Obstructive hypertrophic cardiomyopathy

Pathophysiology and clinical management

by

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2. LIST OF PAPERS

I **Vibeke M. Almaas**, Kristina H. Haugaa, Erik H. Strøm, Helge Scott, Christen P. Dahl, Trond P. Leren, Odd R. Geiran, Knut Endresen, Thor Edvardsen, Svend Aakhus, Jan Peder Amlie. Increased amount of interstitial fibrosis predicts ventricular arrhythmias, and is associated with reduced myocardial septal function in patients with obstructive hypertrophic cardiomyopathy. *Europace. 2013;15:1319–1327.*

II **Vibeke M. Almaas**, Kristina H. Haugaa, Erik H. Strøm, Helge Scott, Hans-Jørgen Smith, Christen P. Dahl, Odd R. Geiran, Knut Endresen, Svend Aakhus, Jan Peder Amlie, Thor Edvardsen. Non-invasive assessment of myocardial fibrosis in patients with obstructive hypertrophic cardiomyopathy. *Heart. 2014;100:631-638.*

III **Vibeke M. Almaas**, Helge Skulstad, Ole-Gunnar Anfinsen, Knut Endresen, Odd R. Geiran, Jan Peder Amlie, Svend Aakhus. Impaired diastolic function in obstructive hypertrophic cardiomyopathy patients with non-successful percutaneous transluminal septal myocardial ablation. *Submitted.*

IV Jensen MK, **Almaas VM**, Jacobsson L, Hansen PR, Havndrup O, Aakhus S, Svane B, Hansen TF, Kober L, Endresen K, Eriksson MJ, Jorgensen E, Amlie JP, Gadler F, Bundgaard H. Long-term outcome of percutaneous transluminal septal myocardial ablation in hypertrophic obstructive cardiomyopathy: a Scandinavian multicenter study. *Circ Cardiovasc Interv. 2011;4:256-265.*

3. ABBREVIATIONS

2D	two-dimensional
AFOG	acid fucsin orange-G
CAD	coronary artery disease
CK	creatine kinase
CK-MB	creatine kinase muscle-brain
ECG	electrocardiogram
EF	ejection fraction
HCM	hypertrophic cardiomyopathy
ICD	implantable cardioverter defibrillator
IVSd	interventricular septal dimension
LGE	late gadolinium enhancement
LGE-CMR	late gadolinium enhancement cardiac magnetic resonance
LV	left ventricle
LVOT	left ventricular outflow tract
MWT	maximal wall thickness
NSVT	non-sustained ventricular tachycardia
NYHA	New York Heart Association
PTSMA	percutaneous transluminal septal myocardial ablation
ROC	receiver operating characteristic
ROI	region of interest

SAM	systolic anterior motion
SCD	sudden cardiac death
SD	standard deviation
STE	speckle tracking echocardiography
VT	ventricular tachycardia

4. INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a common global genetic cardiovascular disease with a heterogeneous clinical spectre. The reported prevalence is 0.02 – 0.2% (1:5000 – 1:500).[1-4] These figures will translate into 1000 - 10 000 individuals affected in Norway, exceeding the number of patients actually discovered. Thus many affected individuals are undiagnosed probably without symptoms and with normal life expectancy, but in some patients the disease progression results in significant complications. [5] The most frightened complication is sudden unexpected death.

An autosomal dominant mutation in a gene encoding thick or thin myofilament proteins or Z-disc proteins of the cardiac sarcomere (i.e. sarcomere-gene mutations) is identified in approximately 60-70% of the HCM-patients with a positive family history, and in approximately 10-50% of the HCM-patients without a family history.[6,7]

Genotype	Phenotype	Clinical manifestation
Positive	Positive	HCM diagnosis: Hypertrophy ≥13 mm
	Negative	Subclinical HCM
Negative	Positive	HCM diagnosis: Hypertrophy ≥15 mm
	Negative	Normal

Figure 4.1. *Genotype positive patients mean that a HCM related mutation is identified, and genotype negative that a HCM related mutation not is identified. Genotype positive patients get the HCM-diagnosis when left ventricle (or right ventricle) wall thickness is measured ≥13 mm. Genotype negative patients get the HCM-diagnosis when wall thickness is measured ≥15 mm.*

The American guidelines from 2011 emphasize that the diagnose HCM is linked to left ventricular hypertrophy *with* a sarcomere mutation identified *or* a genetic substrate unresolved but unexplained left ventricular hypertrophy ≥15 mm without extracardiac or

metabolic findings.[2] Unlike the American guidelines from 2011 the European guidelines from 2014 use a purely morphological disease definition of HCM with the possibility of a number of aetiologies: "*HCM is defined by the presence of increased left ventricle wall thickness (≥15 mm) that is not solely explained by abnormal loading conditions."*[4] In these guidelines HCM is defined by specific morphological criteria and then grouped into familial/genetic or non-familial/non-genetic subtypes, irrespective of the presence of extra-cardiac diseases.

Autopsy-reports from patients suffering sudden cardiac death (SCD) and histological evaluations of explanted hearts from HCM-patients with end-stage heart failure have demonstrated the histopathological hallmarks of HCM with myocyte hypertrophy, regions of myocyte disarray, different types of fibrosis, and small vessel disease.[9] Myocyte disarray is characterized by regions of disorganized myocytes arranged obliquely or perpendicularly to each other with scattered areas of interstitial fibrosis (named plexiform fibrosis by Anderson et al in 1979). [10] Whereas myocyte disarray is present in several cardiovascular diseases, the amount of disarray in HCM is substantially higher (5-10% of the ventricular septum).[11] In the majority of cases, myocyte disarray is extensive and >20% of the myocardium will exhibit disarray in at least two histopathological tissue blocks.[11] It has been argued that myocyte disarray is the arrhythmogenic substrate in young HCM-patients[9], and that increased amount of myocyte disarray is associated with diastolic dysfunction.[12,13] Both macroscopic and microscopic fibrosis are described in HCM-patients.[14] Macroscopic scars are grey or white small patchy or large transmural scars most pronounced in the septum.[15] The myocardial ischemia in HCM-patients is usually unrelated to atherosclerotic coronary artery disease, but rather caused by supply-demand mismatch due to left ventricle (LV) hypertrophy with increased oxygen demand and adverse loading conditions. The myocardial ischemia is followed by medial hypertrophy and luminal narrowing of the intramural arterioles, thickening of these walls, and subsequently, causes compromised intramural blood flow.[16-18]

Anderson et al described different types of microscopic fibrosis: replacement fibrosis, interfibre fibrosis, perivascular fibrosis and plexiform fibrosis in 1979.[19] Until recently the vast majorities of the studies reported total amount of fibrosis and the studies were largely

concerned with the ischemic type of fibrosis (replacement fibrosis). Replacement fibrosis may be a result of small vessel disease, remodelling and longstanding disease, and is increased in patients with heart failure and end stage HCM.[18] Recently, there has been an increased focus on the collagen network that builds up the structural skeletal framework of the extracellular matrix (diffuse interstitial fibrosis).[20] Young HCM-patients have increased amount of collagen and the morphology of the collagen network is abnormal.[21] The role of this diffuse interstitial fibrosis has received less attention, but its role in HCM related arrhythmogenesis is increasingly recognized.

Patients with HCM have obviously reduced myocardial function due to several pathophysiological factors as myocyte hypertrophy, myocardial disarray, myocardial fibrosis, and sarcomere mutations associated with structural abnormalities and possible force generation deficiency. These factors may not influence on myocardial ability of reduction of LV cavity volume, and thereby EF is maintained in these patients.[22] Newer echocardiographic methods as myocardial strain assessment have the ability to detect reduced segmental and global myocardial systolic function.[23-27] Strain-measurements by tissue-Doppler echocardiography were first validated by Urheim et al in 2000.[27] Compared to normal, Yang et al described in 2003 reduced longitudinal Doppler strain in HCM-patients.[28] Additionally, reduced septal longitudinal Doppler strain correlated well with increased wall thickness and degree of asymmetrical septal hypertrophy. In 2004 strain by speckle tracking echocardiography (STE), a more robust and angle independent method using conventional two-dimensional (2D) grey-scale imaging, was validated by Amundsen et al in 2006.[23] Serri et al used this method and compared myocardial function in HCM-patients and normal and described reduced segmental and global 2D strain in HCM patients despite normal EF.[22] In the same paper, in a subgroup analysis of HCM-patients with asymmetrical septal hypertrophy, the authors demonstrated that the strain values in the basal septal segments were lower than other LV segments.[22]

Late gadolinium enhancement cardiac magnetic resonance (LGE-CMR) imaging has become the reference standard for noninvasively assessment of myocardial scarring. A substantial part of the HCM-patients have fibrosis detected with LGE.[29] The distribution of LGE in HCM-patients does not correspond to coronary arteries, but is mainly located in the mid-wall of

the hypertrophic regions and characteristically appears as small punctuate and patchy hyperenhancements.[29] Case reports of HCM-patients have demonstrated that location of myocardial fibrosis post-mortem is similar to that of fibrosis diagnosed by LGE studies,[30,31] but the histological substrate of LGE in HCM-patients is not finally determined. Whether gadolinium-contrast accumulates only in areas with replacement fibrosis, or also accumulates in areas with interstitial fibrosis, is debated.[31] There is an association between the presence of LGE and cardiovascular mortality, heart failure death and all-cause death,[32] and LGE is associated with ventricular arrhythmia on Holter monitoring.[31,33] However, at this time there is still insufficient evidence to support a significant association between the extent of LGE and outcome.[34-37]

