Echocardiographic measurements at Takotsubo cardiomyopathy

To my core family

Michaela, Tedde, David, Simon and Noah…

Örebro Studies in Medicine 111

MICAEL WALDENBORG

Echocardiographic measurements at Takotsubo cardiomyopathy
- transient left ventricular dysfunction

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Abstract

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Takotsubo cardiomyopathy (TTC) is a disease characterized by transient left ventricular (LV) dysfunction and typical wall motion abnormalities in apical parts, without obvious signs of coronary influence. Due to its elusive natural cause and the lack of clarified pathology, further studies are needed. Thirteen patients presented with an episode of TTC, and referred to Örebro University Hospital (USÖ), were prospectively included and investigated by comparisons made at onset (acute phase) against at follow-up three months later (recovery phase). Including echocardiographic measurements, focused on biventricular systolic long-axis function and conventional diastolic function (DF) variables. Systolic improvement was shown, while most DF data were unchanged, suggesting that TTC is mainly a systolic disease affecting both ventricles.

Diagnosis should include multidisciplinary engagement, as TTC associates both with emotional stress and pathological markers of physiological stress. In this thesis, such approach was offered to the aforementioned patients; to see if a common denominator could be found, thus, contributing to better handling. Emotional state was assessed, along with an array of cardiac investigations in addition to echocardiography. Acutely, imbalance in the autonomic cardiac control was shown, as well as a trend toward posttraumatic stress, but specific findings allowing conclusions on differential diagnosis could not be demonstrated.

By adding another 15 TTC patients (i.e. 28 in total), through collaboration with observers from USA, a retrospective echocardiographic analysis could be done to further study DF; concluding that TTC associates with impairment of conventional DF variables which tends to parallel the systolic recovery, in contrary to the initial result but in line with other causes of LV dysfunction.

Magnetic resonance imaging (MRI) is another method of choice at TTC. The USÖ patients had cardiac MRI, thus, a retrospective analysis was done to investigate the effect on LV geometry, both echocardiographic and by MRI; suggesting that TTC is consistently associated with increased LV mass, due to a local impact that seems to follow the change in LV concentric wall motion.

Keywords: Echocardiography, takotsubo, annulus motion, cardiac autonomic function, broken heart, diastolic, ventricular mass, concentric wall motion.

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Table of Contents

LIST OF PAPERS

This thesis is based on the following original papers, which throughout the text will be referred to by their Roman numerals (both in terms of paper or study).

I. Loiske K, Waldenborg M, Fröbert O, Rask P, Emilsson K. Left and right ventricular systolic long-axis function and diastolic function in patients with takotsubo cardiomyopathy. Clin Physiol Funct Imaging 2011;31(3):203-8.

II. Waldenborg M, Soholat M, Kähäri A, Emilsson K, Fröbert O. Multidisciplinary assessment of tako tsubo cardiomyopathy: a prospective case study. BMC Cardiovasc Disord 2011;11:14.

III. Sanjay K/Waldenborg M, Bhumireddy P, Ramkissoon K, Loiske K, Innasimuthu AL, Grodman RS, Heitner JF, Emilsson K, Lazar JM. Diastolic function improves after resolution of takotsubo cardiomyopathy. Clin Physiol Funct Imaging 2014 (doi: 10.1111/cpf.12188).

IV. Waldenborg M, Lidén M, Kähäri A, Emilsson K. Effect on left ventricular mass and geometry in patients with takotsubo cardiomyopathy. Submitted 2014.

Accepted papers (I-III) are reprinted with permission from the publishers, which in this regard are gratefully acknowledged.

Overall ABBREVIATIONS

AMI	Acute Myocardial Infarction
ASE	American Society of Echocardiography
CI	Confidence Interval (a measure for degree of certainty)
CV	Coefficient of Variation (a statistical method)
CW	Continuous-Wave (a Doppler technique)
DF	Diastolic Function
DTI	Doppler Tissue Imaging
ECG / SAECG	ElectroCardioGraphy / Signal-Averaged ECG
EF	Ejection Fraction
GRS	Global RS (the sum of a given number of RS segments)
HRV	Heart Rate Variability
ICD	International Classification of Diseases
LGE	Late Gadolinium Enhancement
LV / LVM	Left Ventricular / Left Ventricular Mass
MADRS-S	Montgomery Åsberg Depression Rating Scale (Self-rated)
MAM	Mitral Annulus Motion
M-mode	Motion mode
MRI	Magnetic Resonance Imaging
PTSS-10	PostTraumatic Stress Scale (10 questionnaire)
PW	Pulsed-Wave (a Doppler technique)
RS	Radial Strain (a segmental tissue-derived variable)
RV	Right Ventricular
RWT	Relative Wall Thickness
SWT	Segmental Wall Thickness
TAM	Tricuspid Annulus Motion
TTC	Takotsubo Cardiomyopathy
TTE	TransThoracic Echocardiography
U.S. / USA	United States of America
USÖ	University Hospital Örebro, Sweden
WMA	Wall Motion Abnormality
WMSI	Wall Motion Score Index
2D	Two-Dimensional
3D	Three-Dimensional

INTRODUKTION

A brief reflection about broken hearts:

> Has anyone here previously encountered expressions like "heartbreak" or "a broken heart"? I think of the tall tales you might have heard, about women who suddenly passed away, shortly after which they have lost someone they love. In my world, in my research, broken female hearts is far from fiction, it's actually the name of the disease that I'am studying, bedside with the patients and by ultrasound, with the aim for increased recognition. The shape of a regular urn could be compared to a normal heart in ultrasound, at least with some imagination, in patients who suffer from broken heart syndrome, however, the heart usually adapts another shape; where the so-called apex becomes rounded, loses contractility and turns out more or less akinetic. In the case of urns, this would be called a "Takotsubo", which is used as an octopus trap in Japan. I can imagine that such catch must sometimes be as hard to accomplish, as compared to within healthcare regarding patients with a broken heart, or Takotsubo, which is the real name of the disease. Of all those seeking care with a suspected heart attack, around two percent are actually suffering from a "Takotsubo-heart". Patients are usually women, over the age of 60, and the presence of some kind of stress is often associated with the onset. At present, we don't know the exact cause, what we agree on, however, is that more research is needed in this field. Thus, the purpose with my work, among others, is to increase the recognition of the syndrome, by the help of ultrasound. The hope is that "broken hearts" will be easier to catch, while patients, as a result, will receive proper diagnosis and treatment. (M. Waldenborg, 27 September 2013)

The above reflection is an English translation of an oral appearance, performed by the undersigned in 2013, in connection with participation in the Swedish contest "Forskar Grand Prix". This is an annual, national contest where scientists, from all kinds of disciplines and fields, compete against each other in terms of being "the best presenter". Each scientist has three minutes to talk about their research in front of a jury and ordinary spectators, which will then vote to determine a winner. The text above refers to the first part of the local subcompetition, arranged by the University of Örebro. Thus, the entire performance (including part one and two), which pretty much summarizes this thesis in a few minutes, can be viewed on the web through the University's own media channel (1, 2). For those of you who dislike taking shortcuts, does not understand Swedish or just happen to have a little more time to spare, the written summary, however, can be read as follows next...

The broken heart syndrome

Takotsubo syndrome (TTC), also known as stress-induced cardiomyopathy or "broken heart syndrome" is a relatively new diagnosis, first described in Japan in the early 1990s (3). The first major U.S. report was released in 2005 (4), while TTC was not highlighted in Europe until 2006 (5). TTC primarily affects post-menopausal women and associates with stress (physiological as well as emotional). At onset, TTC mimics the clinical presentation of an AMI; chest pain, newly ST-segment changes on ECG and increased levels of cardiac enzymes (4, 6-8). A reduction of LV systolic function is usually seen, while its apical area becomes aneurysmal with impaired mobility, corresponding to multiple coronary territories (6, 9). In contrast, hyperdynamic mobility is often seen in the basal parts of the LV, synonymous with so-called outflow obstruction which has been reported in some cases (4). The deformation of the LV resembles a Japanese octopus trap, a "takotsubo", hence the name of the disease (Fig. 1), where the apical ballooning is also the most characteristic finding in conjunction with diagnostic imaging (such as in LV angiography and echocardiography). In the acute phase, TTC is consistent with heart failure, as in AMI. In contrast, however, coronary angiography shows no signs of any significant stenosis, corresponding to the contractile impairment.

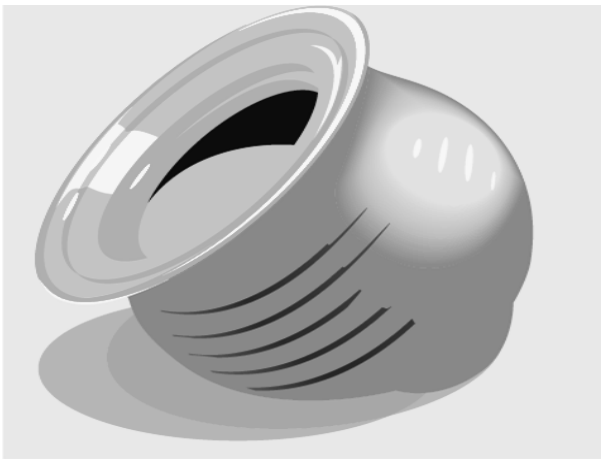

Figure 1. *Takotsubo is the name of a Japanese octopus trap, namely an urn with rounded bottom and narrow neck. Characteristically, the left ventricle adopts a similar look (with apical ballooning) at the onset of takotsubo cardiomyopathy, hence the name of the disease. The image is reprinted from the Swedish journal "Läkartidningen" (number 44, volume 104, 2007), with permission from the responsible publishers, which are gratefully acknowledged in this respect.*

Nor is there any clear evidence of atheromatous, while clinical signs of another obvious cause are missing. Unlike many other heart diseases, the acute findings at TTC are transient and follow-up within three months usually indicates a normalization of the LV systolic function (10), and quite often already within weeks (9). A typical case of TTC, with transient apical ballooning during recovery, is shown in Fig. 2, as depicted both by echocardiography and MRI. The diagnostic criteria for TTC can be summarized in the following four points:

1. Transient LV wall motion abnormalities (e.g. hypokinesis or akinesis) in mid segments with or without apical involvement, where the regional wall motion abnormality extend beyond a single coronary vascular distribution. A stressful trigger is often, but not always, present.
2. Absence of obstructive coronary disease (i.e. significant stenosis) or any obvious signs of acute plaque rupture, at coronary angiography.
3. New ECG abnormalities (either ST-segment elevation and/or T-wave inversion; usually in two or more precordial leads), or modest elevation in cardiac troponin.
4. Absence of other more obvious clinical cause, such as myocarditis or pheochromocytoma.

Several suggestions for alternative criteria have occured, not least because of more or less rare exceptions and reports of atypical cases. In clinical practice, however, the summation above is still considered the most accepted, as proposed by the Mayo Clinic 2010 (11).

The pathophysiology of TTC remains unclear, and several possible causes have been suggested, such as emotional stress, which often (4) but not always precedes the onset (12), as well as physiological stress, for instance changes in the autonomic cardiac function (13). The most prominent hypothesis includes increased plasma levels of catecholamines (i.e. stress hormones, such as adrenaline), as the underlying mechanism; indirectly through induced spasm in cardiac vessels (14, 15), or due to a direct effect with reduced viability and inflammatory damage in the cardiac muscle cells, secondary to calcium overload and changes in the calcium regulation (6, 9, 16). The catecholamine theory could also explain the typical ballooning at onset, due to higher amount of adrenergic receptors apically, along with other known structural changes between the basal and apical parts of the LV (17, 18). Supportingly, 80-90 percent of all cases are elderly women, with reduced protection against stress hormones in terms of decreased estrogen production (9, 20, 21).

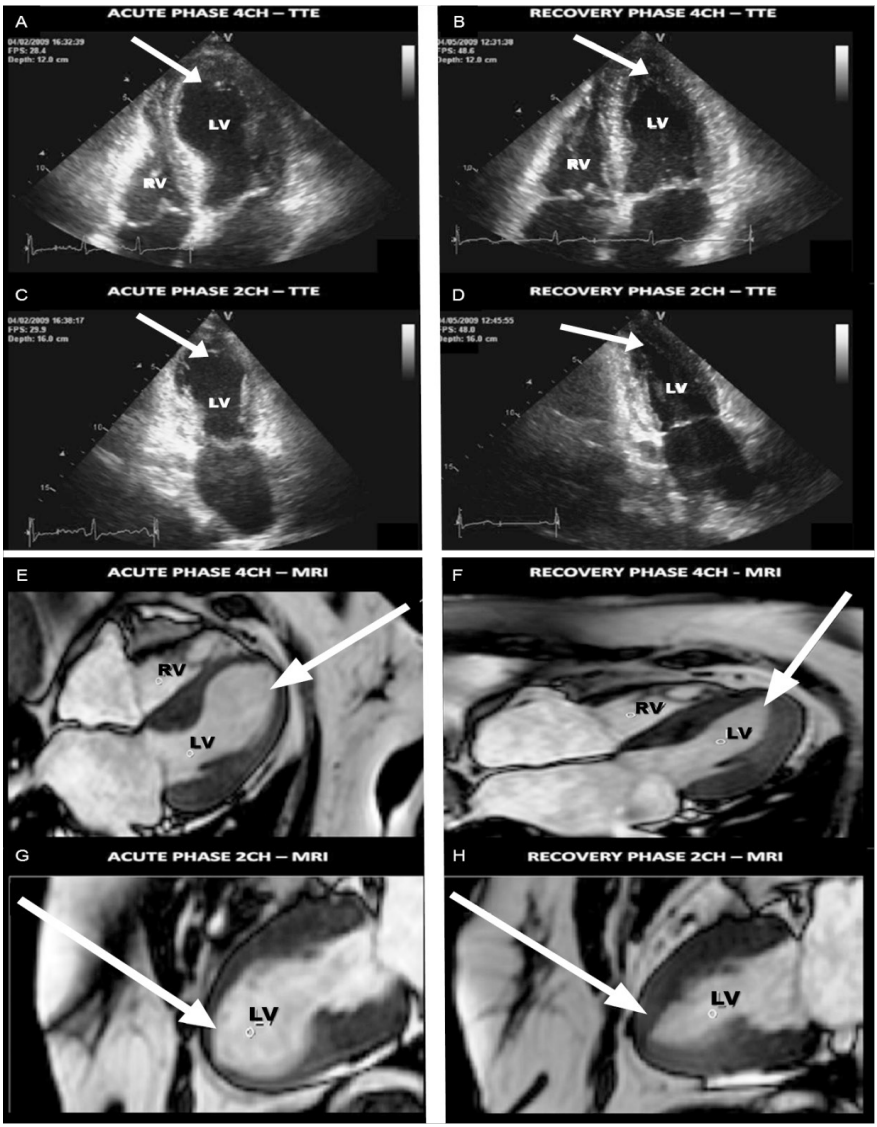

Figure 2. *A typical case of takotsubo cardiomyopathy (TTC), as seen by transthoracic echocardiography (TTE) (upper images, A-D) and at magnetic resonance imaging (MRI) (lower images, E-H). Left images represent the acute phase, while images to the right show the recovery phase (about three months later), during an episode of TTC in a representative woman (age 78 years). All images are taken in left ventricular (LV) contraction phase (i.e. in end-systole); Note the characteristic ballooning of the apical part of the LV (arrows), in the acute phase compared to the recovery, as seen both at TTE and at MRI in the corresponding 4-chamber (4CH) views (A-B, E-F), as well as in the 2-chamber (2CH) views (C-D, G-H). The right ventricle (RV) is also marked in the 4CH images (next to the LV), in purpose to make the orientation easier.*

The prognosis is generally good; few deaths and recurrences are reported, where heart failure and pulmonary edema at onset are the most common complications. So far, customary treatment as in heart failure has been recommended, with emphasis on beta-blockers (12, 19). Relatively few cases are reported and TTC might seem rare; a prevalence around two percent, of all suspected AMIs, have been noted in larger compilations (8, 19, 20). The transient nature, together with lack of knowledge and female domination, may nevertheless be consistent with diagnostic underestimation, i.e. that TTC cases are elusive (21, 22). Despite good prognosis, all-cause mortality has been reported as relatively increased (12), while treatment, e.g. regarding anticoagulants, may be consistent with side effects (19), which are generally unnecessary in TTC as compared to other heart deteriorations. An episode of TTC may still be important to document, while a correct diagnosis is always important in terms of reassurance to those affected. Thus, further studies, with differential diagnostic purpose, are necessary for this disease.

The echocardiographic examination

Echocardiography is a well-established image modality for diagnostic evaluation of cardiac morphology and function, which has been used worldwide for this purpose since the 1960s. Echocardiography can be done in various ways, the most common in adults, however, is with the patient lying in the left lateral recumbent position and by imaging through the chest, that is, as a TTE (Fig. 3). In Sweden, most TTEs are conducted by doctors and biomedical scientists, where TTE currently is a usual examination in cardiac care. (As an example; about 4300 adult TTEs have annually been carried out in the last year, at the Department of Clinical Physiology, USÖ.)

By TTE, LV quantification can be performed with several ultrasonic methods. Not least by conventional and highly available techniques; linear (M-mode) and 2D-derived imaging in terms of size and function, including formula calculations and geometric assumptions for some variables (e.g. volume and mass), as well as Doppler recordings of velocity and direction regarding blood flow (CW and PW) and cardiac muscle/-tissue movements (DTI). Quantification can also be made with volumetric, 3D-derived imaging, not relying on assumptions but with greater demands on image quality (23, 24). Thus, in clinical practice, it is advantageous to be able to access and rely on different methods and variables, for diagnostic purposes.

Quantification of LV systolic function is always of importance in TTE, regardless of the primary issue, where impairment is consistent with heart failure. Systolic function can be expressed both globally, as well as more or less regionally depending on the choice of quantification variable. The most common measure,

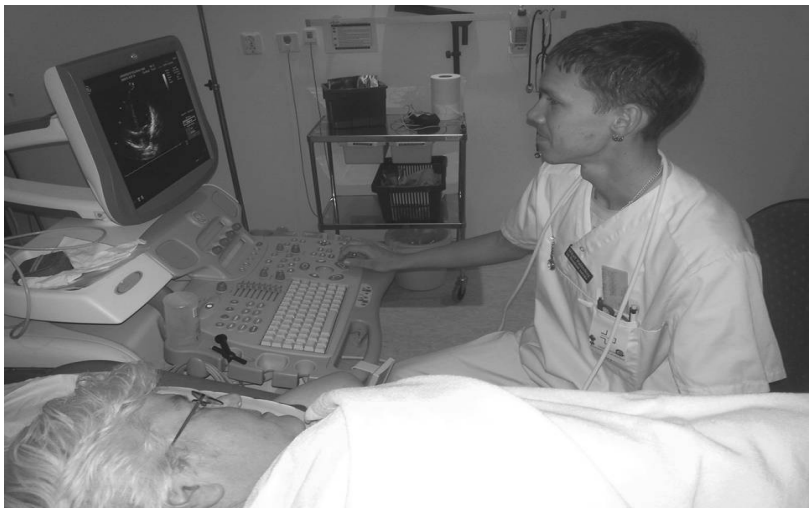

Figure 3. *The image depicts the standard procedure for an echocardiographic examination, with the patient placed in the left lateral position. Note: the image is arranged with a fictitious patient (lying).*

in clinical routine, is probably LVEF; a global measure that depicts the heart's stroke volume in relation to the maximum filling during relaxation (i.e. diastole), expressed in percentages (24). LVEF, in turn, can be estimated by various methods, such as the "Biplane Simpson's method", which is suitable especially in the presence of deformation of the LV (25), such as in TTC.

Traditionally, assessments by TTE are done manually and visually, over time, however, various semi-automatic tools have been developed and are now available on most ultrasound devices, to facilitate the execution and contribute to good reproducibility. Some of these tools enable more sophisticated measurement (i.e. regional portrayal) of LV systolic function, e.g. "2D strain", a speckle/-tissue-tracking software tool with the advantage (compared to Doppler) of being less angular dependent, which is based on moving 2D images (cine images) and subsequent analysis; the LV walls are divided into different segments which can be tracked over time, due to a local spread of echoes resulting in so-called "speckles", and thus, segmental (i.e. local) contraction can be assessed (26). Usually, segmental contraction is referred to as strain, corresponding to the change over time in relation to the starting position (expressed in percentages), while different 2D strain options can be used to track particular movements, where RS, for instance, is a good choice in terms of radial contraction (27).

A great advantage of TTE is its availability and mobility, compared with other modalities such as MRI, which is good in diagnostic purposes, including the investigation of myocardial diseases, and thus also the cases of suspected TTC (19). Evaluation of the heart takes place in real time, and there are no known contraindications related to adult TTE. However, it is not recommended that TTC should only be diagnosed on the basis of TTE (11). Many proposed causes and the absence of a precise such, alleges that TTC is a disease requiring both clinical knowledge and multidisciplinary involvement (19, 20).

Multidisciplinary portrayal of heart disease

Emotional stress

Emotional stress triggers are seen as contributors causing TTC, where, among other things, unexpected death of a close relative is a frequent such (4, 12, 20). Assessment of psychosocial status, including stress and depression, can be done by using appropriate self-rating scales. A validated scale for posttraumatic stress syndrome is PTSS-10, a self-administrated questionnaire including 10 statements about thoughts and feelings, which may occur in connection with a stressful situation; the presence and severity of each statement (i.e. symptom), during the last week, is rated on a scale from one (never) to seven (always). A total score > 35 is consistent with a high probability for posttraumatic stress syndrome, while a score between 27 and 35 is considered as borderline (28).

The MADRS-S is a validated scale for self-assessment of depression; nine depression items (i.e. state of minds) are recorded, and rated between zero and six, according to their intensity. A score > 34 is associated with major depression, while 20-34 is considered borderline (29). Both PTSS-10 and MADRS-S are available in Swedish.

