 36.6%	12 15.6%	27 22.9%
Total		41 100%	77 100%	118 100%

Table 5: Male and female MF patients with and without arterial hypertension

In non-hypertensive patients hypertrophy was more or less evenly spread amongst the different forms (Table 6). However, in hypertensive patients 21 out of 27 (77.8%) developed concentric hypertrophy of the heart, whereas this was only the case in 34% of non-hypertensive patients. Eccentric hypertrophy was the second most common form. Furthermore 3 out of 27 (11.1%) hypertensive patients presented with a normal geometry of the left ventricle, versus 30 out of 91 (33%) non-hypertensive patients. The above strongly suggests an influence of hypertension ($p<0.001$ – chi square) particularly in the development of concentric hypertrophy in MF patients.

Hypertension		No	Yes	Total
Geometry of LV	Normal	30 33%	3 11.1%	33 28.0%
	CR	11 12.1%	0 0%	11 9.3%
	CH	31 34%	21 77.8%	52 44.1%
	EH	19 20.9%	3 11.1%	22 18.6%
Total		91 100%	27 100%	118 100%

Table 6: Different forms of hypertrophy in MF patients with and without hypertension

1.1.10. Basic echocardiographic parameters

The diameter of the aorta was significantly larger in males. On average it was within the norm in both sexes [242, 243]. Aortic dilation however, was found in none of the men and in 1% of women [244]. It is evident from the graph below, that males similarly tended to have a larger diameter of the left atrium (Table 7).

	All	Males	Females	p
n	116	39	77	
Ao [mm]	28.0 ± 6.0 (24.9 – 38.4) 19.1 – 72.0	31.2 ± 4.2 (24.9 – 38.4) 23.6 – 39.2	26.5 ± 6.2 (20.5 – 31.5) 19.1 – 72.0	<0.0001
n	118	41	77	
LAd [mm]	34.1 ± 5.9 (25.2 – 46.0) 21.0 – 51.3	35.5 ± 5.8 (29.3 – 48.7) 26.0 – 51.3	33.4 ± 5.9 (24.7 – 44.3) 21 – 48.7	0.068
LAd_i [mm/m^2]	19.3 ± 3.5 (15.0 – 27.0) 13.0 – 30.0	19.2 ± 3.1 (15.0 – 25.9) 15.0 – 28.0	19.3 ± 3.8 (14 – 27) 13 – 30	NS

Table 7: Basic echocardiographic parameters in male and female MF patients

Yet when indexed to BSA (LAd_i) there was no difference in LA diameter between genders, however the average values were higher than the norm [245]. Evident in Table 8, the mean left ventricular diameters (LVId and LVIs) were within the norm in both women and men [245]. Men had a significantly thicker interventricular wall, measured in systole and diastole (IVSs &IVSd). The mean values for IVSd in males exceeded the reference value of 11mm. Moreover, the mean values for RVAWd, PWd and PWs in men were significantly higher than in women. On average the thickness of the left ventricular posterior wall in diastole (PWd) and the right ventricular anterior wall (RVAWd) in both men and women were above the norm of 11mm and 5mm respectively, which indicates mild hypertrophy [228].

	All	Males	Females	p
n	118	41	77	
IVSd [mm]	11.5 ± 3.9 (6.8 – 21.3) 6.5 – 25.2	12.5 ± 3.9 (7.1 – 22.7) 6.5 – 23.5	10.9 ± 3.8 (6.1 – 21.3) 6.0 – 25.2	0.031
IVSs [mm]	15.7 ± 4.3 (10.0 – 24.0) 5.8 – 30.3	17.2 ± 4.5 (12.0 – 25.4) 6.9 – 28.6	14.9 ± 4.0 (10.0 – 22.3) 5.8 – 30.3	0.005
LVId [mm]	47.0 ± 5.7 (38.6 – 57.4) 33.4 – 63.0	50.6 ± 5.9 (40.8 – 61.0) 39.2 – 63.0	45.1 ± 4.4 (36.9 – 54.1) 33.4 – 56	<0.0001
LVIs [mm]	28.2 ± 4.9 (20.9 – 37.6) 15.9 – 43	30.7 ± 5.1 (23.4 – 41.7) 19.5 – 43	26.8 ± 4.3 (20.2 – 36.5) 15.9 – 39.7	<0.0001
PWd [mm]	11.6 ± 2.6 (7.9 – 16.8) 7.0 – 20.1	12.7 ± 3.0 (8.0 – 18.6) 7.9 – 20.1	11.0 ± 2.6 (7.6 – 15.2) 7.0 – 18.8	0.002
PWs [mm]	17.9 ± 3.4 (12.6 – 24.0) 9.7 – 24.9	19.2 ± 3.7 (13.0 – 24.6) 9.7 – 24.9	17.3 ± 3.0 (12.4 – 23.0) 11.9 – 24.0	0.003
n	75	20	55	
RVAWd [mm]	6.0 ± 1.7 (4.0 – 9.3) 3.0 – 13.0	6.9 ± 2.0 (4.7 – 12.9) 4.7 – 13.0	5.6 ± 1.4 (3.8 – 8.8) 3.0 – 10.5	0.003

Table 8: Echocardiographically measured wall thicknesses in male and female MF patients

1.1.11. Calculated echocardiographic parameters

The parameters derived from the echocardiographic parameters in Table 8, as explained in the chapter ‘methods’, showed a trend of a higher left ventricular average wall thickness (MVWT) in males, compared to females (Table 9). In general, the mean values for MVWT of both genders were above the norm, albeit they did not exceed the limit of 15mm, which would imply severe hypertrophy [224]. Yet 10 males and 6 females had values above 15mm. 2 (12%) of the 6 patients suffered from arterial hypertension, 2 (12%) had repolarization abnormalities in the ECG, 1 of the 16 (6%) patients was graded in diastolic dysfunction grade 1, 4 (25%) patients in grade 2 and 1 (6%) patient in grade 3. Furthermore, there was no significant difference in the relative wall thickness (RWT) with regards to gender, yet both males and females had an increased relative wall thickness compared to the norm (< 0.45).

The left ventricular mass indexed by height did display a small yet insignificant difference between genders. However, the LVM indexed by the body surface area (LVM_i) revealed a significant distinction between the sexes, with higher values in men. Neither the left atrial volume nor the left atrial volume indexed by BSA differed significantly between genders. However regarding the left atrial volume indexed by BSA, which is as robust as by height [246], the mean values in both males and females exceeded the average maximum volume of 24ml/m^2 of healthy individuals [247]

	All	Males	Females	p
n	118	41	77	
MVWT [mm]	11.5 ± 3.1 (6.5 – 21.1) 7.3 – 17.2	12.6 ± 3.1 (7.7 – 19.7) 7.2 – 20.2	11.0 ± 3.0 (7.0 – 17.0) 6.5 – 21.1	0.006
RWT[ ]	0.50 ± 0.15 (0.30 – 0.78) 0.24 – 1.02	0.51 ± 0.15 (0.30 – 0.78) 0.25 – 0.79	0.49 ± 0.15 (0.30 – 0.80) 0.24 – 1.02	NS
LVM_h [g/m$^{2.7}$]	64.2 ± 32.3 (30.2 – 154.1) 23.5 – 201.1	71.9 ± 30.5 (36.7 – 152.9) 23.5 – 189.2	60.0 ± 32.6 (29.4 – 155.6) 25.2 – 201.1	0.057
LVM_i [g/m^2]	149.6 ± 67.0 (73.9 – 316.7) 64.5 – 403.8	179.3 ± 63.9 (105.5 – 324.6) 65.4 – 403.8	133.7 ± 63.4 (73.7 – 310.2) 64.5 – 391.3	<0.0001
LA_Vol[ml]	43.3 ± 24.1 (16.0 – 97.6) 9.3 – 135	48.2 ± 26.2 (25.2 – 115.6) 17.6 – 135.0	40.7 ± 22.6 (15.1 – 86.9) 9.3 – 115.5	0.11
LA_Vol_i [ml/m^2]	24.9 ± 13.6 (9.6 – 52.2) 6.1 – 75.8	26.3 ± 13.8 (13.8 – 61.8) 11.8 – 75.8	24.1 ± 13.5 (8.9 – 51.3) 6.1 – 67.2	NS

Table 9: Calculated echocardiographic parameters in male and female MF patients

1.1.12. Systolic echocardiographic functional parameters

The ejection fraction was similar in males and females. The FS and FSmid did not differ considerably between the sexes. The longitudinal axis shortening however tended to be smaller in males, although the mean values were not below the set lower limit of 45% (Table 10).

	All	Males	Females	p
n	118	41	77	
EF [%]	70.0 ± 8.6	68.8 ± 8.7	70.7 ± 8.5	0.244
LAX [%]	46.6 ± 8.23	45.0 ± 9.6	47.5 ± 7.3	0.146
FS [%]	40.1 ± 7.2	39.3 ± 7.4	40.5 ± 7.1	NS
FSmid [%]	23.1 ± 4.9	22.5 ± 4.6	23.3 ± 5.1	NS

Table 10: Systolic functional echocardiographic parameters in male and female MF patients

1.1.13. Diastolic echocardiographic parameters

There was no significant difference between males and females when considering the diastolic functional parameters, even though IVRT tended to be higher in males compared to females (Table 11).

	All	Males	Females	p
n	118	41	77	
A-wave Vmax [m/s]	0.60 ± 0.15 (0.39 – 0.87) 0.28 – 0.97	0.61 ± 0.15 (0.40 – 0.85) 0.39 – 0.97	0.58 ± 0.15 (0.38 – 0.88) 0.28 – 0.93	NS
E-wave Vmax [m/s]	0.86 ± 0.17 (0.59 – 1.13) 0.52 – 1.5	0.87 ± 0.21 (0.53 – 1.36) 0.52 – 1.5	0.85 ± 0.15 (0.61 – 1.12) 0.57 – 1.18	NS
E/A – Ratio [ ]	1.53 ± 0.48 (0.8 – 2.39) 0.67 – 3.53	1.49 ± 0.45 (0.78 – 2.16) 0.67 – 2.68	1.55 ± 0.5 (0.97 – 2.41) 0.77 – 3.53	NS
n	100	32	68	
IVRT [ms]	86.0 ± 23.2 (49 – 124) 35 – 160	91.3 ± 23.0 (42.9 – 121.4) 39 – 124	83.5 ± 23.1 (49 – 126.2) 35 – 160	0.121

Table 11: Diastolic functional echocardiographic parameters in male and female MF patients

1.1.14. Basic electrocardiographic parameters

There was no significant difference in the P-wave, PQ- and QT- segment between genders (Table 12).

	All	Males	Females	p
n	98	36	62	
P [ms]	101.8± 16.2 (71.9 – 124.1) 62 – 158	102.9 ± 16.5 (71.7 – 129.1) 70 – 158	101.2 ±16.2 (70.9 – 125.7) 62 – 136	NS
n	118	41	77	
PQ [ms]	140.9 ± 25.6 (101.9 – 185.1) 82 – 244	137.1 ± 23.6 (92.4 – 181.8) 82.0 – 185.0	142.9 ± 26.6 (103.8 –192.6) 90 – 244	0.243
QRS [ms]	95.7 ± 16.2 (78 – 122.3) 60 – 184	103.6 ± 18.1 (84.2 – 137.0) 60 – 184	91.6 ± 13.5 (77.8 – 120) 71 – 138	<0.0001
n	117	40	77	
QT [ms]	405.6 ± 37.7 (345.4 – 476.8) 320 – 510	405.1 ± 40.2 (336.2 – 503.7) 334 – 510	405.9 ± 36.5 (347.8 – 476.8) 320 – 508	NS
QTc [ms]	414.6 ± 27.6 (374.8 – 474.2) 362 – 494	406.9 ± 32.3 (363 – 480.3) 362 – 485	418.6 ± 24.1 (384.9 – 474.2) 375 – 494	0.049

Table 12: Basic electrocardiographic parameters in male and female MF patients

Yet of the patients with a P-wave longer than 120ms, 80% were females and 20% were males. The QRS segment was significantly longer in males, 71% of patients with a QRS segment that was longer than 120ms were males, 29% were females (Table 13). On the contrary, the QTc segment was longer in females.

	All	Males	Females	p
n	98	36	62	
P </= 120ms	88	34 (39%)	54 (61%)	NS
P > 120ms	10	2 (20%)	8 (80%)	NS
n	118	41	77	
QRS > 120ms	7	5 (71%)	2 (29%)	NS

Table 13: ECG P-wave and QRS duration in male and female MF patients

Regarding the amplitudes of the ECG, R in V5 and S in V3 were significantly higher in males than in females, which, when considering that R in V5 and S in V3 are variables used for the calculation of hypertrophy, corresponds well with the increased number of male patients with cardiomyopathy compared to in female patients. S in V1, S in V3 and angle alpha did not significantly differ between genders (Table 14).

	All	Males	Females	p
n	95	34	61	
RV5 [mV]	2.1 ± 0.9 (0.9 – 3.5) 0.1 – 5.3	2.7 ± 0.9 (1.3 – 4.6) 1.3 – 5.3	1.8 ± 0.7 (0.7 – 3.4) 0.1 – 3.5	<0.0001
SV1 [mV]	1.2 ± 0.5 (0.4 – 2.0) 0.2 – 3.2	1.3 ± 0.6 (0.5 – 3.1) 0.3 – 3.2	1.1 ± 0.4 (0.3 – 1.9) 0.2 – 2.0	0.288
RaVL [mV]	0.5 ± 0.5 (0.1 – 1.4) 0.0 – 2.7	0.6 ± 0.6 (0.1 – 2.1) 0.0 – 2.6	0.5 ± 0.5 (0.1 – 1.3) 0.0 – 2.7	NS
SV3 [mV]	1.1 ± 0.7 (0.2 – 2.4) 0.0 – 2.9	1.4 ± 0.7 (0.4 – 2.9) 0.3 – 2.9	0.9 ± 0.5 (0.1 – 2.2) 0.0 – 2.5	0.001
n	84	30	54	
Angle alpha [°]	41 ± 32 (-28 – 82) -52 – 95	38 ± 37 (-51 – 82) -52 – 83	43 ± 29 (-23 – 82) -36 – 95	NS

Table 14: ECG: R- and S- amplitudes and angle alpha in male and female MF patients

1.1.15. Calculated electrocardiographic parameters

All the calculated parameters differed significantly between men and women, except for the corrected Cornell index and the corrected Cornell product. Although a tendency of higher values for the corrected Cornell product in men remained. Interestingly, women presented with higher values for the corrected Cornell index, contrasting to the lower values of the Cornell index. The Sokolow-Lyon index was higher in men with a mean value of 4.0mV compared to women with 2.9mV. Furthermore, hemizygous men presented with significantly higher values for the Cornell index, Cornell product, 12 lead and 123 lead than heterozygous women

(Table 15). The Tend-P interval, which was incorporated in the ECG-Index and the ECG-Index itself both showed significantly higher values in men (Table 16).

	All	Males	Females	p
n	95	34	61	
Sokolow Lyon Index [mV]	3.2 ± 1.14 (1.6 – 5.1) 1.1 – 7.0	4.0 ± 1.14 (1.9 – 6.6) 1.7 – 7.0	2.9 ± 0.96 (1.46 – 4.9) 1.1 – 5.2	<0.0001
Cornell Index [mV]	1.63 ± 0.82 (0.47 – 2.84) 0.2 – 5.5	2.02 ± 0.90 (0.8 – 3.85) 0.8 – 5.5	1.42 ± 0.68 (0.31 – 2.7) 0.2 – 3.3	<0.0001
Corrected Cornell Index [mV]	2.15 ± 0.79 (0.98– 3.46) 0.8 – 5.5	2.07 ± 0.94 (0.8– 3.85) 0.8 – 5.5	2.2 ± 0.69 (1.1–3.50) 1.0 – 4.1	NS
Cornell Product [mV*ms]	163.5 ± 103.4 (42 – 314.4) 14.4 – 748	212.3 ± 118.3 (78 – 421) 76.8 – 748	136.2 ± 83.3 (28.8 – 322.3) 14 – 396	<0.0001
Corrected Cornell Product [mV*ms]	211.8 ± 102.5 (85.7 – 411.7) 72 – 748	216.8 ± 119.8 (78 – 421) 76.8 – 748	209.1 ± 92.5 (102.1 – 419.1) 72.0 – 492	NS
12 lead [cm]	24.4 ± 5.8 (12.5 – 32.9) 11.8 – 35.4	24.4 ± 5.8 (12.5 – 32.9) 11.8 – 35.4	17.9 ± 5.9 (11.3 – 31.3) 8.8 – 40.1	<0.0001
123 lead [cm]	4.3 ± 1.3 (2.1 – 6.7) 1.9 – 7.7	4.3 ± 1.3 (2.1 – 6.7) 1.9 – 7.7	3.6 ± 1.3 (2.0 – 6.9) 1.5 – 9.1	0.012

Table 15: Calculated electrocardiographic parameters in male and female MF patients

	All	Males	Females	p
n	118	41	77	
Tend-P [ms]	409.2 ± 147.0 (198.6 – 681.1) 58.9 – 840	469.7 ± 161.3 (216.5 – 792.8) 136.3 – 840	377.0 ± 128.6 (195.0 – 590.6) 58.9 – 670	0.001
ECG-Index [yrs-1]	0.09 ± 0.051 (0.024 – 0.185) 0.015 – 0.232	0.113 ± 0.054 (0.023 – 0.214) 0.020 – 0.232	0.078 ± 0.045 (0.02 – 0.21) 0.015 – 0.215	0.001

Table 16: Calculated electrocardiographic parameters in male and female MF patients

Comparing patients with and without cardiomyopathy

Cardiomyopathy was defined as a LVM indexed by height (LVM_h) ≥50g/m^2.