SCD due to ventricular tachyarrhythmias is most common in young asymptomatic patients <30 year of age.[38] Several deaths in competing athletes have drawn public attention to the disease. In a total HCM-population the over-all annual mortality is ≤1%, but high risk groups closes up to 6%.[39] Implantable cardioverter defibrillator (ICD) is the only effective treatment preventing SCD, but prediction of malignant arrhythmias and SCD based on individual symptoms and characteristics have been of limited success.[40]

It is well established that left ventricular outflow tract (LVOT) obstruction is a major determinant of outcome[41] and often contributes to the heart failure-related symptoms that may occur in HCM.[42] One third of the HCM-patients are obstructive with a peak resting LVOT gradient ≥30 mmHg (*basal obstructive HCM*).[43] The mechanisms of LVOT obstruction are usually related to mitral valve systolic anterior motion (SAM) and basal septal hypertrophy.[44,45] The level of the obstruction in the LV cavity is related to the SAM contact point and the region of the maximal septal hypertrophy. Midcavitary obstruction occurs due to midventricular hypertrophy, hypertrophied papillary muscles[46] or anomalous papillary muscle insertion into the anterior mitral leaflet with the possibility of development of an apical aneurysm.[47] The LVOT gradient (ΔP) is measured as a peak instantaneous LVOT velocity (m/sec) using the simplified Bernoulli formula ($\Delta P=4V^2$). It is important to avoid contamination with the Doppler signal from the mitral regurgitation. Another third of the HCM-patients have dynamic LVOT obstruction with a resting LVOT gradient <30 mmHg and a significant increase of the gradient above 30 mmHg during Valsalva manoeuvre or exercise

testing (*labile obstructive HCM*).[43] The final third of the HCM-patients has a LVOT gradient <30 mmHg both at rest and with provocation (*non-obstructive HCM*).

Resting peak LVOT gradient	Provocable peak LVOT gradient	
<30 mmHg	<30 mmHg	Non obstructive HCM
<30 mmHg	≥30 mmHg	Labile obstructive HCM
≥30 mmHg		Obstructive HCM

Figure 4.2. *HCM-patients are classified as non-obstructive, labile obstructive and basal obstructive due to their resting and/or provocable (Valsalva manoeuvre or exercise induced) peak LVOT gradient[48]. The LVOT gradient (ΔP) is calculated from the peak instantaneous LVOT velocity (m/sec) using the simplified Bernoulli formula ($\Delta P=4V^2$).*

It has clinical important consequences to distinguish between obstructive and non-obstructive patients because treatment strategies rely on these. Marked gradients ≥30-50 mmHg at rest or ≥50 mmHg with provocation, represent the thresholds for septal reduction therapy with either surgical myectomy or percutaneous transluminal septal myocardial ablation (PTSMA), if symptoms cannot be controlled by medications.[5]

Septal reduction therapy is indicated in HCM-patients with severe symptoms despite optimal medical therapy.[2] The patients have to be basal obstructive or labile obstructive with LVOT gradient at rest ≥30-50 mmHg or ≥50 mmHg with physiological provocation, associated with SAM of the mitral valve and septal hypertrophy ≥15-16 mm.

Surgical myectomy was introduced in the 1950s and has been the preferred option to relief LVOT obstruction and symptoms at experienced centres with solid short and long term results.[49-53] Surgical myectomy is done only at selected centres and is therefore less available for a substantial part of symptomatic obstructive HCM-patients. Because of comorbidities and advanced age, some patients are not optimal candidates for surgery, and in 1995 the catheter based procedure PTSMA was introduced.[54] Several studies have reported both short- and long term outcome of PTSMA (small single-centres studies with 50-138 patients

included[55-60], large single-centres studies with 312-629 patients included[61-63], one meta-analysis[64]). In June 2011 an American multi-centre study of long term outcome after PTSMA, reporting 9-year mortality, was published.[65] There are no randomized trials, only observational studies[58,66-72] and meta-analyses[73-76], comparing the outcomes of PTSMA and myectomy. These studies report similar low rates of all-cause mortality and SCD following PTSMA and myectomy. However, the rest-gradient after PTSMA tends to be higher than after myectomy, and some more patients need a permanent pacemaker after PTSMA due to advanced atrioventricular block.[73] Most patient develop right bundle branch block after PTSMA, and left bundle branch block after myectomy.[77] In the latest European guidelines from 2014 PTSMA and myectomy are considered equivalent, but myectomy is recommended if there are additional lesions requiring surgical intervention (mitral valve repair/replacement, papillary muscle intervention, coronary artery by-pass graft surgery or aortic valve replacement).[5] PTSMA may be less effective in patients with severe hypertrophy (≥30 mm) and in patients with extensive scarring on CMR, but systematic data lack.[5] Many patients prefer the less invasive PTSMA procedure. Studies report non-successful treatment of PTSMA in 10-20% of the patients, but there are no established prediction factors according to predict successful PTSMA.

5. AIMS OF THE THESIS

This thesis is based on two main objectives. First, we wanted to evaluate the relation between fibrosis and clinical characteristics in HCM patients. The other main objective was to evaluate the effect of PTSMA and factors associated with non-successful PTSMA.

The aims of the present work were:

1. To investigate the impact of different types of fibrosis on ventricular arrhythmias and on myocardial function (Paper I).
2. To compare the ability of two non-invasive imaging methods to detect myocardial fibrosis (Paper II).
3. To identify patients that do not benefit on PTSMA treatment (Paper III)
4. To analyse the short- and long-term outcome of PTSMA compared to a normal background population (Paper IV).

6. PATIENTS AND METHODS

6.1. Patient population

The majority of HCM patients referred to Oslo University Hospital, Department of Cardiology, Rikshospitalet are either obstructive considering septal reduction therapy or they are referred according to risk stratification considering ICD implantation. Since 2001 PTSMA has been a part of the treatment options at Department of Cardiology, Oslo University Hospital, Rikshospitalet. Septal myectomy was reintroduced as a surgical procedure at Department of Cardiothoracic Surgery, Oslo University Hospital, Rikshospitalet in 2003. PTSMA has been the first line of treatment. Patients with additional significant coronary artery disease (CAD) or heart valvular disease are offered myectomy. If the PTSMA-procedure had to be stopped due to coronary artery anatomy or the delivery area, the patients are offered myectomy.

Between 2001 and 2009 104 patients with HCM were referred to Oslo University Hospital for evaluation for septal reduction therapy and were recruited to this prospective observational study (Paper I, II and III, and the Norwegian part of the patients in Paper IV) (Figure 7.1.). Eighty-four of the 104 patients were considered to fulfil criteria for septal reduction therapy with symptoms (New York Heart Association (NYHA) functional class >2) despite optimal medical treatment and a resting LVOT gradient ≥30 mmHg or a provocable LVOT gradient ≥50 mmHg. The decision of treatment strategy was made after thorough information to the patients about the two intervention methods, and after interdisciplinary team-work with experienced thoracic surgeons and cardiologists with special interest for HCM-patients. Two patients fulfilled the septal reduction therapy criteria, but did not receive septal reduction therapy due to co-morbidity in one and the patient`s own desire in the other. Due to limited basal septal hypertrophy and a serious LVOT gradient and mitral regurgitation with long leaflet and SAM, one patient was operated with mechanical mitral valvular replacement without additional myectomy. One patient had had a prior unsuccessful PTSMA in 2002, at another heart centre, with persistent symptoms and significant LVOT gradient. The patient had a re-do PTSMA in 2004. Forty-six patients were selected for PTSMA and 34 for myectomy. One of the myectomized patients was selected to myectomy based on individual preferences. Twenty-three of the myectomy-patients needed combined surgery with either

additional coronary artery bypass graft surgery and/or heart valvular surgery. In 5 of the patients selected for PTSMA the procedure was stopped due to the coronary anatomy in 2 patients and due to unsatisfactory delivering area in 3. Four of these patients were operated with isolated myectomy, but in one patient myectomy was contraindicated due to co-morbidity. Three months after the attempt of PTSMA this patient died due to acute decompensated heart failure.

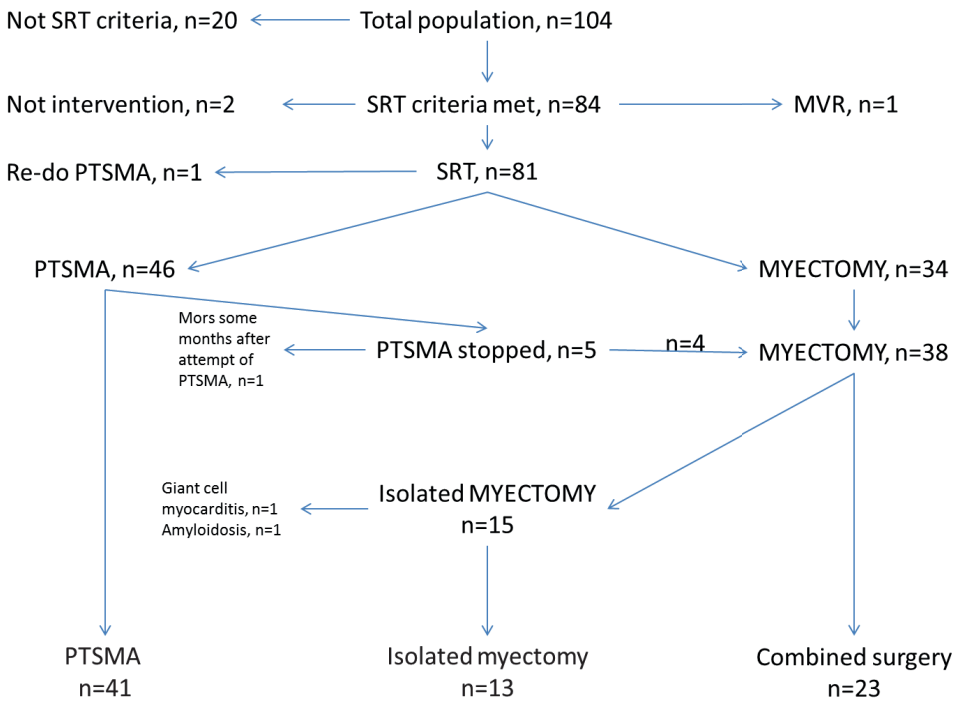

Figure 6.1. *Flow chart of the total population of 104 HCM-patients referred to Oslo University Hospital primarily considering septal reduction therapy between 2001 and 2009. Abbreviations: MVR, mitral valve replacement; PTSMA, percutaneous transseptal myocardial ablation; SRT, septal reduction therapy.*