Physiological cardiac stress

In clinical cardiac care, various biochemical blood markers are frequently used for differential diagnostic purposes. An example is NT pro-brain natriuretic peptide (NTpBNP), a recognized marker of heart failure, in the case of elevated levels, and thus an indirect sign of reduced LV systolic function (i.e. cardiac stress). NTpBNP is often elevated in TTC (20). Other customary markers are: cardiac injury markers (e.g. troponin I), inflammatory markers [e.g. C-reactive protein (CRP)] and markers of increased metabolism (thyroid variables). In TTC, as in other cardiac diseases, a broad array of biomarkers should preferable be used, both in order to find typical patterns, as well as to dismiss other clinical causes, such as AMI etc. (4, 8, 20). Specific markers of increased stress (adrena-

line and noradrenaline) are of particular interest in the diagnosis of TTC, with respect to the aforementioned catecholamine hypothesis, including dismissal of potential pheochromocytoma (8, 20).

A disruption of the homeostasis (physiological balance), e.g. due to changes in autonomous tone (such as in stress), can lead to structural changes in the heart. One manifestation of this is the presence of so-called late potentials, this implies a disturbed propagation of the depolarization ("trigger") of the cardiac muscle. ECG including late potentials is consistent with a "normal" resting ECG, but several hundred heart beats are collected and averaged (that is, an SAECG analysis), after which late potentials can be identified by specific criteria (i.e. cut-off values of certain variables). Previously, SAECG has mainly been used in AMI patients. In clinical practice, SAECG interpretation is mainly based on the merging of three established variables; two pathologically aberrant variables fulfil the criterion of late potentials (30).

Increased stress and changes in autonomous tone also involves the involuntary regulation of the cardiac function. The variability in heart rate over time (that is, an HRV analysis) is an indirect expression of this function. Measurement of HRV is basically a customary long-term recording of ECG, including specific analysis focusing on certain variables, and has previously been used to quantify different types of autonomic disorders (not least regarding cardiovascular diseases) (31). In the clinic, it is recommended to use a broad approach with multiple variables, as well as a self-created material of standard references (specifically both regarding the equipment and the recording duration). There are two main categories of HRV data: time variables (depicts the variability by time quantification, e.g. standard deviations etc.) and frequency variables (depicts the variability as effect over time in various frequency bands). Each category has its pros and cons, while different variables (of both categories) have different purposes. Hence, variables are considered as either "mainly parasympathetic" or "mainly sympathetic", while some depicts the overall autonomic imbalance and are usually referred to as "global" (which may reflect both parasympathetic and sympathetic influences). Thus, assessment can be based both on the summation, as well as from individual variables; an elevated or increased value, in comparison to reference values, is considered as pathological (31, 32).

LV dysfunction can be considered as a state of cardiac stress and is preferable assessed by TTE, not least in TTC due to the typical ballooning (as mentioned). LV function and morphology, however, can also be assessed by MRI. In clinical practice, cardiac MRI often includes a so-called LGE protocol (i.e. imaging with infusion of a specific contrast agent), which also allows for the detection of inflammation and damage to the cardiac muscle (i.e. myocardial viability). Thus,

MRI with LGE protocol might be a useful feature, both in terms of LV quantification and to be included as a differential diagnostic tool (e.g. to dismiss an AMI or myocarditis), which has also been suggested in suspected cases of TTC (19).

Biventricular systolic long-axis function

An alternative way to assess LV systolic function by TTE (i.e. indirect estimate LVEF), is by measuring the systolic shortening of the LV in long-axis direction by measuring either the velocity or the amplitude of the mitral annulus motion, the latter often referred to as MAM (33). MAM can usually be used in spite of reduced image quality and increased demands on the time resolution (e.g. at tachycardia), at least at adequate insonation angle. MAM provides with regional information; measurement usually takes place in four positions, where assessment includes both averaging and regional depiction.

It is known that a failing RV function clearly results in increased mortality. A common way to assess RV function is to measure TAM, i.e. the amplitude of the longitudinal shortening of the RV free wall during systole, using the tricuspid annuli. TAM normally accounts for 80 percent of the systolic function of the RV, and just like MAM, this is a robust variable that is based on M-mode (34). In patients with TTC, it has so far not focused much on MAM, and the same applies to the RV function.

Diastolic heart function

Just as the contraction ability of the LV, its ability to relax and be filled with blood (that is, its DF) is also of importance regarding the overall LV assessment. Many conditions with heart deterioration are consistent with impact on both these abilities; abnormal DF can be considered as a condition where the heart is unable to maintain a normal stroke volume, without compensatory increase in filling pressure (35).

At TTE, DF is usually assessed with multiple methods and variables, in order to identify both the relaxation ability and abnormal filling pattern. Interpretation can be based on individual variables (e.g. in terms of diastolic times and absolute relaxation measurements), although merging and weighting are usually applicable, where the latter also involves indirect properties of DF [for instance the size of the left atrium (LA)]. Weighting of variables allows estimation of the filling degree, as well as grading of DF in various dysfunctional levels, which is primarily of interest clinically (in terms of prognosis and treatment).

TTC usually results in LV systolic dysfunction (8), but its impact on DF is not as fully documented, while the RV function is less focused on overall (as men-

tioned), but particularly with regard to its DF. Previously, acute DF impairment has been shown at TTC, regarding the LV and as assessed by strain measurements (36). However, it is known that strain techniques can sometimes be limiting both in terms of interpretation and regarding comparability between different ultrasound devices, and may thus be difficult to apply in studies that involves more than one centre. Most TTC studies are conducted on relatively small populations, which may partially explain the limited documentation in some respects (such as the DF), and at the same time stress the importance of a requisite collaboration between centres. (This was the origin of study III in this thesis, since study I probably yielded substandard answers regarding DF, because of too few subjects in this specific regard.) Both these aspects, however, emphasizes the advantage of being able to recognize TTC by conventional methods (e.g. M-mode, Doppler and DTI). This is also supported by a more recent report; several customary DF variables were shown to interact with a worse clinical outcome in TTC (37).

Geometric quantification of the LV

Common to cardiomyopathies, including TTC, is that the pathology is localized to the heart muscle itself, and is not primarily due to an external factor, such as high blood pressure (38). Geometric LV quantification is of general importance within cardiac care, in terms of diagnostic contributing information (e.g. regarding treatment). It is known that changes in certain geometric properties are compatible with more distinctive signs of abnormality of the heart muscle. Such as the LV wall thickness, and perhaps even more distinctive, changes in the total LVM. Abnormal enlargement of the heart muscle, as depicted by RWT and LVM measurements, has been proposed as an important predictor in patients with LV hypertrophy (39), as well as in AMI (40).

LVM can preferably be assessed at TTE, and by different methods including both formula-based (M-mode and 2D-derived) as well as volumetric (3D-derived) techniques (24). Each method has its pros and cons, and can thus, be more or less suitable in the clinical practice in different cases.

Cardiac MRI if often considered the gold standard regarding LVM measurements (41), mainly due to the relatively higher image quality as compared to TTE. Geometric LV changes, including measurements of the LVM and LV wall thickness in certain foci (defined as SWT), have been studied at TTC previously (42). The benefits of MRI, as being a diagnostic tool at TTC, have been declared (20). MRI, however, has some well-known back draws. Both regarding general contraindications (e.g. pacemaker), as well as methodological aspects (e.g. that imaging may require long breath holds) and not least, regarding access in the clinic; MRIs are not available in all hospitals and are relatively few in numbers,

while bedside examinations may be prevented due to that they are fixed installations. Besides, cardiac MRI examinations (with LGE protocol) cost approximately three to four times more than a routine TTE. And despite the contributing information by LGE, this protocol cannot always be applied, for instance, in patients with kidney failure (due to inability regarding contrast secretion). Taken together, MRI may sometimes be less beneficial, in terms of accessibility and utility, as compared to TTE.

Two-dimensional strain by TTE has been suggested as a valuable tool regarding viability of the heart muscle, as well as for demonstration of scar formation (as often seen in AMI) (27), although the latter is not generally considered as the main strength. As mentioned, the concentric wall motion of the LV is reflected by RS; this particular strain tool is a measure of the sum of contractions, as a product of systolic shortening of both longitudinal (superficial) and circumferential (profound) heart muscle fibers (27). Thus, RS depicts the condition of the entire myocardial wall, in levels of interest, and its usefulness has been suggested in patients with AMI (43).

The aforementioned study, focusing on LVM at TTC (42), had MRI as their method of choice. Few TTE reports, however, have had the same aim, while the use and natural course by RS is not widely documented at TTC. No previous study has looked at these two components simultaneously. A combined approach, as assessed by TTE, could perhaps be a good clinical setup in patients with TTC, and provide similar diagnostic information as MRI.

AIMS OF THE THESIS

To study and compare the cardiac function during an episode of TTC; mainly by conventional TTE measurements, but also by comparison with other diagnostic variables, as proposed for TTC patients. Hopefully, this will contribute to an increased recognition and better knowledge of the possible causes and management of this disease. Design, layout and main purpose of each substudy was:

- In study I, a prospective approach was used with the aim to investigate biventricular changes in systolic long-axis and diastolic function, between the acute and the recovery phase in patients with TTC. Solely by TTE, due to limited information from previous studies in this respect.

- A prospective, multidisciplinary approach was used in study II, for the same patients as in study I, and similarly, by data collection both at onset and at follow-up. We hypothesized a relation between scores of emotional stress and depression on one side, and cardiac markers of physiological stress on the other. The objective was to find a common pathophysiological denominator and this approach had not been used before.

- Through study III, a retrospective TTE analysis was done, with further focus on the diastolic LV impact, which is not as widely documented at TTC, as the systolic manifestations. We hypothesized that the diastolic function does impair at onset, and recovers in parallel with the systolic function. Thus, several diastolic indices were investigated, during an episode of TTC. Through external collaboration, additional patients were enrolled to the same group as mentioned for Study I-II, which was necessary in this regard, based on previous data (included from study I).

- Based on the aforementioned cohort of patients with TTC (in study I-II), another retrospective, analytical investigation was conducted in terms of study IV. In this substudy, the main objective was to further investigate the effects on LV geometry during an episode of TTC, both by TTE and MRI, including intertechnique comparison. These effects are not thoroughly enough studied at TTC, and few reports have used a multimodal approach. We hypothesized TTE to be in consistency with MRI in this respect, and thus, provide diagnostic information regarding TTC.

SUBJECTS AND METHODS

Study populations

This thesis is based on the following two sub-populations and patient selection:

- The Swedish part; enrolled solely at USÖ (applies to all sub-studies, I-IV). Patients were prospectively selected according to a predetermined protocol; all who met the inclusion criteria, and were referred to USÖ (between January 2008 and March 2010), were screened for TTC[1]. The patients were consecutively enrolled during the whole study time, and thus, those accepted were investigated both in terms of prospective (study I-II) as well as by retrospective analyzes (study III-IV).

- The U.S. part; enrolled by three U.S. centres (only applicable for study III). Patients were retrospectively included by using the ICD-9 code for TTC (between January 2008 and October 2011). Selection was done by one primary observer at each centre; patients were included if they met the inclusion criteria, including a requirement that they would be appropriate matched against the USÖ part (e.g. regarding the timing and quality of the examinations). Thus, analysis was only done retrospectively for the U.S. part (together with the USÖ part, regarding study III).

Patient inclusion was initially (in study I) according to the following criteria: TTC diagnosis defined as acute chest pain, new ECG changes (e.g. ST-elevation and/or negative T-waves), no significant stenosis (≥ 50 %) on coronary angiography and apical LV dysfunction on contrast left ventriculogram. This was in line with the then proposed criteria (17); as depicted by Fig. 4-5, representing two of the Swedish patients and the initial inclusion. In practice, however, the inclusion criteria were the same throughout the thesis (in study I-IV), that is, according to the currently accepted guidelines for TTC (11), as already described in the introduction (page 15). In addition, general exclusion criteria were: earlier history of ischemic heart disease (e.g. AMI) or any coronary intervention (e.g. bypass), as well as suspicion of intracranial bleeding.

[1] Screening was planned to last for up to two year, set from January 2008 for patients with suspected AMI and admitted to acute coronary angiography at the Department of Cardiology, USÖ. Screening and inclusion were done in the catheterization laboratory following coronary angiography and left ventriculography, in the cases of typical findings as in TTC.

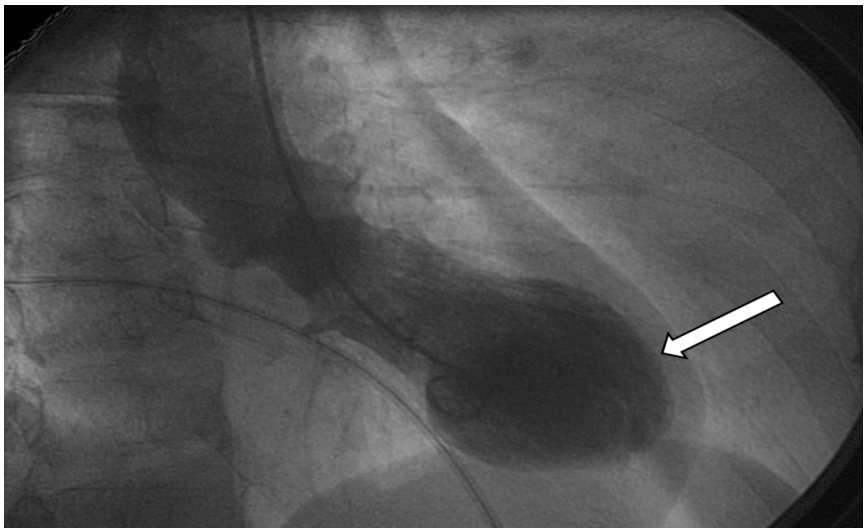

Figure 4. *The image shows an example of a left ventriculogram in 30 degrees right anterior oblique view, from a representative Swedish subject. Note the apical ballooning (arrow), which is typically seen at the onset of takotsubo cardiomyopathy.*

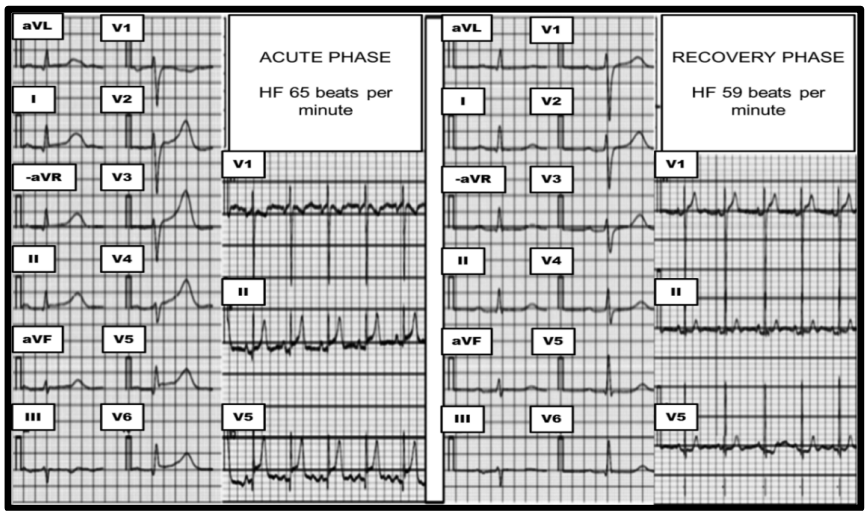

Figure 5. *A typical example of the resting ECG of one of the Swedish subjects (a 76 year old woman), who was included in the USÖ cohort (study I-IV). The figure shows precordial ST-elevations (V2-V6) in acute phase (left), which are normalized around three months later at follow-up (right), as typically seen at takotsubo cardiomyopathy. Heart frequency (HF) is within normal limits at both phases.*

Moreover, specific exclusion criteria were applied for MRI (applicable in study II and IV), regarding contraindications such as pacemaker and kidney impairment, the latter according to the following specification: newly discovered (no MRI at all) or previously known (MRI but without LGE protocol) [based on calculations of glomerular filtration rate (GFR) < 30 ml/min/1.73m^2].

All patients fulfilling these criteria were aimed for inclusion, with initial investigation at onset, after which study-specific examinations were repeated about three months later at follow-up, as further described.

Methods

TTE equipment

- For the Swedish population; a Vivid 7 ultrasound machine (GE Vingmed Ultrasound A/S, Horten, Norway) was used, equipped with a multifrequency phased array transducer (M3S, 1.5-4.0 MHz), in all the TTE examinations (study I-IV). Offline analyzes were done on custom workstations with dedicated software; EchoPAC PC, GE Healthcare, version 8 (study I-II), version 110.1.1 (study III) and version 112 (study IV).

- For the U.S. population (study III); a Philips sonos 5500 ultrasound machine, with 3.5-5.5 MHz and Acuson Sequoia (3.75 MHz) transducers, were used for the TTE examinations. Offline analyzes were performed using a Centricity, GE platform and KinetDx DICOM server.

TTE examinations and measurements

In all sub-studies, initial TTEs were performed within 24 hours after onset (acute phase), while repeated examinations and analyzes refer to the follow-up visits, which generally took place about three months later (recovery phase). All subjects were examined in the left lateral recumbent position. All TTEs (at both phases) were essentially complete, i.e. including assessments of conventional measurements as in clinical routine, generally derived from standardized views and image modes (M-mode, 2D, Doppler etc.). All study-specific measurements were made after the examinations (that is, offline), using digitally stored images (snapshots or cine images) on custom workstations, as above. In overall, study-specific measurements were the average of three collected heart beats, where the majority of the images had been stored at the end of expiration. Images were stored at regular

sinus rhythm, adequate adjustment of the ECG signal was applied (at the collection or offline), while care was taken to optimize the image. Individual measurements that could not be obtained, e.g. due to poor image quality, were excluded. Variables that could not be obtained for less than half of the study population were also excluded, due to lack of additional value to the context. (Study population, in this case, refers solely to the Swedish part (in study I-II and IV), as well as to the total of the Swedish and the U.S. part (in study III).)

For the Swedish population, TTEs were performed at the Department of Clinical Physiology, USÖ; mainly by two independent and experienced biomedical scientists, whereas the clinical routine procedure (in terms of interpretation and reporting of findings) were done by a few independent physiologists (with experience), the latter procedure was done in close proximity to each examination. In addition, and independent of the aforementioned screening protocol, a predetermined and well-planned TTE protocol was applied; initially set for the prospective analysis of study-specific measurements (study I-II), also the retrospective analyzes, as performed separately at later stages (regarding study III-IV), were based on the same protocol (i.e. sets of collected images), in terms of additional offline measurements (including the required re-measurement, e.g. for reproducibility). Final offline compilation and analyzes of study-specific measurements, in all sub-studies, were done solely by one primary biomedical scientist.

For the U.S. population (study III), data sets from the various centres were retrospectively gathered and merged through collaboration between the institutions, in terms of raw data images and/or numbers as derived from the TTEs; final compilation and analyzes of study-specific measurements were done solely by one primary U.S. sonographer (the U.S. centres, with their respective contributions, are clarified in the result section, Table 1).

In overall (study I-IV), basic TTE measurements; not referred to the study-specific aims and depicted without detailed account throughout the remaining thesis [e.g. some LV quantifications and estimation of pulmonary artery systolic pressure (PASP)], can be derived to the current recommendations by the ASE (24, 34), unless otherwise stated. Methods and measurements used for the study-specific aims, however, are summarized as follows:

- M-mode/2D-derived quantification of geometry and function;

In study I, the RV size was measured in 2D-mode as RV inflow tract (RVIT3), in the apical 4-chamber view one third from the base of the RV (24), and as RV outflow tract (RVOT1) from the parasternal long-axis view (44). The amplitudes of MAM were measured by M-mode; recordings from the septal and lateral sites

of the mitral annulus were obtained from the apical 4-chamber view and recordings from the inferior and anterior sites from the apical 2-chamber view. Mean amplitudes of MAM were calculated as the average of the four sites. The amplitudes of TAM were measured at the basal lateral site of the RV (by M-mode), in the apical 4-chamber view. MAM and TAM were measured in line with the recommendations (33, 34); a typical case including both these measurements is depicted in Fig 6. The biventricular lengths were measured in end-diastole, from the epicardial apex to the septal and lateral sites of the mitral annulus (LV), as well as to the septal and lateral sites of the tricuspid annulus (RV), in 2D-mode.

In all sub-studies (i.e. study I-IV), LVEF was measured in 2D-mode by the biplane Simpson's method (25), which is explained in Fig. 7. Geometric LV measurements as follows are solely referring to study IV: 2D-derived apical 2- and 4-chamber views were used for LVM estimation by the biplane Simpson's method, as previously proposed (24). Additionally, a custom method was also used,

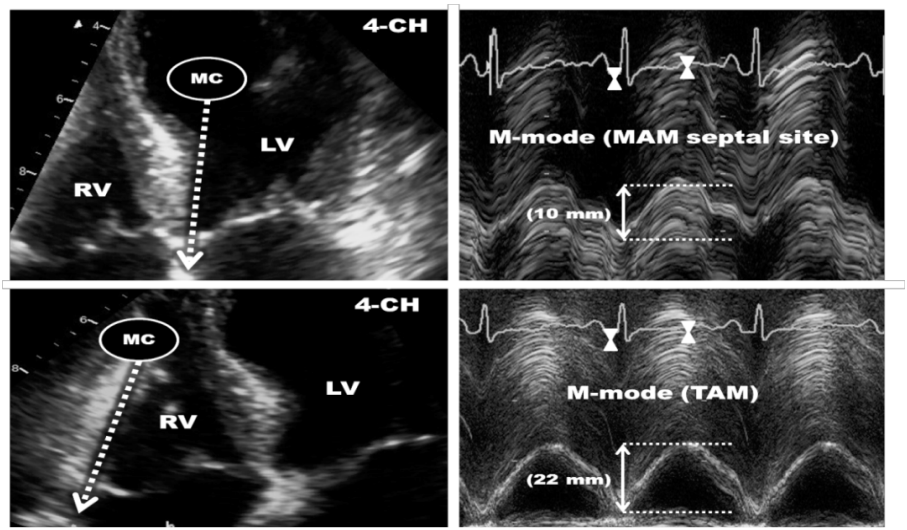

Figure 6. *Measurements of mitral annulus motion (MAM) and tricuspid annulus motion (TAM), a graphical depicture from the acute phase for a representative patient (female, age 71 years), as performed in study I. Both measurements were done in apical 4-chamber view (4-CH, left images), using M-mode recordings (right images) with the M-mode cursor (MC) placed at the septal part of the mitral annulus (for MAM, upper images) and at the basal lateral part of the tricuspid annulus (for TAM, lower images). MAM refers to the left ventricular (LV) systolic long-axis function (33), while TAM represents the right ventricle (RV) in the same manner (34); function in this regard is depicted as maximal longitudinal shortening, i.e. measured amplitudes in millimetres (mm), from each M-mode recording, during the systolic phase (marked on the ECG signals). This example only shows MAM at the septal site. In study I, however, MAM was measured and calculated as the average from four sites, as explained in the text.*

defined as triplane Simpson's, where LVM was estimated according to the same calculation principle as for the biplane method, but with the inclusion of the apical long-axis (APLAX) view. A graphic depiction of both LVM methods by the Simpsons' rule, including more detailed measurement description, is shown in the aforementioned Fig. 7. M-mode estimation of LVM was done in parasternal long-axis view, according to the recommendations and by the use of the geometric cube formula (24), as shown in Fig. 8. Further, LVM estimation was also done by two formula-based 2D-methods: area-length (AL) and truncated ellipsoid (TE). Both AL- and TE-estimates of LVM were performed according to the recommended procedures (24), which together with the respective formula are depicted in Fig. 9. All of the reported LVM values (i.e. in study IV) were normalized for body surface area (BSA, m^2).