Cardiomyopathy		No	Yes	Total
Gender	Males	8 19.5%	33 80.5%	41 100%
	Females	36 46.7%	41 53.3%	77 100%
Total		44 37.3%	74 62.7%	118 100%

Table 17: Male and female MF patients with and without cardiomyopathy

Of the 118 Morbus Fabry patients 74 patients suffered from cardiomyopathy. In females the distribution of affected (53.3%) versus not affected (46.7%) by cardiomyopathy was evenly spread. In males 33 out of 41 (80.5%) Fabry patients suffered from cardiomyopathy (Table 17). This once more illustrates the crucial difference in gender.

1.1.16. Geometry of the left ventricle

The different forms of cardiomyopathy of the left ventricle were studied (Table 18). Hypertrophic cardiomyopathy was the most common form in this patient collective. In males, it accounted for 50% and in females for 39.4% of the cases. Eccentric hypertrophy was more common than concentric remodeling, whereas in males eccentric hypertrophy was twice (28.3%) as common compared to females (13.3%).

Geometry of LV		Normal	CR	CH	EH	Total
Gender	Males	6	2	21	12	41
		17.4%	4.3%	50%	28.3%	100%
	Females	27	9	31	10	77
		34.9%	12%	39.8%	13.3%	100%
Total		33	11	52	22	118
		28.0%	9.3%	44.1%	18.6%	100%

Table 18: Different forms of hypertrophy in male and female MF patients

1.1.17. Basic clinical data and cardiac parameters

Patients with cardiomyopathy were significantly, on average 11.1 years older than patients without cardiomyopathy, which underlines the later onset of the cardiac manifestation. Patients with cardiomyopathy tended to be heavier and smaller than patients without cardiomyopathy. There was no significant difference in the body surface area between the two groups, yet the BMI was significantly larger in patients with cardiomyopathy with an average BMI of 24.6 ± 4.6 kg/m² compared to patients without cardiomyopathy with an average of 22.1 ± 3.8 kg/m² (Table 19).

In patients with severe cardiomyopathy, defined as LVM_h≧ 75g/$m^{2.7}$, there was an even more distinct difference in age between the groups with and without severe cardiomyopathy, on average patients with severe cardiomyopathy were 16 years older than patients without severe cardiomyopathy. There was no significant difference in weight and BSA between the two groups, yet patients with severe cardiomyopathy were significantly taller and had a larger BMI than the compared group (Table 20).

Cardiomyopathy	No	Yes	p
n	44	74	
Age [yrs]	31.2 ± 10.1 (18.1 – 49.4) 18.1 – 59.6	42.3 ± 14.1 (22.9 – 68.2) 18.2 – 70.8	<0.0001
Weight [kg]	64.2 ± 12.5 (50.8 – 89.8) 47.1 – 107.5	69.1 ± 13.4 (47.8 – 96) 46 – 101.2	0.051
Length [cm]	170.2 ± 8 (160 – 186) 158 – 194.5	167.8 ± 9.5 (153.8 – 184.5) 148.5 – 189	0.151
BSA [m²]	1.71 ± 0.17 (1.5 – 2) 1.49 – 2.36	1.75 ± 0.18 (1.47 – 2.07) 1.43 – 2.22	0,296
BMI [kg/m²]	22.1 ± 3.8 (16.8 – 30.8) 16.6 – 31.8	24.6 ± 4.6 (18 – 35.2) 15.9 – 37.4	0.003

Table 19: Basic clinical data in MF patients with and without cardiomyopathy

Severe cardiomyopathy (LVM_h ≧ 75g/m$^{2.7}$)	No	Yes	p
n	87	31	
Age [yrs]	34 ± 11.4 (18.2 – 58.1) 18.1 – 64.6	50 ± 13.3 (28 – 70.6) 26.8 – 70.8	<0.0001
Weight [kg]	66.1 ± 12.4 (50.3 – 89.5) 46 – 107.5	70.5 ± 15.1 (47.6 – 98.1) 47 – 101.2	0.111
Length [cm]	169.9 ± 8.5 (157.5 – 186) 154 – 194.5	165.4 ± 9.7 (148.6 – 183) 148.5 – 184	0.018
BSA [m²]	1.73 ± 0.17 (1.49 – 2.02) 1.44 – 2.36	1.74 ± 0.19 (1.45 – 2.11) 1.43 – 2.16	NS
BMI [kg/m²]	22.9 ± 3.9 (16.9 – 31) 15.9 – 31.8	25.8 ± 5.3 (17.9 – 36.4) 17.8 – 37.4	0.002

Table 20: Basic clinical data in MF patients with and without severe cardiomyopathy

Cardiomyopathy	No	Yes	p
n	44	74	
RRsys [mmHg]	121.1 ± 9 (108 – 134.8) 102 – 142	131.6 ± 16.3 (104.5 – 158.3) 84 – 167	<0.0001
RRdias [mmHg]	73.3 ± 9 (59.5 – 93.3) 56 – 97	73.3 ± 9.8 (55.8 – 89.3) 50 – 98	NS
RRmid [mmHg]	89.2 ± 8 (76.4 – 105.4) 74.7 – 107	92.7 ± 10.3 (74.1 – 109.9) 64.7 – 118	0.056
n	42	72	
HF [s^{-1}]	74.2 ± 13.4 (53.3 – 101.7) 50 – 106	65.6 ± 13.6 (50.7 – 90.7) 44 – 134	0.001

Table 21: Basic cardiac parameters in MF patients with and without cardiomyopathy

As established in the previous chapter, there was no significant difference in blood pressure between genders, which leads to the conclusion that the effect of cardiomyopathy on blood pressure disregards gender. In patients suffering from cardiomyopathy the average systolic blood pressure is significantly higher by on average 10.5 mmHg. The mean arterial blood pressure tended to also be higher in patients with cardiomyopathy, whereas the heart rate was significantly lower in this group. There was no significant difference in diastolic blood pressures between the two groups (Table 21).

Patients with severe LVH had a significantly higher systolic blood pressure, on average 10.8 mmHg higher than patients without severe LVH. The mean blood pressure was significantly higher and the heart rate significantly lower in patients with severe LVH. There was no significant difference in diastolic blood pressures between the two groups (Table 54).

Of the 44 patients that did not present with cardiomyopathy, 3 (6.8%) patients developed hypertension. In the group with cardiomyopathy 24 out of 74 (32.4%) patients had hypertensive values (Table 22). This leads to the conclusion, that there is a significant link between the development of cardiomyopathy and the subsequent development of higher blood pressures ($p<0.001$).

Hypertension		No	Yes	Total
Cardiomyopathy	No	41	3	44
		93.2%	6.8%	100%
	Yes	50	24	74
		67.6%	32.4%	100%
Total		91	27	118
		77.1%	22.9%	100%

Table 22: MF patients with and without cardiomyopathy and the occurrence of arterial hypertension

1.1.18. Basic echocardiographic parameters

Patients with left ventricular hypertrophy had a significantly larger diameter of the left atrium and aortic root compared to patients without cardiomyopathy (Table 23). Similarly, in patients with severe cardiomyopathy the left atrial diameter was also significantly larger (39 ± 6.3mm) and the aortic root diameter tended to be larger (30 ± 9.1mm) than in the group without severe cardiomyopathy (Table 55).

Cardiomyopathy	No	Yes	p
n	44	72	
Ao [mm]	26.4 ± 4 (20.5 – 34.9) 20.2 – 36.1	28.9 ± 6.8 (21.6 – 37.9) 19.1 – 72	0.031
n	44	74	
LAd [mm]	30.5 ± 4.2 (23.8 – 38.1) 21 – 42	36.2 ± 5.8 (28.4 – 48) 23.8 – 51.3	<0.0001

Table 23: Aortic and LA diameter in MF patients with and without cardiomyopathy

Illustrated in Table 24, the interventricular wall thickness (IVSd and IVSs) as well as the posterior wall thickness (PWd and PWs) in diastole and systole and the right ventricular anterior wall thickness (RVAWd) were significantly larger in patients with cardiomyopathy and exceeded the reference values [228]. Patients with cardiomyopathy showed significantly larger values for LVId and no significant difference between the groups for LVIs, both did not exceed the reference values [245]. When considering the group with severe cardiomyopathy it was apparent that the values for the different wall thicknesses were significantly higher in the group with severe cardiomyopathy, with one exception, the LVIs. There was no significant difference between the group with and without severe cardiomyopathy (Table 56).

Cardiomyopathy	No	Yes	p
n	44	74	
IVSd [mm]	8.5 ± 1.6 (6 – 11.8) 6 – 12	13.2 ± 3.9 (8.3 – 23.2) 8 – 25.2	<0.0001
IVSs [mm]	12.5 ± 2.4 (7.6 – 16.3) 5.8 – 19	17.6 ± 4 (12.1 – 25.6) 11 – 30.3	<0.0001
LVId [mm]	45.2 ± 4.8 (37.4 – 56.6) 36.9 – 61	48.1 ± 5.9 (38.9 – 60) 33.4 – 63	0.006
LVIs [mm]	28.1 ± 4.5 (21.2 – 37.4) 19.9 – 43	28.3 ± 5.2 (20.2 – 39.3) 15.9 – 42	NS
PWd [mm]	9.4 ± 1.7 (7.1 – 11.8) 7 – 16.6	12.9 ± 2.5 (9.2 – 18.8) 8 – 20.1	<0.0001
PWs [mm]	15.6 ± 2.7 (11.9 – 20.5) 9.7 – 21.6	19.3 ± 2.9 (14.9 – 24.1) 13 – 24.9	<0.0001
n	30	45	
RVAWd [mm]	5 ± 1.1 (3.1 – 7.6) 3 – 8	6.6 ± 1.7 (4.8 – 10.5) 4.6 – 13	<0.0001

Table 24: Echocardiographically measured wall thicknesses in MF patients with and without cardiomyopathy

1.1.19. Calculated echocardiographic parameters

Derived from the above parameters the mean ventricular wall thickness, relative wall thickness and left ventricular mass indexed by height were significantly increased in patients with cardiomyopathy. Similarly, the left atrial volume and the left atrial volume indexed by BSA were significantly larger and above the reference value of 24ml/m² in patients with cardiomyopathy compared to patients without cardiomyopathy (Table 25). This was also the case in patients with severe cardiomyopathy (Table 57), moreover the average of the calculated cardiac parameters was unanimously higher in patients with severe cardiomyopathy (LVM_h ≧ 75g/m$^{2.7}$) compared to patients with cardiomyopathy (LVM_h ≧ 50g/m$^{2.7}$).

Cardiomyopathy	No	Yes	p
n	44	74	
MVWT [mm]	8.9 ± 1.4 (6.6 – 11.4) 6.5 – 12.8	13 ± 2.8 (9.4 – 20) 9 – 21.1	<0.0001
RWT [ ]	0.4 ± 0.08 (0.26 – 0.57) 0.24 – 0.63	0.55 ± 0.15 (0.33 – 0.81) 0.3 – 1.01	<0.0001
LVM_h [g/m$^{2.7}$]	39.2 ± 6.9 (25.7 – 49.6) 23.5 – 49.6	79 ± 32.3 (51.9 – 170.7) 50.2 – 201.1	<0.0001
LA_Vol [ml]	30 ± 12.5 (13.4 – 55.5) 9.3 – 74.1	51.2 ± 25.8 (22.9 – 110.3) 13.5 – 135	<0.0001
LA_Vol_i [ml/m²]	17.3 ± 6.5 (8.4 – 30.1) 6.1 – 38.6	29.4 ± 14.7 (13.2 – 65.6) 8.1 – 75.8	<0.0001

Table 25: Calculated echocardiographic parameters in MF patients with and without cardiomyopathy

1.1.20. Systolic echocardiographic parameters

The ejection fraction was significantly higher ($p<0.036$) in patients with cardiomyopathy. The long axis shortening of the left ventricle was significantly lower ($p<0.003$) in patients with cardiomyopathy. Compared to standard values [31] 12.2 % of the patients with cardiomyopathy had a LAX value below the norm, this was the case in merely 4.5% of the patients without CM. The fractional shortening of the heart however was significantly higher in patients with CM, 14.1% of these patients exceeded the maximum norm value, whereas only 1 patient (0.01%) without CM exceeded the maximum norm value. There was no significant difference in FSmid between the two groups (Table 26).

Cardiomyopathy	No	Yes	p
n	44	74	
EF [%]	68 ± 7.5 (54.7 – 79.5) 40.3 – 80.3	71.3 ± 9 (56.8 – 86.9) 51.3 – 88.9	0.036
LAX [%]	49.5 ± 6.7 (35.9 – 60.9) 32.3 – 64.4	44.9 ± 8.7 (24.9 – 56) 23.8 – 65.7	0.003
FS [%]	38.1 ± 5.7 (28.3 – 47.9) 19.6 – 48.9	41.3 ± 7.8 (29.6 – 56.5) 26.5 – 59.4	0.020
FSmid [%]	22.3 ± 3.9 (17.1 – 28.2) 6.5 – 28.4	23.5 ± 5.4 (15.3 – 32.3) 13.4 – 37.8	0.175

Table 26: Systolic functional echocardiographic parameters in MF patients with and without cardiomyopathy

	Number out of 118 patients	Number of these patients that have CM
LAX < 35%	11	9
LAX > 63%	2	1
FS < 28%	2	1
FS >48%	15	14

Table 27: Number of patients with values for LAX>63%, LAX<35% and FS>48%, FS<28%

When comparing patients classified as having severe cardiomyopathy to the patients without severe hypertrophy there was no significant difference in EF. The LAX is significantly lower, and FS merely showed a tendency for higher values in patients with severe hypertrophy. Yet in FSmid there was no longer any difference between the two groups (Table 28).

Severe cardiomyopathy (LVM_h ≥ 75g/m$^{2.7}$)	No	Yes	p
n	87	31	
EF [%]	69.4 ± 7.8 (56.5 – 80.1) 40.3 – 88.9	71.7 ± 10.5 (53.0 – 87.1) 51.3 – 88.2	NS
LAX [%]	48.6 ± 7.1 (36.9 – 60.4) 23.8 – 65.7	41.1 ± 8.8 (24.5 – 53.5) 24.3 – 54.0	<0.0001
FS [%]	39.4 ± 6.4 (29.5 – 48.6) 19.6 – 59.4	41.9 ± 9.0 (27.6 – 57.0) 26.5 – 58.3	0.094
FSmid [%]	22.8 ± 4.3 (15.8 – 29.8) 6.5 – 32.5	23.9 ± 6.3 (14.5 – 36.9) 13.4 – 37.8	NS

Table 28: Systolic functional echocardiographic parameters in MF patients with and without severe cardiomyopathy

1.1.21. Diastolic echocardiographic parameters

The maximum velocity of the A-wave over the mitral valve was higher in patients with CM, whereas there was no difference in the two patient groups regarding the maximum velocity of the E-wave over the mitral valve. Furthermore, the E/A Ratio was significantly lower and the IVRT was significantly ($p<0.0001$) longer in the group with CM (Table 29). In patients with severe cardiomyopathy very similar results were observed (Table 58).

Cardiomyopathy	No	Yes	p
n	44	74	
E-wave Vmax [m/s]	0.86 ± 0.14 (0.62 – 1.12) 0.61 – 1.18	0.86 ± 0.19 (0.57 – 1.15) 0.52 – 1.50	NS
A-wave Vmax [m/s]	0.53 ± 0.12 (0.32 – 0.78) 0.28 – 0.85	0.63 ± 0.15 (0.42 – 0.89) 0.39 – 0.97	<0.0001
E/A-Ratio [ ]	1.71 ± 0.54 (1.04 – 3.06) 0.78 – 3.53	1.42 ± 0.41 (0.78 – 2.14) 0.67 – 2.68	0.001
n	38	62	
IVRT [ms]	73.5 ± 17.0 (38.8 – 108.2) 35 – 112	93.7 ± 23.3 (56.6 – 127.4) 45 – 160	<0.0001

Table 29: Diastolic functional echocardiographic parameters in MF patients with and without cardiomyopathy

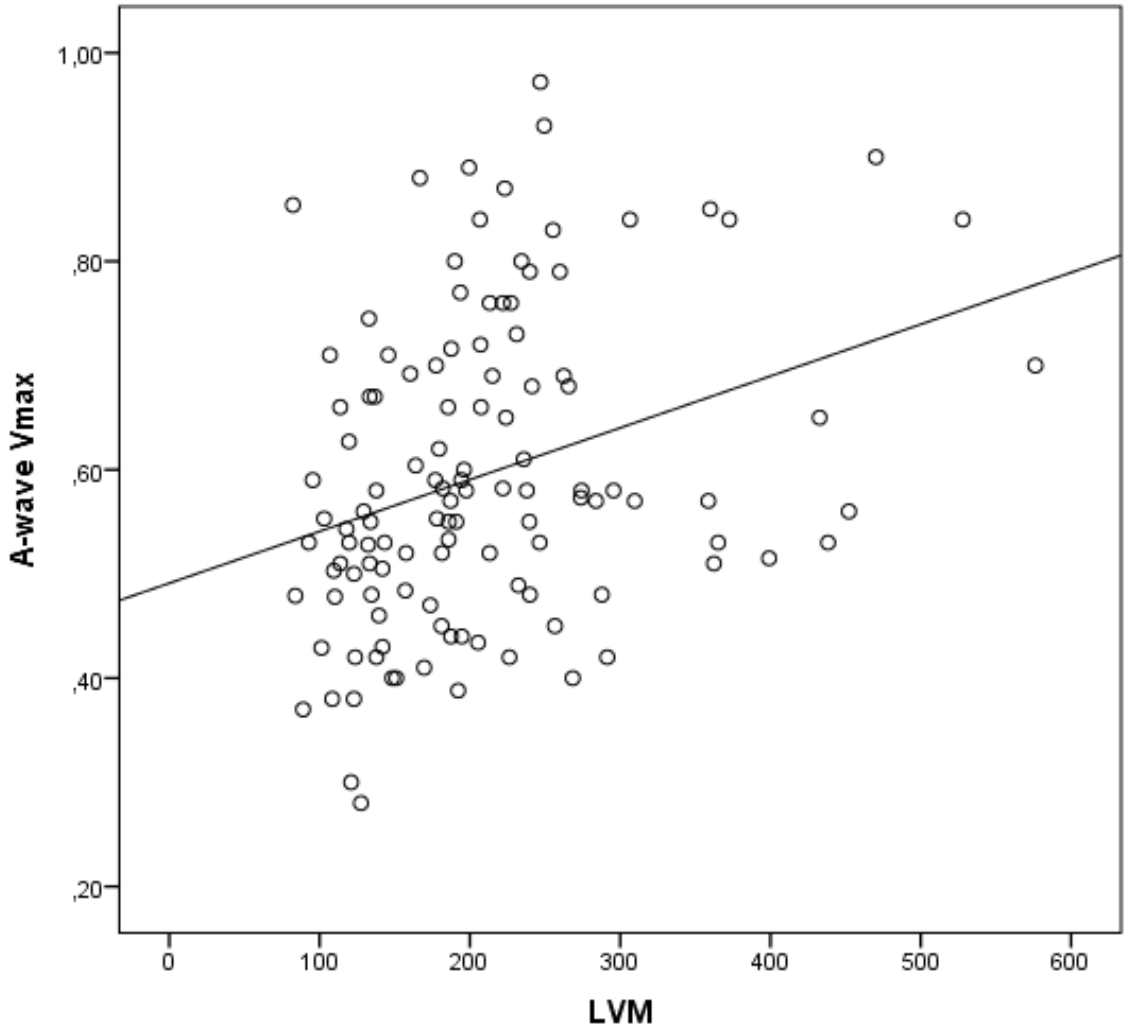

Figure 13: Correlation of the A-wave Vmax [m/s] and LVM [g] in MF patients.