6.2. Scandinavian HOCM database

The Scandinavian HOCM database (www.scand-hocm.org) was established in collaboration among heart centres at Copenhagen University Hospital, Rigshospitalet, Denmark, Copenhagen University Hospital, Gentofte, Denmark, Karolinska University Hospital, Stockholm, Sweden, and Oslo University Hospital, Rikshospitalet, Norway, in 2006. The database is defined as a quality register and was built up in collaboration with all the participating countries. The register complies with data registry regulations within the European Union and in Norway, and was approved by relevant data authorities and committees for ethics in medicine. According to the Regional Committee for Medical Research Ethics in Norway and the Privacy Policy at Oslo University Hospital, the register at Rikshospitalet in Oslo was defined as a combined quality and research register requiring written and oral informed consent from the participants. Two participants from the 3 countries constitute the steering board of the database.

In Paper IV all PTSMA-treated patients from 1999 to 2010 in the 4 centres were included, of which 35 Norwegian patients. The patients were referred from a background population of approximately 50% of the Scandinavian population corresponding to 10 million citizens. In 2007 retrospective data for this study were merged in the database, and since 2007, prospective data from new patients and follow-up data have been included. Data retrieved from patients' files, echocardiography reports, and survival registries, were registered at baseline and annually during follow-up. Data were extracted from the Scandinavian HOCM database on 4th of April 2010.

All the centres used the same HCM-diagnose definition and the same septal reduction therapy criteria, unless the peak resting LVOT gradient which was ≥30 mmHg in Oslo and ≥50 mmHg in the other centres. The PTSMA procedure and monitoring of the patients after the procedure were the same at the 4 centres.

6.3. Genetic testing

All patients included in this study were tested for mutations in 6 regions encoding cardiac sarcomere-proteins. A total of 86 polymerase chain reaction products together spanning the translated exons with flanking intron sequences of the sarcomere genes myosin-binding protein C (MYBPC3), β myosin heavy chain (MYH7), regulatory and essential light chains of myosin (MYL2 and MYL3), and cardiac troponin T (TNNT2) and I (TNNI3) were subjected to DNA sequencing using Version 3.1 of BigDye terminator cycle-sequencing kit and a Genetic Analyzer 3730 from Applied Biosystems (Foster City, CA). Pathogenicity of identified mutations was assessed by the use of the bioinformatics programs PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/).

6.4. Exercise test

A maximal symptom limited exercise test was performed at baseline, and 3 and 12 months after septal reduction therapy using an electrically braked bicycle ergometer (Jaeger ER900, VIASYS Healthcare GmbH, Hochberg, Germany). A protocol starting at 20 watt with 20 watt increase every other minute was chosen and repeated for all patients. Oxygen consumption (VO_2), CO_2 production (VCO_2), and ventilation (VE) were measured in 38% (39/104, 2007 - 2009) of the patients on a breath-to-breath basis (MVmax 229, VIASYS Healthcare GmbH, Germany). Heart rate and electrocardiogram (ECG) were continuously recorded, and blood pressure was recorded at baseline, at every step, and every minute after ended exercise. Peak VO_2 and respiratory exchange ratio (RER) were registrated at the end of the test.

6.5. Holter monitoring

Forty-eight hours ECG monitoring with 5 electrodes (Medilog, ScanMed AS) was performed prior to and 3 and 12 months after the septal reduction therapy. Type of rhythm, episodes of bradycardia, and supraventricular (atrial fibrillation and other forms of supraventricular arrhythmias) and ventricular arrhythmias (non-sustained ventricular tachycardia (NSVT) and ventricular tachycardia (VT)) were registered.

6.6. Risk stratification and definition of ventricular arrhythmia

Risk stratification in order to prevent SCD and evaluation of indication of ICD were performed prior to, and 3 and 12 months after septal reduction therapy. According to guidelines from 2003 for risk stratification, patients with survived heart arrest or documented sustained VT were offered a secondary preventive ICD.[3] NSVT was defined as 3 or more ventricular complexes with heart rate ≥120 and duration <30 seconds. If two or more of the following risk factors were available, implantation of ICD was evaluated: (1) family history of premature SCD, (2) recent unexplained syncope (suspected arrhythmogenic syncope and/or syncope during exercise), (3) documented NSVT, (4) LV hypertrophy ≥30 mm, (5) abnormal blood pressure response during exercise testing. Premature SCD in the family was defined as sudden unexpected death without any known heart disease and/or HCM-related sudden death in first- and/or second degree relatives, and age < 40 year. In Paper I and II ventricular arrhythmia was defined by ≥1 of the following criteria: (1) prior cardiac arrest with documented ventricular arrhythmias, (2) documented NSVT at Holter monitoring, (3) unexplained syncope.

6.7. Echocardiography

Transthoracic echocardiography was performed at baseline, and 3 and 12 months after septal reduction therapy, using a commercially available digital ultrasound scanner (Vivid 7 with the M3S transducer, GE Vingmed Ultrasound, Horten, Norway). Images were obtained in two parasternal and three standard apical views and the recordings and analyses were performed according to standards.[78] In Paper I and II maximal wall thickness (MWT) and septum-posterior wall ratio were assessed from parasternal short-axis plane at the level of the papillary muscle. Additionally, in Paper III, maximal basal septal hypertrophy (end-diastolic interventricular septal dimension (IVSd)) was evaluated in 2D greyscale images from parasternal long-axis plane. In Paper IV measurements of IVSd was performed either with 2D greyscale images or M-mode from parasternal long-axis view.

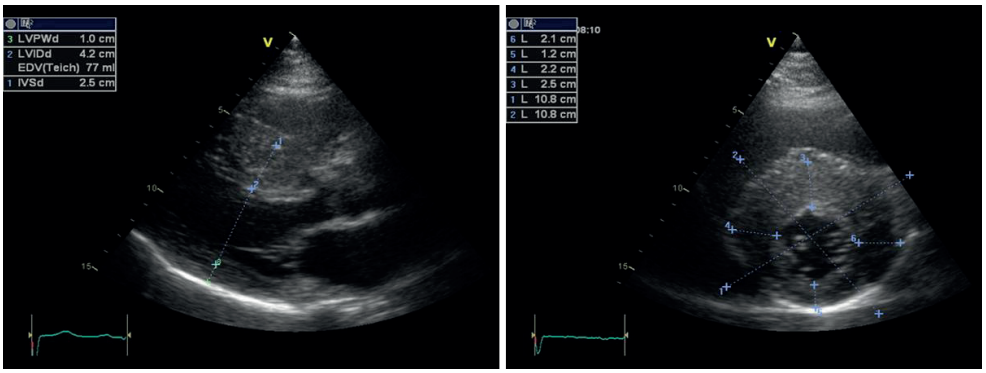

Figure 6.2. *End-diastolic interventricular septal dimension (IVSd)) was evaluated in two-dimensional greyscale images from parasternal long-axis plane (left). Maximal wall thickness (MWT) and septum-posterior wall ratio were assessed from parasternal short-axis plane at the level of the papillary muscle (right).*

To measure the extent of LV hypertrophy we used Spirito-Maron index and Wigle's score (Paper II).[79,80] Peak resting LVOT gradient was measured by continuous wave Doppler. When resting LVOT gradient was <30 mmHg exercise induced LVOT gradient was measured by ergometric stress-echocardiography. Ergometric stress-echocardiography was done in a subset of 63 patients (61%) with a semi-recumbent exercise bike (Ergoline) in a tilted position (Figure 7.2.). A protocol starting at 20 watt with 20 watt increase every second minute was chosen and repeated for all patients. During the test continuous rhythm registration (12-lead ECG) and blood pressure measurements every second minutes were done. Peak LVOT gradient was measured at rest, after 4-6 minutes exercise, and at maximal exercise. Peak exercise induced LVOT gradient was defined as maximal LVOT gradient measured during exercise.

Figure 6.3. *The semi-recumbent exercise bike (Ergoline) used for ergometric stress echocardiography.*

2D strain by STE was performed in the three standard image planes from the apex. The endocardial contour was traced on an end-diastolic frame, and the software automatically tracked the contour, and with of region of interest (ROI) was adjusted to fit the average of the myocardial thickness. If segments were automatically excluded by the software, the width of ROI and/or the endocardial contour were manually adjusted to achieve an optimal speckle tracking. Global longitudinal strain was defined as an average of peak systolic strain in a 16-segments model. Septal longitudinal strain was defined as an average of peak systolic strain of basal- and mid-septal segments from the apical 4-chamber view (Paper I: 2-segments septal strain) and from apical long-axis view and apical 4-chamber view (Paper II: 4-segments septal strain).