Further, in study IV, parasternal short-axis views were used to acquire SWT of the LV, by 2D-mode measurements at end-diastole, as suggested (24). At each examination, three SWT sites were defined and measured: basal septal (at the level of the mitral valve leaflets), apical septal as well as apical inferior (below the papillary muscle level). Fig. 10 depicts an example of the SWT measurement, from a representative patient (images to the right are TTE). RWT was calculated, for each examination, based on M-mode measurements at end-diastole in parasternal long-axis-view (depicted by the aforementioned Fig. 8), after which LV hypertrophy (LVH) geometries could be categorized as: normal geometry, concentric remodeling, and as either concentric or eccentric hypertrophy. RWT calculations and LVH categorization were performed as suggested (24), where the latter procedure is based on RWT and LVM data.

- Doppler/DTI-derived functional evaluation and DF categorization;

In study I, PW Doppler mitral and tricuspid diastolic flow velocities were recorded from the apical 4-chamber view by placing the sample volume between the leaflet tips; the transmitral/-tricuspid peak rapid filling velocity (E), peak atrial filling velocity (A), and E-wave deceleration time (DT) were measured, while the E/A ratio was also obtained. The LV isovolumetric relaxation time (IVRT) by PW Doppler was recorded from the apical 5-chamber view by simultaneous recording of the aortic and mitral flows. The IVRT of the RV by PW Doppler was measured as previously described (45); using parasternal short-axis (at the level of the aortic orifice) and apical 4-chamber views, where IVRT was calculated as the difference between the onset of tricuspid flow and the end of the pulmonic flow (with the R-wave on the ECG as a common time reference). In general, the heart rate was quite similar, in the respective views, at each examination.

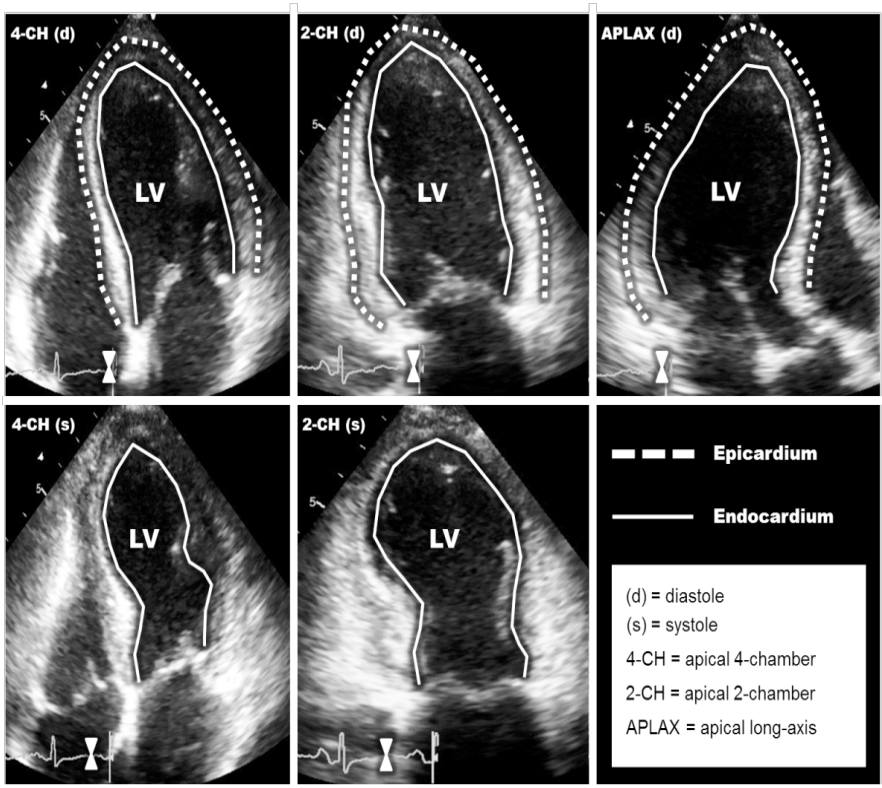

Figure 7. *A graphic depiction; measurement of the left ventricular (LV) global systolic function (study I-IV) and estimation of the LV mass (LVM, study IV), for a representative female patient (age 77 years). In apical 4- and 2-chamber views the LV endocardial borders (solid lines) were traced, both in diastole (upper images) and in systole (lower images). LV systolic function was calculated as the volumetric difference divided by the diastolic volume, expressed as ejection fraction (EF %), according to the biplane Simpson's method (25). LVM estimation in this regard is also based on Simpson's method; both the endocardial and epicardial (dashed lines) borders were traced, in this case only in diastole, after which biplane LVM was calculated as the volumetric difference (between the endo- and epicardium) summed from each of the two apical views and multiplied by 1.05 (i.e. the myocardial density, in g/ml), in line with previous procedure (24). Similar approach was used for the self-defined Simpson's triplane method, by adding the volumetric difference (of endo- and epicardial tracing in diastole) from the apical long-axis view (APLAX). Thus, LVM was calculated according to the same principle; by multiplying the summed volumes, from each of the three apical views, with the density factor 1.05. The papillary muscles were considered as a part of the LV cavity, for all the mentioned measurements. All the above images are from the onset, hence the typical apical ballooning seen in systole. Markers on the ECG signals refer to the diastolic and systolic phases.*

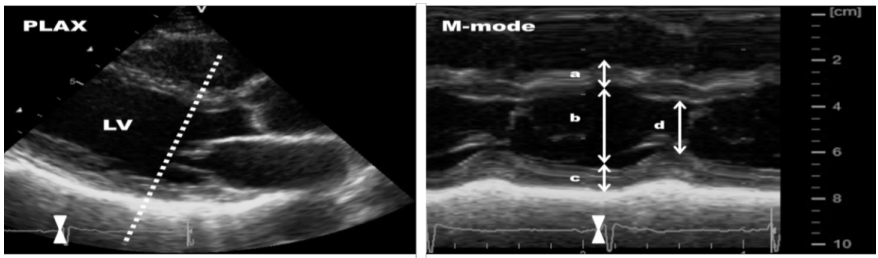

Figure 8. *Left ventricular mass (LVM) estimation by the M-mode method, for a representative woman (age 76 years), as carried out in study IV. The measurement was done in parasternal long-axis view (PLAX, left image), by placing the cursor just below the mitral leaflet tips. Simultaneously by M-mode recording (right image), left ventricular (LV) septal (a) and posterior (c) wall* thicknesses were measured, as well as the LV internal diameter (b), in end-diastole (marked on the ECG signals). *LV internal diameter was also measured in systole (d). Thus, LVM (in grams (g)) was obtained by the cube formula derived from Devereux 1986; LVM = 0.8 x (1.04[(b+c+a)³ – (b)³]) + 0.6. From the same M-mode recordings, relative wall thickness (RWT) could also be calculated; RWT = (2 x c) / b). The RWT data was used for categorization in different LV hypertrophy geometries, which was also performed in study IV, as mentioned and further explained in the text. All above measurements and used formulas were according to the recommendations (24).*

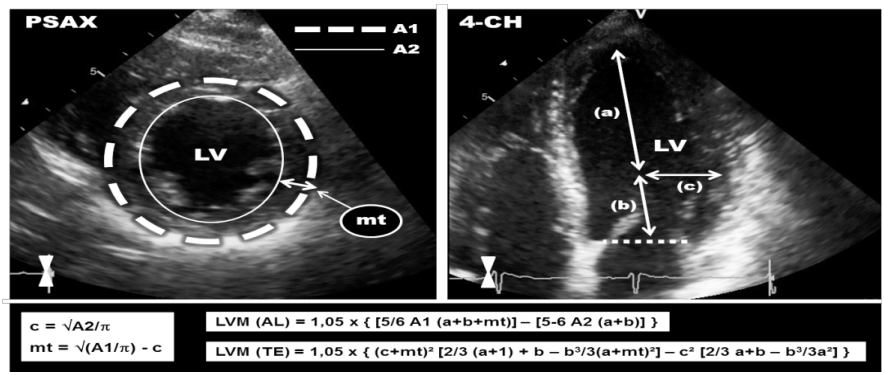

Figure 9. *A graphical display of the left ventricular mass (LVM) estimation by the area-length (AL) and truncated ellipsoid (TE) 2D-methods (24), used in study IV, for a representative woman (age 76 years). In parasternal short-axis (PSAX, left image) the borders of the left ventricular (LV) epicardium (A1) and endocardium (A2) were traced, at the level of the papillary muscles, which were considered as a part of the LV cavity. In the apical 4-chamber (4-CH, right image) the long semi-major axis was measured (a = from the widest minor-axis diameter to LV apex), as well as the truncated semi-major axis (b = from the widest short-axis diameter to mitral annulus plane). The LV radius is automatically computed (c = back calculated from the PSAX endocardium area, by the formula below the images) and a mean wall thickness is derived (mt = from the PSAX epi- and endocardium areas). LVM could then be obtained with the specific formulas for each of the AL- and TE-methods, both formulas include factor 1.05 which refers to myocardial density (in g/ml). All measurements were obtained at end-diastole (marked on the ECG signals).*

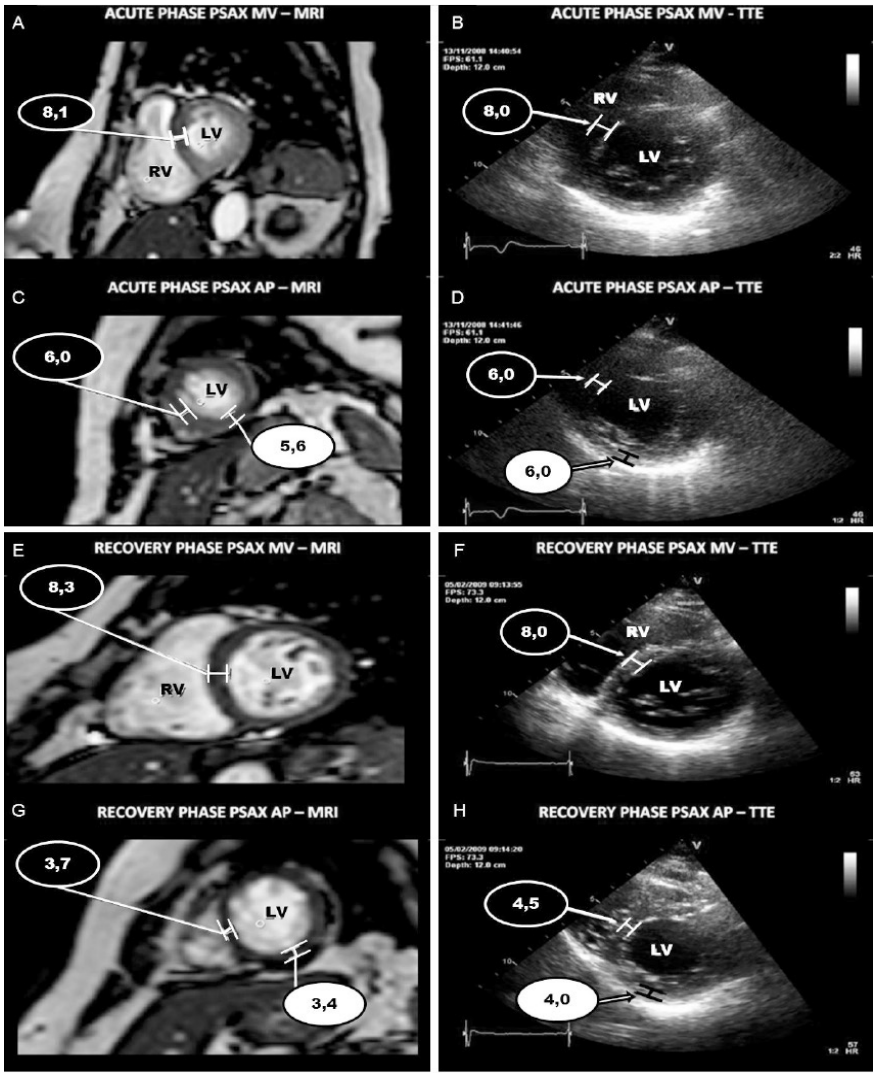

Figure 10. *A graphical clarification of segmental wall thickness (SWT) measurement of the left ventricle (LV), conducted in study IV, depicting both acute phase (upper images, A-D) and the recovery phase (lower images, E-H), from a representative patient (female, age 76 years). To the left are the magnetic resonance images (MRI), and to the right the corresponding images by transthoracic echocardiography (TTE). All measurements were made from parasternal short-axis (PSAX) views in LV relaxation phase (i.e. in end-diastole). Thus, SWT was obtained in the basal level at the mitral valve (MV) leaflets (A-B, E-F), as well as apically (AP) below the papillary muscle level (C-D, G-H). Numbers within the circles refers to SWT (in mm) measured in each level and at each SWT point (marked in the images with solid lines and arrows); black circles with white text are the septal sites, whereas white circles with black text refers to the inferior sites of the LV. The position of the right ventricle (RV) is helpful in this regard (i.e. to determine the measurement points).*

Further (in study I), the velocities at the mitral annulus were measured by PW-DTI and 2D color DTI from four sites; recordings from the septal and lateral sites were obtained from the apical 4-chamber view, while the inferior and anterior sites were obtained in the apical 2-chamber view. Mean velocities were calculated as the average of the four sites. PW-DTI measurements of both the mitral and tricuspid annuli were done as previously described (46), thus, including measuring of both systolic (s') as well as early- (e') and late-diastolic (a') tissue velocities. For the LV, the E/e' ratio was calculated, where mean velocities of e' (by PW-DTI) were calculated as the average of the septal and lateral sites (i.e. only as derived from the 4-chamber view); according to the recommendations (35). The measurements of tissue velocities by 2D color DTI were done as recommended (47). The IVRT at the both annuli was also measured, as previously described, both by PW-DTI (48) as well as by 2D color DTI (49).

As in study I, PW Doppler measurements of mitral diastolic flow velocities were also done in study III, in the exact same ways regarding the following variables: E, A, DT and E/A ratio (for the U.S. part). Additionally (for both populations, study III), LA mitral flow duration (Adur) was measured, as well as atrial reversal flow duration (Arevdur), the latter was obtained from the upper right pulmonary vein (also in apical 4-chamber view); which allowed estimation of LV filling pressure (by calculations of the difference between Arevdur and Adur).

In study III, LV IVRT by Doppler (in the 5-chamber view) was measured in a similar way, but with the use of CW Doppler (for the U.S. part). Additionally, for both populations in study III, the diastolic filling time was also measured in the 5-chamber view (by PW in Sweden and CW in the U.S. part), as previously done (50). PW-DTI measurements of the mitral annulus were performed in study III, similarly as in study I (regarding the velocities of s' and e'), but only calculated and expressed as the average of the septal and lateral sites (i.e. only by measuring in the 4-chamber view); due to lack of PW-DTI images from the 2-chamber view (in the U.S. part), since measuring of four sites is not necessarily recommended in the clinical practice (35, 51). Thus, in study III, mean s' was re-calculated for the Swedish part, while mean e' (of septal and lateral sites) could be obtained from the earlier calculations of the E/e' ratio (where E/e', as in study I, was calculated for both populations in study III). A case from study III, demonstrating some representative Doppler-measuring, is depicted in Fig. 11.

Further, in study III, DF was categorized into different stages (normal, grade I to III) according to the recommendations (35), among other things, based on LA size; measured by 2D-tracing solely in 4-chamber view (LA area) and by subsequent tracing in 4- and 2-chamber views (LA volume), both LA size methods were in line with the guidelines (24), where the latter refers to the area-length method.

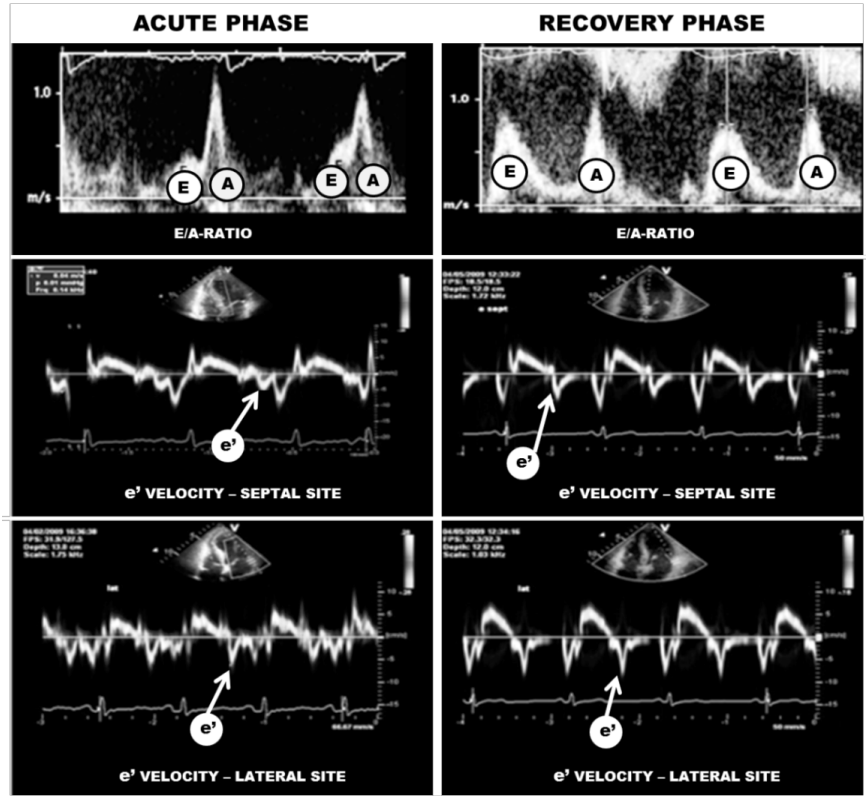

Figure 11. *Pulsed-wave Doppler of mitral inflow (upper images) and tissue Doppler imaging at the mitral annuli (middle and lower images). For a male patient in study III, at the acute phase compared to recovery. E wave refers to early-diastolic inflow velocity and A is late-diastolic inflow due to atrial contraction. In this case in the acute phase, the E velocity is 52 cm/sec which increases to 78 cm/sec during recovery. Early-diastolic tissue velocity is shown as e', septal site in the middle (increases from 5 cm/sec to 7 cm/sec during recovery), while bottom images show the lateral site (increases from 6 cm/sec to 9 cm/sec during recovery). At the acute phase heart rate is about 70 beats per minute (bpm), and at the recovery around 65 bpm.*

Full accounting of the DF categorization is depicted in Fig. 12 (Note that Arevdur and Adur are expressed as "Ar" and "A", respectively, in Fig. 12).

For the Swedish population (applicable both in study I and III), the following Doppler-based settings were applied: insonation angle < 20 degrees with an image sector set as narrow as possible; in general, 2D color DTI images were obtained at a frame rate > 130/sec (scale was adjusted just above the level of aliasing). The sample volume used for PW Doppler/-DTI was set around 5.0 mm. The sample

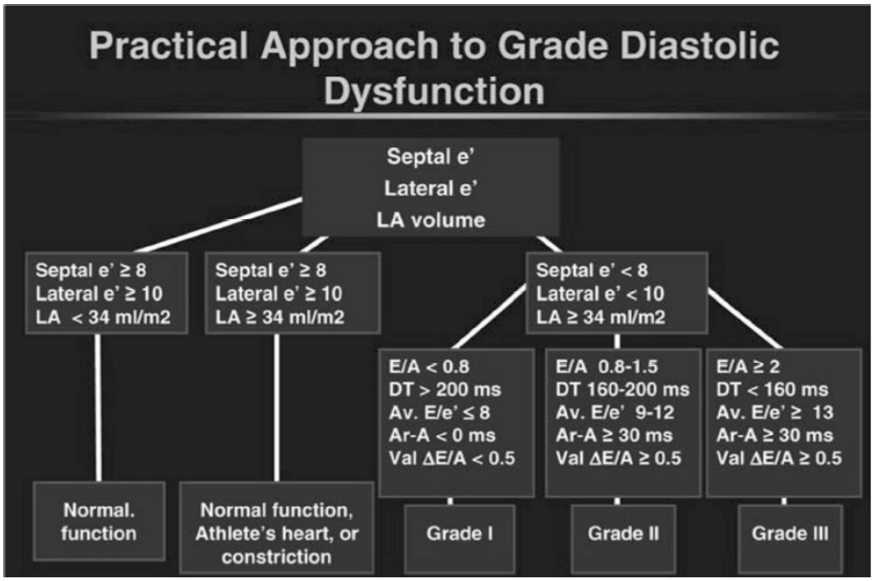

Figure 12. *Scheme for grading of the diastolic function; according to the recommendations (35) and which was used in study III. The image is reprinted from the "Journal of the American Society of Echocardiography" (number 2, volume 22, February 2009), with permission from the responsible publishers, which are gratefully acknowledged. Abbreviations are: Av = Average. LA = Left atrium. Val = Valsalva (non-mentioned abbreviations are clarified in the body text).*

region for color 2D DTI was used as default (offline set 6.0x6.0 mm), and manually tracked throughout each heart cycle. PW Doppler recordings from the LV outflow tract were used for timing of the aortic valve opening and closure. No detailed information, for the Doppler-settings used in the U.S. part, was documented (regarding study III), but the U.S. observers were instructed to consider the general guidelines for Doppler measurements (27, 35), by which all Doppler-based methods used (both in study I and III) may be derived. Thus, the image quality, including Doppler-settings, was matched against the USÖ part (as stated).