The A-wave Vmax and IVRT strongly correlated with LVM. As the values for the LVM increased, the values for A-wave Vmax (Figure 13) and IVRT (Figure 14) increased, with a correlation coefficient of $r^2 = 0.316$ ($p<0.0001$) and $r^2 = 0.541$ ($p<0.0001$) respectively.

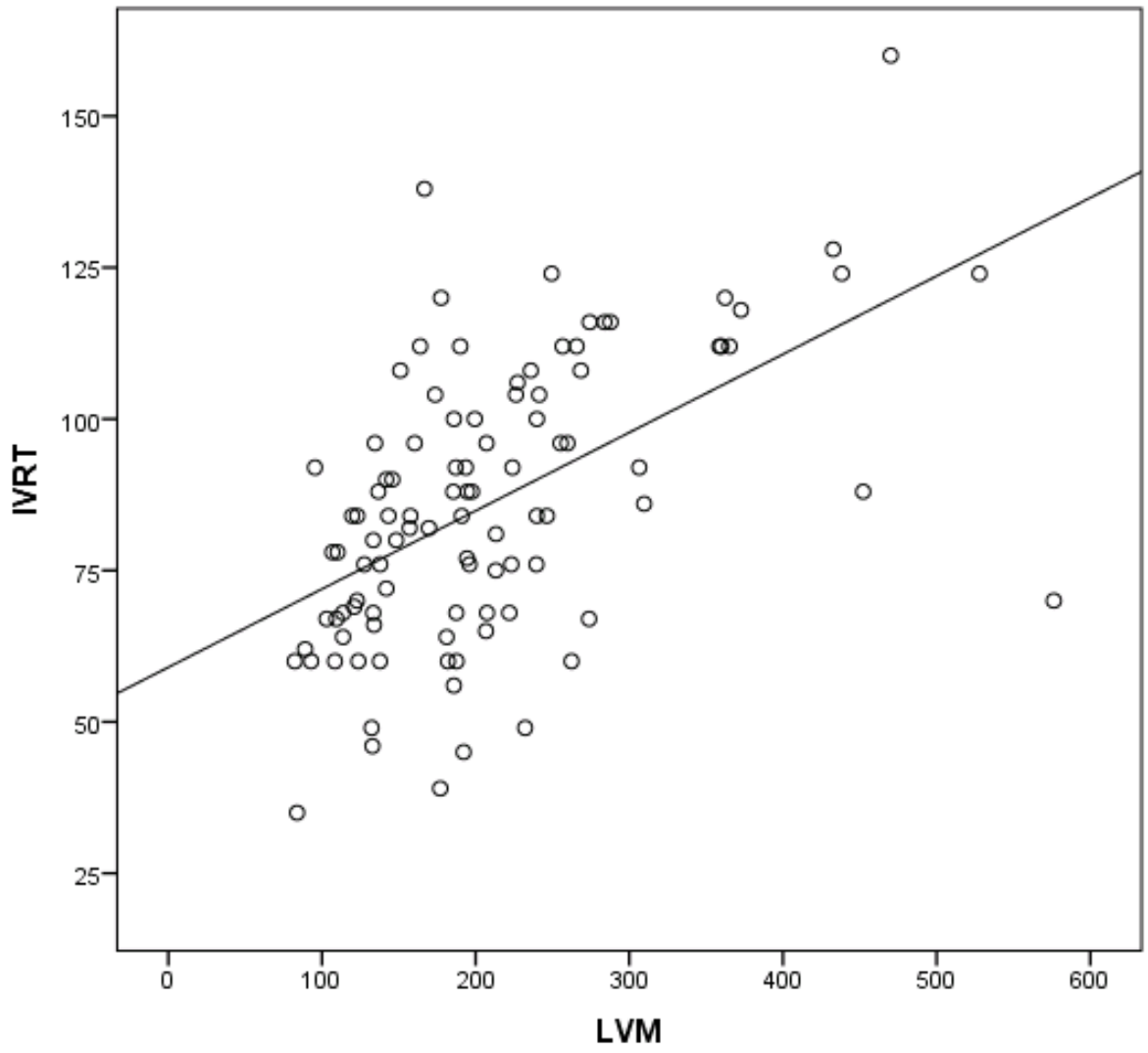

Figure 14: Correlation of the IVRT [ms] and LVM [g] in MF patients.

1.1.22. Basic electrocardiographic parameters

Patients with cardiomyopathy tended to have a longer P-wave and QTc duration, the duration of the P-wave exceeded the norm in patients with cardiomyopathy, whereas the QTc duration was within the norm. Furthermore, patients with cardiomyopathy had a significantly longer QRS and QT duration compared to patients without cardiomyopathy, the values for QRS were just above the maximum values of the norm. There was no significant difference in the PQ duration between the two groups, the values were within the norm (Table 30).

Cardiomyopathy	No	Yes	p
n	36	62	
P [ms]	97.7 ± 17.6 (68.8 – 124) 62 – 158	104.2 ± 15 (73.2 – 125.7) 64 – 136	0.055
n	44	74	
PQ [ms]	139.1 ± 21.2 (104 – 170.8) 100 – 198	142 ± 28 (95 – 187.5) 82 – 244	NS
QRS [ms]	87.1 ± 10.1 (71.3 – 107.5) 60 – 110	100.9 ± 17 (80 – 130.5) 76 – 184	<0.0001
n	43	74	
QT [ms]	388.4 ± 26.1 (333.6 – 429.2) 320 – 431	415.6 ± 39.9 (347.5 – 492.5) 334 – 510	<0.0001
QTc [ms]	409 ± 18.2 (377 – 440) 363 – 453	417.8 ± 31.5 (372.8 – 477.3) 362 – 494	0.058

Table 30: Basic electrocardiographic parameters in MF patients with and without cardiomyopathy

The values for R in V5, R in aVL and S in V3 were significantly higher in patients with cardiomyopathy. The angle alpha was significantly lower in the group of patients with cardiomyopathy compared to patients without cardiomyopathy. Patients with cardiomyopathy tended to have higher values for S in V1 (Table 31).

Cardiomyopathy	No	Yes	p
n	36	59	
RV5 [mV]	1.6 ± 0.6 (0.7 – 2.6) 0.6 – 3.4	2.4 ± 0.9 (1 – 4.2) 0.1 – 5.3	<0.0001
SV1 [mV]	1.1 ± 0.4 (0.3 – 1.7) 0.2 – 1.8	1.3 ± 0.6 (0.6 – 2) 0.3 – 3.2	0.056
RaVL [mV]	0.3 ± 0.2 (0.1 – 0.8) 0 – 0.8	0.7 ± 0.6 (0.1 – 1.9) 0 – 2.7	<0.0001
SV3 [mV]	0.9 ± 0.5 (0.1 – 2) 0 – 2.4	1.2 ± 0.7 (0.3 – 2.5) 0 – 2.9	0.010
n	32	52	
Angle alpha [°]	53 ± 21 (13 – 89) 8 – 95	34 ± 35 ([-41] – 81) [-52] – 83	0.003

Table 31: ECG: R- and S- amplitudes and angle alpha in MF patients with and without cardiomyopathy

1.1.23. Calculated electrocardiographic parameters

Considering the above values and implementing them in the following indexes, it is evident that patients with CM had higher values for the Sokolow Index, Cornell Index, corrected Cornell Index, Cornell Product and the corrected Cornell Product as well as for the 12lead and 123lead, which all indicate hypertrophy (Table 32). Furthermore, the value for Tend-P was increased in patients with cardiomyopathy. The ECG-Index did not yield a significant difference between the two groups (Table 33).

Cardiomyopathy	No	Yes	p
n	40	61	
Sokolow-Lyon Index [mV]	2.4 ± 1.1	3.5 ± 1.3	<0.0001
Cornell Index [mV]	1.06 ± 0.7	1.84 ± 0.86	<0.0001
Cornell Product [mV*ms]	94.9 ± 66.9	192.3 ± 111.9	<0.0001
n	39	61	
Corrected Cornell Index [mV]	1.73 ± 0.6	2.32 ± 0.86	<0.0001
Corrected Cornell Product [mV*ms]	146.6 ± 66.6	236.2 ± 118.4	<0.0001
n	36	59	
12 lead [cm]	16.1 ± 4.3	22.7 ± 6.5	<0.0001
123 lead [cm]	3 ± 0.7	4.4 ± 1.4	<0.0001

Table 32: Calculated electrocardiographic parameters in MF patients with and without cardiomyopathy

Cardiomyopathy	No	Yes	p
n	44	74	
Tend-P [ms]	351.2 ± 143 (103.4 – 595.5) 58.9 – 840	443.6 ± 139.2 (228.1 – 700.3) 186.9 – 795.6	0.001
ECG-Index [yrs-1]	0.09 ± 0.05 (0.02 – 0.18) 0.02 – 0.21	0.09 ± 0.05 (0.02 – 0.19) 0.02 – 0.23	NS

Table 33: Calculated electrocardiographic parameters in MF patients with and without cardiomyopathy

Comparing patients with normal and impaired diastolic function

Out of 17 patients that presented with diastolic dysfunction, 16 (94.1%) also presented with cardiomyopathy. Furthermore, of the patients without DD approximately half (42.6%) of them did not present with CM. Out of the 74 patients with CM 16 (21.6%) also presented with DD, whereas only 1 out of 44 (2%) patients without CM presented with DD. Evidently there is a significant link between CM and the development of DD ($p < 0.004$).

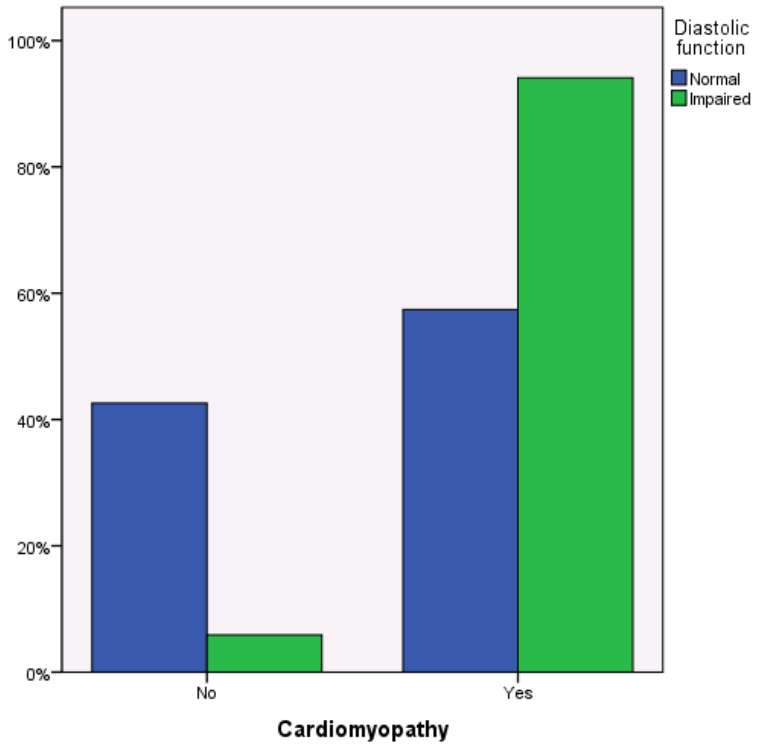

Figure 15: Percentage [%] of patients with or without cardiomyopathy and the occurrence of diastolic dysfunction

In patients with normal diastolic function, the most common geometry of the left ventricle was concentric hypertrophy (37.6%). In patients with impaired diastolic function it was also the most common form of hypertrophy. However, the percentage was much higher at 82.3% (14 out of 17). Not a single patients with impaired diastolic function presented with a normal geometry of the left ventricle (Table 34).

Geometry of LV		Normal	CR	CH	EH	Total
Diastolic function	Normal	33	10	38	20	101
		32.7%	9.9%	37.6%	19.8%	100%
	impaired	0	1	14	2	17
		0%	5.9%	82.3%	11.8%	100%
Total		33	11	52	22	118
		28.0%	9.3%	44.1%	18.6%	100%

Table 34: Different forms of hypertrophy in MF patients with normal and impaired diastolic function

1.1.24. Basic clinical data and cardiac parameters

Patients with diastolic dysfunction were significantly older, taller and have a larger BMI, which is comprised of weight divided by BSA, compared to patients with normal diastolic function. However there was no significant difference between the two groups with regard to the weight and the BSA of the patients (Table 35).

Diastolic function	Normal	Impaired	p
n	101	17	
Age [yrs]	36.0 ± 12.7	51.0 ± 14.0	<0.0001
	18.1 – 68.0	26.8 – 70.8	
Weight [kg]	66.8 ± 13.1	69.8 ± 13.8	NS
	46.0 – 107.5	47.0 – 96.0	
Length [cm]	169.5 ± 8.6	163.8 ± 10.0	0.010
	148.5 – 194.5	148.7 – 184.0	
BSA [m²]	1.74 ± 0.18	1.73 ± 0.19	NS
	1.44 – 2.36	1.43 – 2.16	
BMI [kg/m²]	23.3 ± 4.3	26 ± 4.9	0.018
	15.9 – 35.7	18 – 37.4	

Table 35: Basic clinical data in MF patients with normal and impaired diastolic function

Considering values for the blood pressure, there was no significant difference between the two groups, yet the systolic blood pressure tends to be higher in patients with diastolic dysfunction. This group of patients also showed significantly lower values for the heart rate (Table 36).

Diastolic function	Normal	Impaired	p
n	101	17	
RRsys [mmHg]	126.6 ± 14.5 84 – 161	133.9 ± 16.1 112 – 167	0,144
RRdias [mmHg]	73 ± 9.4 50 – 97	75.1 ± 10.2 60 – 98	NS
RRmid [mmHg]	90.8 ± 9.4 64.7 – 112.7	94.7 ± 10.4 80.7 – 118	NS
n	100	14	
HF [s-1]	69.8 ± 14.4 44 – 134	61.4 ± 8.5 53 – 80	0.023

Table 36: Basic cardiac parameters in MF patients with normal and impaired diastolic function

1.1.25. Basic echocardiographic parameters

The left atrial diameter was significantly ($p<0.0001$) larger in patients with impaired diastolic function, with an average difference of 11.3mm. The aortic root was however only insignificantly ($p< 0.290$) larger in patients with diastolic dysfunction (Table 37). The values for IVSd, IVSs, PWd, PWs, the ventricular wall thicknesses, of patients with impaired diastolic function were significantly higher compared to patients with normal diastolic function. However, the right ventricular anterior wall thickness and the left ventricular diameter in systole and diastole did not differ significantly between the two groups (Table 38).

Diastolic function	Normal	Impaired	p
n	99	17	
Ao [mm]	27.8 ± 6.2 19.1 – 72	28.7 ± 4.6 21.7 – 38.4	0.290
n	101	17	
LAd [mm]	32.5 ± 4.5 21 – 49	43.8 ± 3.5 37.4 – 51.3	<0.0001

Table 37: Aortic and LA diameter in MF patients with normal and impaired diastolic function

Diastolic function	Normal	Impaired	p
n	101	17	
IVSd [mm]	10.7 ± 3.2 6 – 23.1	15.6 ± 5 8 – 25.2	<0.0001
IVSs [mm]	14.9 ± 3.7 5.8 – 25.5	20.3 ± 4.5 15 – 30.3	<0.0001
LVId [mm]	46.9 ± 5.3 36.8 – 61	47.8 ± 7.6 33.4 – 63	NS
LVIs [mm]	28 ± 4.6 15.9 – 43	28.9 ± 6.6 20.2 – 42	NS
PWd [mm]	11.2 ± 2.6 7 – 20.1	14.1 ± 2.8 10 – 18.8	<0.0001
PWs [mm]	17.6 ± 3.4 9.7 – 24.9	19.8 ± 2.6 16.3 – 24	0.010
n	64	11	
RVAWd [mm]	5.8 ± 1.6 3 – 13	6.8 ± 2.2 4 – 10.5	0.179

Table 38: Echocardiographically measured wall thicknesses in MF patients with normal and impaired diastolic function

1.1.26. Calculated echocardiographic parameters

The relative and mean values for the wall thickness (MVWT and RWT) were both significantly higher in patients with impaired diastolic dysfunction. The left ventricular mass indexed by height also showed significantly ($p<0.0001$) higher values in patients with impaired diastolic function. The volume of the left atrium and the left atrial volume indexed by BSA were also significantly ($p<0.0001$) elevated in patients with impaired diastolic function (Table 39).