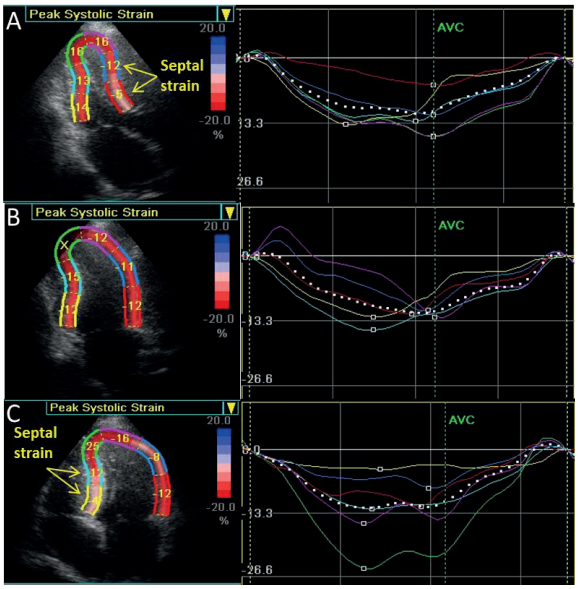

Figure 6.4. *Sixteen-segments model of longitudinal strain by speckle tracking echocardiography. Septal longitudinal strain was defined as an average of peak systolic strain of basal- and mid-septal segments from the apical longaxis (**A**) and 4-chamber view (**C**). **A** shows apical longaxis view, **B** apical 2-chamber view, and **C** apical 4-chamber view.*

Global circumferential strain was obtained from short axis view at the papillary muscle level, averaging peak systolic strain from six segments. Septal circumferential strain was defined as an average of peak systolic strain from two septal segments. In a subset of 18 patients (Paper II) global longitudinal strain was additionally evaluated with tissue Doppler imaging-derived strain.

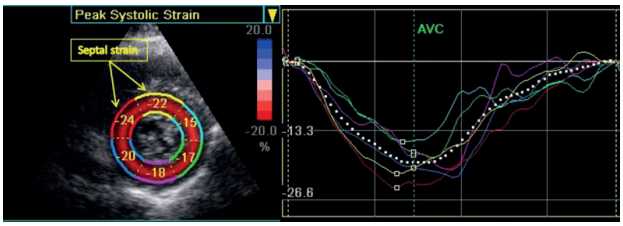

Figure 6.5. *Six segments model of circumferential strain by speckle tracking echocardiography. Septal circumferential strain was defined as an average of the anteroseptal and septal segments from the parasternal shortaxis-view.*

6.8. Cardiac magnetic resonance

Cardiac magnetic resonance (CMR) was performed at baseline in 77 of the 104 patients. In 69 patients late LGE-CMR was available. CMR was performed using a 1.5 tesla scanner (Siemens, Erlangen, Germany) and a phased array body coil. Using an electro cardiogram-triggered segmented balanced gradient echo technique, breath-hold long axis and short axis cine images were acquired. After 10-20 minutes intravenous injection of 0.2 mmol/kg gadopentetate dimeglumine were injected. The LGE images were obtained with multiple short axis slices covering the entire LV with a slice thickness of 7 mm and an interslice gap of 3 mm. With an inversion time of 210 – 260 msec a breath-hold segmented magnetization-prepared turbo gradient echo sequence was used.

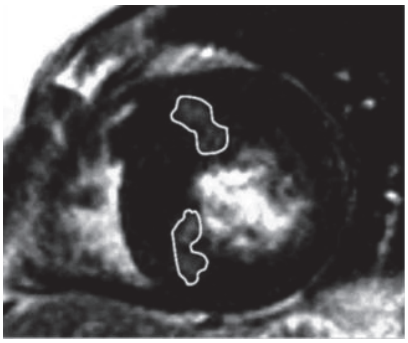

Figure 6.6. *LGE-CMR short-axis image of a patient with obstructive hypertrophic cardiomyopathy with LGE present in the basal anteroseptal (upper area) and inferoseptal*

segments(lower area). Adapted by permission from BMJ Publishing Group Limited (Almaas et al, Heart 2014;100:631-638).[81]

EF was calculated using end-diastolic volumes and end-systolic volumes. The extent of LGE was quantified in a subset of 25 myectomized patients, by a visual appreciation using a 17-segments model.[82] For each segment, the areas of the LGE and the area of the entire myocardium were manually drawn. The amount of LGE was calculated as percentage of the actual segment. Global LGE was defined as the percentage LGE of LV. Septal LGE was defined as the percentage LGE of the basal and mid antero- and inferoseptal segments.[83] The LV mass was calculated by adding the areas of all 17 segments and multiplying by 1.04 g/cm3.[84] All the CMR analyses were performed by an experienced radiologist (HJS) blinded to all clinical data.

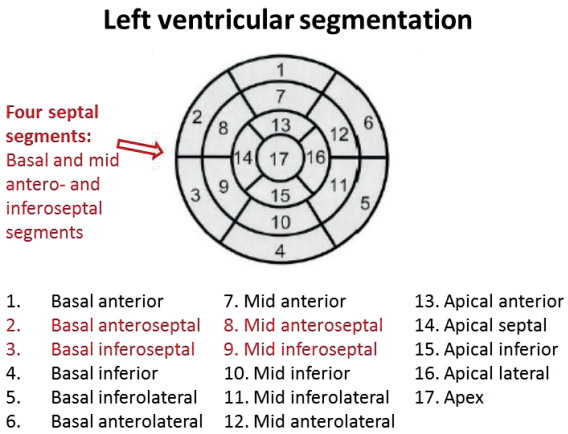

Figure 6.7. *An illustration of the 17 segments CMR model. The percentage LGE in the basal and mid antero- and inferoseptal segments was defined as septal LGE.*

6.9. PTSMA

In the first 29 patients both catheter measured and transthoracic echocardiographic LVOT gradient was determined prior to the procedure, and in the last 18 patients only

echocardiographic measured LVOT gradient was used. The left main coronary artery was intubated and visualized, after which the first septal branch was probed. Then, an over the wire angioplasty balloon catheter was placed in the proximal part of the septal branch. After the balloon was inflated to a pressure of 8 bars, an injection of contrast medium through the balloon central lumen was done. Simultaneously transthoracic echocardiography was used to confirm the delivery area to only the target myocardium, which was defined as the septal region corresponding with SAM-contact point and peak LVOT gradient. If the target myocardium was not reached, a more distal branch or the second septal branch was tried. In some few patients the contrast enhanced other regions (papillary muscles) and the procedure had to be stopped. When the contrast enhancement confirmed delivery area to the target myocardium, about 1-2.5 ml of 96% alcohol was injected. Immediately after the alcohol-injection the LVOT gradient, measured by echocardiography, was reduced. The balloon catheter was deflated after 10 minutes. If the LVOT gradient was not reduced to <16 mmHg, an additionally smaller dosage of alcohol (0.5 – 1 ml) was injected in a more distal septal branch or in the second septal branch after controlling the delivery area. After the PTSMA-procedure the patients were carried out in the intensive care unit for 24 hours for ECG monitoring and determination of creatine kinase (CK), creatine kinase muscle-brain fraction (CK-MB), and Troponin T values 6 hours after the procedure and the following morning, followed by daily ECG check-ups including evaluation of development of bundle branch block and measurements of PQ-time according to development of atrioventricular block. The patients were transferred to the cardiologic ward for 3-5 days with continued ECG monitoring, unless they had developed atrioventricular block in need of pacing. The temporary pacing wire inserted prior to the ablation was left there for at least 48 hours. A transthoracic echocardiographic examination was done before discharge

6.10. Myectomy

The interventricular septum was exposed through an aortic incision, protecting the aortic leaflets. After visual inspection and digital exploration the myocardial resection was started below the right coronary cusp moving distally and leftwards. The dissection proceeded distal to the mitral valve contact with the septum, taking care to remove fibrous tissue and

aberrant muscle bundles towards the apex. Valve function, extent of resection and relief of LVOT obstruction as well as unexpected findings was evaluated by transoesophaegal echocardiography, and guided the surgeon whether to resume surgery or complete the operation.

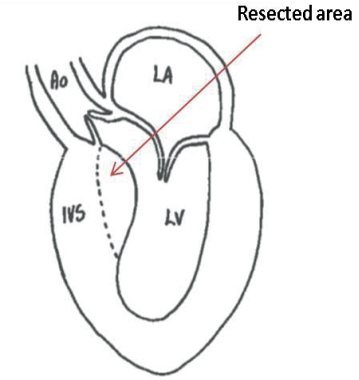

Figure 6.8. *An illustration of the basal septal area resected during myectomy. Ao, aorta; IVS, interventricular septum; LA, left atrium; LV, left ventricle.*

6.11. Histologic examination of the operation specimens

The operation specimens were fixed in 10% formalin, paraffin-embedded and sectioned for light microscopy. Two staining methods were used for light microscopy; hematoxylin-eosin-saffron and acid fucsin orange-G (AFOG). The last method was used to highlight connective tissue. To exclude storage disease a small proportion of each specimen was fixed in glutaraldehyd, embedded in epon and processed for electron microscopy.

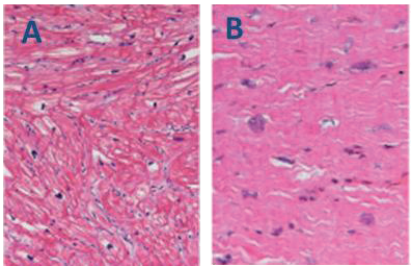

Figure 6.9. *Hematoxylin-eosin-saffron-stained sections form myectomy specimens.* ***A****: Myocyte disarray.* ***B****: Myocyte hypertrophy without disarray. Reprinted with permission form European Cardiology 2010;6:88-91.*[85]

We classified the fibrosis as interstitial, replacement, perivascular or subendocardial.[14] Confluent fibrosis substituting the myocytes were classified as replacement fibrosis, and thin strands of fibrous tissue encircling the myocytes were classified as interstitial fibrosis. Perivascular fibrosis and myocyte disarray were assessed as being present or not. Subendocardial fibrosis was excluded from the analyses.