- 2D-derived assessment of LV wall motion;

The clinical reporting procedure for the Swedish population (made in proximity to each examination, as mentioned), among other things, included visual assessment of WMA. Thus, in study IV, WMSI could be derived and documented based on these initial assessments. Both WMA assessment and WMSI scoring were done according to the guidelines, by the use of a well-established 16 segment

model of the LV (24). A typical case with WMSI scoring, at each examination phase, is depicted in Fig. 13 (upper images, A-B).

Analysis of RS was done in parasternal short-axis views (2D cine images at papillary muscle and apical levels), using dedicated software package (2D strain, GE Healthcare; integrated in the aforementioned offline system), which is commercially available and based on measuring through speckle-tracking. In the analysis, endocardial borders were manually traced (in each short-axis level), after which the software automatically divided the LV walls into six segments. The segments were manually adjusted and approved by the observer, after which peak systolic RS could be obtained for each segment. The software has an integrated control against unfavorable measuring conditions (e.g. inappropriate frame rate and/or tachycardia), and thus, only segments that were accepted in this respect, were included in the analysis. The whole analysis procedure is in line with the software description (26), while a more detailed description is presented in the original paper (study IV, method section). A graphical example, of a representative case including RS analysis, is depicted in Fig. 13 (images C-F).

Based on averaging of all segmental RS values, as obtained at each examination (i.e. a total of 12 segments per sample), mean RS was calculated and defined as GRS. Aortic valve closure was determined by PW Doppler recordings (at LV outflow tract) and linear drift compensation was applied, as recommended (27). RS values were documented without so-called systolic pre-stretching or post-systolic shortening, in the presence of such phenomenon (52).

- Reproducibility tests;

In study III, intra- and interobservative variability was tested for DF variables: E/A ratio and mean e' velocities, retrospectively for all examinations (i.e. both populations). Intraobservative tests were performed approximately 12 months apart, separately for each population, by two observers [that is, the primary biomedical scientist at USÖ (for the Swedish part) and the primary U.S. sonographer (for the U.S. part)]. Interobserver control was carried out by letting another U.S. sonographer, as well as another Swedish biomedical scientist, repeat the same measurements (for the U.S. and Swedish parts separately). The latter observers were unaware of the previously measured data.

In study IV, variability tests were done for all study-specific variables. Intraobserver control was done, solely by the primary biomedical scientist; applicable variables (LVM, SWT, RWT and GRS) were repeated approximately three months apart. Interobserver control was done for linear variables: LVM (by M-mode) and RWT, by comparing M-mode measurements performed separately in

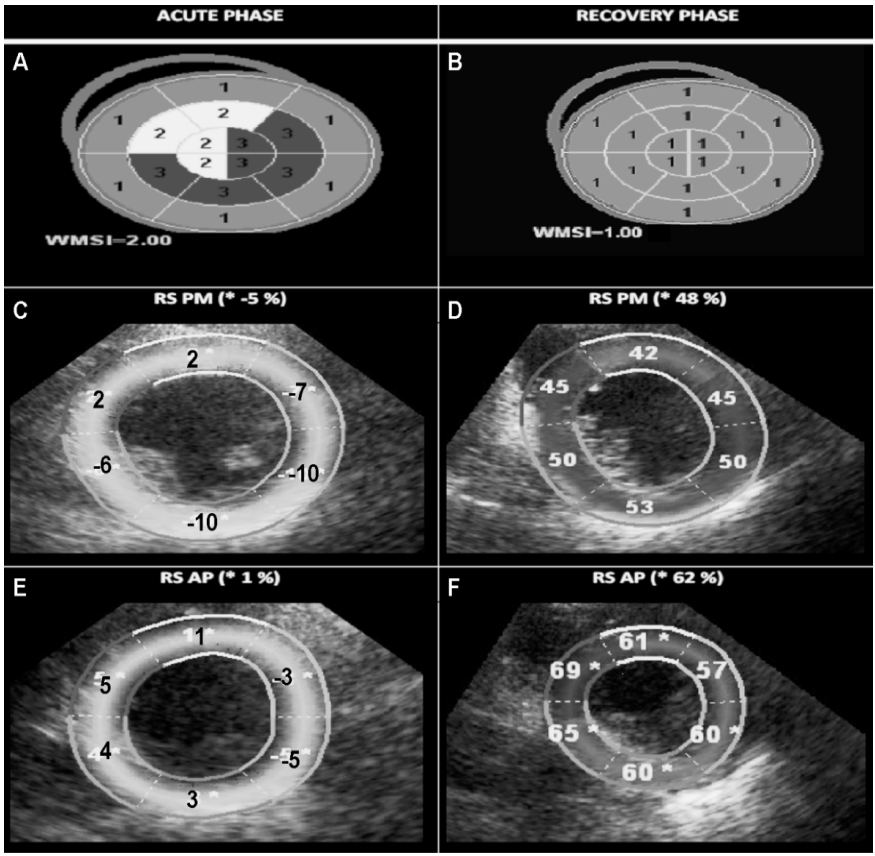

Figure 13. *Echocardiographic 2D assessments of wall motion abnormality, a graphic display from study IV, depicting both the acute (left images) and the recovery phase (right images). All images are from the same woman (age 77 years). Assessment included visual inspection of image clips in parasternal short-axis views, by which wall motion score index (WMSI) was documented (images A-B); using an established 16 segment model of the left ventricle (LV), each segment is given a number corresponding to either normal ("1") or abnormal ("2" or higher) wall motion. Segments are summarized into a global WMSI, where "1.00" equals normal in overall, while higher values reflect the presence of some kind of regional abnormality (24). Assessment also included measurement of radial strain (RS), from short-axis cine images at papillary muscle (PM) and apical (AP) levels (images C-F); using dedicated software (2D strain, GE Healthcare), a semi-automatic tool based on 2D speckle-tracking. The operator manually marks the region of interest (i.e. the wall borders), after which the software automatically identifies the LV and calculates RS (divided into six segments in each level), where strain is defined as maximum wall thickening (during systole) divided by the relaxed thickness (in diastole), i.e. segmental LV contraction (given in percent) (26). The RS data are shown both for each wall segment, and as an average per level, like in the above examples (numbers within brackets refers to each RS average).*

the clinical reporting procedure (by the independent physiologists), with those from the study-specific analysis (by the primary biomedical scientist). Similar, interobserver comparison (i.e. between the clinical reporting and the study-specific analysis) was done for WMSI. Thus, including interrater reliability test for WMA assessment, categorized by self-definition as: no typical TTC look (no WMA), typical TTC look (distinct WMA apically seen or a probable TTC variant), or less typical TTC look (WMA but not as usually described in TTC). The interobserver control was performed without mutual knowledge among involved observers, until compilation of data was performed.

Other equipment and investigations

- Posttraumatic stress and depression;

In study II, the patients answered self-rated questionnaires about posttraumatic stress (PTSS-10) and about the presence of depression (MADRS-S) (28, 29), within 48 hours after the onset as well as at follow-up three months later. Final interpretation of the completed scales was done by one experienced psychiatrist (Department of Psychiatry, USÖ), with the help from two responsible nurses at the Department of Cardiology, USÖ (K. Persson and P. Haglund), where the response/-collection process took place. Patients were assigned the questionnaires by the nurses, who assisted with the necessary assistance and instructions, after which the patients were given sufficient time to answer both scales.

- Biochemical markers;

In Study II, sampling of biochemical markers was done within 48 hours after the onset; conventional markers as in the clinical cardiac routine (e.g. NTpBNP, CRP and troponin I) were taken initially (maximum value within 24 hours) and at 48 hours, while complementary sampling was done in the morning after the onset: lipids (e.g. cholesterol), thyroid, and a specific sample of catecholamines (i.e. for adrenaline and noradrenaline). The additional samples were repeated three months later at the clinical follow-up. Sampling was done at the Department of Cardiology, USÖ, as well as the final compilation and clinical interpretation of the analyzed values. All samples were analyzed at the Department of Clinical Chemistry, USÖ, apart from the catecholamine samples, which were analyzed at the Department of Clinical Chemistry, Karolinska Hospital (Huddinge, Sweden), through referral from USÖ. All analyzes, including the type of equipment, were performed according to the prevailing clinical practice, at the respective clinic.

- Analyzes by ECG;

In study II, recordings of SAECGs and HRVs were carried out, within 48 hours after the onset, as well as three months later at the follow-up. The SAECGs were performed with a Megacart ECG recorder (Siemens-Elema AB, Solna, Sweden); following a normal resting ECG, a total of 300 heart beats were analyzed (with 0.3 μV noise reduction and a correlation of 0.98). The bipolar X-, Y- and Z-lead system was used. Three established diagnostic criteria (i.e. SAECG variables), for evaluation of late potentials, were assessed: fQRS, RMS 40 and LAS 40 (30) (explained in the "Results" section). HRV was recorded with a solid-state recorder (DXP1000, Braemar, Eagan, USA); continuous recording of ambulatory ECG was carried out for 24 hours, using a set-up of seven electrodes and three ECG leads (patients live as normal at home during the recording, and are asked to note any symptoms). Obtained data were analyzed using an Aspect Holter system with HRV-module (Danica Biomedical, GE, version 3.81); following manual editing, i.e. sorting out any artifacts and arrhythmias, the HRV data were derived from the residual normal rhythm. The whole HRV procedure was in line with the recommendations (32). Eight time and four frequency HRV variables, often used in clinical practice, were analyzed (individually clarified in the "Results" section).

All SAECGs and HRVs were performed at the Department of Clinical Physiology, USÖ, by a few experienced biomedical scientists (of which one took care of all specific analyzes of the HRV variables), while interpretation, of all ECG data, was done mainly by one experienced physiologist.

- Cardiac MRI;

Cardiac MRI (applicable in study II and IV) took place at the Department of Radiology, USÖ, within 72 hours after the onset and at the follow-up (three months later). The MRIs were conducted as in the clinical routine; using a 1.5 Tesla scanner (Philips Achieva, Philips Medical Systems), equipped with a phased array cardiac coil for imaging. Repeated breath-holds and ECG gating were applied during recording. Balanced Turbo Field Echo (bTFE) cine images were acquired (as single 8.0 mm slices), in standardized long-axis, 3- and 4-chamber views. Full ventricular cover was achieved with 15-20 sequential short-axis slices (of 5.0 mm thickness). Further, LGE images were acquired; by breath-hold inversion-recovery TFE using 10 slices of 10.0 mm (in the same three views as above), about 15 short-axis slices were acquired for ventricular coverage. The LGE protocol was applied 15 minutes after intravenous contrast injection (Gadiodiamid, 0.15 mmol/kg) and was primarily of interest in study II (in terms of differential

diagnostic information during the screening/-inclusion period). All images were stored digitally for offline analysis, performed with dedicated software (ViewForum, Philips Medical Systems), by experienced radiologists, blinded to the TTE analyzes in each of the study II and IV.

Standard techniques and images were used for manual measurements of LV dimensions and function, performed in proximity to each examination (solely by one radiologist). Thus, including LVEF assessment (by Simpsons' rule, used in study II), as well as end-diastolic measurement of both SWT and RWT (used in study IV); left short-axis images in Fig. 10 depict an example of the SWT measurements by MRI (beside the corresponding TTE images to the right, as already highlighted). RWT was derived from long-axis images (by the same principle and formula as noted for TTE in Fig. 8). SWT and RWT sites had been pre-agreed, in an earlier stage, with the biomedical scientists who later on made the corresponding TTE measurements.

Assessment of LVM, only applicable in study IV, was measured separately at a later stage (by another radiologist who was independent of the first), from the same sets of short-axis slices; LV borders were manually traced in end-diastole, after which LVM was calculated as the sum of the difference, between epi- and endocardial volumes, multiplied by the density factor of cardiac tissue (41). The obtained LVM values were normalized for BSA (m^2).

Statistical analyzes and method comparisons

Test of normality for continuous variables (individual for separate data and on differences regarding comparisons) was performed with the Kolmogorov-Smirnov test (study III) and the Shapiro-Wilk's test (study I-II and IV). In study I-II and IV, as a significant portion of the variables were found to be non-normally distributed (and/or consistent with outliers by visual inspection of box plots); Wilcoxon signed-rank test was used for pairwise comparisons, as well as for intertechnique biases between TTE and MRI (the latter only regarding study IV).

In study IV, comparisons between groups were analyzed using Mann Whitney U-tests. (Distributions were similar upon visual inspection of the group-based histograms.) In study III, all continuous data were normally distributed; differences between the study phases (within the group) were compared using paired Student's t-test, while unpaired t-test was used for comparisons between subgroups. (There were no extreme outliers and all variances were similar)

In study III-IV, categorical variables were represented as number and proportions (percentage); comparisons of dichotomous proportions (within the groups and between study phases) were performed with McNemar's test, while Pearson's

chi-squared test was used (solely in study III) for comparison of data between the study populations (or Fisher's exact test when appropriate).

In study II and IV, Spearman's rank correlation coefficient was used for non-parametric assessment of interactions between two variables. Thus, applicable in study II regarding emotional versus physiological stress markers, while the following purposes were of interest in study IV: intertechnique agreement (between TTE and MRI), correlations of simultaneous TTE changes (during recovery) and intra/-interobserver variability tests (for the TTE part). In study III, Pearson's coefficient was used for parametric correlations of diastolic versus systolic TTE variables (with linear regression analysis), as well as for the intra/-interobservative variability tests. In study III-IV, regarding the variability, the CV was also calculated; as a measurement of percentage errors on repeated measurements (53). Further (solely in study IV), Cohen's Kappa test was applied (for interrater reliability regarding the WMA assessment), while limits of agreement (between TTE and MRI regarding LVM estimation) were determined by Bland-Altman analysis (53). In study IV, BSA calculations were done by the DuBois & DuBois formula (54).

In general (study I-IV), differences were considered statistically significant at P values < 0.05. In study III, this was only applicable after Bonferroni correction for multiple comparisons regarding pairwise data (55), otherwise, the difference was considered as tending (i.e. when significance at a P level < 0.05 only was reached before correction, but not afterwards). The same principle has been applied in this thesis; correction has been applied (retrospectively) regarding the other sub-studies (i.e. except study III), and thus, pairwise differences in the results section are generally presented with and without correction (although no correction was initially done in study I-II or IV). Thus, in the remainder of the thesis, all pairwise differences (from all sub-studies) are described as tending, where applicable. Generally, good correlation (Spearman's and Pearson's) was considered as ≥ 0.70, while CV < 10 % was seen as acceptable (in terms of variability). Interrater reliability, in study IV, was considered as good when Cohen's $K \geq 0.80$.

In the original sub-studies, the choice of statistical method, with the presented significance of the difference, was based on the dominant data character, with respect to all variables included in each study. In this thesis, however, some variables are presented as mean±SD, which are shown as median (interquartile range) in the original papers (study II and IV), because those specific variables passed the normality tests. The same principle applies to the Wilcoxon's tests in study I; individual variables with normal distribution are re-assayed by parametric method. This explains why certain data in the thesis may look different compared to the original papers [regarding presentation (study II and IV), as well as some P values (study I)]. Thus, in the remainder of the thesis, presented data have been consid-

ered as statistically individual in higher extent; some variables (or differences) that passed the normality test have been analyzed by parametric methods (shown as mean±SD), otherwise a non-parametric method has been applied [shown as median (interquartile range or absolute range, the latter is clarified where applicable)], regardless of the presentation in the respective original paper. Any supplementary statistics, carried out and presented solely in the thesis (in addition to the sub-studies), have been done by the same principles as above; choice of presentation and method have consistently been based on the character of the components (in conjunction with each analysis), while considerations (regarding interpretation) were similar.

For the Swedish population, a statistical power analysis was done in proximity to the onset of the screening/-inclusion period at USÖ; based on reported changes in LVEF at TTC, from previous studies (4, 9). An assumption was set to reach the same standard deviation (20 %), with statistical power of 0.80 and $\alpha = 0.05$, including the aim to find a difference in LVEF of 20 %. Ten participants were required to find this difference (based on paired Student's t-test calculation). With regard to the loss of some data, the hope was to include about 15 patients.

Data were analyzed using dedicated statistical software; SPSS version 17 (SPSS Inc., Chicago, IL, USA, in study I and III), SPSS Statistics version 21 (IBM Corp., in study IV) and SigmaStat 3.5 (Systat, San Jose, Ca, USA, in study II). In all sub-studies, statistical advices have been acquired from a biostatistician at the Department of Clinical Epidemiology and Biostatistics, USÖ (A. Magnuson), when the need has existed.

Ethical aspects

All Swedish patients fulfilling the aforementioned criteria for inclusion, had to give their written informed consent to participate, before final acceptance and enrollment were applied; after which they became fully documented (included in terms of study-specific demographics) and underwent complete investigation as described. Customary procedures regarding the care and follow-up, as in clinical practice at the Department of Cardiology, USÖ, were applied to all patients during the study period (including the management of any pathological findings in addition to the TTC diagnosis). Complete study procedure, applicable in all sub-studies (I-IV) and with regard to their separate purposes, was approved by the Regional Ethical Committee in Uppsala, Sweden.

For the U.S. population (regarding study III), patients' consent and their inclusion were based on the policy of the Review Board at each Institution (IRB). All U.S. cases were IRB exempted; the use of de-identified data, in the way that was done in study III, was approved without requirement for any further permission.

RESULTS

Study populations

At USÖ (applicable in study I-IV), the nearly 2-year screening period yielded a total of 15 female patients (from April 2008, when the first enrollment occurred), who met the inclusion criteria. Two patients declined participation while 13 patients (average 68 years) were willing to participate. Another woman with suspected TTC, from a TTE perspective, was asked to participate (mainly aimed for study I); detection occurred at a cooperating institution (Karlskoga Hospital, Sweden), although the patient later on declined participation. Thus, the total number of USÖ enrollment (13 patients) was similar in all sub-studies, and apart from study III, this group represents the total population as being studied; in the remainder of the thesis, reference to the Swedish/-USÖ part will solely refer to these 13 patients (regardless of any study referencing).

In study III, the population consisted of 28 patients with TTC diagnosis (average 64 years, females 24), where 15 patients (average 60 years, females 11) were retrospectively identified by the U.S. centres (the remainder were previously enrolled at USÖ, as above). Obtained data, derived by study III, is partially reported as divided in this thesis (by each sub-population). However, this is merely for clarity, the focus of the original study III was towards the defined total population; in the remainder of the thesis, reference to the total population in study III will refer to the compiled group of 28 patients (derived from the two sub-populations), unless otherwise stated.

Median time, during recovery, was 13 weeks (range 12-15) for the USÖ part, while similar numbers, for the total population in study III, was median 13 weeks (range 1-26). For the USÖ patients, acute hospitalization was four days in median (range 2-4), and the following ECG findings were reported at onset: nine patients had ST-elevations in two or more leads, three had poor R-wave progression and concomitant T-wave changes in precordial leads, while one had precordial ST-depressions (Fig. 5 shows a typical case with ST-elevations, as already highlighted). During recovery, all 28 patients (in both sub-populations) received customary treatment as in AMI (including beta-blockers), as recommended (20).

Complete accounting of patient demographics, applicable in all sub-studies (I-IV), is presented in Table 1 (the data is partially divided, for each sub-population, regarding variables as derived from study III). Note that the individual U.S. centres (and their respective contribution) are made clear in the table text.