Diastolic function	Normal	Impaired	p
n	101	17	
MVWT [mm]	10.9 ± 2.7 6.5 – 20	14.9 ± 3.3 9.5 – 21.1	<0.0001
RWT [ ]	0.47 ± 0.13 0.24 – 0.8	0.64 ± 0.18 0.32 – 1.01	<0.0001
LVM_h [$g/m^{2.7}$]	57.5 ± 23.2 23.5 – 157.3	103.8 ± 48.1 47.4 – 201.1	<0.0001
LA_Vol [ml]	36.2 ± 16 9.3 – 117.6	85.4 ± 20.7 52.3 – 135	<0.0001
LA_Vol_i [ml/m^2]	20.7 ± 8.5 6.1 – 63	49.6 ± 11.9 36.5 – 75.8	<0.0001

Table 39: Calculated echocardiographic parameters in MF patients with normal and impaired diastolic function

1.1.27. Systolic echocardiographic parameters

Merely the LAX was significantly shorter in patients with DD compared to patients with a normal diastolic function. The values for the other markers for systolic function did not differ significantly between the two groups (Table 40).

Diastolic function	Normal	Impaired	p
n	101	17	
EF [%]	70.2 ± 8.3 40.3 – 88.9	69.1 ± 10.6 51.3 – 82.8	NS
LAX [%]	47.6 ± 7.5 23.8 – 65.7	40.8 ± 10.4 24.2 – 55.3	0.015
FS [%]	40.2 ± 7 19.6 – 59.4	39.6 ± 8.6 26.5 – 51.8	NS
FSmid [%]	23.3 ± 4.8 6.5 – 37.8	21.8 ± 5.3 13.4 – 32.3	NS

Table 40: Systolic functional echocardiographic parameters in MF patients with normal and impaired diastolic function

1.1.28. Diastolic echocardiographic parameters

Considering the markers for diastolic function, the A-wave Vmax was increased and the E/A ratio was decreased in patients with impaired diastolic function compared to patients with normal diastolic function. No significant difference could be noted between the two groups with and without DD regarding the E-wave Vmax and the IVRT (Table 41).

Diastolic function	Normal	Impaired	p
n	101	17	
E-wave Vmax [m/s]	0.86 ± 0.16 0.52 – 1.38	0.89 ± 0.21 0.65 – 1.5	NS
A-wave Vmax [m/s]	0.58 ± 0.15 0.28 – 0.97	0.67 ± 0.13 0.48 – 0.9	0.017
E/A-Ratio [ ]	1.56 ± 0.49 0.67 – 3.53	1.37 ± 0.43 0.92 – 2.68	0.074
n	86	14	
IVRT [ms]	84.5 ± 22.2 35 – 138	95.1 ± 27.9 60 – 160	0.259

Table 41: Diastolic functional echocardiographic parameters in MF patients with normal and impaired diastolic function

1.1.29. Basic electrocardiographic parameters

In this study, no significant difference in the duration of the P-wave and PQ segment was noted between patients with and without diastolic dysfunction. The QRS duration was within the norm in both groups, however significantly longer in patients with DD. This was also the case with regards to the QT and QTc duration which were longer in patients with DD compared to patients with normal diastolic function (Table 42).

Diastolic function	Normal	Impaired	p
n	82	16	
P [ms]	101.3 ± 15.5 62 – 158	104.1 ± 19.9 72 – 136	NS
n	101	17	
PQ [ms]	140.9 ± 22.9 96 – 200	140.7 ± 39 82 – 244	NS
QRS [ms]	93.4 ± 13.4 60 – 138	109.8 ± 23.5 86 – 184	0.001
n	100	17	
QT [ms]	399.9 ± 34.6 320 – 510	439.4 ± 38.1 383 – 508	<0.0001
QTc [ms]	411.1 ± 25.3 362 – 481	435.1 ± 32.2 373 – 494	0.003

Table 42: Basic electrocardiographic parameters in MF patients with normal and impaired diastolic function

Considering the amplitudes, R in V5 tended to be higher ($p<0.059$) and R in aVL was significantly ($p<0.0001$) higher in patients with impaired diastolic function. There was no significant difference in S in V1 and S in V3 between the two groups compared. The angle alpha however was significantly ($p<0.009$) reduced in the group of patients with impaired diastolic function (Table 43).

Diastolic function	Normal	Impaired	p
n	81	14	
RV5 [mV]	2 ± 0.9 0.1 – 5.3	2.5 ± 1 1 – 4.2	0.059
SV1 [mV]	1.2 ± 0.5 0.2 – 3.2	1.2 ± 0.5 0.6 – 2	NS
RaVL [mV]	0.4 ± 0.4 0 – 2.6	1 ± 0.7 0.2 – 2.7	<0.0001
SV3 [mV]	1.1 ± 0.7 0 – 2.9	1 ± 0.7 0 – 2.4	NS
n	74	10	
Angle alpha [°]	44 ± 32 -52 – 95	20 ± 27 -18 – 68	0.009

Table 43: ECG: R- and S- amplitudes and angle alpha in MF patients with normal and impaired diastolic function

1.1.30. Calculated electrocardiographic parameters

Patients with diastolic dysfunction tended to have higher values for the Sokolow Index, Cornell Index, corrected Cornell Index, Cornell product and corrected Cornell Product, as well as 123lead. Furthermore, there was a tendancy for higher values in 12lead in patients with diastolic dysfunction(Table 44). The values for Tend-P were significantly higher in patients with DD, whereas the ECG-Index did not differ between the two patient groups (Table 45).

Diastolic function	Normal	Impaired	p
n	87	14	
Sokolow-Lyon Index [mV]	3 ± 1.4 0 – 7	3.8 ± 1.1 1.8 – 5.2	0.024
Cornell Index [mV]	1.45 ± 0.9 0 – 5.5	2.07 ± 0.5 1.2 – 2.8	0,002
Cornell Product [mV*ms]	141 ± 107.8 0 – 748	233 ± 64.1 124.7 – 324	<0.0001
n	86	14	
Corrected Cornell Index [mV]	2 ± 0.81 0.8 – 5.5	2.64 ± 0.67 1.2 – 3.5	0.002
Corrected Cornell Product [mV*ms]	186 ± 107.1 0 – 748	295.1 ± 79.9 124.7 – 420	<0.0001
n	81	14	
12 lead [cm]	19.6 ± 6.2 8.8 – 35.4	23.8 ± 8.1 11.8 – 40.1	0.072
123 lead [cm]	3.7 ± 1.2 1.5 – 7.7	4.8 ± 1.9 2.2 – 9.1	0.015

Table 44: Calculated electrocardiographic parameters in MF patients with normal and impaired diastolic function

Diastolic function	Normal	Impaired	p
n	101	17	
Tend-P [ms]	397.4 ± 144.8 58.9 – 840	479.3 ± 144.7 255.9 – 795.6	0.023
ECG-Index [yrs-1]	0.09 ± 0.05 0.02 – 0.22	0.08 ± 0.06 0.02 – 0.23	NS

Table 45: Calculated electrocardiographic parameters in MF patients with normal and impaired diastolic function

Cardiac involvement and clinical effects

1.1.31. NYHA

The patients were categorized according to the New York Heart Association (NYHA) classification.

NYHA Grade	Description
1	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnoea.
2	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnoea.
3	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnoea.
4	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

Table 46: NYHA grading {Herold, 2016 #868}

In this study all patients without cardiomyopathy had either no symptoms at all or merely mild shortness of breath, palpitation, fatigue and slight limitations during physical activity (NYHA≤2). However, in patients with CM, 77% (57 out of 74) were classified in NYHA≤2. Furthermore 23% (17 out of 74) of patients with CM presented with NYHA scores of ≥3. This means these patients experienced mild to severe limitations during minimal physical activity (like walking short distances) or symptoms at rest (NYHA≥3) (Table 47).

NYHA		≤ 2	3	4	Total
Cardiomyopathy	No	44 100%	0 0.0%	0 0.0%	44 100%
	Yes	57 77.0%	16 21.6%	1 1.4%	74 100%
Total		101 85.5%	16 13.5%	1 1%	118 100%

Table 47: NYHA Scores of MF patients with and without cardiomyopathy

As illustrated in Table 48 all patients with a normal geometry of the left ventricle, concentric remodelling or eccentric hypertrophy had no or mild symptoms during ordinary activity (NYHA scores ≤2). Yet out of 52 patients diagnosed with concentric hypertrophy 17 (17.5%) experienced severe limitations during minimal activity or in rest (NYHA scores ≥3).

NYHA		≤2	3	4	Total
Geometry of LV	Normal	33 100%	0 0.0%	0 0.0%	33 100%
	CR	11 12.9%	0 0.0%	0 0.0%	11 100%
	CH	35 67.3%	16 30.7%	1 2%	52 100%
	EH	22 100%	0 0.0%	0 0.0%	22 100%
Total		101 85.6%	16 13.6%	1 0.8%	118 100%

Table 48: LV Geometry in MF patients in relation to NYHA scores

The majority of patients with normal diastolic function presented with only mild symptoms of heart insufficiency (NYHA≤2). Merely 8.9% of patients with a normal diastolic function had NYHA scores of ≥3. In contrast 47% of the patients diagnosed with impaired diastolic function reported symptoms compatible with NYHA scores of ≥3 (Table 49).

NYHA		≤ 2	3	4	Total
Diastolic function of the LV	Normal	92 91.1%	8 7.9%	1 1%	101 100%
	Impaired	9 53%	8 47%	0 0.0%	17 100%
Total		101 85.6%	16 13.6%	1 0.8%	118 100%

Table 49: Normal and impaired diastolic function in MF patients in relation to NYHA scores

1.1.32. CCS

The Canadian Cardiovascular Society (CCS) score records the occurrence and degree of angina pectoris at different levels of activity.

CCS Grade	Description
0	No cardiac symptoms.
1	Ordinary physical activity (such as walking and climbing stairs) does not cause angina. Angina with strenuous or rapid or prolonged exertion at work or recreation.
2	Slight limitation of ordinary physical activity.
3	Marked limitation of ordinary physical activity.
4	Inability to carry on any physical activity without discomfort, anginal syndrome may be present at rest.

Table 50: CCS grading {Campeau, 1976 #865{Campeau, 2002 #1072}}

In the group of patients with cardiomyopathy roughly 47.9% suffered from angina during activity and experienced slight to marked limitations in daily life (CCS=1-3). The other half of patients was asymptomatic. However, in patients without CM 90.3% (37 out of 41) of patients were asymptomatic (Table 51).

CCS		0	I	2	3	Total
Cardiomyopathy	No	37	2	1	1	41
		90.3%	4.9%	2.4%	2.4%	100%
	Yes	38	13	9	13	73
		52.1%	17.8%	12.3%	17.8%	100%
Total		75	15	10	14	114
		65.8%	13.1%	8.8%	12.3%	100%

Table 51: CCS Scores of MF patients with and without cardiomyopathy

Of the patients with a normal geometry of the ventricle, one patient reported angina pectoris during strenuous physical activity (CCS=2) and one patient during moderate activity (CCS=1). The remaining 29 patients did not report angina (CCS=0). 4 patients with eccentric hypertrophy suffered from angina during strenuous physical activity, the remaining 18 patients did not have angina. Most (8 out of 10) of the patients with a concentric remodelling had a CCS score of 0. The majority of patients (60.8%) with concentric hypertrophy illustrated clinical symptoms such as angina during moderate, strenuous and vigorous activity(CCS=1-3).

Of the 114 patients in total, 14 reported a score of CCS=3 of which all of them had concentric hypertrophy, with the exception of one patient that had concentric remodelling. 9 out of the 10 patients that reported angina consistent with CCS=2 were likewise found to have concentric hypertrophy. The majority of patients (9 out of 15) that reported a score of CCS=1, also had a left ventricle which was concentrically hypertrophied, the other 4 out of 15 had eccentric hypertrophy (Table 52).

CCS		0	I	2	3	Total
Geometry of LV	Normal	29	1	1	0	31
		93.6%	3.2%	3.2%	0%	100%
	CR	8	1	0	1	10
		80%	10%	0%	10%	100%
	CH	20	9	9	13	51
		39.2%	17.65%	17.65%	25.5%	100%
	EH	18	4	0	0	22
		81.8%	18.2%	0%	0%	100%
Total		75	15	10	14	114
		65.8%	13.2%	8.8%	12.2%	100%

Table 52: LV Geometry in MF patients in relation to CCS scores

In patients with impaired diastolic function 56.2% of patients complained of angina during daily activities or vigorous physical activity (CCS 2-3). Whereas this was only the case in 15.3% of patients with a normal diastolic function (Table 53).

CCS		0	I	2	3	Total
Diastolic function of the LV	Normal	69	14	6	9	98
		70.4%	14.3%	6.1%	9.2%	100%
	Impaired	6	1	4	5	16
		37.5%	6.3%	25%	31.2%	100%
Total		75	15	10	14	114
		65.8%	13.1%	8.8%	12.3%	100%

Table 53: Normal and impaired diastolic function in MF patients in relation to CCS score

ECG-Index, ECG changes as a sign of diastolic dysfunction

In the following chapter the relationship between the ECG-Index and age, LVM, MVWT, RWT and diastolic echocardiographic parameters was examined. Furthermore, the ECG-Index and further electrocardiographic parameters were examined by means of binary logistic regression regarding the presence of DD.

1.1.33. Correlation of the ECG-Index and age

Figure 16 shows that the ECG-Index decreased with increasing age and presented with a correlation coefficient of $r^2 = -0.595$ ($p<0.0001$).

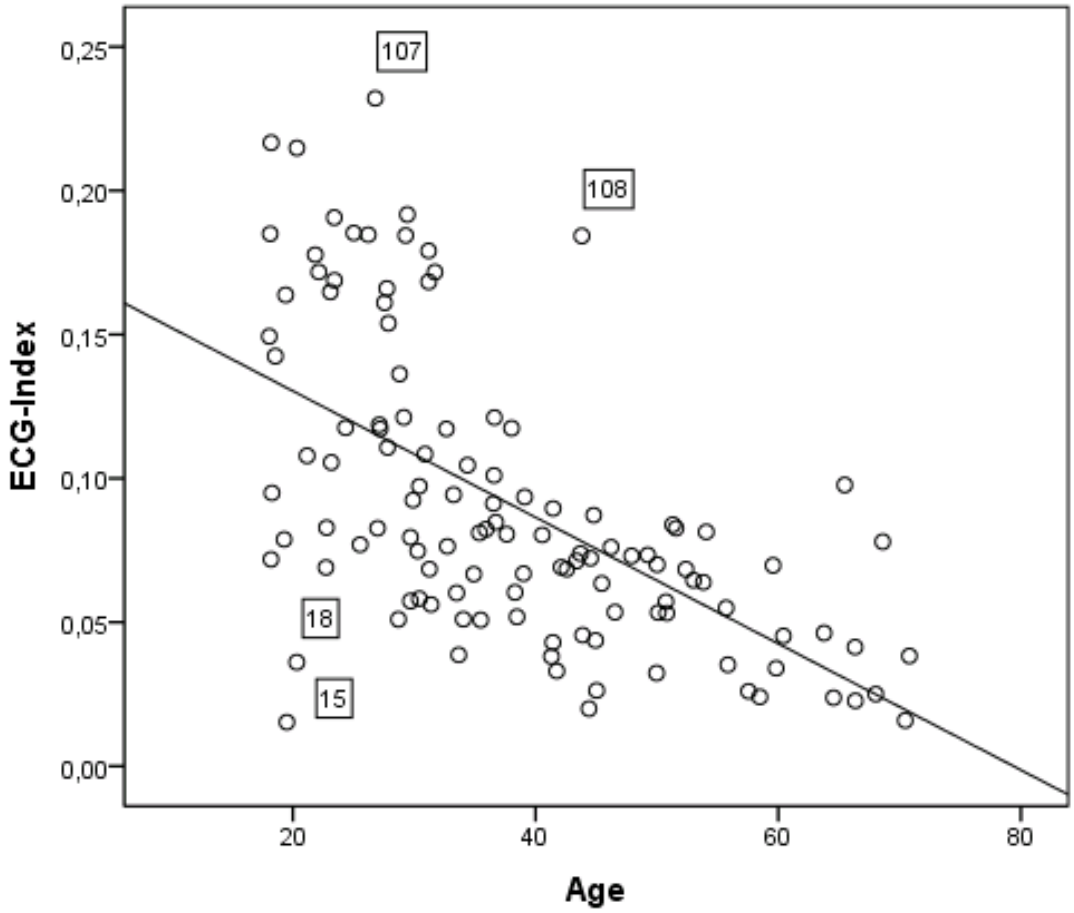

Figure 16: Correlation of the ECG-Index [ ] and age [yrs] in MF patients.

The two outliers above the line were men and both presented with CH, left atrial dilation and DD. One patient had a reduced LAX (38%) and NYHA score of 3, whereas the other did not have impaired systolic function and reported a NYHA score of 1.

The two outliers below the line were two females and had no systolic or diastolic functional impairment, except for a marginally decreased LAX of 43% in one patient. Furthermore they did not present with LV hypertrophy or LA dilation, yet both reported a NYHA score of 1.

There is no visible pattern of lower values for the ECG-Index pointing specifically to DD, systolic impairment or clinical signs as apparent from these figures, however the correlation between the ECG-Index and age remains highly significant.

1.1.34. Correlation of the ECG-Index and left ventricular mass

In this group of Fabry patients there was no significant correlation between the ECG-Index and LVM (Figure 17), with a correlation coefficient of $r^2 = -0.011$ ($p<0.906$).