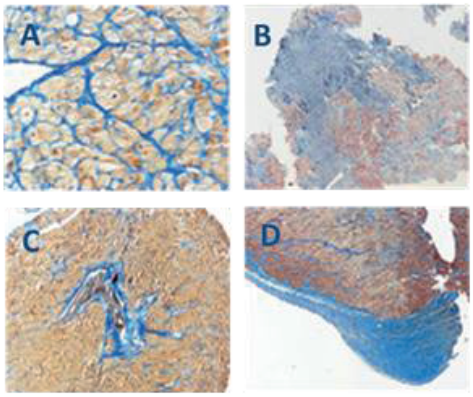

Figure 6.10. *AFOG-stained sections from myectomy specimens. Collagen fibrils (fibrosis) are staining blue, nucleus black, erythrocytes and cytoplasm pink-orange, elastic fibrils pale pink or unstained, and protein deposits red.* ***A****: Interstitial fibrosis.* ***B****: Replacement fibrosis.* ***C****: Perivascular fibrosis.* ***D****: Subendocardal fibrosis. Reprinted with permission form European Cardiology 2010;6:88-91.*[85]

Interstitial and replacement type of fibrosis were quantified in AFOG-stained sections by two observers (EHS and HS) simultaneously. The observers were blinded for clinical data and outcome. They made a visual estimate of the percentage area affected by interstitial and replacement fibrosis of the total specimen. Total amount of fibrosis was an addition of interstitial and replacement fibrosis. After 12 weeks a repeated blinded quantification of replacement fibrosis was performed.

6.12. Statistical analysis

Unpaired Student's t-test was used to compare normally distributed continuous data presented as mean ± standard deviation (SD), and Mann-Whitney U test to compare non-normally distributed data presented as medians (25%, 75% percentiles). Pearson Chi Square or Fischer's Exact test were used for comparisons of proportions. Two-sided p-values <0.05 were considered statistically significant. Reproducibility was expressed by intraclass correlation coefficient in Paper I and II. All statistical analyses were performed with SPSS IBM, New York (version 18 in Paper I and II, and version 21 in Paper III),

In Paper I univariate linear regression analyses were used to identify explanatory variables of reduced septal longitudinal strain. In the total population (n=63) and in the histology population (n=24) univariable logistic regression analyses were used to identify predictors of arrhythmic events. In the total population multivariable analysis was performed including significant variables (p<0.05) from the univariable analyses. Receiver operating characteristic (ROC) analyses were used to assess the ability of global and septal longitudinal strain and amount of histological fibrosis to discriminate patients with ventricular arrhythmias.

In Paper II non-parametric correlation test (Spearman's rho) was used to analyse the relation of %area of histological fibrosis, %LGE, and echocardiographic strain. To identify predictors

of histological fibrosis ≥15% (50% percentile) univariable logistic regression analyses were performed. Modifying factors as CAD, MWT, and peak LVOTG, were included in the multivariable logistic regression analysis.

In Paper IV “baseline” refers to pre-PTSMA data and “most recent follow-up” to the most recently available data after PTSMA. When normally distributed, data was presented as mean±SD and when non-normally distributed as median (interquartile range). Proportions were presented as percentages (%). Student’s t-test or Wilcoxons Rank Sum tests were used for unpaired comparisons of the data, depending on normally or non-normally distribution.Student’s t-test was performed for paired comparisons of continuous variables. Proportionswere compared with Chi-square test or Fishers exact test when unpaired, and McNemar testwhen paired. In the survival analyses death of all causes was considered an event. KaplanMeier estimates of survival were calculated, and time-to-event curves were compared performing Log Rank test. Univariate Cox regression model was performed to predict death of all causes, and predictors showing association ($p \leq 0.1$) with decreased survival entered a multivariate Cox regression model. All statistical analyses were performed with SAS® statistical software package version 9.1.

7. SUMMARY OF RESULTS

7.1. Paper I

In this study 63 HCM-patients (mean age 57±13 years) were examined by strain by STE prior to septal reduction therapy with either PTSMA (n=37) or basal septal myectomy (n=26). Fifteen patients (24%) had experienced ventricular arrhythmias defined as documented VT or arrhythmogenic suspected syncope. Patients with ventricular arrhythmias had significantly higher MWT, and reduced global and septal longitudinal strain compared to those without ventricular arrhythmias. By univariate linear regression analyses, ventricular arrhythmias (R^2=0.12, p=0.006), MWT (R^2=0.28, p<0.001), and the presence of a sarcomere mutation (R^2=0.10, p=0.01) were significantly associated with reduced septal longitudinal strain. In the multiple regression analysis MWT was independently associated with reduced septal longitudinal strain (p=0.005). (Reduced septal longitudinal strain, increased MWT, and genotype-positive status significantly predicted ventricular arrhythmias. In the multivariable analyses septal longitudinal strain was an independent predictor of ventricular arrhythmias. Operation specimens were available in 24 of the myectomized patients (histology population). Six of these patients (25%) experienced ventricular arrhythmias. In the histology population the patients with ventricular arrhythmias had a greater extent of total fibrosis (47(30, 71)% vs. 10(6, 15)%, p=0.001), interstitial (44(23, 61)% vs. 7(2, 12)%, p=0.001) and replacement fibrosis (7(3, 14)% vs. 3(1, 3)%, p=0.02) compared to the patients without ventricular arrhythmias. There was a linear association between septal longitudinal strain and percentage area of total (R^2=0.31, p=0.006) and interstitial (R^2=36, p=0.003), but not with replacement fibrosis (R^2=0.03, p=0.43). Univariable logistic regression analysis of septal strain, total, interstitial and replacement fibrosis, showed that reduced septal longitudinal strain and increased amount of total and interstitial fibrosis significantly predicted ventricular arrhythmias.

These results suggest that total amount of fibrosis is associated with ventricular arrhythmias in obstructive HCM patients. Interstitial fibrosis was more important compared with replacement fibrosis in arrhythmogenesis, and was related to reduced septal myocardial function. These findings suggest that interstitial fibrosis may play an important role as the

arrhythmogenic substrate, and that strain echocardiography might be useful in risk assessment.

7.2. Paper II

In this study the abilities of LGE and strain echocardiography to detect type and extent of myocardial fibrosis in 32 obstructive HCM-patients (age 60±10 years) undergoing septal myectomy were assessed. The percentages of total, interstitial and replacement fibrosis were 15(7, 31)%, 11(5, 24)%, and 3(1, 6)%, respectively. LGE was present in 13 out of 21 patients (62%), and these patients had 9(2, 10)% septal LGE. There were no significant differences in echocardiographic and histological parameters between the patients with and without septal LGE. No correlations were found between percentage septal LGE and total, interstitial, or replacement fibrosis. There was a linear correlation between septal longitudinal strain and total and interstitial fibrosis, but not with replacement fibrosis. To predict histological fibrosis ≥15% (median value) the following parameters were used: age at intervention, sex, additional diseases, CAD, HT, MWT, peak LVOT gradient, EF, global longitudinal strain, septal longitudinal strain, global circumferential strain, and septal circumferential strain. Septal longitudinal strain predicted histological fibrosis ≥15% in both univariable analyses and in the multivariable model.

Eight patients (25%) experienced ventricular arrhythmias. These patients had a higher amount of total and interstitial fibrosis, and a lower septal longitudinal strain compared to the patients without arrhythmias (n=24). In univariable logistic regression analyses and in the multivariable model, septal longitudinal strain and interstitial fibrosis predicted ventricular arrhythmias significantly. LGE did not predict ventricular arrhythmias. No significant differences were demonstrated between patients with (n=4) and without (n=9) ventricular arrhythmias with respect to percentage global and septal LGE.

Thus, in myectomised HCM-patients the extent of total fibrosis correlated with preoperative reduced septal strain, but not with the amount of septal LGE. Echocardiographic strain might add valuable information in HCM-patients.

7.3. Paper III

In this study we evaluated whether pre-procedural parameters were associated with non-successful PTSMA. Echocardiographic and clinical examinations were performed before procedure and one year after. At one year follow-up we used three definitions of successful intervention. *Symptomatic success* was defined as a reduction of ≥1 NYHA functional class. *Hemodynamic success* was defined as rest LVOT gradient <30 mmHg or ≥50% reduction of exercised induced LVOT gradient. *Combined symptomatic and hemodynamic success* was defined as a combination of symptomatic and hemodynamic success.

Forty obstructive HCM-patients (age 56±14 years) treated with PTSMA were enrolled. Two patients died, two patients were lost to follow-up, and one labile obstructive patient was not evaluated by ergometric stress-echocardiography at one year follow up. Thus, 35 patients were evaluated one year after PTSMA.

One–year survival was 95%, and mean improvement of NYHA functional class 1.0±0.7. The reduction of peak LVOT gradient was 37±24 mmHg. Eight out of 35 patients (23%) had symptomatic non-successful PTSMA, 9 out of 35(26%) had hemodynamic non-successful PTSMA, and 12 out of 35 (34%) had combined symptomatic and hemodynamic non-successful PTSMA. Non-successful PTSMA (symptomatic, hemodynamic, and combined symptomatic and hemodynamic) was associated with younger age at intervention and a higher residual LVOT gradient at follow-up than successful PTSMA ($p<0.01$ for all the comparisons). Patients with non-successful hemodynamic response to PTSMA had higher transmitral E/A-ratio (1.66±0.91 vs. 1.13±0.38, $p=0.03$) and lower E-wave transmitral deceleration time (201±46 msec vs. 263±76 msec, $p=0.04$) at baseline compared to hemodynamic successful PTSMA.