Table 1. *Demographic display of the included patients, with the declaration of certain findings of interest, in terms of potential influencing factors, obtained at transthoracic echocardiography (TTE). The first part of the table (below) is transnational, with separate data for each study population (i.e. for the U.S. and the Swedish patients), as well as in overall numbers (the rightmost column), while the second part of the table (on the next page) shows additional data only for the Swedish population. Grey coloured data columns (below in the first part; USA and Overall) are only applicable for study III, while the white data columns, in both parts of the table, are relevant to all sub-studies (i.e. study I-IV). Values are mean±SD or number (percentage). No significant difference ($p<0.05$) was seen between the two countries, when comparing the populations with respect to the variables listed in the transnational table part (unpaired t-test, Pearson's chi-squared or Fisher's exact test). Non-clarified abbreviations are: IHD = ischemic heart disease. PASP = pulmonary artery systolic pressure. USA = United States institutions: SUNY Downstate Medical Center – Brooklyn, New York (contribution of seven patients), Parma Community General Hospital – Cleveland, Ohio (contribution of five patients), New York Methodist Hospital – Brooklyn, New York (contribution of three patients). USÖ = University Hospital Örebro. n = number of patients. WHO = World Health Organization. IPM = implantable pacemaker. TAM = tricuspid annulus motion.*

Variables - transnational	USA (n = 15)	Sweden, USÖ (n = 13)	Overall (n = 28)
Age (years)	60±12	68±10	64±10
Height (cm)	156±5	158±9	158±7
Weight (kg)	62±10	70±20	66±15
Women, n (%)	11 (73)	13 (100)	24 (86)
History of hypertension, n (%)	5 (33)	5 (38)	10 (36)
Diabetes mellitus, n (%)	4 (27)	0 (0)	4 (14)
Current smoker, n (%)	0 (0)	1 (8)	1 (4)
Family history of IHD, n (%)	1 (7)	3 (23)	4 (14)
Absence of significant heart valve disease, both in the acute and follow-up TTE, n (%)*	14 (93)	13 (100)	27 (96)
Absence of arrhythmias (such as atrial fibrillation), both in the acute and follow-up TTE, n (%)**	15 (100)	12 (92)	27 (96)
Absence of right ventricular systolic dysfunction, both in the acute and follow-up TTE, n (%)†	15 (100)	13 (100)	28 (100)
Absence of significant increased PASP, both in the acute and follow-up TTE, n (%)††	8 (89)	7 (88)	15 (88)

Variables – additionally only for the Swedish (USÖ) population	(n = 13)
Prevalence of obesity, according to the WHO definition; Body mass index (BMI) ≥ 30, n (%)	4 (31)
Alcohol habits; amount of patients declaring an alcohol intake of more than once per week (based on the epicrisis documented at onset, according to the usual clinical routine), n (%)	0 (0)
History of chronic lung disease (oxygen-demanding), or prevalence of any significant pulmonary disorder (e.g. embolism) throughout the study period, n (%)	0 (0)
History of chronic kidney failure with or without anemia, or diagnosed with acute kidney failure throughout the study period, n (%)	0 (0)
Definite trauma within two weeks at admission (based on the epicrisis documented at onset, according to the usual clinical routine), n (%)‡	6 (46)
Recurrence in takotsubo cardiomyopathy, during the study period (i.e. between onset and follow-up), or afterwards until the last check (spring 2014), n (%)	0 (0)
Reported as deceased since the study period, according to the last check (spring 2014), n (%)§	2 (15)
Prevalence of IPM, n (%)Δ	2 (15)
Absence of obvious signs of pericardial effusion, both in the acute and follow-up TTE, n (%)	13 (100)
Absence of signs of obstruction in the left ventricular outflow tract, in the acute TTE, n (%)	13 (100)

*Defined as significant valve stenosis and/or regurgitation (≥ grade 2/4), according to guidelines (56-58). **Brief episodes of IPM-rhythm in one of the patients. †Defined as TAM < 16 mm, according to guidelines (34). ††Defined as PASP ≥ 50 mmHg, according to guidelines (34) - [NOTE; complete PASP-data (i.e. from both study phases) only from nine U.S. versus eight Swedish patients (17 overall)]. ‡Trauma defined as; newly discovered, severe disease in a family member (in two patients), physical activity consistent with panic feeling (in two patients), or elements of threats and violence (in two patients). §No report including heart disease as a cause. ΔOne patient had IPM before the onset (due to history of arrhythmia), while one patient received a IPM during recovery (due to brief episodes of complete heart blockages on the ECG, during the acute hospitalization)

Echocardiographic measurements

Regarding the USÖ population, most TTEs were performed by M. Waldenborg and a colleague at that time[1], while the former was the one taking care of the final offline work (which occurred blinded to the results from the MRI analyzes, regarding study II and IV). For the U.S. population (regarding study III), a doctor at a hospital in New York conducted the compilation and analytical work[2] (in consulting with M. Waldenborg regarding the compilation with the Swedish data).

Standard TTE variables from all sub-studies (I-IV), as usually assessed in the clinical practice, are thoroughly presented in Table 2, including additional properties as often obtained (e.g. estimation of PASP as well as documentation of heart rate and blood pressure). Both study phases are represented, with the significance of the difference there between, as well as study-specific correction for multiple comparisons. Note that the rightmost column (in Table 2) depicts matched normal values for each variable, while the presented data is partially divided regarding variables that can be derived from study III; the Swedish numbers are shown both individually (solely for the USÖ population) and as compiled with the U.S. population (as overall numbers for both sub-populations), where the separate USÖ numbers (in these cases) mainly refer to study I-II and IV.

In the Swedish population, the prevalence of a pacemaker was applicable in two patients, where brief episodes of pacemaker-rhythm were observed in the acute phase. None of these patients, however, were completely pacemaker-dependent. All study-specific TTE images were consistent with sinus rhythm and the absence of any significant arrhythmia (e.g. atrial fibrillation) (Table 1), while the average heart rate, at both examination phases, was within normal limits (applicable in study I-IV).

Several study-specific TTE variables and results can be derived from Table 2 (at least regarding study I-III). Clarifications of the separate main results, however, are summarized in the following subheadings:

Biventricular systolic long-axis function

Among the main findings, in study I, were that the LV systolic long-axis shortening, in terms of the amplitude of MAM (total of the four sites), tended to increase during recovery [9.6±2.2 (acute) versus 11.2±1.9 mm (at recovery), Table 2 page 54]. Regarding the four different sites of measuring MAM, an overall difference (i.e. increase) was seen, although the septal site could not be proven as significant (regardless of any correction, depicted in paper I, Table 2).

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Further, in study I, the velocity of s' measured by PW-DTI (total of four sites), showed a tendency to increase between the study phases [5.9±1.5 (acute) versus 7.2±1.9 cm/sec (at recovery), uncorrected p=0.02]. However, no similar trend was seen by using 2D color DTI (mean s' was 5.3 cm/sec at both phases, depicted in paper I, Table 2). Besides, an additional comparison, of the separate mitral annulus sites, demonstrated higher values of s' as measured by PW-DTI (at all sites and at both study phases), than by using 2D color DTI. In study III, similar trend as in study I was demonstrated, regarding the velocity of s' by PW-DTI (mean of septal and lateral sites), that is, an acute impairment (Table 2 page 54).

During recovery, in study I, there was also a tended increase in the amplitude of TAM [21.3±3.6 (acute) versus 24.1±2.8 mm (at recovery), Table 2 page 53)], while the s' velocities (by both DTI techniques) remained unchanged and with overall greater numbers regarding PW-DTI (as for the LV) (paper I, Table 3).

Diastolic heart function

In study I, the diastolic tissue velocities (that is, e' and a') by PW-DTI (total of four sites) tended to increase during recovery (uncorrected p=0.04 for both variables), no similar trend, however, was demonstrated by 2D color DTI (depicted in paper I, Table 2). At both study phases and for all measurement positions, an additional comparison yielded overall greater e'- and a' velocities by PW-DTI (compared to 2D color DTI). Besides the diastolic tissue velocities of the LV, no further change in diastolic variables could be demonstrated during recovery, a similar trend was applicable regarding the RV [Table 2, page 53 for the RV and page 55 for the LV (all DF variables are depicted in paper I, Table 2-3)].

In study III, the total velocity of e' (mean of septal and lateral sites) improved during recovery [6.7±2.6 (acute) versus 8.7±3.1 cm/sec (at recovery), Table 2 page 55]. A tended improvement in DF, among other things, was also seen as a decrease in the E/e' ratio [10.3±4 (acute) versus 8.9±3 (at recovery)] as well as an increase in the E/A ratio [0.9±0.3 (acute) versus 1.1±0.5 (at recovery)] (Table 2 page 55). No further differences (either corrected or uncorrected), were obtained for the other DF variables as investigated in study III. Variables of pulmonary vein flow (including Arevdur) could not be obtained in more than half of the total population; therefore they were left out of the analysis in study III.

Further, in study III, comparison of DF stages was possible for a total of 19 patients, in whom complete data for classification could be obtained at both study phases. This comparison showed an overall shift towards improved DF stage during recovery (in terms of normal versus abnormal DF), as shown in Fig. 14.

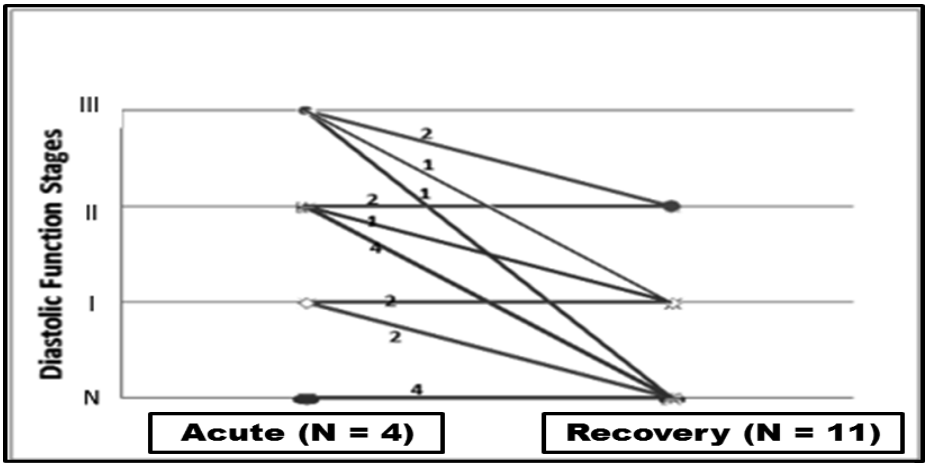

Figure 14. *Improvement of diastolic function (DF) stages from the acute to recovery phase, for the 19 patients in study III, which could be classified in this regard on both occasions. The classification was done according to the recommendations (35), where DF was assessed as either normal (N) or abnormal (grade I-III), as shown in more detail in Fig. 12. Acutely, four patients (21 %) had normal DF, compared to 11 patients (58 %) at recovery (digits in the lower headers). This difference was statistically significant (p=0.016, McNemar's test). Digits in the chart refer to the number of patients, who either maintain or improve their DF stage during recovery.*

Table 2. *Overall list of usual measurements, from all sub-studies (study I-IV), often included in the clinical echocardiographic routine. Both baseline (acute) and follow-up (recovery) data are shown, with the significance of the difference (including 95 % CI and multiple comparison correction), as well as depiction of normal values (the rightmost columns). Values are overall shown as mean±SD (based on paired Student's t-tests), although single variables are non-normally distributed (clarified with a footnote). At the next page data for the right ventricle (RV) and some additions are shown, while data for the left ventricle (LV) are shown on the following two pages. Rows highlighted in grey refer to variables with separate data both for the overall population (USA and Sweden, Table 1) as well as for the Swedish part, while non-marked rows solely refer to the latter. Variables within black squares are systolic measurements, the ones written in italics are diastolic and the rest refer to dimensions and additions. LV mass is only relevant for study IV and highlighted variables (with the U.S. part) are only relevant for study III, otherwise, all variables (at least for the Swedish part) are of relevance in all sub-studies (although LV ejection fraction was the only variable of interest in study II). Non-mentioned abbreviations are: TTE = transthoracic echocardiography. CI = confidence interval. n = number of patients with complete data at both study phases. n.s. = not significant. 2D = two-dimensional. TAM = tricuspid annulus motion. s' = systolic tissue Doppler velocity. PW = pulsed-wave. E/A = early/late-diastolic velocity. DTI = Doppler tissue imaging. e' = early-diastolic tissue Doppler velocity. USÖ = University Hospital Örebro. USA = U.S. institutions (Table 1). MAM = mitral annulus motion.*

TTE variables; right ventricular - RV (n)	Acute phase	Recovery phase	95 % CI of difference	P (Corrected*)	Reference values**
End-diastolic RV inflow tract diameter (RVIT3), by 2D in basal site, mm	29.7±5.2	30.0±2.9	-2.9, 2.3	n.s.	Normal RVIT3 (basal RV part); 22-36 mm (in women)
End-diastolic RV outflow tract (RVOT1), by M-mode in parasternal long-axis (PLAX), mm (13)	27.0±4.2	28±4.2	-2.4, 0.5	n.s.	Normal RVOT1 (PLAX) ≤ 33 mm
TAM by M-mode, mm (13)	21.3±3.6	24.1±2.8	-5.0, -0.5	0.021 (n.s.)	Normal TAM ≥ 16 mm
Lateral annulus s' by PW-DTI, cm/sec (13)	12.2±2.2	12.5±2.3	-1.8, 1.2	n.s.	Normal s' lateral RV annulus ≥ 10 cm/sec
E/A ratio by PW, (13)	1.3±0.5	1.1±0.4	-0.1, 0.6	n.s.	Normal E/A ratio; 0.8-2.1
Deceleration time (DT) by PW, msec (13)	195±59	201±42	-49, 37	n.s.	Normal DT; 120-229 msec
Isovolumetric relaxation time (IVRT) by PW, msec (13)	85±28	63±26	-5, 48	n.s.	Normal IVRT; 23-73 msec
Lateral annulus e' by PW-DTI, cm/sec (13)	11.3±3.6	11.8±2.8	-3.0, 2.5	n.s.	Normal e' lateral RV annulus; 8-20 cm/sec

TTE variables; other findings-/additions (n)	Acute phase	Recovery phase	95 % CI of difference	P (Corrected*)	Reference values or recommendations**
Left atrial volume by 2D area-length method, ml					
-Swedish (USÖ) population (13)	45±16	48±21	-10, 4	n.s.	Normal volume of left atrium; 22-52 ml (in women)
-Overall population, USA and Sweden (21)	55±26	52±26	-5, 11	n.s.	
Estimated pulmonary artery systolic pressure (PASP), mmHg†					
-Swedish (USÖ) population (8)	38±13	33±6	-3, 14	n.s.	Normal PASP ≤ 35 mmHg (may be slightly higher in the elderly and obese)
-Overall population, USA and Sweden (17)	35±10	31±9	0.5, 9	0.04 (n.s.)	
Average heart rate during TTE, beats per minute					
-Swedish (USÖ) population (13)	70±13	64±12	-4, 15	n.s.	Heart rate of 50-100 beats per minute is generally considered as normal (at rest)
-Overall population, USA and Sweden (28)	75±15	68±16	-2, 9	n.s.	
Systolic/Diastolic blood pressure, mmHg (13)	124±22 / 75±12	127±16 / 75±7	-7, 1 / -4, 4	n.s.††	130/80 mmHg is often considered to be upper limit (at rest)

TTE variables; left ventricular - LV (n)	Acute phase	Recovery phase	95 % CI of difference	P (Corrected*)	Reference values**
End-diastolic internal diameter (EDD) by M-mode, mm (13)	43.7±4.6	43.1±3.2	-1.6, 2.8	n.s.	Normal EDD; 39-53 mm (in women)
End-systolic internal diameter (ESD) by M-mode, mm (13)	27.9±6.1	26.9±3.0	-2.5, 4.6	n.s.	Normal ESD ≤ 40 mm (in women)
End-diastolic septal wall thickness by M-mode, mm (13)	10.7±2.5	10.4±2.6	-0.2, 0.9	n.s.	Normal septal wall thickness ≤ 12 mm (in women)
End-diastolic posterior wall thickness by M-mode, mm (13)	9.1±1.1	9.0±1.4	-0.4, 0.6	n.s.	Normal posterior wall thickness ≤ 11 mm (in women)
LV mass (LVM) by 2D truncated ellipsoid method, g/m² (13)	62±6	58±7	1, 7	0.014 (n.s.)	Normal LVM by 2D ≤ 88 g/m² (in women)
End-diastolic volume (EDV) by biplane Simpson's method, ml -Swedish (USÖ) population (13) -Overall population, USA and Sweden (25)	 77±20 100±29	 63±17 91±25	 7, 21 -2, 21	 0.001 n.s.	Normal EDV; ≤ 104 ml (in women)
End-systolic volume (ESV) by biplane Simpson's method, ml -Swedish (USÖ) population (13) -Overall population, USA and Sweden (24)	 37±12 44±31	 24±8 31±14	 9, 17 1, 24	 <0.001 0.03 (n.s.)	Normal ESV; ≤ 49 ml (in women)
Total MAM (average of four annulus sites) by M-mode, mm (13)	9.6±2.2	11.2±1.9	-2.8, -0.3	0.017 (n.s.)	Normal MAM (mean of four sites); 15±2 mm (60±4 years)
Ejection fraction (EF) by biplane Simpson's method, % -Swedish (USÖ) population (13)† -Overall population, USA and Sweden (23)	 53±10 44±16	 63±4 60±7	 -5, -15 -20, -10	 0.001 <0.001	Normal LVEF ≥ 55 %
Mean s' (average of septal-lateral annulus) by PW-DTI, cm/sec -Swedish (USÖ) population (13) -Overall population, USA and Sweden (16)	 5.2±1.2 7.8±5	 6.5±1.7 8.7±4	 -1.9, -0.6 -1.9, 0.11	 0.001 0.02 (n.s.)	Normal s' (mean of septal-lateral); 8.7±1.6 cm/sec (65-75 years)

TTE variables; left ventricular - LV (n)	Acute phase	Recovery phase	95 % CI of difference	P (Corrected*)	Reference values**
E/A ratio by PW					
-Swedish (USÖ) population (13)	1.2±0.7	0.9±0.3	-0.24, 0.72	n.s.	Normal E/A ratio; 0.96±0.18 (> 60 years)
-Overall population, USA and Sweden (22)	0.9±0.3	1.1±0.5	-0.41, -0.02	0.04 (n.s.)	
Mean e' (average of septal-lateral annulus) by PW-DTI, cm/sec					
-Swedish (USÖ) population (13)	7.1±2.8	7.5±2.9	-2.3, 1.4	n.s.††	Normal e' (mean of septal-lateral); 9.0±1.4 cm/sec (65-75 years)
-Overall population, USA and Sweden (19)	6.7±2.6	8.7±3.1	-3.3, -1.2	<0.001	
Mean E/e' ratio (average of septal-lateral annulus)					
-Swedish (USÖ) population (13)	12.6±7	11.9±10	-1.6, 3.2	n.s.††	Normal E/e' ratio (mean of septal-lateral); 7.6±1.8 (65-75 years)
-Overall population, USA and Sweden (19)	10.3±4	8.9±3	0.10, 2.7	0.03 (n.s.)	
Isovolumetric relaxation time (IVRT) by PW, msec					
-Swedish (USÖ) population (13)	92±19	91±17	-14, 15	n.s.	Normal IVRT; 87±7 msec (> 60 years)
-Overall population, USA and Sweden (19)	102±33	105±23	-19, 13	n.s.	
Deceleration time (DT) by PW, msec					
-Swedish (USÖ) population (13)	206±92	250±69	-89, 2	n.s.	Normal DT; 200±29 msec (> 60 years)
-Overall population, USA and Sweden (19)	202±88	226±75	-53, 6	n.s.	

*Correction with Bonferroni for study-specific, multiple comparisons. **Normal values according to U.S., European and Swedish TTE compilations (24, 33, 35, 51, 59, 60), focused on the elderly and women with regard to the demographics of the patients (Table 1), or general recommendations within cardiac care (heart rate and blood pressure). †Complete data, where estimation was possible in both study phases, only for eight Swedish patients and 17 overall. ††Non-normally distributed data, analyzed and reported on the basis of a non-parametric test in the respective original paper (Wilcoxon signed-rank test), significances reported in the table, however, are not different from the study results

Quantification of LVEF and LV geometry

The LV systolic function, expressed by LVEF (Simpson's method), was reduced at onset, while complete normalization was shown at the recovery phase; both regarding the USÖ population [53±10 (acute) versus 63±4 % (at recovery), applicable in study I-II and IV] as well as for the overall population in study III [44±16 (acute) versus 60±7 % (at recovery)] (Table 2 page 54). In study I, no difference in the end-diastolic LV length could be demonstrated (between the study phases), while the same trend (i.e. no difference) was seen regarding the measurements of LV diameter (Table 2 page 54). Similarly as for the LV, no differences (during recovery) were found regarding the size of the RV, neither in terms of width (expressed as RVIT3 and RVOT1, Table 2 page 53) nor regarding the measurements of RV length, as carried out in study I.

The geometric LV measurements, as conducted in study IV, yielded the following main findings when comparing the acute phase against the recovery:

* LVM decreased, by the two formula-based methods (i.e. the AL/-TE-method), while M-mode and the bi-/triplane Simpson's methods (despite tantamount reduction) remained unchanged. The TE-method yielded following numbers: 62±6 (acute) versus 58±7 g/m^2 (at recovery) (Table 2 page 54).

* SWT decreased in the two apical sites [median 7.0 (6.0-8.0) versus 7.0 (6.0-8.0) mm at septal site (p=0.031, Z=-2.157), and 7.0 (6.0-7.0) versus 6.0 (6.0-6.0) mm at inferior site (p=0.006, Z=-2.739)], while the basal septal site, as well as RWT, remained unchanged. The aforementioned Fig. 10 (right images) shows an example of changes in SWT during the recovery.

* WMA improved, a decrease in WMSI was documented [median 1.50 (1.25-1.56) versus 1.00 (1.00-1.00) %, p=0.002 (Z=3.068)], while a shift was demonstrated regarding the visual WMA assessment; at recovery, no patient were considered to have typical look as in TTC (apical WMA and/or ballooning), compared with 11 patients at onset (p=0.001). Moreover, GRS improved [16±14 (acute) versus 34±12 % (at recovery), p=0.006 (95 % CI = -30, -6)].

* For each study phase, the obtained data for RWT and normalized LVM from the TE-method were used for categorization into LVH geometries (8); using 0.42 as the RWT cut-off and 95 g/m^2 for normalized LVM (in women), 10 patients (77 %) were classified as having a normal geometry while the reminders turned out as having a concentric remodeling geometry. This classification yielded the exact same, invariant LVH geometry division at each phase.

Reproducibility and feasibility

The results from the intra/-intervariability tests in study III, regarding the main variables of E/A ratio and e', are shown in Table 3. The intra/-intervariability results from study IV, regarding geometric LV variables, are shown in Table 4.

In study IV, a total of 156 segments were included in the GRS analysis (at each study phase); 135 (acute) and 131 (at recovery) were accepted; yielding a non-significant feasibility of 87 % versus 84 % (p=0.125). The median frame rate (for all cine images used in the GRS analyzes) was 62 Hz (55-67), and the median time required for offline analysis was 3.0 minutes (absolute range 2.0-4.0). The median time required for measuring of LVM was (in minutes): 1.5 (M-mode and AL/-TE-methods), 3.0 (biplane Simpson's) and 4.5 (triplane Simpson's).