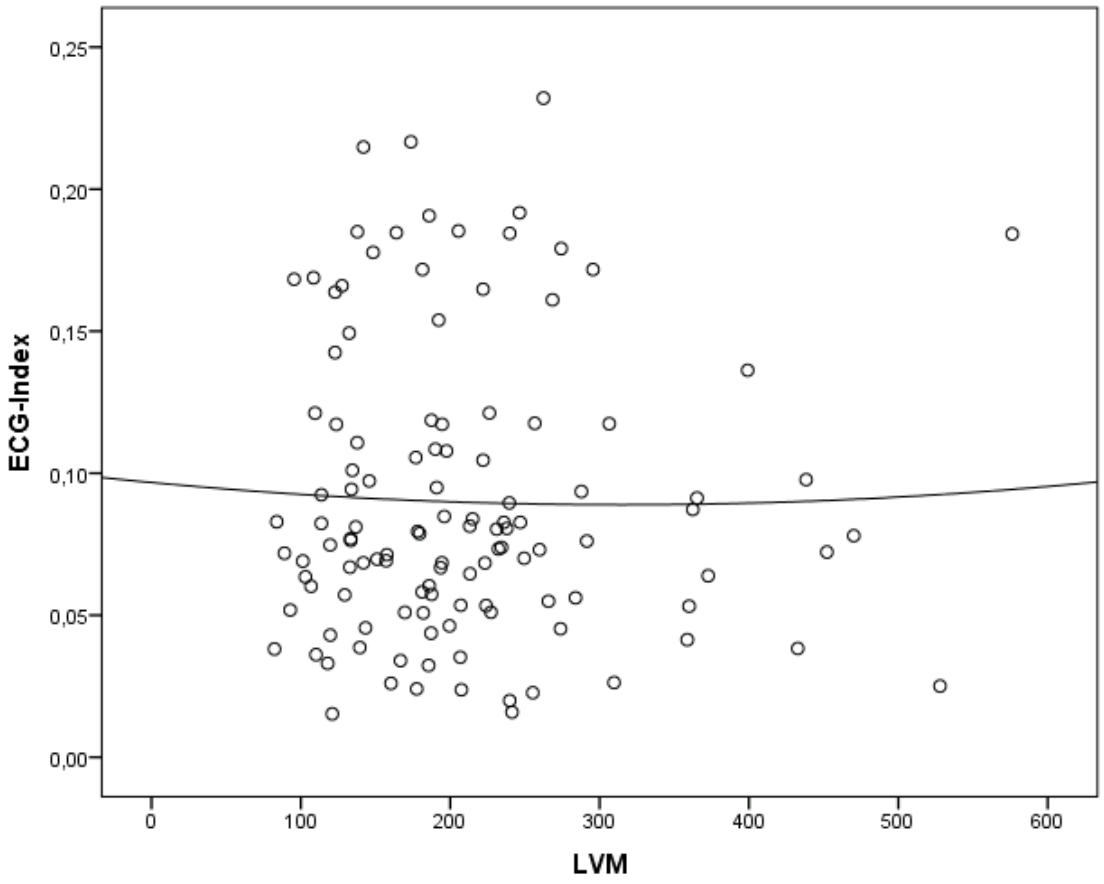

Figure 17: Correlation of the ECG-Index [ ] and LVM [g] in MF patients.

1.1.35. Correlation of the ECG-Index and mean left ventricular wall thickness

There was a weak correlation between the ECG-Index and MVWT (Figure 18), with a correlation coefficient of r^2 = -0.174 ($p<0.060$). As the MVWT increased the value for the ECG-Index decreased.

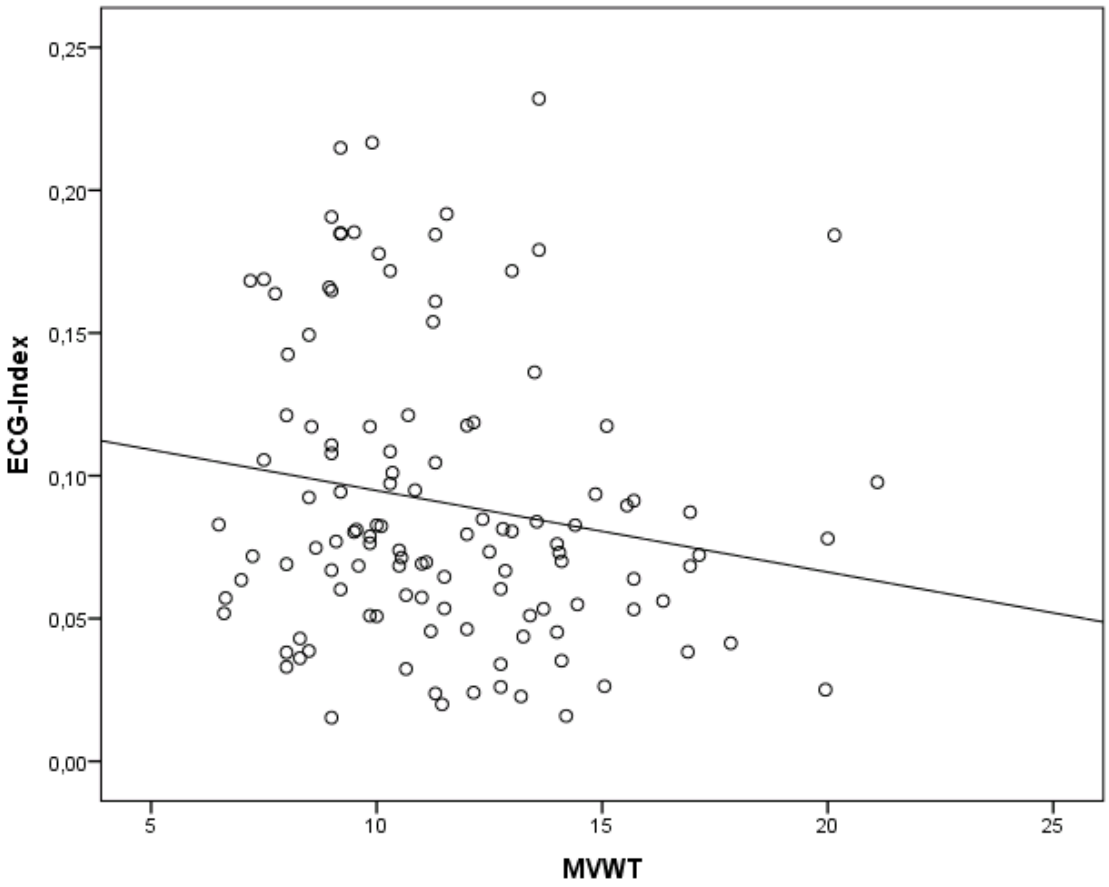

Figure 18: Correlation of the ECG-Index [ ] and MVWT [mm] in MF patients

1.1.36. Correlation of the ECG-Index and relative wall thickness

Furthermore there was a mediocre correlation between the ECG-Index and RWT (Figure 19), with a correlation coefficient of $r^2 = -0.288$ ($p<0.002$). With an increasing RWT, signifying the progression of the disease, the ECG-Index decreased. This corresponded well to the trend seen above, considering the MVWT.

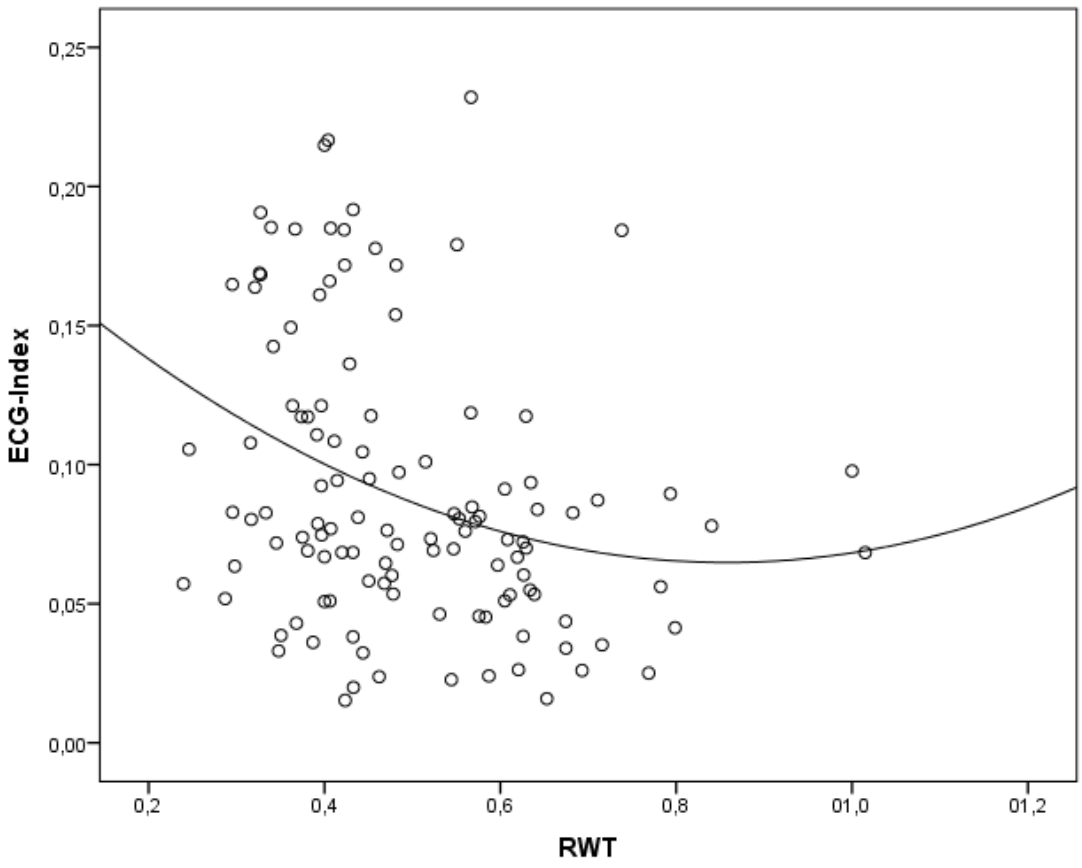

Figure 19: Correlation of the ECG-Index [ ] and RWT [ ] in MF patients.

The correlation of the ECG-Index and echocardiographic diastolic functional parameters

1.1.37. Correlation between the ECG-Index and the E-wave Vmax

In this patient collective, the E-wave Vmax positively correlated with the ECG-Index and revealed a correlation coefficient of r^2 = 0.220 ($p<0.017$). The ECG-Index increased as the E-wave Vmax increased (Figure 20).

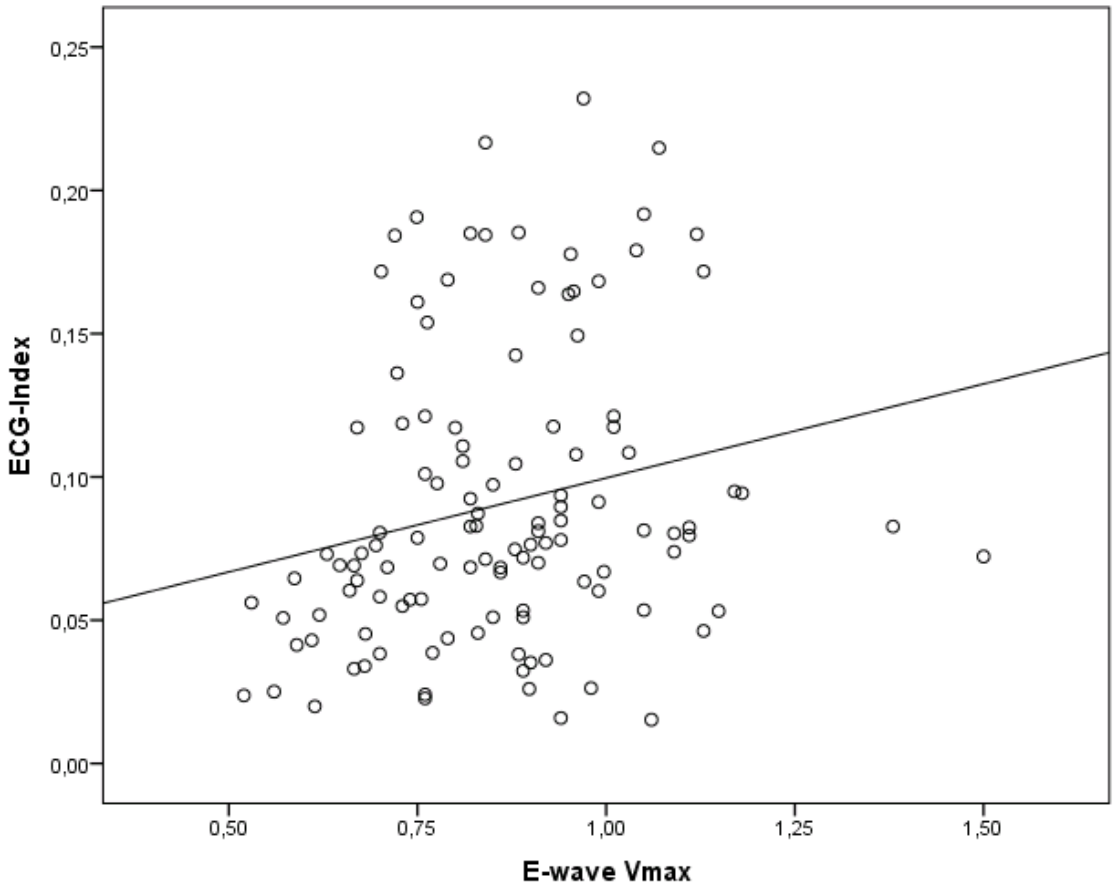

Figure 20: Correlation of the ECG-Index [ ] and E-wave Vmax [m/s] in MF patients.

1.1.38. Correlation between the ECG-Index and the A-wave Vmax

In this study cohort the A-wave Vmax negatively correlated with the ECG-Index and presented a correlation coefficient of r^2 = – 0.367 ($p<0.0001$). The ECG-Index decreased as the A-wave Vmax increased (Figure 21). This correlation is stronger compared to the correlation between the E-wave Vmax and the ECG-Index.

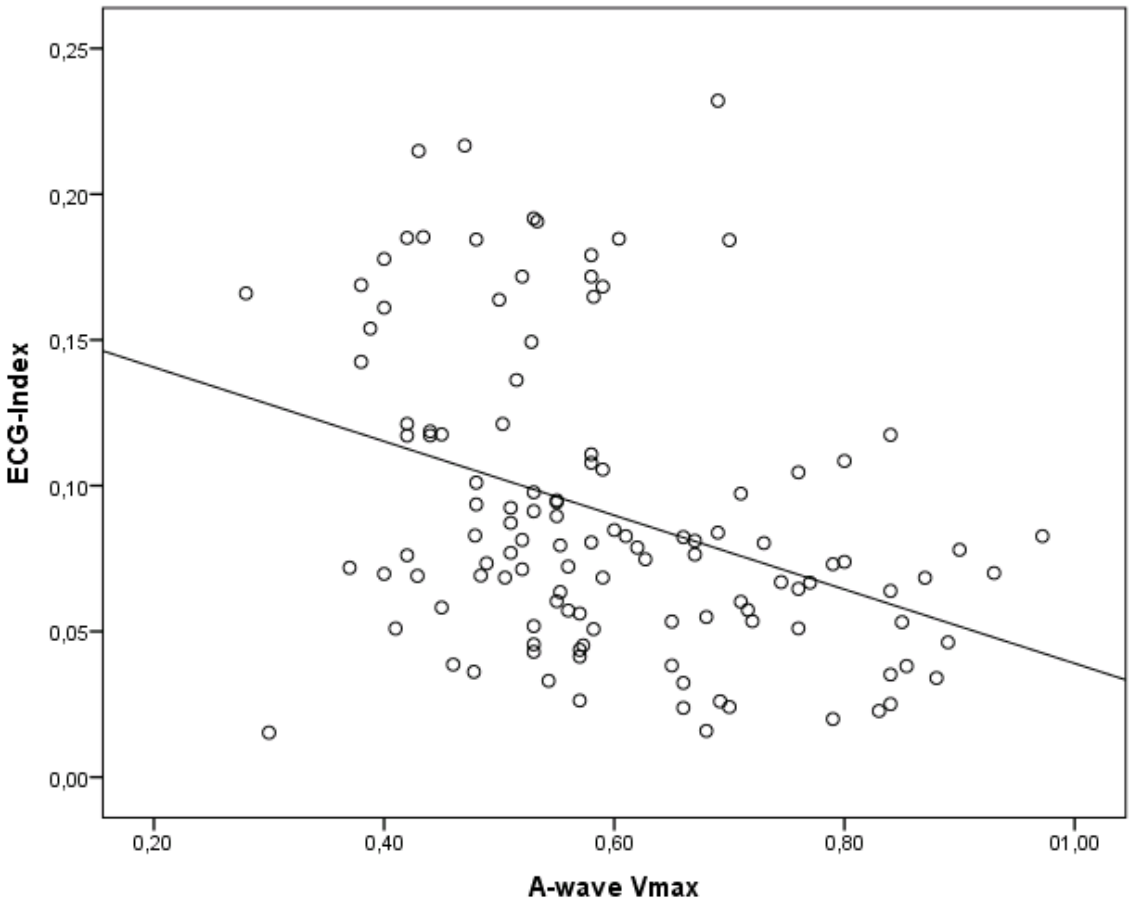

Figure 21: Correlation of the ECG-Index [ ] and A-wave Vmax [m/s] in MF patients.

1.1.39. Correlation between ECG-Index and the E/A ratio

The correlation between the ECG-Index and the E/A ratio was positive (diagram x), with a correlation coefficient of $r^2 = 0.416$ ($p<0.0001$). The ECG-index increased as the E/A ratio increased (Figure 22). This parameter showed the strongest correlation to the ECG-Index out of the diastolic echocardiographic parameters.