Using all the three definitions of successful PTSMA there were no differences in pre-procedural peak LVOT gradient, in volume alcohol injected during the procedure and of infarction size evaluated with maximum CKMB when comparing successful and non-successful PTSMA.

Thus, patients with non-successful hemodynamic response of PTSMA had pre-procedural worse diastolic dysfunction than the patients with successful PTSMA. Patients with non-successful PTSMA (symptomatic, hemodynamic and combined symptomatic and hemodynamic) were associated with younger age at intervention.

7.4. Paper IV

In this international multi-centre study we evaluated long-term outcome after PTSMA in 279 obstructive HCM-patients (mean age 59±14 years) at four Scandinavian high-volume PCI-centres/moderate-volume PTSMA-centres. Long-term outcome of 313 PTSMA procedures were reported. Mean time of follow-up were 2.9±2.6 years.

The resting peak LVOT gradient was reduced from 58(34-89) mmHg at baseline to 12(8-24) mmHg at one year follow-up and to 12(8-21) mmHg ($p<0.001$) at most recent follow-up (2.9±2.6 years after first PTSMA). The proportion of patients in NYHA functional class III/IV decreased from 94% at baseline to 21% ($p<0.001$) at one-year follow-up and to 21% ($p<0.001$) at most recent follow-up.

88% of patients achieved symptomatic improvement defined as decreased NYHA- or CCS-class or disappearance of syncope at last follow-up. Two years after the procedure 14% of the patients were treated with a re-do septal reduction therapy (26 PTSMA, 6 myectomy).

In-hospital mortality was 0.3%, and 30-day mortality was 0.6%. Twenty percent of the 230 patients with no permanent pacemaker or ICD prior to PTSMA-procedure, had a permanent pacemaker implanted after PTSMA.

Median observational time used in the survival analyses was 3.7 years (IQR, 1.4 to 6.3 years). Overall mortality rate was 3% per year. The 1-, 5-, and 10-year survival rates after PTSMA were 97%, 87%, and 67%, respectively, compared to 98%, 90%, and 78% in the age- and sex-matched background population ($p=0.06$). Patients aged <60 years (48±9 years, n=141) had 1-, 5-, and 10-year survival rates of 99%, 94%, and 88% compared to 100%, 97%, and 93% ($p=0.12$) in the age- and sex-matched background population. In patients aged ≥60 years (70±7 years, n=138) the 1-, 5- and 10-year survival rates were 95% , 79% , and 46%

compared to 97%, 83%, and 63% (p=0.09) in the age- and sex-matched background population. At most recent follow-up 11% had an ICD. Age at baseline, sex, chronic obstructive pulmonary disease, and sum of arrhythmic events during in-hospital monitoring showed p-values ≤1 in univariable cox regression analyses and were associated with reduced survival. In a multivariable model only age at baseline showed significant association to survival (HR, 1.07; 95% CI, 1.03 to 1.1; p=0.001).

Thus, in this multicentre study, the in-hospital morality after PTSMA was low. The symptomatic and hemodynamic effects were sustained. The 10-year survival rate was not significantly different from that in a gender- -and age-matched background population.

8. DISCUSSION

HCM is the most prevalent genetic cardiac disease and affects many patients. The disease-spectrum is broad, ranging from non-discovered symptom-free patients to severe cardiomyopathy and sudden death. Selecting the right patient to the right treatment, prediction of treatment-response, and identification of patients at risk are crucial. In this thesis we have examined several facets of this topic. In Paper I and Paper II we demonstrated that reduced longitudinal septal strain correlated with ventricular arrhythmias. As reduced strain also was interrelated with increased fibrosis this may provide an explanation for this association. Septal reduction therapy has a central role in management of HCM, but the role of myectomy and PTSMA has not been finally determined. In paper IV we demonstrated that PTSMA may be performed with satisfactorily results. The results in Paper III indicate that younger patients should be offered myectomy as these patients are less likely to get good results from PTSMA. Young age at the time of symptom debut may reflect a more aggressive disease. In line with this we found that genotype positive patients were younger at symptom onset and at intervention, and had a higher frequency of ventricular arrhythmias (Paper I). Furthermore, patients with non-successful PTSMA had a worse pre-procedural diastolic dysfunction. Thus pre-procedural assessment is important for selection of the patients to the right treatment.

8.1. The HCM population - one diagnosis - several diseases?

The European guidelines from 2014 use a purely morphological disease definition of HCM with the possibility of a number of aetiologies: "*HCM is defined by the presence of increased LV wall thickness that is not solely explained by abnormal loading conditions.*" This means that the definition of HCM include patients with and without sarcomere-mutations, and patients with deposition (e.g. amyloidosis) and metabolic (e.g. Anderson-Fabry) diseases. Although these patients meet the diagnosis requirements according to LV wall thickness and loading conditions, the underlying pathophysiology could be quite different. The variation in the aetiological background included in this broad definition may translate into subgroup

differences in risk factors and therapy outcome within the same disease. Thus the clinicians have to be conscious about what kind of HCM-population they are dealing with.

Initially only the PTSMA-treated patients were included in this study. From 2005 all the HCM-patients referred to Rikshospitalet considering septal reduction therapy were included. Patients who were myectomized before this time were retrospectively included. These factors influence on the composition of the HCM-population in this study. Thus in the first years primarily obstructive PTSMA- and myectomy-treated patients were included. From 2005 there is a more mixed population of obstructive and non-obstructive HCM-patients. In the final population of 104 patients, 84 (81%) are obstructive. This does not reflect a general HCM-population, and for this reason, we have concentrated on the obstructive patients (n=84). Primarily 41 patients were treated with PTSMA, and 38 with myectomy (15 isolated myectomy, and 23 with combined surgery). Fifty-five percent of the patients (46/84) had additional CAD and/or HT and/or other additional diseases, 31% (25/81) of the patients tested for a HCM related mutation was genotype positive (unpublished results). Due to different research questions and hypotheses different subgroups of the total population of 84 patients with obstructive HCM are investigated in Paper I, II and III. Paper I contains 63 patients accepted for PTSMA or myectomy, Paper II includes 32 myectomized patients, and Paper III and IV 40 and 279 PTSMA-treated patients, respectively. Due to the intervention-selection-criteria at Oslo University Hospital, Rikshospitalet, the population treated with myectomy contained markedly more patients with additional cardiovascular diseases than the PTSMA population. In Paper I 46% (29/63) had additional CAD and/or HT and 38% (24/63) were genotype positive. In Paper II 72 % (23/32) had additional diseases with CAD, and/or myocardial infarction, and/or HT, and/or diabetes mellitus without organ complication, and/or other diseases, and only 13% (4/32) were genotype positive. In Paper III 3% had additional CAD, 35% HT, and 45% were genotype positive.

HCM is a common cardiovascular disease, and in clinical practice a substantial part of the HCM-patients have common additional diseases as CAD and HT. In many HCM-studies patients with additional diseases are excluded. The reason for limiting investigations to a narrowly-defined homogenous HCM-population is to address the pathophysiological changes directly related to the sarcomere-disease. However, HCM-patients with additional

diseases constitute an important part of the HCM population. Consequently, one must take the reservation that results derived from studies where HCM patients with additional diseases are excluded cannot be extrapolated to the total HCM-population where additional cardiovascular diseases are common. We did not want to exclude patients with common additional diseases. Our studies are performed on HCM-populations that include patients with additional CAD and HT to reflect the populations that we face in clinical practice.

There are some differences between the studies in this thesis with respect to inclusion of patients with CAD and myocardial infarction. In Paper I all the patients with CAD and myocardial infarction were excluded (n=4), but in Paper II only patients with CAD and myocardial infarction in the basal septal region were excluded (n=0). Consequently, 4 patients with myocardial infarction in Paper II were not excluded. Both in Paper I and II patients *with CAD without myocardial infarction* were not excluded. With respect to ventricular arrhythmias, these differences did not influence on septal myocardial function and the importance of interstitial type of fibrosis.

To get more knowledge about the effect of an isolated sarcomere-disease and how the disease presentation and development are influenced by additional cardiovascular disease, a population with pure sarcomere-disease and a population with sarcomere-disease and additional cardiovascular disease had to be compared. Another factor that has not been studied in this thesis is whether the HCM disease in itself influences that development of CAD and whether CAD influences the progression of a HCM disease. The frequency of CAD in the obstructive HCM group exceeds the rate found in an age-matched population by far. Whether this reflects that the HCM disease increases the risk of CAD or whether this is merely a reflection of the selection of the patient groups is undetermined. The lower incidence of CAD in the genotype positive HCM population may be explained (at least partly) that these patients are younger at the time of intervention. The interrelationship between CAD and HCM disease should be studied further.