Table 3. *Intra- and interobserver variability for each country (from study III), presented as CV, Pearson's correlation test (with P values) and mean±SD of the difference (or bias), regarding repeated measurements of variables E/A ratio and mean e', at respective study phase. Abbreviations are: n = Number of patients. USA = all three U.S. centres. E/A ratio = the quotient between the E- and A wave velocities. mean e' = mean of septal and lateral e' velocities (cm/sec). USÖ = University Hospital Örebro. CV = coefficient of variation (percentage). Mean diff (SD) = mean difference with the standard deviation for repeated measurements, expressed as the absolute difference from acute phase to recovery.*

	Intraobserver control			**Interobserver control**		
Country (n), variable and examination phase	CV %	Pearson's correlation (P value)	Mean diff (SD)	CV %	Pearson's correlation (P value)	Mean diff (SD)
USA (n = 15) E/A ratio acute phase	8.1	0.96 (<0.001)	-0.1 (0.1)	8.5	0.97 (<0.001)	0.1 (0.1)
USA (n = 15) E/A ratio recovery phase	8.3	0.97 (<0.001)	-0.1 (0.1)	10.7	0.96 (<0.001)	0.1 (0.1)
USA (n = 15) mean e' acute phase	3.5	0.99 (<0.001)	-0.2 (0.3)	5.5	0.99 (<0.001)	-0.1 (0.3)
USA (n = 15) mean e' recovery phase	4.5	0.99 (<0.001)	-0.1 (0.4)	5.4	0.97 (<0.001)	-0.2 (0.4)
Sweden, USÖ (n = 13) E/A ratio acute phase	6.6	0.93 (<0.001)	0.2 (0.7)	11.5	0.90 (<0.001)	0.3 (1)
Sweden, USÖ (n = 13) E/A ratio recovery phase	4.6	0.95 (<0.001)	-0.1 (0.1)	6.7	0.94 (<0.001)	0.1 (0.1)
Sweden, USÖ (n = 13) mean e' acute phase	2.6	0.97 (<0.001)	-0.1 (0.1)	4.8	0.95 (<0.001)	-0.1 (0.5)
Sweden, USÖ (n = 13) mean e' recovery phase	2.9	0.97 (<0.001)	0.1 (0.3)	5.2	0.95 (<0.001)	0.2 (0.7)

Table 4. *Reproducibility results from echocardiographic measurements of left ventricular mass and geometry, as carried out in study IV; intra- and/or interobserver variability for continuous variables and Cohen's Kappa test for proportions regarding interrater variability for WMA-assessments, at each study phase for all 13 USÖ patients. Intra- and interobserver variability are presented as the CV (percentage) and Spearman's rank correlations (with P values), for repeated measurements (intraobserver data are highlighted in grey, while non-highlighted data relate to interobservative such). Interrater variability is shown separate (bottom without highlighting) with the Kappa coefficient. Abbreviations are: WMA = wall motion abnormalities. CV = coefficient of variation. rs = correlation coefficient. LVM = left ventricular mass. ASE = American Society of Echocardiography. 2D = two-dimensional. AL = area-length method. TE = truncated ellipsoid method. RWT = relative wall thickness. GRS = global radial strain. WMSI = wall motion score index. TTC = takotsubo cardiomyopathy. CI = confidence interval. USÖ = University Hospital Örebro, Sweden.*

Continuous variables	**Acute phase**				**Recovery phase**			
	CV %		Spearman's, rs (P)		CV %		Spearman's, rs (P)	
LVM, M-mode ASE-method (g/m²)*	2.2	2.7	0.94 (<0.001)	0.74 (0.004)	2.5	4.2	0.98 (<0.001)	0.96 (<0.001)
LVM, 2D AL-method (g/m²)	2.2	-	0.96 (<0.001)	-	2.0	-	0.96 (<0.001)	-
LVM, 2D TE-method (g/m²)	2.0	-	0.96 (<0.001)	-	2.5	-	0.95 (<0.001)	-
LVM, 2D biplane Simpson's method (g/m²)	3.9	-	0.94 (<0.001)	-	4.1	-	0.89 (<0.001)	-
LVM, 2D triplane Simpson's method (g/m²)	6.3	-	0.86 (<0.001)	-	4.3	-	0.91 (<0.001)	-
Segmental wall thickness, basal septal site (mm)	1.6	-	0.99 (<0.001)	-	2.5	-	0.94 (<0.001)	-
Segmental wall thickness, apical septal site (mm)	3.3	-	0.97 (<0.001)	-	2.1	-	0.99 (<0.001)	-
Segmental wall thickness, apical inferior site (mm)	2.0	-	0.99 (<0.001)	-	4.5	-	0.87 (<0.001)	-
RWT, index*	3.8	4.5	0.96 (<0.001)	0.96 (<0.001)	3.3	2.9	0.87 (<0.001)	0.90 (<0.001)
GRS; average of 12 papillary muscle and apical segments, (%)	8.1	-	0.98 (<0.001)	-	4.4	-	0.93 (<0.001)	-
WMSI (%)	-	6.3	-	0.84 (<0.001)	-	3.9	-	0.74 (0.004)

Proportions of WMA-assessments	**Acute phase; Cohen's Kappa (95 % CI of Kappa) (P)**	**Recovery phase; Cohen's Kappa (95 % CI of Kappa) (P)**
Interrater variability**	0.711 (0.348, 1.07) (0.001)	1.00 (0.99, 1.01) (<0.001)

*A few comparisons (interobserver) were made between M-mode and two-dimensional measurements. **Between study-specific assessment by one observer (M. Waldenborg) and clinical assessment by independent observers. WMA was defined as; no typical TTC-look (no WMA), typical TTC-look (WMA mid-ventricular with or without apical involvement, or a probable TTC-variant), or less typical TTC-look (WMA but not as typically seen at TTC)

Other measurements

Posttraumatic stress and depression

In study II (for the USÖ population), assessment of posttraumatic stress and depression were made by one involved psychiatrist[1]; two patients fulfilled criteria for posttraumatic stress syndrome at admission (by the PTSS-10 questionnaire), while seven patients were in the borderline zone. At three months follow-up one patient still fulfilled the criteria for posttraumatic stress, while four had borderline scores (individual PTSS-10 scores are depicted in Fig. 15.). Using the MADRS-S questionnaire; one patient was in the borderline zone for depression at admission, while the remainders (acutely) as well as the entire group (at follow-up) had scores within the normal range. For the entire group there were no differences in rating scale scores when comparing the acute phase and follow-up: PTSS-10 yielded median 30 (25-32) versus 24 (17-33) (p=0.30), while MADRS-S scores were median 8 (4-10) versus 10 (5.5-11) (p=0.49).

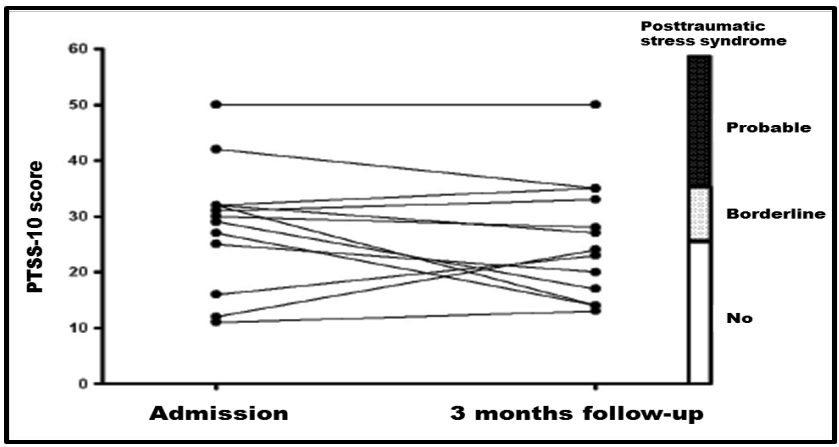

Figure 15. *Inventory of Posttraumatic Stress Scale 10–Questionnaire (PTSS-10), individual scores in the acute phase (admission) compared to the recovery (three months follow-up), for the 13 women being diagnosed with takotsubo cardiomyopathy and investigated in study II. Scoring criteria for PTSS-10 are illustrated on the right-hand y-axis. The difference in PTSS-10 score, between the two phases, was not statistically significant (p=0.30, Wilcoxon signed-rank test).*

[1] M. Soholat, MD. Department of Psychiatry, USÖ.

Biochemical markers

In study II (regarding the USÖ population), the results from the biochemical screening can be summarized as follows: acute markers of myocardial damage and heart failure (i.e. troponin I, creatinine kinase MB and NTpBNP) were slightly to moderately elevated (in all patients). Catecholamines (adrenaline and noradrenaline) and thyroid variables were normal and remained unchanged from acute hospitalization to the three months control visit. Further, cholesterol levels were slightly above the normal and were also unchanged during follow-up.

The overall results from the biochemical screening are presented in detail in Table 5, including depiction of matched normal references (rightmost column).

Analyzes by ECG

In study II, the majority of the SAECG and HRV analyzes were done by one biomedical scientist[1], while the clinical interpretation (of all analyzes) were mainly done by one physiologist[2]. The results from the SAECGs demonstrated an acute reduction of fQRS, as compared to the follow-up at three months, while the variables of RMS 40 and LAS 40 were unchanged. In the HRV analyzes, two time variables were altered: SDNN and SDANN, which were both shorter acutely as compared to the follow-up. (SDNN and SDANN are "global variables", where SDANN is considered as the relatively most sensitive regarding increased sympathetic activity.) No other HRV variables were altered. The majority of the ambulatory ECG recordings (as preceding the HRV analysis) did not yield any significant arrhythmias, while no patient reported any serious symptoms (e.g. fainting).

The overall results, in terms of individual numbers for all SAECG/-HRV variables used in study II, are shown in detail in Table 6; including description of the separate variables, and depiction of matched normal references (a self-created material of normal values was used for the HRV variables, which is clarified in Table 6). The distribution of the specific variables of fQRS, SDNN and SDANN, at both study phases and as compared against separate normal references, are graphically depicted in Fig. 16. The results from the clinical assessment of the SAECG/-HRV analyzes, in terms of normal versus predominant presence of pathological findings (based on summation of variables), are depicted in Fig. 17 (for the patients where interpretation was possible, as clarified in the figure text).

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Table 5. *Biochemical variables and findings of the 13 USÖ patients, as carried out in study II. Depicting both acute values (within 48 hours at onset) and at follow-up (three months), with the significance of the difference (where appropriate) and the standardised test statistics (based on Wilcoxon signed-rank tests). Values are median (interquartile range). All analyzes were done at the Department of Clinical Chemistry (USÖ), except those for catecholamines (bottom highlighted in grey), which were performed at the Department of Clinical Chemistry at Karolinska Hospital (Huddinge, Sweden), through referral from USÖ. Depicted references (in the rightmost column) are according to the guidelines of each department (i.e. Karolinska Hospital for the catecholamines), except for NT pro-brain natriuretic peptide (clarified with a footnote). Abbreviations are: USÖ = University Hospital Örebro, Sweden. n.s. = not significant. n.r. = not relevant. AMI = acute myocardial infarction.*

Variable	Initial findings	48 hours	3 months	P (Corrected†)	Z-score	Reference values or normal range
Troponin I (µg/L)*	2.70 (0.45-2.90)	0.42 (0.17-0.76)	Not measured	0.002	-3.110	AMI limit > 0.10††
Creatine kinase µkat/L	2.55 (1.58-3.15)	1.50 (1.00-1.90)	Not measured	0.034 (n.s.)	-2.118	0.6-3.5 (women)
Creatine kinase MB (µg/L)	11.60 (5.35-22.33)	4.40 (2.80-5.10)	Not measured	0.005 (n.s.)	-2.824	AMI limit > 3.5 (women)
NT pro-brain natriuretic peptide (ng/L)	389 (228-447)	319 (229-751)	Not measured	n.s.	-1.067	Upper limit around 300 (women older than 65 years)‡
High-sensitivity C-reactive protein (mg/L)	4.10 (2.90-6.70)	10.50 (7.10-19.60)	Not measured	0.021 (n.s.)	2.313	Normally < 2.0
Hemoglobin (g/L)	141 (133-144)	Not measured	Not measured	n.r.	n.r.	117-153 (women)
Plasma creatinine (µmol/L)	66 (56-78)	Not measured	Not measured	n.r.	n.r.	45-95 (women)
Thyroid-stimulating hormone (mIU/L)	1.20 (0.68-1.70)	Not measured	1.10 (0.90-1.50)	n.s.	0.078	0.4-4.0
Thyroxine (pmol/L)	14.30 (13.60-15.50)	Not measured	14.00 (13.30-15.00)	n.s.	-0.628	9-19
Cholesterol (mmol/L)	6.10 (5.30-6.60)	Not measured	5.40 (4.40-6.00)	n.s.	-0.628	3.9-7.8 (< 5.0 in elderly > 50 years)
Low-density lipoprotein cholesterol (mmol/L)	3.60 (2.70-4.40)	Not measured	3.00 (2.10-3.50)	n.s.	-0.628	2.0-5.3 (< 3.0 in elderly > 50 years)
High-density lipoprotein cholesterol (mmol/L)	1.50 (1.20-1.50)	Not measured	1.40 (1.30-1.60)	n.s.	1.449	1.0-2.7 (≥ 1.2 in women)
Triglyceride (mmol/L)	1.60 (1.20-2.20)	Not measured	1.50 (1.20-2.40)	n.s.	0.939	0.45-2.6 (preferably < 2.0)
Plasma adrenaline (nmol/L)**	0.30 (0.30-0.40)	Not measured	0.30 (0.30-0.30)	n.s.	0.000	< 0.7
Plasma noradrenaline (nmol/L)**	3.70 (2.60-5.50)	Not measured	3.30 (2.50-3.40)	n.s.	-1.527	< 4.8 (non-resting value)

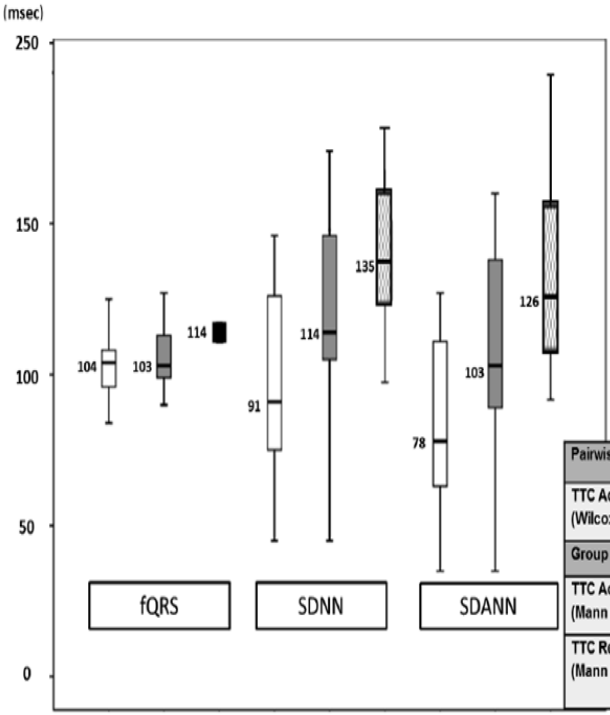
*Corrected measurement unit from the original paper (study II), where troponin I mistakenly was stated in mg/L. **Complete data (i.e. obtained at both study phases) only from seven patients. †Correction with Bonferroni for multiple comparisons. ††AMI limit for troponin I has been updated and adjusted to > 0.070 µg/L as of December 2010 (i.e. since the study was completed). ‡Upper limit according to general recommendations within cardiac care

Table 6. *Data of late potentials (SAECG) and HRV analyzes, as performed for the 13 USÖ patients in study II. Both acute findings and recovery data are shown, with the significance of the difference and the standardized test statistics (Wilcoxon signed-rank tests). Values are median (interquartile range). Rightmost column depicts matched references, with respect to the demographics (Table 1). HRV rows marked in grey are frequency variables, while non-marked are time variables. Abbreviations are: SAECG = signal-averaged ECG. HRV = heart rate variability. USÖ = University Hospital Örebro, Sweden. fQRS = duration of filtered QRS complex. RMS 40 = Root mean square voltage of signal in the last 40 milliseconds (msec) of fQRS. LAS 40 = Duration of low amplitude signal below 40 µV in the last fQRS part. Total = all effect ≤ 0.4 Hz. VLF = very low frequency, LF = low frequency, HF = high frequency. SDNN = standard deviation of all the normal-to-normal beat (RR) intervals. SDANN = standard deviation of RR-interval mean values in five minute segments. RMSSD = mean squared differences of successive RR-intervals. SDNNi (index) = mean of standard deviation of all the RR-intervals in five minute segments. SDSD = standard deviation of successive differences. NN50 count = the number of interval differences of successive RR-intervals >50 msec. pNN50 = percentage of adjacent RR-interval differences >50 msec. TINN = triangular interpolation of RR-intervals. (g) = global variable of the overall autonomic balance. (s) = mainly sympathetic variable. (p) = mainly parasympathetic variable. n.s. = not significant.*

SAECG variables*	**ACUTE**	**RECOVERY**	**P (Corrected††)**	**Z-score**	**Reference values‡**
fQRS, msec	104 (96-108)	103 (99-113)	0.01	2.403	Cut-off for late potentials > 114 msec
RMS 40, µV**	31 (16-38)	30 (10-34)	n.s.	-1.575	Cut-off for late potentials < 20 µV
LAS 40, msec	31 (27-40)	35 (30-46)	n.s.	1.887	Cut-off for late potentials > 38 msec

HRV variables†		**ACUTE**	**RECOVERY**	**P (Corrected††)**	**Z-score**	**Normal range 60-69 years [women only]§**
Total, msec^2	(g)	1300 (764-2241)	1881 (1276-2065)	n.s.	1.223	2003 (1379-2859) [1615 (1153-2809)]
VLF, msec^2	(s)	929 (563-1463)	948 (875-1352)	n.s.	1.013	1433 (889-2016) [1143 (839-1668)]
LF, msec^2	(s)	207 (154-469)	315 (186-558)	n.s.	1.433	432 (324-796) [380 (264-769)]
HF, msec^2	(p)	155 (60-216)	132 (109-519)	n.s.	0.594	125 (94-209) [139 (92-212)]
SDNN, msec	(g)	91 (75-126)	114 (105-146)	0.008 (n.s.)	2.434	135 (121-174) [141 (125-174)]
SDANN, msec	(g)	78 (63-111)	103 (89-138)	0.008 (n.s.)	2.550	126 (107-162) [129 (116-163)]
RMSSD, msec	(p)	28 (21-35)	24 (22-41)	n.s.	0.550	24 (20-31) [22 (20-32)]
SDNNi, msec	(p)	41 (31-51)	47 (40-49)	n.s.	1.468	49 (39-58) [44 (39-58)]
SDSD, msec	(p)	28 (21-35)	24 (22-41)	n.s.	0.550	24 (20-31) [22 (20-32)]
NN50 count	(p)	4157 (967-5085)	2913 (1448-12500)	n.s.	0.874	2215 (1259-5092) [1696 (1259-5468)]
pNN50, %	(p)	3.96 (2.38-6.39)	3.02 (1.66-14.47)	n.s.	0.800	2.00 (1.00-6.00) [2.00 (1.00-7.00)]
TINN, msec	(s)	390 (290-600)	480 (400-610)	n.s.	1.883	620 (510-740) [650 (560-750)]

*One patient had partial pacemaker rhythm during the SAECG recording at recovery. **Corrected measurement unit from the original paper (study II), where RMS 40 mistakenly was stated in mV. †One patient had increased amount of extra systoles (acutely), while two patients had elements of pacemaker rhythm (one at both stages and the other at recovery), during the HRV recordings. ††Correction with Bonferroni for multiple comparisons (separately for SAECG/-HRV variables). ‡General cut-off values for late potentials at SAECG (30). §Normal data for HRV, provided by the Department of Clinical Physiology, Norrlands University Hospital, Umeå Sweden (2004), with permission to present (personal communication, P. Rask (MD, PhD), June 2014); values represent the age group 60-69 years, for both genders (41 participants, left) and separately for the women included (21 participants, to the right within square brackets)



Pairwise comparisons	fQRS	SDNN	SDANN
TTC Acute vs. recovery (Wilcoxon's test)	p=0.01†	p=0.008†	p=0.008†
Group comparisons		**SDNN**	**SDANN**
TTC Acute vs. reference* (Mann Whitney U-test)		p<0.001†	p<0.001†
TTC Recovery vs. reference* (Mann Whitney U-test)		p=0.049	p=0.033

Figure 16. *The distribution of filtered QRS duration (fQRS), standard deviation of all normal-to-normal beat (RR) intervals (SDNN), and standard deviation of RR-interval mean values in five minute segments (SDANN); which in study II were seen to be reduced acute, compared with the recovery, in the takotsubo cardiomyopathy (TTC) cohort (upper part of the table). Upper and lower box borders represents interquartile ranges, digits next to the boxes relates to each median value (marked with solid lines), where all three variables are measured in milliseconds (msec). Additionally, the SDNN/-SDANN data are compared against an age-appropriate normal reference; with signs of relatively lower numbers, at both phases, in the TTC-group (lower part of the table). Further, fQRS is also shown in contrast to what is considered to be normal (black square, the digit next by refers to the upper limit of normal). Abbreviations are: n = number of patients (TTC) or number of controls (reference group regarding SDNN and SDANN). vs. = versus. *Normal data; as collected and compiled at the Department of Clinical Physiology, Norrlands University Hospital, Umeå Sweden (2004), with permission to present (personal communication, P. Rask (MD, PhD), June 2014). **Upper limit of fQRS (30). †Statistical significance (p<0.05 level), even after Bonferroni correction for multiple comparisons (done separately for pairwise and group-based tests, in the table portion).*

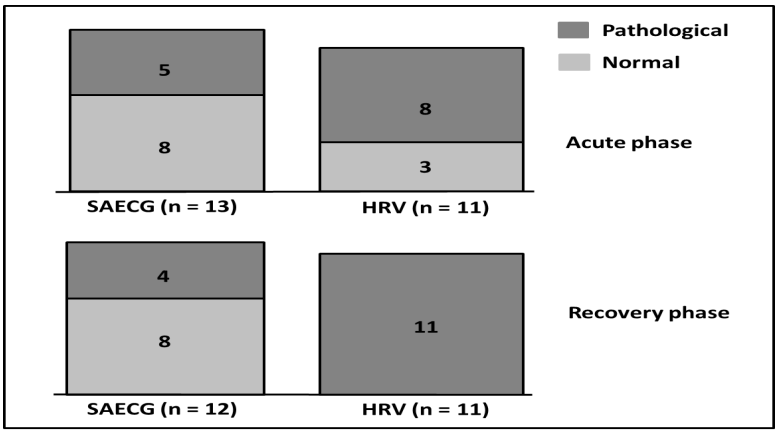


Figure 17. *Distribution of signal-averaged ECG (SAECG, left stacks) and heart rate variability (HRV, right stacks) analyzes during acute and recovery phase, for the USÖ patients (study II). Digits refer to number of patients, in terms of analyzable (bottom) and assessed as normal or pathological findings (within the stacks). Assessment, in this respect, is based on summation of all variables included in the respective SAECG and HRV analysis. One patient had a pacemaker before onset, one had frequent extra systoles at the acute HRV recording and another patient had a pacemaker during recovery, which is why only 12 SAECGs at the recovery and 11 HRVs in overall were considered as interpretable (although individual variables may still be interpreted). Most analyzes were done by the same observer (J. Myrin, MSci), while the assessments mainly were performed by another observer (P. Rask, MD, PhD), both with good experience in this field. None of the distributions are significantly different between phases (by separate McNemar's tests, solely for 12 patients regarding SAECG).*

Cardiac MRI

Regarding the MRIs, for the USÖ population and as applicable in study II and IV, four patients could not undergo MRI at all, while one of the patients only underwent MRI acutely; the presence of a pacemaker was the cause for two individuals (as mentioned, Table 1), one patient had elevated serum creatinine (discovered during acute hospitalization) and two suffered from claustrophobia. Thus, nine patients had MRI acutely versus eight patients at the recovery. Besides, the additional LGE protocol could not be applied in one of the patients, who underwent the main MRI protocol (at any phase). (This was due to earlier history of elevated serum creatinine, which allowed for the standard protocol, in contrast to the aforementioned patient with newly discovered elevation in this regard.) Thus, eight patients had additional LGE protocol acutely versus seven at the follow-up.