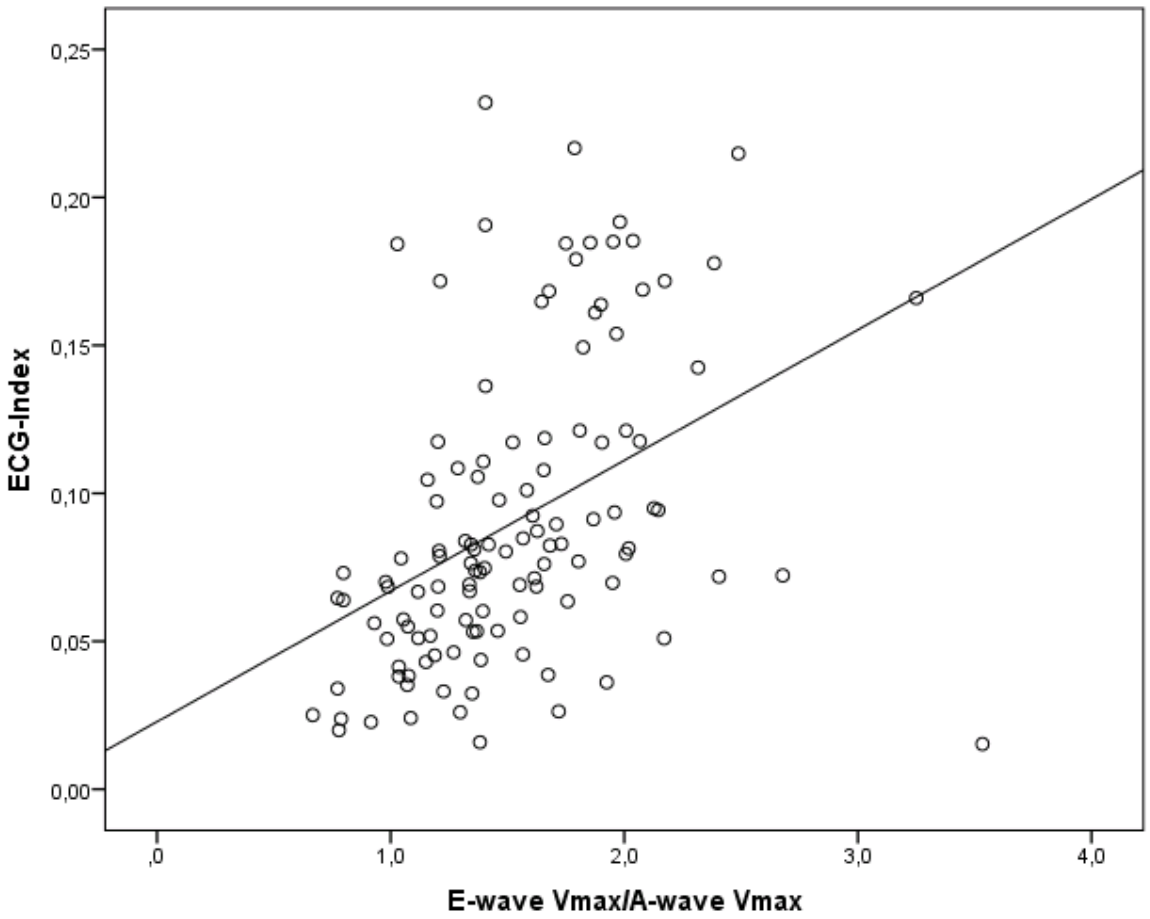

Figure 22: Correlation of the ECG-Index [ ] and E-wave Vmax/ A-wave Vmax [ ] in MF patients.

1.1.40. Correlation between the ECG-Index and isovolumetric relaxation time

The IVRT showed a rather weak negative correlation to the ECG-Index. As the values for the ECG-Index decreased, the values for IVRT increased (Figure 23), with a correlation coefficient of $r^2 = -0.157$ ($p<0.118$).

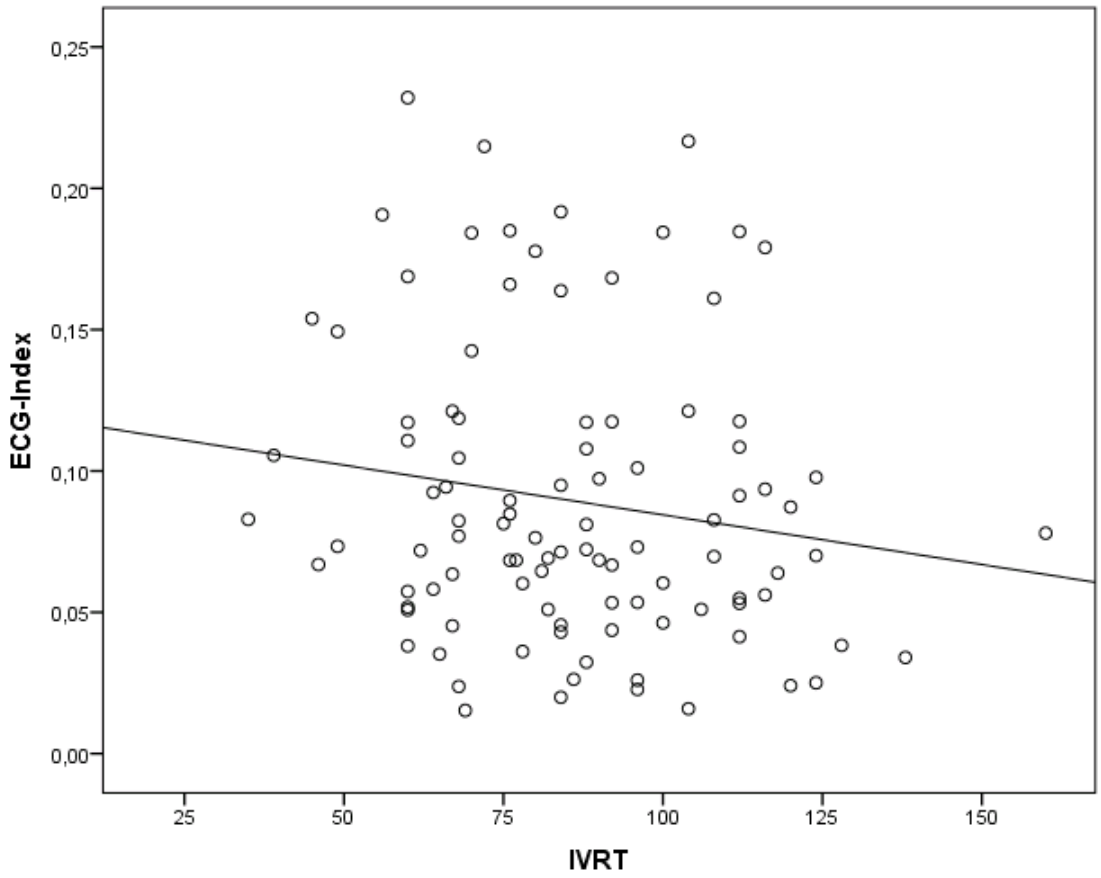

Figure 23: Correlation of the ECG-Index [ ] and IVRT [ms] in MF patients.

1.1.41. Correlation between the ECG-Index and the left atrial volume

The correlation between the ECG-Index and the left atrial volume was weak in this group of patients (Figure 24). The correlation coefficient amounted to $r^2 = 0.052$ ($p<0.579$). There was no clear pattern to describe the relationship between these two parameters.

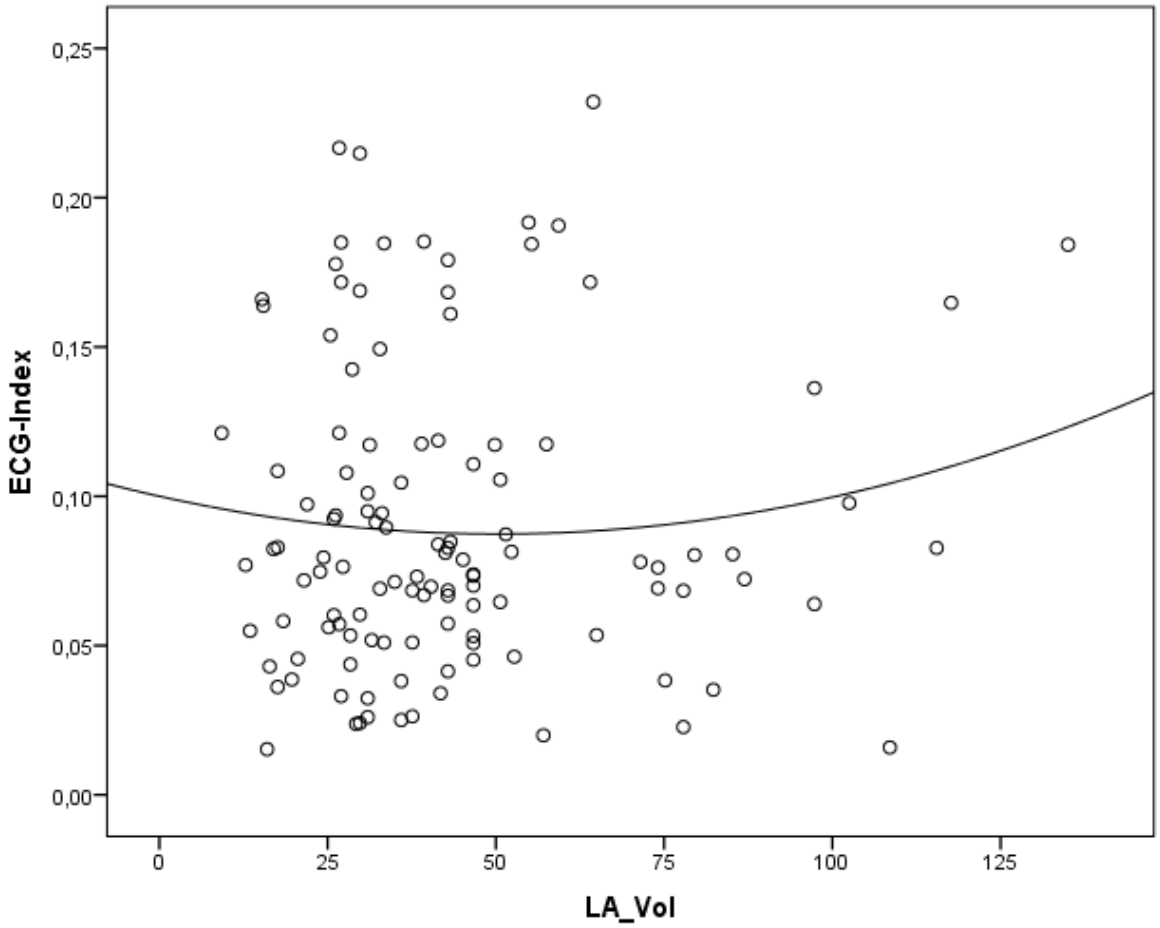

Figure 24: Correlation of the ECG-Index [ ] and LA Volume [ml] in MF patients.

ECG-Index and diastolic dysfunction

When comparing the values for the ECG-Index between the group of patients with and without DD, the group of patients with DD tended to present with lower values for the ECG-Index than patients not affected by DD (Figure 25). However, the significance is practically negligible (p<0.576).

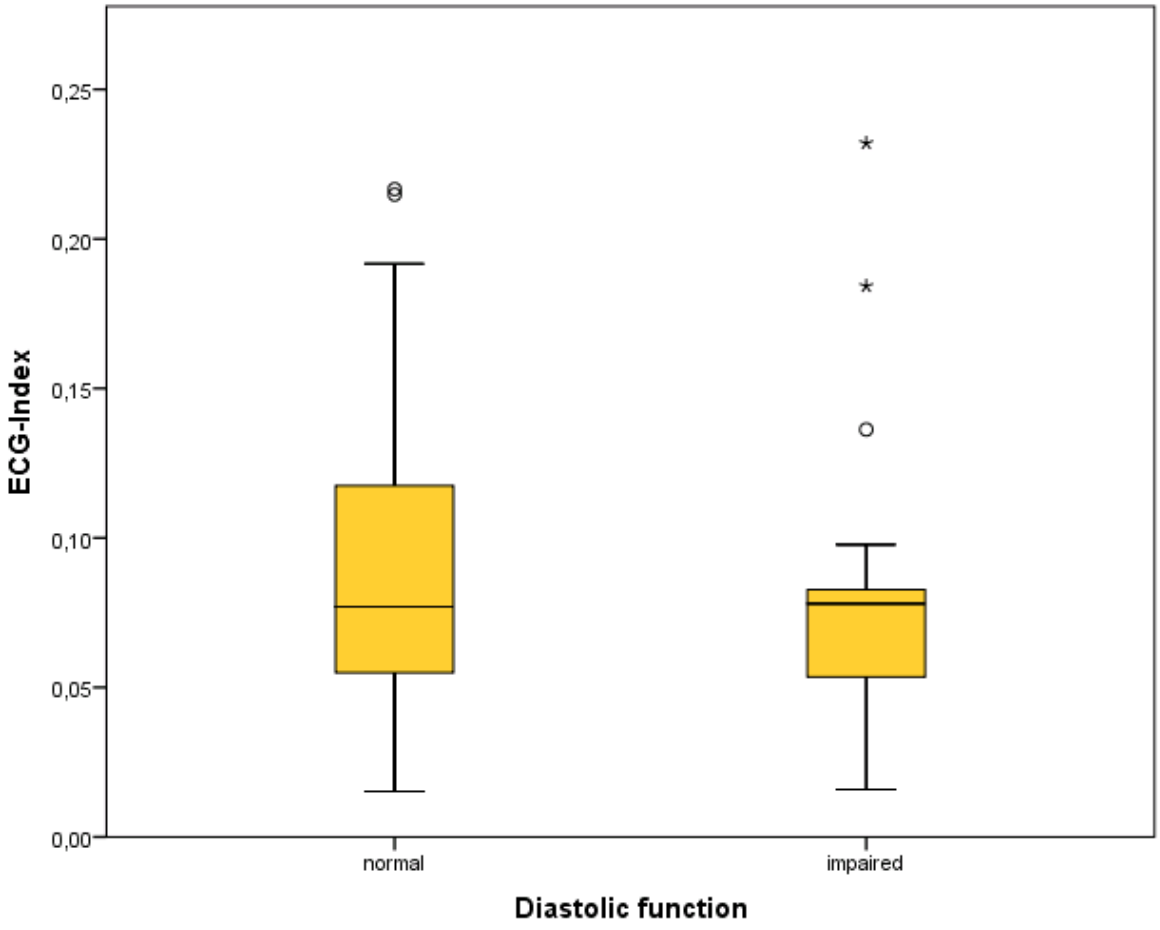

Figure 25: Normal and impaired diastolic function in MF patients in relation to the ECG-Index [ ].

Considering the different genders, males with DD contrary to anticipation presented with tendencially higher values (p<0.235) for the ECG-Index (Figure 26). This is probably due to the limited number of patients in this group (n=5). Females with impaired diastolic function tended to have lower values for the ECG-Index (p<0.130) which corresponded well with the decreasing values for the ECG-Index parallel to the increasing values for MVWT and RWT, reflecting the progression of the disease.

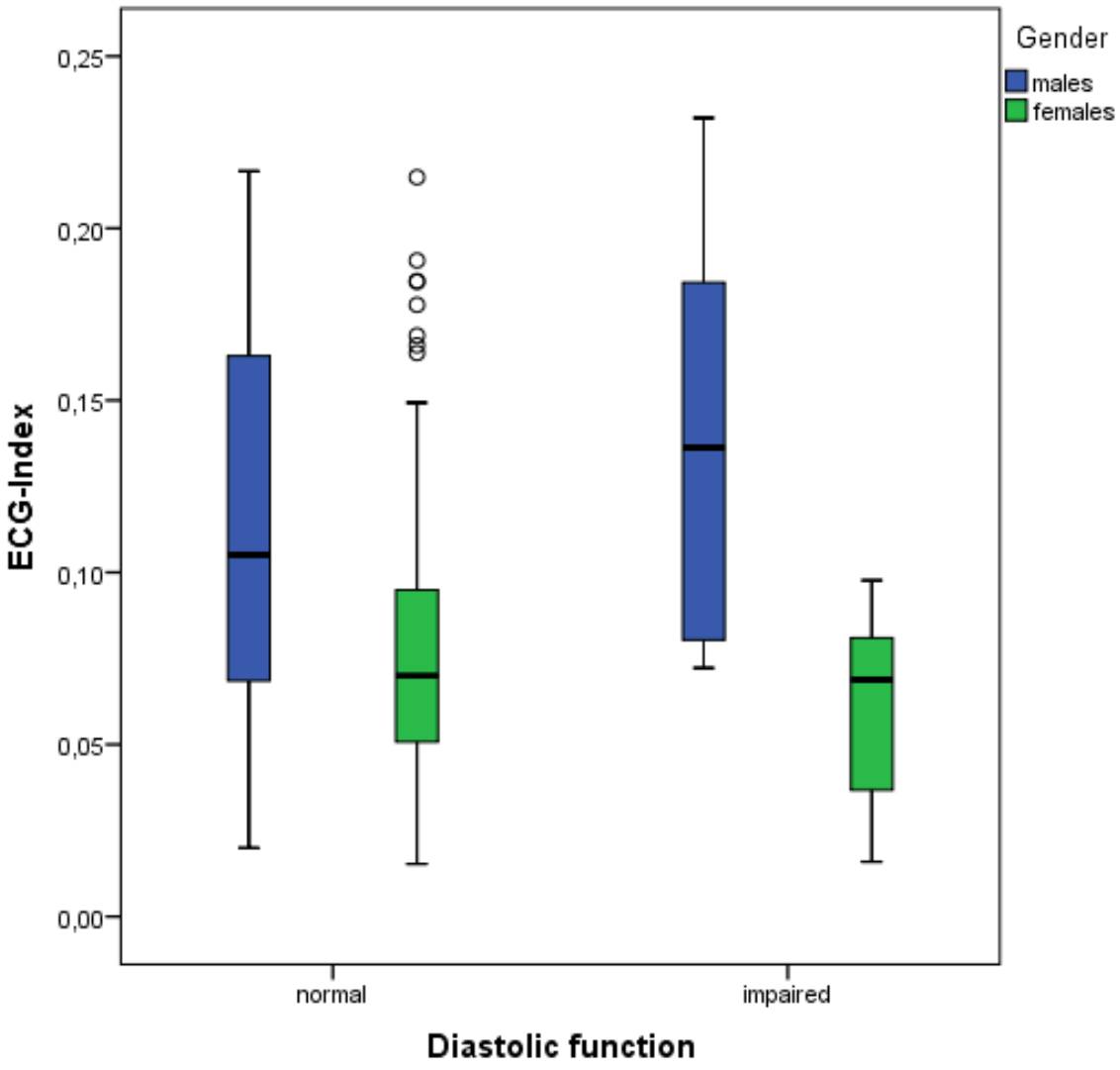

Figure 26: Normal and impaired diastolic function in male and female MF patients in relation to the ECG-Index [ ].