In addition to the rate of obstructive HCM patients and the number of patients with additional diseases other factors as age at debut of symptoms and genotype status are important characteristics. In Paper I we found that the genotype positive patients were younger at symptom onset and at intervention, and had a higher frequency of ventricular

arrhythmias. Additionally, in Paper III we found that younger patients had less favourable effects of PTSMA compared to older patients. This may reflect that the disease progression is different according to age at symptom debut and genotype status.[86,87]

More than 1400 mutations in 11 or more sarcomere-genes are known to definitively cause HCM. Of the genotype positive patients, about 70% are found to have mutations (of either definitive or uncertain pathogenicity) in the most common genes, beta myosin heavy chain (MYH7) and myosin binding protein C (MYBPC3), while other genes including troponin T (TNNT), troponin I (TNNI), alpha tropomyosin, actin, regulatory light chain, and essential light chain, each account for a small proportion of the patients (1-5%).[6,8] In 1990 the first description of a specific HCM mutation (beta myosin heavy chain) was reported[88], but the molecular mechanisms by which HCM-associated mutations cause the disease remain unclear and controversial.[7] Initially, impaired myofibrillar contractile function was suggested to be the most important mechanism accounting for compensatory hypertrophy and diastolic dysfunction, but the altered contractility caused by various sarcomeric gene mutations is not consistently found. A variety of functional defects, including altered calcium cycling and sarcomeric calcium sensitivity, increased fibrosis, disturbed biomechanical stress sensing and impaired energy homeostasis have been revealed.[7,89] In the past some HCM genes and variants have been reported to convey more severe disease than others, but on the basis of large cohorts of genetic tested unrelated HCM-patients there are now consensus that a specific HCM mutation cannot predict clinical outcome.[5,6] Anyway, irrespective of a defined HCM-mutation, genotype positive HCM-patients have increased risk of adverse events compared to genotype negative HCM-patients.[86,87] Up to 5% of the patients carry at least two independent mutations (compound or double heterozygous). Triple gene mutations in sarcomeric proteins has been reported with an incidence of 0.8%.[7] Double and triple mutations are associated with early onset and a severe phenotype. In line with earlier published studies, our results indicate that genotype positive HCM-patients (sarcomere-mutations) have a poorer prognosis compared to genotype negative patients.[86,87] The composition of the patient populations should be carefully considered when different studies are compared.

8.2. Fibrosis

Paper I (n=24) and II (n=32) included overlapping, but slightly different, populations as patients with additional diseases suspected to produce hypertrophy were excluded in paper I, but not in paper II. However, the presence of total (100% vs 97%), interstitial (100% vs 94%), and replacement fibrosis (100% vs 97.%), and the median amount of total (14% and 15%), interstitial (10% vs 11%) and replacement fibrosis (3% vs 3%) were not different in the two studies. Moravsky et al have published similar levels of fibrosis in HCM patients with mean amount of total fibrosis (16%), interstitial fibrosis (14%) and replacement fibrosis (2%)[90] In Paper I and II we used AFOG as a myocardial fibrosis staining method. AFOG has been the preferred fibrosis-staining method at our laboratory due to visibility and contrast to other co-stains used. Collagen fibrils are staining blue, nucleus black, erythrocytes and cytoplasm pink-orange, elastic fibrils pale pink or unstained, and protein deposits red. Various staining methods for assessment of fibrosis in HCM patients have been used, including Picro Sirius[21] and different trichrome methods.[90] In the recent years Picro Sirius has been the most frequently used fibrosis staining method.[91] None of the methods are specific for collagen as other connective tissue components are also stained. Studies comparing the methods are scarce. Despite different staining methods both Moravsky et al and Shirani et al reported fibrosis in HCM patients at the same level as we did. The latter study included however younger HCM patients so the populations are different. Nevertheless we have no indications that these differences in staining methods constitute major differences in the interpretation of the level of fibrosis.

Studies of different cardiac diseases have shown remodelling of the extracellular matrix components resulting in enhanced stiffness of the myocardium and diastolic and systolic dysfunction as well as increased electrical instability.[92-98] Ho et al examined 77 genotype positive HCM-patients, 38 with overt HCM and 39 without LV hypertrophy (genotype positive/phenotype negative) and 30 controls, with serum biomarkers of collagen metabolism.[95] All the 77 patients had increased amount of serum biomarkers indicating increased myocardial collagen synthesis. The fact that all the 39 genotype positive patients without LV hypertrophy had increased levels of serum biomarker for collagen synthesis indicated that myocardial interstitial fibrosis was an early disease manifestation in patients

with HCM. These findings correspond well with Shirani et al who examined 16 previously asymptomatic children and young adults with HCM who died suddenly, and found increased amount of and changed structure of microscopic analysed interstitial matrix collagen (interstitial fibrosis) compared to controls . None of these patients had myocardial replacement fibrosis. Our results in Paper I and II indicate the same, that increased amount of both total and interstitial fibrosis is associated with ventricular arrhythmias. The association between myocardial fibrosis and arrhythmias make non-invasive assessment methods assessing HCM related fibrosis, possible candidates to predict arrhythmias.

8.3. Arrhythmia, LGE and strain

Prediction of risk for malignant arrhythmias and SCD is important in the care of HCM patients. In the papers included in the thesis we found that 24% (Paper I), 25% (Paper II) and 23% (Paper III) of the obstructive HCM patients were diagnosed with ventricular arrhythmias (including arrhythmia suspect syncope) before septal reduction therapy. These figures are in line with other studies.[31,33,37,99,100] Patients with one or several of the traditionally 5 risk markers have increased risk of SCD.[2-4] Unfortunately, these 5 risk markers have low positive predictive value, and many of the patients who have an ICD implanted never experience ICD-discharge. On the contrary, the absences of these 5 risk factors has a negative predictive value of >95% for malignant arrhythmias. In 2014 a novel risk prediction model, using 7 predictors (age, maximum LV wall thickness, LA size, max LVOT gradient, family history of SCD, NSVT and unexplained syncope), for SCD in HCM-patients was described.[101] The risk prediction model make a individualize 5-years low (<4%), intermediate (≥4% and <6%) or high (≥6%) risk estimate for the probability of SCD.[3,101] However, the effort to improve the prediction models continuous in order to pick the right patients for ICD and several other factors such as myocardial fibrosis determined by LGE-CMR, LV apical aneurysm and the inheritance of multiple sarcomere protein gene mutations have been suggested.[2,5] LGE-CMR has been associated with ventricular arrhythmias and development to heart failure and end-stage disease.[31,33,102] Available data is currently not considered sufficient for LGE to be judged as an independent risk marker for adverse prognosis and SCD in HCM in addition to or replacing the other traditional risk factors.[5,35] In Paper II we did not find any significant

differences in amount of global or septal LGE between patients with or without ventricular arrhythmias. However, this may be due to the relatively low number of patients. In a recent published study of 1293 HCM-patients Chan et al showed for the first time that increased extent of LGE was associated with an increased risk of SCD.[35] They demonstrated a 2-fold increase in SCD risk in those patients otherwise considered to be at lower risk. However, it has to be mentioned that McKenna and Nagueh pointed out that 16 of the 20 patients (80%) who died suddenly or experienced a cardiac arrest had LGE ≤5%.[103] This indicates that while there is an association between LGE and arrhythmias this tool is not yet sensitive enough to predict ventricular arrhythmias. One factor that may explain this is the fact that the histological substrate representing LGE is not finally characterized, and there is an ongoing discussion whether the interstitial fibrosis can be detected with the LGE-method at all.[20] Gadolinium is an extracellular agent accumulating in areas with interstitial expansion due to myocardial fibrosis, oedema or infiltration.[104] In animal models delayed enhancement corresponds well with ischemic/fibrotic infarction area due to CAD.[104] There are only some few case-reports comparing LGE and corresponding microscopic fibrosis in explanted hearts from HCM-patients.[30,31] The lack of existing data linking LGE-CMR with histology was the background for performing the study in Paper II where we found no relation between amount of fibrosis and LGE. Furthermore, enhanced LGE was not, in contrast to longitudinal septal strain, associated with arrhythmias.

Different grey-scale thresholding methods have been used to define areas of LGE.[90] The presence and amount (percentage of total LV mass) of LGE may either be judged visually or by using dedicated software.[104] The quantification methods rely on the fact that LGE makes the scar brighter than normal myocardium. LGE has been defined as signal intensity of ≥2 SD above normal myocardium. In HCM-patients it has been stated that using the 2SD-method LGE will be overestimated, and thus, several studies now use the 5-6SD-method to measure LGE in HCM-patients.[35,90,105] Moravsky et al described a good correlation between 6SD-method and visual estimate.[90] In the above-mentioned study by Chan et al, the authors used visual estimate to demonstrate an association between LGE and an increased risk of SCD.[35]

In Paper II we used a visual estimate to measure the presence and amount of LGE and our results (presence of global LGE of 62%) corresponds well with others (presence of LGE of

56% and 41%).[33,37] The presence of basal septal LGE of 52% in Paper II (11 of 21 patients) is also in the same range as the findings in a study by Kwon et al of 63% (38 of 60 patients).[83] In our study 7 of the 8 patients without visualized LGE had microscopic fibrosis in the myectomy specimens. This means that visually estimated LGE-CMR does not reflect the total amount of myocardial fibrosis which may be responsible for malignant arrhythmias in HCM-patients. However, whether new methods are better correlated with both fibrosis and ventricular arrhythmias are yet to be answered. A new LGE-CMR method, T1-mapping and extracellular volume quantification, may be useful for the assessment of diffuse interstitial fibrosis.[105] This method was not available at the time of our project.