The MRIs were initially conducted by one MRI-savvy radiologist[1], who also did the majority of the offline analyzes (used both in study II and IV), while the specific LVM measurements regarding study IV was done by another radiologist[2], (also experienced); no detailed analysis time was documented for the LVM estimation by MRI, but most of the measurements took about 15-20 minutes.

Complete accounting for the MRI data, both in terms of execution and the study-specific results, are shown in Fig. 7, including depiction of matched normal references (rightmost column). The main findings of the respective sub-study are summarized as follows:

* In study II, LVEF was moderately reduced at hospitalization and improved to normal values for all patients [54 (45-64) acutely versus 73 (66-74) % at recovery, Table 7]. In none of the patients undergoing MRI, with additional LGE protocol, could any signs of myocardial necrosis or fibrosis be demonstrated.

* In study IV, a decrease in LVM was found during the recovery [60±14 (acute) versus 56±12 g/m² (at recovery), p=0.012 (95 % CI = 2, 12)] (Note; LVM is shown as in the original paper IV in Table 7, that is, as median (interquartile range).)

* Further, in study IV, SWT altered in the two apical sites from acute phase to the recovery [median 6.1 (5.8-7.6) versus 5.3 (4.8-6.0) mm at septal site (p=0.012, Z=-2.521), and 5.6 (5.3-8.0) versus 5.7 (4.4-6.1) mm at inferior site (p=0.021, Z=-2.313)], while the basal septal site as well as RWT values remained unchanged. An example of the SWT alterations by MRI is depicted in Fig. 10 (left images).

Interaction and coherence of measurements

In study II, acute associations were tested between PTSS-10 score and major variables of cardiac function (e.g. LVEF, NTpBNP, troponin I, CRP, thyroid markers, plasma catecholamines and all the variables of the SAECG/-HRV analyzes); yielding an inverse correlation between PTSS-10 score and fQRS obtained by the SAECG (rs= -0.66), as shown in Fig. 18. Supplementary analyzes yielded the following two interactions regarding LVEF as obtained by TTE versus by MRI: a positive correlation for the nine patients with complete data at acute phase (rs=0.70, p=0.035), as well as for the eight patients with complete data at both study phases (rs=0.76, p=0.028), where the latter refers to the difference (i.e. increase) from acute phase to the recovery.

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[2] M. Lidén, MD, PhD. Department of Radiology, USÖ.

Table 7. *Some left ventricular (LV) findings on cardiac magnetic resonance imaging (MRI), carried out for the USÖ patients, with relevance in study II and IV (non-marked rows refer primarily to study II, while rows marked in grey are mainly related to study IV). The data only represents the patients who could undergo MRI: nine subjects acute versus eight at recovery phase (of the total 13 patients, see text for exclusion declaration). Study-specific values are median (interquartile range) or number (percentage), with the significance of the difference and the standardized test statistics (where appropriate). For continuous data Wilcoxon signed-rank test was used (based on eight patients with complete data at both phases), while McNemar's test was used for proportions. The rightmost column shows matching references (61-63), where values are mean±SD. Abbreviations are: USÖ = University Hospital Örebro, Sweden. n.s. = not significant. n.r. = not relevant. SSFP = steady-state free precession. bTFE = balanced Turbo Field Echo (which is a kind of SSFP-protocol). LGE = late gadolinium enhancement (with intravenous injection of Gadiodiamid contrast). GFR = Glomerular Filtration Rate.*

Cardiac MRI variable, by bTFE protocol	ACUTE [n = 9 (69 %)]	RECOVERY [n = 8 (62 %)]	P (Corrected*) [n.s.]	Z-score [n.r.]	Normal references, with SSFP protocol in adults (≥ 20 years)
End-diastolic volume (EDV), by modified Simpson's method, ml	134 (120-143)	129 (117-140)	n.s.	-1.965	mean±SD of EDV 134.9±19.3 ml (based on 30 female subjects)
End-systolic volume (ESV), by modified Simpson's method, ml	50 (45-86)	34 (31-55)	0.016 (n.s.)	-2.383	mean±SD of ESV 48.9±10.7 ml (based on 30 female subjects)
Ejection fraction (EF), by modified Simpson's method, %	54 (45-64)	73 (66-74)	0.016 (n.s.)	-2.380	mean±SD of EF 64.0±4.9 % (based on 30 female subjects)
End-diastolic diameter (EDD), measured on long-axis images, mm	49 (42-51)	45 (43-48)	n.s.	-1.169	mean±SD of EDD 46±4.0 mm (in long-axis, based on 44 subjects of which 15 female)
Absence of signs of myocardial necrosis demonstrated by additional LGE protocol, n (%)**	8 (100)	7 (100)	n.s.	n.r.	n.r.
LV mass (LVM), calculated through summation of sequential, volumetric short-axis slices, g/m^2	60 (56-67)	53 (48-60)	0.036 (n.s.)	-2.100	mean±SD of LVM 52.0±7.7 g/m^2 (based on 30 female subjects)
Segmental wall thickness (SWT); -Basal septal site, measured on short-axis images, mm	9.5 (8.7-9.8)	8.6 (8.3-10.8)	n.s.	-1.893	mean±SD of SWT basal septal; 5.4±1.0 to 8.6±1.6 mm (in short-axis, based on 169 female subjects)
-Basal posterior site, measured on long-axis images, mm	8.0 (7.0-7.0)	8.0 (7.0-7.0)	n.s.	0.340	mean±SD of SWT basal posterior; 5.7±1.0 to 7.7±1.3 mm (in long-axis, based on 169 female subjects)

*Study-specific correction with Bonferroni for multiple comparisons. **One patient undergoing bTFE protocol (i.e. the main MRI part) could not undergo the additional LGE routine (at any phase), due to exceeded LGE limit for serum creatinine (GFR < 30 ml/min/1.73m^2)

In study III, a positive correlation, during recovery, was demonstrated between the increase in LVEF and the increase in mean e' velocity (septal-lateral sites), and these simultaneous changes were found to be linearly related, as shown in Fig. 19. Similarly, the change in mean s' tended to correlate with the change in mean e' (r=0.45, p=0.05). Further, a group wise analysis regarding the difference in the recovery of E/A ratio yielded a parallel response versus the degree of systolic impact at onset, which is depicted in Fig. 20.

The main results from the interactions and coherences as analyzed in study IV, between geometric assessments by TTE and by MRI, are summarized as follows:

* Comparison of the five methods for LVM estimation by TTE, for the nine patients with complete data acutely; yielded no intertechnique correlation, while the relatively best was seen for the TE-method (rs=0.63). All methods by TTE overestimated LVM (versus MRI), where M-mode was relatively worst (bias of 19 g/m^2). Bland-Altman analyzes yielded quite similar 95 % limits of agreement (LOA) for the other methods, where the TE-method had the lowest mean difference against MRI (1.0 g/m^2).

* Similar comparison as above, for the eight patients with complete data at recovery; demonstrated a positive correlation to MRI, for the TE-method (rs=0.71, p=0.048). LVM was overestimated by all TTE-methods, where the TE-method was the only one without a bias. The 95 % LOA by Bland-Altman yielded similar pattern as in the acute phase, the TE-method, however, was the only one without a significant mean difference compared to MRI.

The intertechnique agreement regarding LVM estimation, between TTE and MRI, by phase and specifically for the TE-method, is depicted in Fig. 21, while complete numeric accounting in this respect (i.e. between all five methods by TTE as compared to MRI), are presented in the top of Table 8 (section A).

* Further, comparisons of the three SWT sites and RWT data, at onset (nine patients) and at recovery phase (eight patients) were also made; correlations were found for all the septal SWT measurements, where the apical site had the relatively best intertechnique correlation [rs=0.79, p=0.011 (acute) and rs=0.85, p=0.007 (at recovery)], while no correlation (at any phase) was seen for the apical inferior site. The RWT data was also positively correlated, both acutely (rs=0.85, p=0.004) as well as at the recovery (rs=0.72, p=0.043).

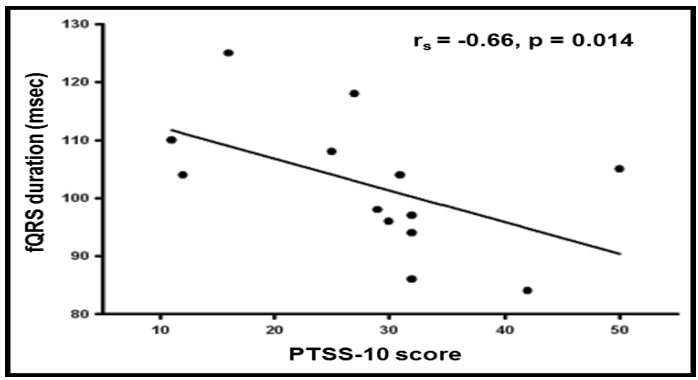

Figure 18. *Inverse correlation (Spearman's test) between individual inventories of Posttraumatic Stress Scale 10-Questionnaire (PTSS-10) scores, and filtered QRS (fQRS) duration obtained by signal-averaged ECG. For the 13 Swedish women, at the acute phase after being diagnosed with takotsubo cardiomyopathy, from the multi-based analysis conducted in study II (including test of associations between PTSS-10 scores and other variables of cardiac function). The complete analysis yielded no other significant correlation, apart from the one shown above.*

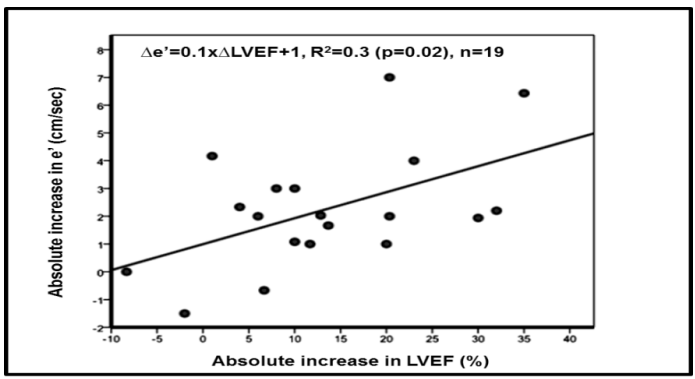

Figure 19. *Association between the increase in left ventricular (LV) ejection fraction (EF, absolute %) and in mean e' (i.e. early-diastolic tissue velocity, averaged from septal and lateral LV sites, cm/sec), from the interaction tests between systolic and diastolic variables as performed in study III (Pearson's coefficient and linear regression analysis). The changes displayed are from acute to the recovery phase (around three months apart); where "n" refer to the 19 patients included in the actual analysis (with respect to complete e' data at both occasions). As shown, the positive correlation (r=0.52) were found to be linearly related (the equation at the top of the chart). No further associations, between systolic and diastolic LV variables, were found to be of significance in study III.*

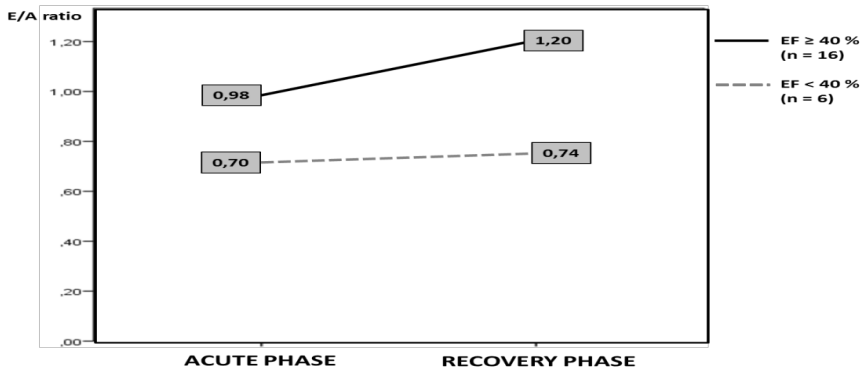

Figure 20. *A graphic display of the changes in E/A ratios from the acute to the recovery phase, in patients with reduced left ventricular ejection fraction (defined as EF < 40 %, dashed line) compared to the rest (EF ≥ 40 %, solid line), obtained in study III. The improvements between phases, in each group, are not significantly different (p=0.09, unpaired t-test), indicating that diastolic impact parallels systolic ditto at takotsubo cardiomyopathy. Digits within the boxes refer to mean values of E/A ratio (at each group and phase), while "n" refer to the number of patients with measurable E/A ratio in each group.*

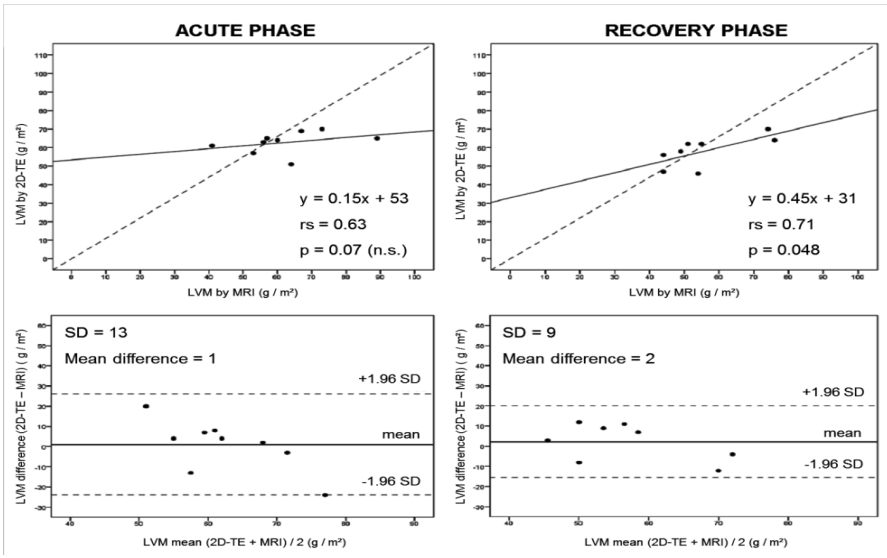

Figure 21. *Comparison of left ventricular mass (LVM, g/m²) obtained by a two-dimensional (2D) echocardiographic method [truncated ellipsoid (TE) formula], and with magnetic resonance imaging (MRI), at acute phase (nine patients, left images) and at the recovery phase (eight patients, right images). Depicted analyzes are Spearman's correlations (at the top) and the Bland-Altman plots (bottom) obtained from study IV. The same scale levels apply for each analysis.*

The main results from the interactions as analyzed in study IV, regarding simultaneous geometric measurements by TTE, are summarized as follows:

* No correlation was found, by comparing the acute increase of SWT in the apical level, with the acute increase in LVM (TE-method), although the septal site showed relatively better interaction than the inferior site (rs=0.47 versus rs=-0.44).

* During recovery, a negative correlation was found between the simultaneous improvements in GRS and WMSI, for the 12 patients with complete data in this respect (rs=-0.66, p=0.018) (depicted in Fig. 13, as already highlighted).

* Analyzes of improved GRS against the decrease in LVM (TE-method) and SWT (septal apical site), as well as against LVEF improvement; yielded no co-correlation. Repeated testing, after GRS was defined by short-axis level (i.e. as divided into six segments at papillary muscle and apical levels respectively); yielded a negative correlation between the GRS improvement (apical level) and the LVM reduction (TE-method), while no further interaction was found for any combination possible. The interactions between apical GRS versus LVM (TE-method) and SWT (apical septal site) are shown in Fig. 22, while numerical depiction, for all GRS correlations tested as above, are shown in Table 8 (section B).

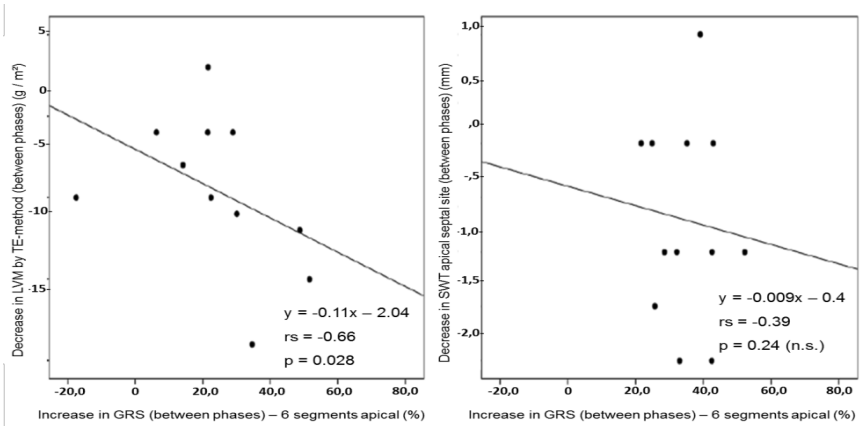

Figure 22. *Simultaneous changes obtained by echocardiography during recovery (study IV); global radial strain (GRS, %) apical level (six segments) versus left ventricular mass (LVM, g/m²) by the truncated ellipsoid (TE) method (left image, 11 patients with complete data at both phases), and GRS apical level versus segmental wall thickness (SWT, mm) at apical septal site (right image, 12 patients with complete data at both phases). Depicted analyzes are separate Spearman's correlations, where GRS is an overall measure (average of six segments) obtained from short-axis views at apical level.*

Table 8. *Complete accounting from study IV; for estimation of left ventricular mass (LVM), with phase divided comparison between five different methods by transthoracic echocardiography (TTE) against magnetic resonance imaging (MRI) (upper section, A), and for simultaneous geometric changes of the left ventricle (LV), obtained at TTE during recovery (lower section, B). Analyzes include correlations (section A and B), Bland-Altman results and pairwise test results for intertechnique bias (section A). Significances are flagged (highlighted in grey). In overall "n" refers to the number of patients included in each analysis, with regard to available data for the individual variables. Abbreviations are: M-mode = motion mode. 2D = two-dimensional. AL = area-length formula. TE = truncated ellipsoid formula. diff.(SD) = difference with standard deviation. n.s. = not significant. GRS = global radial strain. SWT = segmental wall thickness.*

(A) **LVM estimation; method comparison by TTE versus MRI**	Acute phase [n = 9]			Recovery phase [n = 8]		
	Spearman's correlation (P value)	Mean diff.(SD) Bland-Altman analysis, g/m²	Wilcoxon's test for bias, P value	Spearman's correlation (P value)	Mean diff.(SD) Bland-Altman analysis, g/m²	Wilcoxon's test for bias, P value
M-mode versus MRI	0.08 (0.83) (n.s.)	19.0 (19.0)	0.021**	0.21 (0.63) (n.s.)	18.0 (17.0)	0.036**
2D AL-method versus MRI	0.50 (0.170) (n.s.)	7.0 (13.0)	0.155 (n.s.)	0.683 (0.062) (n.s.)	9.0 (9.0)	0.049**
2D TE-method versus MRI	0.63 (0.07) (n.s.)	1.0 (13.0)	0.594 (n.s.)	0.71 (0.048)*	2.0 (9.0)	0.528 (n.s.)
2D biplane Simpson's versus MRI	0.57 (0.109) (n.s.)	6.0 (12.0)	0.097 (n.s.)	0.66 (0.073) (n.s.)	12.0 (9.0)	0.025**
2D triplane Simpson's versus MRI	0.54 (0.130) (n.s.)	4.0 (12.0)	0.374 (n.s.)	0.64 (0.091) (n.s.)	11.0 (10.0)	0.025**

(B) **Simultaneous TTE changes in LV geometry, during the recovery**	Spearman's correlation (P value)		Regression line slope (interception)	
	Decrease in LVM (g/m²) by 2D-TE [n]	Decrease in SWT (mm) at apical septal site [n]	Decrease in LVM (g/m²) by 2D-TE [n]	Decrease in SWT (mm) at apical septal site [n]
Increase in GRS (%) - 12 segments; mid-ventricular and apical levels, versus:	-0.33 (0.29) (n.s.) [n = 12]	-0.011 (0.97) (n.s.) [n = 12]	-0.08 (-2.68) [n = 12]	-0.004 (-0.63) [n = 12]
Increase in GRS (%) - 6 segments; mid-ventricular level, versus:	-0.20 (0.54) (n.s.) [n = 12]	0.21 (0.50) (n.s.) [n = 12]	-0.05 (-3.64) [n = 12]	0.002 (-0.73) [n = 12]
Increase in GRS (%) - 6 segments; apical level, versus:	-0.66 (0.028)* [n = 11]	-0.39 (0.24) (n.s.) [n = 12]	-0.11 (-2.04) [n = 11]	-0.009 (-0.4) [n = 12]

*Significant correlation (p<0.05). **Significant difference (i.e. bias) between methods (p<0.05)

DISCUSSION

Methodological considerations

Reproducibility at TTE

In study III (regarding the tests of variability by population, Table 3), all obtained CV numbers were generally low (< 10 %) except for the interobserver control of E/A ratio; slightly higher values were seen acute for the Swedish part (11.5 %), as well as at the recovery phase for the U.S. part (10.7 %). In overall, for the entire intra/-interobserver control, good correlations (r≥0.90) were demonstrated for the repeated measurements of E/A ratio and mean e'.