Discussion

Arterial blood pressure and hypertension

Arterial hypertension is historically not a pathognomonic feature of Fabry disease.

However, with evolving knowledge a moderate form of hypertension has been described as a common characteristic in Fabry's nephropathy [31, 248]. This is unlike other nephropathies, which are accompanied by severe hypertension [249, 250]. Hypertension has multiple definitions in various studies which results in different data concerning the prevalence of hypertension in Fabry patients.

However, in general a higher percentage of male Fabry patients (14-57%) compared to female Fabry patients (6-47%) is found to be hypertensive [59, 75, 103, 248, 251, 252]. This is explained by the higher prevalence of rapid and severe progression of renal dysfunction in males compared to females [248, 252, 253]. The prevalence of hypertensive patients in this thesis was on average lower than in previous studies. In total 22.9% of the patients were hypertensive, 36.6% of men and 15.6% of women. This may be explained by the strict definition of hypertension in this thesis (systolic blood pressure ≥140mmHg) and the fact that antihypertensive medication was not taken into account when considering blood pressure values.

To create a better comparison and avoid diverging definitions, it is advantageous to consider the blood pressure values themselves. In literature, the average systolic and diastolic blood pressure in Fabry patients is approximately 123-128mmHg and 71-77mmHg respectively, with generally similar diastolic and systolic values in both genders. This study presented an average blood pressure of 128/73mmHg with a tendency ($p<0.121$) for higher systolic values in men (131/74mmHg), than in women (126/73mmHg) and no significant difference in diastolic values between genders. The data collected in this study shows higher systolic values in men compared to values in literature, the diastolic values however coincide largely with the values in literature [29, 82, 169, 248].

Furthermore, in this study patients with CM had significantly higher systolic blood pressures (131.6mmHg) than patients without CM (121.1mmHg). This is due to structural and geometrical changes in the LV, which resulted in altered LV loading

and pressure conditions. This consequently may induce accelerated hypertrophic development and progression [254]. The diastolic blood pressure was not elevated in patients suffering from cardiomyopathy compared to patients without CM. This may be explained by the absence of peripheral vasculopathy in Fabry patients.

Approximately half of the non-hypertensive patients in this study developed cardiomyopathy and the other half did not. However, 88.9% of hypertensive patients suffered from cardiomyopathy, this reflects the strong link between hypertension and CM in Fabry patients. Yet, the high percentage of patients with CM and hypertension may also include the patients suffering from primary hypertension influencing the development of hypertrophy independent of Morbus Fabry.

Morbus Fabry associated cardiomyopathy

Cardiomyopathy in Fabry patients is one of the major manifestations and occurs in up to 69%of Fabry patients [82, 171]. In this study cardiomyopathy was classified as LVM_h $\geq$ 50g/m$^{2.7}$. CM was also found in the current patient group, particularly marked in males compared to females, this is due to the late onset of the disease in females compared to males. This is underlined by the significantly higher average age in women (42.3 years) compared to men (31.2 years) included in this study. Furthermore, patients with CM in this study are on average 11 years older than patients without CM, which accentuates the later onset of the cardiac manifestation. This coincides with current literature available [39, 59, 60, 82, 119]. Similarly, as reported in previous studies, the aortic root diameter [83], anterior right ventricular wall thickness (RVAWd) and the mean end-diastolic wall thickness (MVWT) of the ventricle [167], the latter takes the interventricular and posterior wall into account, are also significantly increased in men compared to women, this corresponds to the increase in LVM.

The most common geometry of the left ventricle in hypertensive and non-hypertensive patients is concentric hypertrophy, which coincides with current literature and indicates that the deposition of Gb3 occurs ubiquitous in the septal and posterior myocardial wall [31, 59, 145].

Echocardiographic parameters

This study is a prospective study dating from the mid 1990's, which means that the parameters and data obtained for this study, correspond with the established parameters of that time and were collected by sonographic apparatus that partly also date from the mid 90's.

1.1.42. Systolic function and myocardial changes

Systolic function can be defined by multiple parameters of which a few have emerged as useful parameters. EF, FS reflect the load independent radial function, FSmid is a reliable parameter for the radial function independent of left ventricular geometry. LAX represents the longitudinal function of the left ventricle. The radial and longitudinal function make up the global systolic function of the left ventricle. Previous studies have shown that the deterioration in the longitudinal function occurs before the radial function in Fabry patients [29, 81, 100, 165, 255]. In this study the systolic functional parameters were similar in males and females, however the percentage of long axis shortening (LAX) tended to be smaller in males compared to females. This may again be due to earlier and more grave disease progression, specifically the development of cardiomyopathy in males [82]. Patients with cardiomyopathy presented with significantly decreased LAX compared to patients without CM. Contributing factors may be fibrosis, necrosis and the loss of contractile fibres [62] leading to the deterioration of the systolic function. Additionally, these factors may influence compensatory mechanisms such as a significantly increased FS and EF, as is the case in patients with CM in this study. However, the increase in EF may also be the result of the increased wall thickness, which decreases the volume of blood in the ventricle and then may lead to a relatively larger EF (in percentage) yet a decreased net volume compared to the total volume in the ventricle. These possible compensatory mechanisms however seem to reach a limit, as in patients with severe CM there is no significant difference in EF, a less significant difference in FS and a distinctly higher significance in LAX. There was no significant change in EF, FS and FSmid in patients with and without DD. However, the LAX was also significantly smaller in patients with DD, indicating that DD may be accompanied by systolic dysfunction.

1.1.43. Diastolic function and myocardial changes

Moderate and severe DD are viewed as important predictors of adverse outcomes, particularly in patients with heart failure and preserved EF. The early diagnosis is essential as patients are likely to benefit from heart failure treatment [256].

In this study diastolic dysfunction was a frequent characteristic. 14.4% (17/118) of the patients in this study presented with DD of Grade 2 or higher.

No substantial differences between gender were noted regarding diastolic echocardiographic parameters, this is in agreement with previous studies [59, 167].

In patients with DD the values for the A-wave Vmax were increased ($p<0.017$) and the values for E/A ratio tended to be smaller ($p<0.074$) compared to patients without DD. This is plausible when considering the definition of DD in this study was set according to two parameters of which one of them is the E/A ratio. However, the values for E-wave Vmax do not differ significantly between the two groups, which indicates that the E-wave Vmax does not have an impact on the change of the E/A ratio. Subsequently the question arises, whether the A-wave Vmax as a single parameter may be more useful in the diagnosis of DD in Fabry patients.

In patients with cardiomyopathy, the A-wave Vmax ($p<0.0001$) and IVRT ($p<0.0001$) are both increased significantly compared to patients without CM, this is in line with previous studies [31, 73, 104, 155, 167, 257]. The IVRT positively correlated with the increasing LVM (Figure 14), as the LVM increased the IVRT increased. The E/A ratio decreased ($p<0.001$) in patients with CM in the current study and there was no significant difference in E-wave Vmax between the group of patients with and without CM. Previous studies found similar results, however not always significant [31, 73, 104, 167]. Nevertheless, one study found the E-wave Vmax to tendentially decrease in patients with CM [155]. The above results illustrate that CM is closely linked to DD ($p<0.001$ chi quadrat). Besides, more than half of the patients suffering from CM in this study also showed impaired diastolic function (Figure 15).

Furthermore, in the current study the left atrial volume indexed by BSA in patients with DD was more than twice as large as in patients with normal diastolic function. The same applies to patients with CM, which likewise presented with nearly double as large LA_Vol as patients without CM. Namdar et al. reported the same in Fabry

patients in early stages of the disease [135]. The volume of the LA in this clinical survey was calculated with the single dimension cubic formula, which has a high rate of observer variability and low rate of reproducibility [241]. Ultimately it is not the most accurate method of quantification available as the left atrial volume is consistently underestimated with this formula {Khankirawatana, 2002 #30}. However, this enhances the significance of the above results indicating a strong correlation between LA enlargement and DD, as reported in numerous studies [150, 151, 154, 241, 258], yet in disagreement with Putko et al. who found similar LA volumes in Fabry patients compared to healthy controls [153].

Frankly the above changes in parameters thus signify the following in Fabry patients; DD associated with hypertrophy and fibrosis is linked to normal or tendentially decreased passive emptying of the left atrium (normal or tendentially decreased E-wave Vmax). Increased wall stiffness, impaired relaxation of the left ventricle (increased IVRT) and decreased compliance consequently lead to increased LV filling pressures (increased A-wave Vmax vs IVRT, see Figure 27) [31]. The thin left atrial wall is sensitive to pressure changes in the left ventricle and therefore amenable to the enlargement and stretching of the left atrium. Hence, increased left atrial wall thickness, as a compensatory mechanism, and the left atrial function are valuable markers for the severity and chronicity of diastolic dysfunction [31, 150, 151, 154, 258]. Additionally atrial enlargement induces pathophysiological changes of the atrial fibrils, which in combination with inconsistent fibrosis of the left atrial wall interrupts the conduction of sinus impulses [31, 149, 150, 152, 259, 260] and increases the risk for electrophysiological abnormalities such as atrial fibrillation [261, 262], congestive heart failure [263], stroke and cardiac death [264]. This reiterates the clinical importance of early diagnosis and treatment of diastolic dysfunction.

Diastolic function and left ventricular geometry

In patients with normal diastolic function, 41% had a normal configuration of the left ventricle and 35% presented with concentric hypertrophy. The most common form of cardiomyopathy in patients with diastolic dysfunction was concentric hypertrophy, this is in agreement with previous studies [31] and underlines the fact that the most common form of hypertrophy in Fabry patients is concentric hypertrophy [31, 59, 82, 86].

1 out of 44 patients (patient nr. 264) with DD did not present with CM, there was no significant age difference between the female patient compared to the rest of the Fabry patients in this study, this patient did not take any medication, neither did she suffer from hypertension or have any electrocardiographic abnormalities, she did however present with concentric remodelling. This implies that DD in Fabry patients may occur before the development of LVH. This is concurrent with previous studies [155, 164]. Consequently the measurements of diastolic function are important parameters that may significantly improve the diagnosis of cardiac manifestation in Fabry patients before the development of LVH.

Diastolic dysfunction and electrocardiography

In recent years, the electrocardiogram has received increased attention in the diagnosis and prediction of DD as it is non-invasive, accessible, operator independent, highly reproducible, and less susceptible to hemodynamic changes compared to echocardiography [265, 266]. Specifically, the following parameters have been discussed as important markers for the prediction of DD: the P-wave duration, P-wave dispersion, PQ interval, QT interval, QT dispersion and corrected QT interval [206, 260, 267].

In previous studies the P-wave and PQ interval were both found to be significantly prolonged in patients with DD compared to controls, Murata et al. suggest this to be due to the adherence of glycosphingolipids to a pre-existing accessory pathway specifically described in his case report, which may have reduced the pathway's conductivity [206, 260, 268]. Furthermore, Wilcox et al. reported the PQ-interval to be twice as sensitive as the indexed left atrial volume for the identification of diastolic dysfunction. This may suggest that the PQ prolongation, as an expression of left atrial remodelling, is an early and more sensitive electrocardiographic parameter, which may be identified before the morphological changes can be detected by imaging techniques [260, 206]. Moreover, P-wave dispersion was increased in patients with DD, this parameter has likewise been associated with remodelling of the left atrium and an increased risk of atrial fibrillation [149, 206, 269]. In the current study the P-wave and PQ interval did not show a significant difference between patients with and without DD. The P-wave dispersion was not examined in this study.

The QTc duration in particular was found to correlate with Doppler-derived parameters of DD in two studies [260, 270]. However, Sauer et al. suggests the Tpeak-Tend interval may be the key element in the QTc prolongation in DD [271]. Albeit Namdar could not verify this in his study [206].

In the current study the QRS interval, QT and QTc interval as well as the Tend-P interval correlated significantly with echocardiographically defined DD. Similarly the calculated electrocardiographic parameters (multiple ECG-Indexes and Products, Table 44), which are markers for hypertrophy, significantly correlated with the echocardiographically defined DD, which indicates that most patients with diastolic function also suffered from hypertrophy. This correlates to the data collected by echocardiography. However the specific ECG-Index by Namdar et al. [206] did not present significantly different values between the two groups with and without DD (Table 45). The QT dispersion was not examined in this study.

Nevertheless, when considering the correlation between the ECG-Index and different parameters the following can be established. With age progression, the ECG-Index yields lower values, which indicates that lower values coincide with an increased disease progression (Figure 16). Yet, the value for the ECG-Index does not change with increasing LVM and left atrial volume (Figure 17, Figure 24). However, contradictingly, the MVWT and RWT increase as the ECG-Index tends to yield lower values. As there is no uniform trend regarding hypertrophy and the ECG-Index, the ECG-Index does not serve as a useful tool for the diagnosis of hypertrophy. Nevertheless, in this study the ECG-Index positively correlates with the following echocardiographic parameters: E/A ratio and E-wave Vmax (Figure 22, Figure 20). It yields a moderate, negative correlation to the A-wave Vmax and IVRT (Figure 23, Figure 21). This means, as the maximum velocity of the passive diastolic filling of the left ventricle mildly decreases and on the other hand the IVRT and the maximum velocity of the active filling of the left ventricle in diastole increase, values for the ECG-Index decreases. These changes are typical for diastolic dysfunction, which suggests that the ECG-index may be suitable for the diagnosis of DD in Fabry patients. Further research in the form of a prospective study is required to examine whether the electrocardiographic parameters expose DD before echocardiographic parameters do so.

Besides electrophysiological changes seen in the ECG, sympathetic denervation may be detected using speckle tracking echocardiography and ^{123}I-meta-iodobenzylguanidine imaging, which may occur before the development of fibrosis or reduction of LV ejection fraction and therefore may be another method for early detection of diastolic dysfunction [164, 272].

Clinical cardiac manifestation

1.1.44. Clinical cardiac manifestation with regards to diastolic functional parameters

Diastolic dysfunction seems to have an impact on the clinical cardiac symptoms, such as dyspnoea, as more patients with impaired diastolic function, relatively, were graded in NYHA 3 or higher, than patients with normal diastolic function. Furthermore, 62.5% of Fabry patients with impaired diastolic function had a CCS score of 1 or more (experienced angina pectoris regularly) and only 29.6 % of patients with a normal diastolic function reported a score of CCS 1 or more.

1.1.45. Clinical cardiac manifestation with regards to systolic functional parameters

Patients with cardiomyopathy more frequently had physical limitations in daily life measured by the NYHA score than Fabry patients without CM. Additionally, only Fabry patients suffering from concentric hypertrophy had a NYHA score of 3 and above. With regard to angina pectoris similar findings were recorded, approximately half of the patients with CM experienced angina chest pain regularly (CCS score of 1 or more), whereas this was the case in only 10% of patients without LVH.

Study limitations

This study has several limitations that should be acknowledged. The incomplete or erroneous documentation and measurement would decrease the potentiality of subgroup analysis. Additionally, the data extraction began in the 1990's which means that the manner of recording variables and the variables themselves may be overhauled. More exact measurement methods and apparatus are currently the goldstandard. The NYHA and CCS scores are based on subjective estimations and therefore may be a source of error [273-275]. The diagnosis of hypertension in this study was made based on the mean values of two measurements recorded 5 minutes apart on the same day, which does not comply with the current guidelines for the diagnosis of arterial hypertension, which is comprised of a minimum of three measurements on alternating arms, on three different days at three different times of the day. Medication, specifically anti-hypertensive medication was not considered when interpreting the blood pressures, nor were diabetes and non-Fabry associated hypertension considered, even though they may influence cardiac function and affect the development of LVH [39, 93, 144]. This may lead to attribution bias.

Despite the modest size of the patient group, considering the rareness of the disease and the fact that this was a single-center study, the number of patients in some subgroupings was too small to make meaningful comparisons and to use these results to make valid statements regarding the general population. Furthermore, due to the lacking number of patients, diastolic function could not be graded into four groups, instead it was divided into two groups, this may have prevented better insight and understanding of diastolic dysfunction in Morbus Fabry patients.

Matching of patients with a control group was not performed.

And lastly, participation in the study was voluntary which limits the analysis, as only a subset of the total patient population in Mainz and its surroundings have their data recorded, which may suggest selection bias.

Outlook

Prevention is more effective than treatment of already damaged organs, this means earliest diagnosis possible is essential and furthermore early, effective treatment is mandatory.