LGE-CMR is limited in clinical use both by methodological difficulties and restricted availability. MS Maron and Pandian pointed out in an editorial-comment of Di`Salvo et al`s echocardiographic study of 93 HCM-patients, "Is 2D echocardiographic strain ready for prime time?"[106,107] In the study of Di`Salvo et al the patients with NSVT (26%) had lower longitudinal septal strain compared to the patients without NSVT, and the presence of longitudinal strain ≥-10% in >3 of 16 segments predicted NSVT independently with a sensitivity of 81% and specificity of 97%. These findings corresponds well with our findings both in Paper I and II, where patients with NSVT (24% and 25%, respectively) had longitudinal septal strain in the same area as Di`Salvo. In Paper I and II the echocardiographic findings were compared with microscopic fibrosis in operation specimens from the myectomized patients. The results demonstrated that patients with ventricular arrhythmias had higher amount of microscopic fibrosis compared to patients without arrhythmias, and when separating into replacement and interstitial type of fibrosis there was a significant association between reduced longitudinal septal strain and interstitial fibrosis. In Paper I reduced longitudinal septal strain was an independent predictor of ventricular arrhythmias in the total population of 63 patients, and in the histology-population of 24 patients both longitudinal septal strain and increased amount of interstitial fibrosis predicted ventricular arrhythmias significantly in a univariable logistic regression analysis. Although the numbers of patients are low in Paper I and II, longitudinal septal strain significantly predicted ventricular arrhythmias and detected microscopic fibrosis. This was in contrast to LGE-CMR, indicating that longitudinal septal strain is a more powerful tool to predict ventricular arrhythmias than visual estimated LGE.

8.4. Myocardial function

Myocardial function evaluated by EF is normal or often supra-normal in patients with HCM. Patients with HCM have obviously reduced myocardial function due to several pathophysiological factors as myocyte hypertrophy, myocardial disarray, myocardial fibrosis, and sarcomere mutations associated with structural abnormalities and possible force generation deficiency. These factors may not influence on myocardial ability of reduction of LV cavity volume, and thereby EF is maintained. In all the four Papers mean EF was normal. Therefore, it is necessary with several non-invasive modalities to quantify myocardial function in HCM-patients. Normal LV systolic function is the result of deformation caused by synchronous contraction of myocardial fibres in different orientations.[108] The LV long-axis is oriented from apex to base, the radial axis is perpendicular to the epicardium away from the cavity, and the circumferential axis is perpendicular to the radial and longitudinal directions and is orientated counterclockwise around the short-axis. The fibre orientation changes gradually from a right-handed helix (+60°) in the subendocardium to a left-handed helix (-75°) in the subepicardium.[109] The midwall-fibres are circumferentially oriented. During systole endo- and epicardial longitudinal fibre components contribute to long-axis contraction, while the midwall circumferential fibre components contribute to the reduction of the short-axis diameter. Both these mechanisms contribute to systolic wall thickening and a reduction of LV cavity volume. Additionally, there is long-axis torsion due to contraction of the obliquely orientated fibres. The most widely used markers of global and regional LV function, ejection fraction (EF) and wall motion score index, utilize the reduction of cavity volume during LV systolic ejection, and visual evaluation of LV regional wall motion and thickening, respectively. Strain is a measure of deformation. In cardiac mechanics strain represents the fractional or percentage change in dimension, and is defined as tissue elongation in relation to end-diastolic length. Positive strain refers to elongation and negative strain to shortening. Strain is a vector that can be measured along any given axis. Quantification of myocardial strain along longitudinal and circumferential shortening and radial thickening, represent a measure of contractile function. In line with several other studies, the results in Paper I and II demonstrated that longitudinal function is

reduced[22,28,110-112], and circumferential function is maintained.[110,111] Some studies report decreased values of global circumferential function in HCM.[22,112] In Paper I, II and III (unpublished results) mean global and septal longitudinal strain were reduced, although the level differed due to different patient populations. Global and septal circumferential strain reported in Paper II, were increased. In line with our results, Urbano-Moral et al found decreased longitudinal and increased circumferential 3D-strain and concluded that the extent of hypertrophy, not the fibrosis detected by LGE, was the primary factor altering global myocardial mechanics.[113]

In an animal model with myosin-binding protein C-knockout mice there was substantial myoarchitectural disarray located in the mid-myocardium-subendocardium, and impairment of the transmural progression of helix angles from subepicardium to subendocardium.[114] The mid-wall circumferential fibreorientation was retained. Urbano-Moral et al speculated that the longitudinal subendocardial muscle layers, which are responsible for most of the longitudinal deformation, are the most impaired functionally due to changed fibreorientation.[113]

8.5. Septal reduction therapy

The latest European guidelines equate PTSMA and myectomy as septal reduction therapy for obstructive HCM-patients with a resting or provoked LVOT gradient ≥50 mmHg and with symptoms (NYHA functional class III-IV) despite maximum tolerated medical therapy.[5] Myectomy is favoured if there are additional lesions requiring surgical intervention (mitral valve repair/replacement, papillary muscle intervention, CAD, or moderate to serious aortic valve disease). Due to the less invasive nature of PTSMA this procedure has gained popularity and in the recent years PTSMA has been performed more frequently than myectomy.

In Paper IV we report persistent low LVOT gradient and symptomatic improvement, and good short and long outcome in a Scandinavian population of 279 obstructive HCM-patients. At publication time for this article (May 2011), this was the first international multi-centre long term outcome study of PTSMA. The main findings of this study, done at high-volume

PCI-centres and low/moderate-volume PTSMA-procedures (minimum of 10 PTSMA-procedures per year), was low in-hospital mortality rate (0.3%) comparable to other PTSMA-studies.[62,63] In June 2011 Nagueh et al published a multi-centre North-American long term outcome study of 874 PTSMA-treated patients.[65] They reported 1-, 5-, and 9-years survival estimates of 97%, 86%, and 74%, comparable of the 1-, 5-, and 10-years survival estimates in Paper IV of 97%, 87%, and 67%. Ommen et al reported in 2005 1-, 5-, and 10-year overall survival after myectomy (n=289) of 98%, 96%, and 83%.[52] In the same study non-operated obstructive HCM-patients (n=228) had 1-, 5-, and 10-years overall survival estimates of 90%, 79%, and 61%.[52]

There are no randomized trials comparing myectomy and PTSMA, but several meta analyses show equal symptom response and equal short- and long-term outcome.[115] The residual LVOT gradient tends to be higher after PTSMA, and there are a higher proportion of patients needing a permanent pacemaker after PTSMA. Thus if myectomy was available in every centre for all patients; should all the obstructive HCM-patients be operated with myectomy?

In the European guidelines myectomy is recommended if there are serious hypertrophy (≥30mm) and in young patients and children. Considering the nature of the PTSMA- and myectomy-procedure, it is reasonable to assume that myectomy is preferable in the patients with serious septal hypertrophy, but few studies support this statement. The guidelines recommend myectomy for children and young adults with obstructive HCM. As a consequence the literature lacks studies of PTSMA-procedures done at children and young adults.

At Oslo University Hospital, Rikshospitalet, both PTSMA and myectomy are established procedures, and according to guidelines, the selection of treatment option is result of a comprehensive discussion among experienced HCM-experts. However, it is often difficult to predict the most suitable procedure, and there are no established prediction-factors favouring PTSMA or myectomy. In Paper III we used three different success-definitions based on earlier published studies; symptomatic, hemodynamic, and combined symptomatic and hemodynamic success one year after PTSMA.[61,115,116] PTSMA is primarily a symptomatic treatment and studies report symptomatic improvement in 80-90% of the patients short and long time after PTSMA.[61,62] When combining both optimal symptomatic response and

improvement of exercise capacity Faber et al published successful PTSMA in 43% of the patients.[61]

LV relaxation, filling and compliance are frequently impaired in patients with HCM, and these factors are a major contributor to symptoms in these patients.[117-119] Diastolic dysfunction is a result of the underlying disease process in HCM and may represents a complex interaction of prolonged ventricular relaxation, myocardial fibrosis, and increased chamber stiffness leading to chronically elevated left ventricular filling pressures, which in turn result in left atrial enlargement. Maron et al described in 1987 impaired diastolic dysfunction assessed by Doppler echocardiography compared to normal.[120] Catheter measured LV end-diastolic pressure in obstructive HCM-patients is reported to be 22-25 mmHg.[121-123] In contrast to other cardiac diseases, in HCM-patients there is only a modest correlation between Doppler echocardiographic estimates of LV filling pressure using transmitral flow and mitral annular velocities, and directed catheter measured LV filling pressure.[117]

In the total population in Paper III age at intervention and baseline echocardiographic diastolic parameters were in the same range as earlier studies.[117,120,124,125] In Paper III we conclude that non-successful hemodynamic response was associated with worse diastolic function and younger age at intervention time. These parameters indicate that patients with more serious phenotype of HCM are more un-responsive of successful intervention with PTSMA. Sitges et al compared echocardiographic diastolic parameters before and 5 months after PTSMA and myectomy.[124] The parameters showed equal improvement of diastolic function after removal of LVOT obstruction after PTSMA and myectomy. To our knowledge we are the first to publish worse baseline diastolic parameters in patients with hemodynamic non-successful PTSMA.

8.6. Future perspective

One of the main finding in this thesis was the importance of differentiation between different types of fibrosis. Our studies indicate that different types of fibrosis have different clinical effects. Thus it is not sufficient to report total amount of fibrosis. As the type of fibrosis is associated with clinical effects, the pathophysiology of different types of fibrosis

should be examined at a molecular level. In our hands strain by STE was a powerful method to assess fibrosis and to predict ventricular arrhythmias. This should be performed in larger studies.

Given the current guidelines it is almost impossible to perform randomised controlled trials comparing myectomy and PTSMA. However, there might be sub-groups which might benefit on PTSMA and future studies should aim to identify these patients.

9. CONCLUSIONS

The main conclusions from this thesis are as follows:

- In HCM reduced septal myocardial function and interstitial fibrosis predicts ventricular arrhythmias.

- Reduced interventricular septal strain correlates with the extent of histological fibrosis in HCM-patients.

- Younger age at intervention and pre-procedural diastolic dysfunction is associated with non-successful PTSMA.

- PTSMA can be performed with low in-hospital mortality and with sustained symptomatic and hemodynamic effects with 10-year survival rate comparable to background population.

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