The most effective screening method would be the sequencing of the A-GAL A gene by mutation analysis in the course of the newborn screening. The screening is already implemented in Germany and includes the most common, severe yet treatable hereditary diseases. However, the mutation analysis is also the most costly manner to diagnose Morbus Fabry, yet the only reliable way to diagnose the disease in females. In males the determination of α-GAL A activity is sufficient and currently the goldstandard in the diagnosis of Morbus Fabry. Moreover, it is by far more cost-efficient (60 Euros). The above two options should be reviewed as well as the possibility of a screening in late adolescence, i.e. during the supplementary J2 screening (16-17 years) examination, which should then evidently become obligatory and include the examination of a urine sample, an ECG and echocardiography as a large group of adolescents suffering from Morbus Fabry already have manifest symptoms such as LVH and proteinuria. Currently in Germany a urine sample is examined during the U8 screening examination (at age 46-48 months) and an ECG is conducted during the supplementary U10 screening examination (at age 7 – 8 years), the latter is not paid for by all health insurances. Naturally, awareness of the disease and its multiple, unspecific symptoms are the prerequisite and foundation in the process of diagnosis and should be propagated.

Once an early diagnosis has been made, treatment should commence. ERT is currently the therapy of choice, yet the incomplete response, high cost and lifelong dependency are factors that cannot be neglected. The search for new more effective therapies has produced Migalastat HCl, the chaperone protein, explained above which has shown promising effects. Furthermore, substrate reduction therapy, which is an orally administered drug and entails blocking the formation of glycosphingolipids and has shown to reduce levels of Gb3 uniformly in organs such as the kidneys, liver and heart of murine models [276]. It is more efficacious when combined with ERT compared to either therapies administered individually and is more effective in reducing levels of tissue Gb3 in younger mice than in older mice, which underlines

the importance of early diagnosis [277, 278]. Furthermore, the combination of substrate reduction therapy and ERT may improve quality of life, by less frequent and thereby reduced dependency on ERT infusions [279]. Moreover, in a recent exploratory trial in Fabry patients substrate reduction therapy has been shown to reduce levels of glycosphingolipids [280].

A further method involves the introduction of retroviral vectors to insert a normal alpha galactosidase A gene into bone marrow hematopoietic stem, progenitor cells and stromal cells. The cells are transplanted into an alpha Gal A deficient murine Fabry model which in the course of time have shown increased α-GAL A levels and reduced levels of accumulated Gb3 in multiple organs [281-284]. Furthermore, as a result of secondary transplantation of bone marrow, where mononuclear cells from a transplanted murine model are transplanted into a second alpha Gal A deficient murine Fabry model, correction of the metabolic defect in non-transduced cells was observed [285-288]. In 2016 the first phase 1 trial was initiated. A Fabry patient received an autologous stem cell transplantation with transduced cells (Lentivirus alpha Gal A). The first results presented in 2017 showed that within 45 days of the administration of a single dose, alpha Gal A levels were in a normal range and the level was maintained within this range for the duration of the 6-month trial. No study related severe adverse events were recorded within this period [289]. Funding for a Phase 2 trial has been granted [290].

Summary regarding the aims of this study

Comparison of systolic and diastolic echocardiographic changes at the baseline examination before treatment, between hemizygous males and heterozygous females.

There was no significant difference between male and female Fabry patients in systolic and diastolic echocardiographic parameters.

Comparison of ECG changes at the baseline examination before treatment between hemizygous males and heterozygous females.

The QRS interval was significantly longer in males and the QTc was longer in females. Furthermore, males had higher values for RV5 and SV3 than females. This was also the case when regarding the values for the Sokolow-Lyon Index, the Cornell Index, the Cornell Product, the 12lead and the 123lead. The ECG-Index as well as the Tend-P produced higher values in males than in females.

Presentation of systolic and diastolic echocardiographic changes and ECG changes in patients with cardiomyopathy compared to without cardiomyopathy at the baseline examination.

In patients with cardiomyopathy the EF and FS was higher and the LAX presented with lower values than in patients without cardiomyopathy. Considering the diastolic echocardiographic parameters, patients with cardiomyopathy had higher values for the A-wave Vmax and IVRT and lower values for the E/A ratio. Regarding the ECG values, the P-wave and the QTc interval tended to be longer and the QRS interval and the QT interval were significantly longer in Fabry patients with cardiomyopathy. The hypertrophy specific indexes (Sokolow-Lyon Index, the Cornell Index, the Cornell Product, the corrected Cornell Index, the corrected Cornell Product, the 12lead and the 123lead) were naturally all higher in patients with cardiomyopathy. The Tend-P interval was longer in patients with cardiomyopathy, however the ECG-Index did not differ between the groups with and without cardiomyopathy.

Presentation of systolic and diastolic echocardiographic changes and ECG changes in patients with normal diastolic function compared to patients with impaired diastolic function at the baseline examination.

The only systolic echocardiographic parameter that differs between the groups is the LAX, which showed significantly lower values in patients with impaired diastolic function. Two diastolic echocardiographic parameters significantly differed between the two groups; the A-wave Vmax was increased and the E/A ratio was reduced in the group of patients with diastolic dysfunction. Moreover, the group of patients with impaired diastolic function presented with significantly higher values for the QRS, the QT and QTc interval and the Tend-P interval.

Performance and diagnostic value of the ECG-Index regarding diastolic dysfunction in Morbus Fabry patients.

The values for the ECG-Index did not show a significant difference between the groups with and without diastolic dysfunction. However, this may also be due to the small patient collective, as with increasing age, MVWT, RWT, A-wave Vmax and IVRT, the ECG-Index tends to decreased. And as the E-wave Vmax and the E/A ratio decreased, the ECG-Index decreased. These are indicators that there may be a connection between diastolic dysfunction and the ECG-Index, this however needs to be examined in a larger collective of patients.

Zusammenfassung

Vergleich der systolischen und diastolischen echokardiographischen Veränderungen zwischen hemizygoten Männern und heterozygoten Frauen zum Zeitpunkt der Baseline-Untersuchung.

Es gab keinen signifikanten Unterschied zwischen weiblichen und männlichen Morbus Fabry Patienten bezüglich der systolischen und diastolischen echokardiographischen Parametern.

Vergleich der EKG Veränderungen zwischen hemizygoten Männern und heterozygoten Frauen zum Zeitpunkt der Baseline-Untersuchung.

Bei Männern war der QRS Intervall signifikant verlängert und bei Frauen die QTc Zeit verlängert. Zudem hatten Männer höhere Werte für RV5 und SV3 als Frauen. Die Werte für den Sokolow-Lyon Index, Cornell Index, Cornell Produkt, 12lead und 123lead waren ebenfalls erhöht bei den Männern. Auch der EKG Index sowie Tend-P ergaben höhere Werte bei den Männern verglichen mit Frauen.

Vergleich der systolischen und diastolischen echokardiographischen und EKG Veränderungen zwischen Morbus Fabry Patienten mit und ohne Kardiomyopathie zum Zeitpunkt der Baseline-Untersuchung.

In Patienten mit Kardiomyopathie verglichen mit Patienten ohne Kardiomyopathie ergaben sich höhere Werte für die EF und FS und niedrigere Werte für die LAX. Bezüglich der diastolischen echokardiographischen Parameter hatten Patienten mit Kardiomyopathie höhere Werte für A-wave Vmax und IVRT und niedrigere Werte für das E/A Verhältnis. Im Hinblick auf die EKG Werte tendierten Patienten mit Kardiomyopathie zu verlängerten P-Wellen und QTc Intervallen und außerdem zu signifikant verlängerten QRS- und QT-Intervallen. Die Hypertrophie spezifischen Indices (Sokolow-Lyon Index, Cornell Index, Cornell Produkt, korrigierter Cornell Index, korrigiertes Cornell Produkt, 12lead und 123lead) waren selbstverständlich höher in den Patienten mit Kardiomyopathie. Der Tend-P Intervall war verlängert in Patienten mit Kardiomyopathie, jedoch gab es keinen bemerkenswerten Unterschied

zwischen den Patienten mit und ohne Kardiomyopathie bezüglich des EKG Index.

Vergleich der systolischen und diastolischen echokardiographischen und EKG Veränderungen zwischen Patienten mit einer normalen und Patienten mit einer beeinträchtigten diastolischen Funktion zum Zeitpunkt der Baseline-Untersuchung.

Der einzige systolische echokardiographische Parameter der sich in den beiden Gruppen unterschied ist die LAX, diese zeigte signifikant niedrigere Wert in Patienten mit einer beeinträchtigten diastolischen Funktion. Zwei der diastolischen Funktionsparameter zeigten einen signifikanten Unterschied zwischen den beiden Gruppen; die Werte für die A-Welle Vmax waren erhöht und die Werte für das Verhältnis E/A waren vermindert in den Patienten mit beeinträchtigter diastolischen Funktion. Zudem ergaben sich signifikant höhere Werte für QRS, QT und QTc Intervalle sowie für den Tend-P Intervall in dem Patientenkollektiv mit beeinträchtigter diastolischen Funktion.

Bedeutung und diagnostischer Wert des EKG-Index bezüglich einer Beeinträchtigung der diastolischen Funktion in Morbus Fabry Patienten.

Zwischen den Patientengruppen mit einer normalen und beeinträchtigten diastolischen Funktion zeigte sich kein signifikanter Unterschied bezüglich der Werte für den EKG-Index. Jedoch ergibt sich dieses Ergebnis möglicherweise durch die begrenzte Zahl der Patienten, denn je höher die Werte für das Alter, MVWT, RWT, A-Welle Vmax, IVRT, desto kleinere Werte ergeben sich für den EKG-Index. Je niedriger die Werte für die E-Welle Vmax und das Verhältnis E/A, desto niedrigere Werte ergeben sich für den EKG-Index. Dies spricht für eine Verbindung zwischen dem EKG-Index und der Beeinträchtigung der diastolischen Funktion, jedoch muss dies in einem größeren Patientenkollektiv erforscht werden.

Addendum

Severe Cardiomyopathy	No	Yes	p
n	87	31	
RRsys [mmHg]	124.8 ± 12.8 (106.8 – 147) 97 – 161	135.6 ± 17.4 (101.4 – 161.6) 84 – 167	0,003
RRdias [mmHg]	72.9 ± 9.3 (56 – 88) 50 – 97	74.2 ± 10 (57.4 – 95.6) 55 – 98	NS
RRmid [mmHg]	90.2 ± 8.7 (75.4 – 105.1) 69.7 – 109	94.6 ± 11.2 (74.3 – 116.4) 64.7 – 118	0,028
n	84	30	
HF [s^{-1}]	71 ± 14.6 (52 – 98.5) 44 – 134	62.5 ± 10.6 (49.6 – 89) 49 – 100	0,004

Table 54: Basic cardiac parameters in MF patients with and without severe cardiomyopathy

Severe Cardiomyopathy	No	Yes	p
n	86	30	
Ao [mm]	27.3 ± 4.2 (20.7 – 35.1) 20 – 39.2	30 ± 9.1 (20.5 – 53.5) 19.1 – 72	0,121
n	87	31	
LAd [mm]	32.3 ± 4.7 (25 – 41.3) 21 – 49	39 ± 6.3 (27.1 – 49.7) 23.8 – 51.3	<0,0001

Table 55: Aortic and LA diameter in MF patients with and without severe cardiomyopathy

Severe Cardiomyopathy	No	Yes	p
n	87	31	
IVSd [mm]	10 ± 2.5 (6.3 – 15.3) 6 – 19	15.6 ± 4.3 (10.4 – 24.2) 9 – 25.2	<0.0001
IVSs [mm]	14.2 ± 3.2 (10 – 19.7) 5.8 – 24	19.9 ± 4.2 (13.8 – 29.3) 12 – 30.3	<0.0001
LVId [mm]	46.5 ± 5.6 (38.1 – 58.9) 36.8 – 61	48.4 ± 5.5 (38.4 – 58.8) 33.4 – 63	0.1
LVIs [mm]	28.2 ± 4.7 (20.5 – 37) 15.9 – 43	28.1 ± 5.6 (20.8 – 40.6) 20 – 42	NS
PWd [mm]	10 ± 2 (8 – 14) 7 – 17	15 ± 2 (12 – 19) 12 – 20	<0.0001
PWs [mm]	17 ± 3 (12 – 22) 10 – 25	21 ± 3 (17 – 24) 16 – 25	<0.0001
n	56	19	
RVAWd [mm]	5 ± 1 3 – 8	7 ± 2 5 – 13	<0.0001

Table 56: Echocardiographically measured wall thicknesses in MF patients with and without severe cardiomyopathy

Severe Cardiomyopathy	No	Yes	p
n	87	31	
MVWT [mm]	10.2 ± 2 (7.1 – 13.9) 6.5 – 15.6	15.3 ± 2.6 (11.4 – 20.5) 10.5 – 21.1	<0.0001
RWT [ ]	0.4 ± 0.1 (0.3 – 0.7) 0.2 – 0.8	0.6 ± 0.1 (0.4 – 1) 0.4 – 1	<0.0001
LVM_h [g/m$^{2.7}$]	49.8 ± 12.7 (28.1 – 70.6) 23.5 – 73.5	104.4 ± 36.6 (75.8 – 194) 75.7 – 201.1	<0.0001
LA_Vol [ml]	36 ± 16.8 (15.7 – 70.4) 9.3 – 117.6	63.8 ± 29.3 (20.5 – 123.3) 13.5 – 135	<0.0001
LA_Vol_i [ml/m²]	21 ± 9 (10 – 39) 6 – 63	37 ± 17 (12 – 71) 8 – 76	<0.0001

Table 57: Calculated echocardiographic parameters in MF patients with and without severe cardiomyopathy

Severe Cardiomyopathy	No	Yes	p
n	87	31	
E-wave Vmax [m/s]	0.86 ± 0.16 (0.61 – 1.13) 0.52 – 1.38	0.85 ± 0.21 (0.55 – 1.29) 0.53 – 1.5	NS
A-wave Vmax [m/s]	0.57 ± 0.14 (0.38 – 0.84) 0.28 – 0.97	0.66 ± 0.15 (0.46 – 0.91) 0.42 – 0.93	0.007
n	86	31	
E/A-Ratio [ ]	1.61 ± 0.48 (0.86 – 2.4) 0.77 – 3.53	1.35 ± 0.42 (0.75 – 2.28) 0.67 – 2.68	0.013
n	74	26	
IVRT [ms]	73.5 ± 17.0 (38.8 – 108.2) 35 – 112	93.7 ± 23.3 (56.6 – 127.4) 45 – 160	<0.0001

Table 58: Diastolic functional echocardiographic parameters in MF patients with and without severe cardiomyopathy

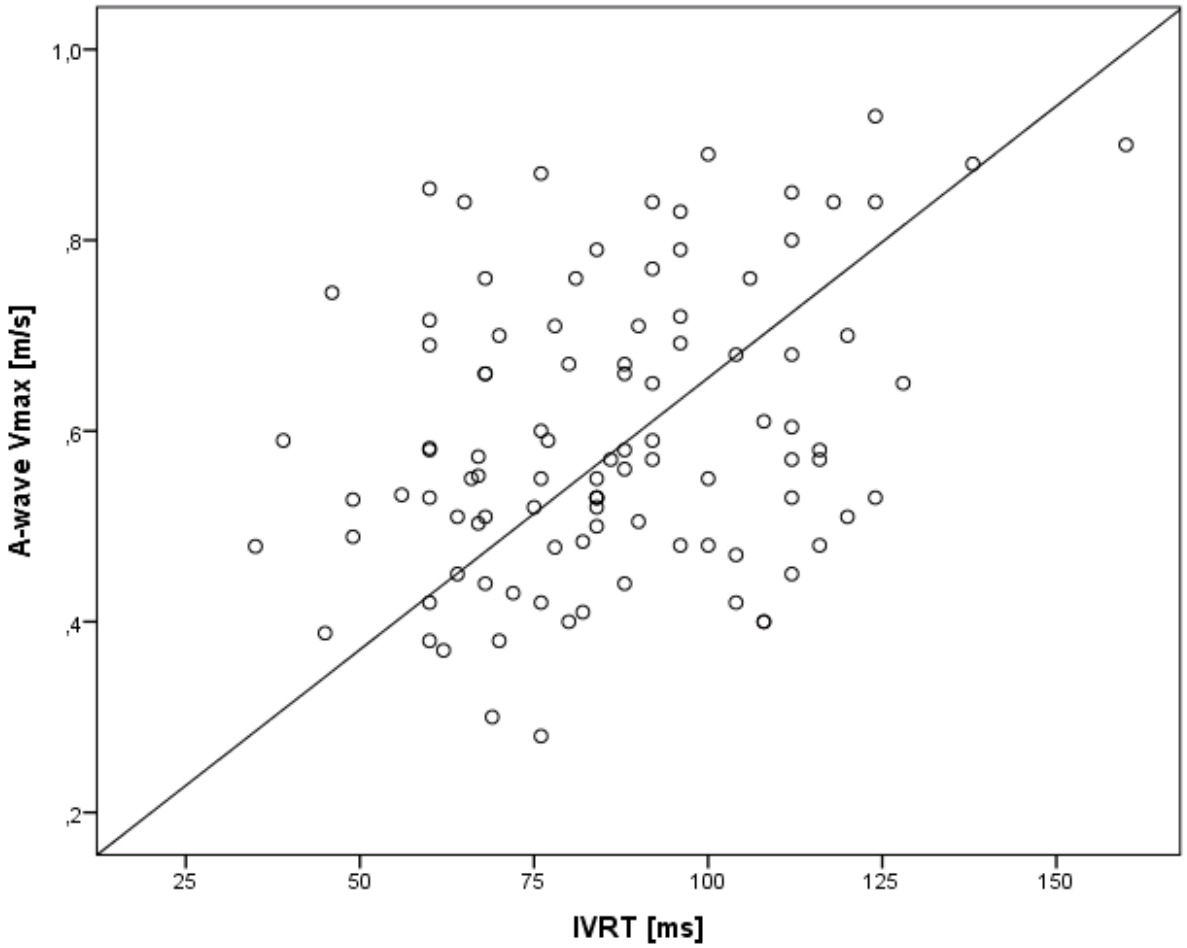

Figure 27: Correlation of A-wave Vmax [m/s] and IVRT [ms] in MF patients

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