As shown in Table 4, all repeated measurements of continuous variables, referring to the geometric LV assessment as performed in study IV, yielded good correlations (rs≥0.74). Acceptable values were obtained for the CV calculations (in overall < 10 %), while the majority of the data ended up around or even below 5 %. Cohen's Kappa test, regarding the interrater reliability for WMA assessment, yielded acceptable agreement, both at onset (K=0.71) as well as at follow-up (K=1.00). Complete reproducibility, i.e. intra/-interobservative tests for all variables in Table 4, was not possible to achieve because of practical reasons (mainly limited research space). Note, in some cases the interobservative tests (for LVM by M-mode and RWT) were made between M-mode versus 2D measurements. However, a combination of M-mode and 2D (in this respect) is not considered as forbidden (24); as long as one is aware that the normal values are based on M-mode. Besides, in study IV, the main aim was to investigate the relative differences, between phases, rather than as compared to normal.

Coherence between TTE and MRI

In study IV, the demonstrated effect on LVM; an acute increase simultaneously obtained with both TTE and MRI, is in line with previous findings (42). Admittedly, some studies have shown the benefit with 3D-derived TTE-methods in this respect (24), and thus, the use of 3D should perhaps have been considered as well. However, such equipment was not an available option at the time of enrollment, and besides (according to own knowledge and experience), most of the available 3D techniques are still based on 2D, while 3D is not yet widely used in the clinical TTE routine. Further, 3D-derived TTE imaging has generally higher demands on image quality and can sometimes be consistence with challenging acquirement, e.g. in terms of sequential technology (24). Hence, lack of 3D-derived LVM estimation, as with the TTE-methods used in study IV, still seems

to be an accessible and sensible option at the clinical management of TTC. The average time used, for offline analysis, also supports the usefulness of 2D in this respect, as an acceptable choice versus MRI.

Assessment of emotional and cardiac stress

Although the information collected by the emotional stress scores, in study II, did not show any overwhelming signs of acute stress, the actual scores in themselves might have had a bearing, in terms of interpretation difficulties etc. (including lack of proper instructions to the patients), it might have been more useful if the patients had been more thoroughly examined for acute stress disorder, such as by interviews or at least a semi-structured qualitative approach, which might have changed the outcome of the results. However, in study II, such approach was not an available option; due to limited resources in the qualitative field (i.e. psychologist access), as well as lack of knowledge among the other investigators involved.

Further, regarding study II and the biochemical screening as performed; only one blood sample of catecholamines was taken at each sampling occasion (in contrast to the recommendation of two samples), while the routine use of intravenous diazepam at acute catheterization (USÖ) might have yielded a reduction in catecholamine excretion (64).

Regarding the HRV analyzes in study II, it is known that the results are highly influenced in terms of equipment and analysis procedure, while a general consideration is the lack of overall references. In study II, however, the same technical setup was used throughout the whole study period, while analyzes and interpretations were done by a few experienced investigators. Admittedly, the normal material used was self-created, but the material is well-established (at least in Sweden) and has been used in other studies before this thesis; the material was based on the same equipment as used at USÖ at that time, and should thus be considered as an appropriate choice according to the recommendations (31). Further, regarding the SAECGs/-HRVs in study II, the presence of a pacemaker in two subjects, as well as frequent extra systoles during the acute recording by HRV in one patient, may have influenced these analyzes. Thus, retrospective re-calculations were performed in this regard. Acute versus recovery data were tested (Wilcoxon) for HRV data (without all three subjects as above), while SAECGs were re-analyzed without one of the pacemaker patients (who had pacemaker rhythm at the recovery). Further, acute correlations were tested (Spearman's) between all HRV variables (not applicable for SAECG) against scores of PTSS-10 and MADRS-S, where two of the three subjects as above (as applicable in this regard) were excluded in the re-analysis. The results from these additional analyses can be summarized as follows: fQRS duration still tended to decrease acutely (uncorrected

p=0.02), while the SDNN/-SDANN data also remained acutely lowered (p=0.002), in addition the isolated HRV variable of TINN (mainly sympathetic variable) also turned out as shortened (p=0.004). The acute correlations yielded two additional interactions [between RMSSD/-SDSD and PTSS-10 (rs=-0.66, p=0.027)], where RMSSD/-SDSD are considered as parasympathetic indicators (both these variables where still unchanged, between phases, and overall within normal limits even after the re-analysis). Otherwise, there were no differences observed as compared to the initial analysis. Taken together, the re-analyzes did not change the interpretation regarding the original results, while the overall conclusions in study II remains, despite the highlighted presence of pacemakers and extra systoles.

In study IV, the use of RS may be a consideration. Normal values are quite spread, while there is some disagreement regarding the data interpretation (11, 17). Irrespectively, the most important interpretative factor is consistency (11), and thus, the same analysis procedure was applied for all RS assessments in study IV (as described in the "Methods"). Gender, age and choice of equipment are other important factors in this regard (11, 17). However, all USÖ patients were elderly women, while the same equipment (from one vendor) was used both regarding image acquisition and offline analysis. Thus, RS should be considered as an applicable tool in this respect.

Statistical choices and multiple comparisons

Overall in this thesis (study I-IV), many statistical tests have been performed, which is known to be consistent with the risk of mass significance (i.e. statistical type two error). In this respect, however, correction for multiple comparisons was applied (at least retrospectively). Thus, the main findings in study I-II should be considered as appropriate, despite that no correction was done initially nor by the fact that some variables only showed a tendency (after retrospective correction), according to the revealed numbers for CI 95 % as well as Z-scores for these main variables (all within appropriate limits). Besides, regarding the lack of initial correction in study I, this was corrected by actually perform study III, since several variable more or less were the same in these two sub-studies, while Bonferroni was taken in consideration from the start regarding study III.

In study II, the lack of initial correction could partly be nullified by the retrospectively performed correction, since changes in fQRS duration still turned out as significant even after correction, while the overall findings in study II, despite only tended significance in many cases, could be supported by similar findings in other reports. Further, in this respect, the multidisciplinary approach in study II

allowed to compensate for ambiguous findings, by interdisciplinary comparisons, which thus, also made the initial lack of corrections less importantly.

In study IV, statistical analyzes were made continuously over time, which is why no final correction was made for multiple comparisons, in the original paper. However, the resulting Z-scores of the main findings in study IV were all acceptable in this regard (depicted in Table 2 in the original paper IV). Besides, an additional analysis as carried out retrospectively (by paired Student's t-tests) regarding changes between study phases for the main variables of LVM (by TE-method and MRI) as well as for GRS (averages of both 12 segments and the six apical segments); which all yielded acceptable CI 95 % limits. Thus, the above reflections suggesting that at least these main findings are not coincidental, and should be considered as appropriate despite the lack of initial corrections.

Limitations of the studies

The use of a retrospective, analytical design can be considered as limiting; with potential risk of missing certain properties due to the lack of a common study protocol, as was the case in study III, where several centres and different ultrasound devices may also be seen as limiting elements. However, conventional Doppler techniques as used are known to have good reproducibility (35), which was also revealed by the results from the variability tests as performed (Table 3). Thus, at least the main variables of study III should be considered reasonable in this respect. In study IV, no reproducibility control was performed regarding MRI data, which may have had a bearing; MRI was considered as a reference method. Lack of MRI-savvy radiologist was the main reason for this. Nevertheless, test of variability regarding TTE (Table 4) was considered as more important in this perspective, due to relatively lower image quality.

Follow-up examinations were not performed repeatedly (i.e. on more than one occasion), thus, the complete recovery may therefore have been missed for some variables, including the main findings in the respective sub-study. However, the overall findings in this thesis (study I-IV) indicate that the majority of the patients, at least were not acutely affected at the time of follow-up. All patients were treated similarly, no patient had recurrence and no further cardiovascular event (in addition to the TTC diagnosis) were reported during recovery. Most cases of TTC are usually reported as recovered, within the stipulated time frame of around three months (10), as applied regarding follow-ups in the thesis. Besides, a recent MRI study reported a difference at TTC, regarding the recovery in systolic and diastolic variables by comparing short- versus long-term follow-ups, where complete recovery was considered to be applicable only at the latter (which was around three months) (65). Taken together, the timing of follow-up in this thesis should

be considered appropriate, while the use of several follow-ups had not certainly added any value, at least not within the period of three months.

Although the bulk of literature on TTC is confined to case reports, the studies in this thesis are limited by its population size, and apart from study III, by the fact that data come from a single center (solely based on the 13 USÖ patients). Still, regarding the USÖ part, the numbers of patients that were included were within the expected range; in line with the statistical power analysis, as initially carried out, as well as according to the predetermined time set for the screening plan, in contrast to the number of expected AMI cases. Regarding the U.S. part (study III), no such detailed power analysis was carried out, while the relatively small sample size (15 patients) could probably be secondary due to substandard inclusion (e.g. incomplete or incorrect diagnostic coding).

Other aspects could be that no controls were used, in any sub-study, as well as the fact that only a subset of patients (USÖ part, study II and IV) underwent MRI and had plasma catecholamine samples. The former issue was mainly due to the aims of each sub-study, main focus (study I-IV) was mainly investigations regarding relative differences (between phases), rather than as compared against normal, while such design does not necessarily require controls. Further, complete data on MRI and stress hormones, totally seen, are unlikely to have changed the outcome in none of the applicable sub-studies (i.e. II and IV). With respect to the sizes of the populations in which this thesis are based on, the results must be carefully considered in this regard. Nevertheless, the combined investigation approach, not least for the USÖ part and applicable for all sub-studies (I-IV), is still one of the most comprehensive and detailed of a TTC cohort.

Theoretical implications and influences

Biventricular systolic long-axis function

In study I, the biventricular systolic long-axis shortening, in terms of amplitudes of MAM and TAM, as well as the LV systolic velocity of s' (by PW-DTI, average of four mitral annulus sites) improved from the acute to the recovery phase.

Our findings of lower amplitudes of MAM in the acute phase indicate that the longitudinal fibers are affected in TTC. These fibers are mostly located subendocardially and this part of the myocardium has the highest risk of ischemia (66). One might therefore surmise that atherosclerotic coronary disease-mediated myocardial ischemia could be a cause to the decrease in systolic long-axis shortening. That theory, however, is not supported by, for instance, MRI findings; earlier reports have rejected evidence of focal perfusion abnormalities corresponding to a specific coronary vessel territory (67), which is also supported by the findings at

LGE in study II. Despite the isolated finding regarding stress hormones in study II (not elevated acutely), the catecholamine hypothesis could not be rejected, and thus, could also explain the findings regarding long-axis function in study I, based on the known impact on myocardial viability and overload in this regard (9).

The non-significant difference of MAM at the septal site could be explained by fewer longitudinal fibers at septum than at the other anatomical locations; septum is formed by subendocardial fibers from both ventricles with a middle layer composed of circumferential fibers in continuity with those from the corresponding layer of the LV free wall, while circumferential septal fibers are lacking towards the apex (66). When using PW-DTI the systolic velocities of s' are measured at the basal parts of the LV. The increase in the systolic long-axis shortening (i.e. MAM) may therefore also explain the increase in this variable, in study I. However, the same velocities, as measured by 2D color DTI, remained unchanged despite that these measurements were done at the same locations. Further, velocities by 2D color DTI were lower than the velocities obtained by PW-DTI; the former technique is based on autocorrelation (resulting in peak mean velocities), while PW-DTI is computed with an algorithm transformation technique as resulting in measuring peak velocities, which might explained this difference (68). The placement and size of the sample tools, as well as other DTI related settings (e.g. insonation angle) may also cause the differences, although the same general procedure was applied (for the separate techniques) in each examination.

In study III, the same trend (i.e. increase in s' during recovery) was seen regarding the PW-DTI measurements, also when the average was based on two sites (i.e. only septal and lateral), and by adding more patients (where s', solely for the USÖ part, reached a significant change even after Bonferroni correction, Table 2). This implies, regardless of any technical influences, that the obtained improvement of s', in study I, probably was not a coincidence.

Further in study I, as for MAM, there was also a difference concerning TAM, which implies a biventricular impact at TTC. The mechanisms explaining the involvement of RV in TTC are sparse documented and the involvement, among other things, argues against LV outflow tract obstruction, which can be seen in some cases (4). The findings in study I, however, suggest that the RV impact in TTC, as for the LV, involves longitudinal fibers. Probably most in the apical regions, with respect to the fact that none of the USÖ patients had significant RV dysfunction in the acute phase (defined as TAM < 16 mm, measured at the basal part, Table 2), while the same trend was applicable also for the U.S. population in study III (Table 1).

The measurements of s' velocities, by both DTI techniques (i.e. PW-DTI and 2D color DTI) were unchanged during recovery, with overall greater numbers for

PW-DTI (similar as for the LV), the latter issue is probably due to different technical principles (as for the LV), while the overall lack of sensitivity, in the DTI measurements, could be secondary to measurement difficulties (e.g. regarding the insonation angulation, which has higher impact on Doppler techniques as compared to M-mode) as well as due to hyper dynamics in the basal region; DTI velocities reflect the contractility, compared to TAM, which is more of a measurement of the total contraction of the RV free wall.

Markers of emotional and cardiac stress

The main findings of study II were that in addition to clinical and laboratory indices of heart failure, cardiac autonomic function is altered at TTC, while a relation between emotional stress and QRS duration could be demonstrated.

The emotional stress scoring (mainly regarding PTSS-10) revealed two patients with definite posttraumatic stress, and borderline in another seven (at onset). Posttraumatic stress is not uncommon in patients following AMI, which has been reported previously (69). Among other things, psychological stress was reported to be secondary to chest pain and acute heart failure; this should also apply to TTC, and may very likely explain the results from the emotional assessment in study II. One patient had a history of mental illness; manio-depressive psychosis, as diagnosed several years before the onset of TTC. Besides, her manio-depressive disease was considered stable at admittance, and should thus not have had any major influence on the overall results [at both phases, she was scored as "borderline" (PTSS-10 scale) as well as "no depression" (MADRS-S scale)].

Biochemical markers of myocardial damage were slightly to moderately elevated, in the acute phase (study II), and this corresponds with previous findings in patients with TTC (4, 7). As already mentioned, and in contrast with the expected from previous, we did not find any elevation in plasma catecholamines in study II. However, both the sample procedure, as well as the use of diazepam during catheterization, may have influenced in this regard (as mentioned).

Although the overall values, as derived from the SAECGs (study II), were within the limits of normal (30), an acute reduction of the fQRS duration could be demonstrated. This variable is under the influence of autonomic tone (30), thus, the use of beta-blockers (regarding the treatment during recovery) might have had an impact on fQRS, although this is less likely (70). Besides, as applicable both for the SAECGs as well as the HRVs, there was no obvious bias in terms of different levels of beta-blockers between study phases; the beta-blockers were introduced at admission and all the ECGs were made over the next two days, while no modification in terms of dosing occurred during the recovery. The variable of fQRS has been associated with major disturbances in terms of ventricular electro-

physiology, such as dyssynchrony (71), and is reduced in healthy subjects due to mental stress (72). The finding of reduced fQRS duration at onset, despite reduced LVEF and presumable susceptibility to prolonged duration and ventricular arrhythmias, as previously observed at TTC (73), supports that emotional stress had more impact on this variable than LV dysfunction.

The HRV results, i.e. acute reductions in the variables of SDNN and SDANN (study II), is in line with previous findings (74). The "global" variable of SDANN reflects both a sympathetic and parasympathetic heart rate modulation, while a depressed SDANN is thought to indicate a relative sympathetic dominance (30). In studies of heart failure patients SDNN and SDANN have been found to correlate inversely with plasma noradrenaline (75), while acute pain had no effect on SDNN in a study of healthy males (76). In a study of 138 patients in the early phase of AMI Doulalas et al. found similar SDNN values as in study II [mean 86±35 (SDNN) and 74±29 (SDANN)] (77). Emotional stress has been seen as a major contributor to causing TTC and emotional stress also affects HRV. In one study a mental stress task significantly reduced SDNN index (i.e. SDNNi according to Table 6, which is considered as relatively more sympathetic compared to SDNN) in patients undergoing AMI (78), while an insignificant trend towards reduced SDNNi was seen at onset. In other words, both emotional stress as well as LV dysfunction can cause the kind of HRV effect that was demonstrated in study II.

LVEF improved during recovery (similarly by TTE and MRI), in line with the bulk of literature regarding TTC. The values of LVEF as reported in the acute TTE examinations [53±10 % (USÖ part) and 44±16 % (total population in study III)] may appear high compared to previous reports (4, 7); leading one to surmise that the recovery had already began. However, other studies have reported similar LVEF at the onset of TTC (79). There are case reports of normalized LVEF within days (80), but it is unlikely that this is applicable in this thesis (study I-IV) since all acute examinations were performed within 24 hours after onset. By own knowledge, no patient with TTC has been reported to recover in LVEF within this time. Probably, the acute preservation is likely due to the compensating, hyperdynamic wall motion in the basal parts, as often described in TTC (4).

Diastolic heart function

In study I, no change was observed in any of the conventional diastolic variables, from acute phase to the recovery, except for the LV diastolic velocities of e' and a' as measured by PW-DTI (average of four mitral annulus sites), both increasing during the recovery. However, in study I, the same velocities (obtained at the same sites), were generally lower and could not be proven as acutely impaired,

when measured by 2D color DTI. This trend was similar as for the s' measurements, and just as in the case of s', the obtained differences regarding diastolic tissue velocities could most probably be explained by different algorithms (i.e. technical principles for PW-DTI respectively 2D color DTI), while measurement deviations cannot be totally excluded (despite the use of a common setup regarding Doppler-settings).

In study III, velocities of e' (by PW-DTI) were re-calculated as the average of two sites (septal and lateral); recommended as necessarily enough in the clinical practice (35). This yielded the same result regarding mean e', for the total population in study III (i.e. a demonstrated acute decrease), while the separate e' data for the USÖ part, turned out as non-significant after the re-calculation (regardless of any correction) (Table 2). This implies that the results of the DF measurement, in study I, may also have been secondary due to the relatively lower population size (as compared to study III). Despite a larger population and in contrast to e', most conventional DF variables could not be proven as acutely affected in study III either (after Bonferroni correction), neither in terms of differences (between phases) or seen as absolute numbers at the onset of TTC; with respect to the fact that indicators of diastolic relaxation impairment can be considered as age-appropriate findings over the age of 60 (35). In this regard, measurement difficulties cannot be excluded as a possible explanation, or the fact that an even bigger sample might have changed the outcome of the results in study III.

It is known that several diastolic indicators are influenced by heart rate and RR-interval. However, no sustained arrhythmia was observed, at any examination, and the difference in average heart rate during the TTEs (between phases) did not reach significance for any of the study populations (Table 2 page 53). Another important aspect, especially regarding DF, is the follow-up duration [average 89 days (range 1-188), for the total population in study III]. However, as already mentioned (in "Limitations"), the applied timing of follow-up cannot be considered as a major bias, thus, applicable also regarding the DF variables as measured in study I and III.

In study III, several findings implied an existing interaction between systolic and diastolic impact at TTC, that is, the typical transient LV dysfunction seems to involve both these properties in the same degree of severity; not least as depicted by the correlations and comparisons between LVEF and the conventional variables of E/A ratio and mean e' (Fig. 14 and Fig. 20 respectively). On one side this is not surprisingly, as this is in accordance with other causes of LV deterioration, but on the other side this pattern must also be taken in consideration in terms of bias; a previous report has shown that both loading conditions, as well as LV systolic function, may have influence on the e' velocity (81). Although the systolic

improvement cannot be excluded in this regard, it is less likely that differences in terms of relaxation and loading conditions have had a major cause on the results in study III; time- and frequency-related influences as derived to heart rhythm were overall similar and within normal limits at both phases (as stated), while the same was applicable regarding other known loading influences (such. as PASP and presence of a significant valve disease), as clearly shown in Table 1.

As seen in Table 1, earlier history of hypertension and diabetes were present in a total of 14 patients (overall population, study III), where both these risk factors may influence DF. However, the frequencies in these properties did not differ significantly between the sub-populations, while the overall frequencies (regarding study III) are comparable with other studies in TTC (19), as well as with the expected prevalence among adults as globally seen (82). Thus, the presence of diabetes and history of hypertension should not be considered as a selection bias in this regard.

Effect on LVM and geometry

In study IV, a decrease in LVM was shown during recovery, as measured both by MRI and TTE. The MRI finding is in line with previous (42), confirming a probable trend in TTC. Despite similar trend with TTE, reductions of significance were reached only for the formula based AL- and TE-methods, where relatively better coherence (against MRI) was shown for the TE-method. M-mode has previously been shown to overestimate LVM compared to MRI (83); the poor coherence in study IV is thus not surprising. Overall, the Simpson's methods had less coherence and reproducibility compared to the formula-methods, likely due to measurement difficulties. The formula applied for the TE-method takes into account that the wall thickness of the LV differs slightly along its length, compared to the AL-method, in which the formula assumes the same thickness throughout the LV (24). Thus, better adapted formula could explain the relatively higher intertechnique coherence, for the TE-method. As depicted clearly in Fig. 7, the typical apical ballooning seems most distinct in systole, while the LVM measuring occurs in diastole, which is probably one reason why the TE-formula is applicable at TTC; contrary to the recommendation not to use this method in the cases of LV deformations, due to multiple calculation steps (where small measurement errors are consistent with high risk of over/-underestimations) (24).

An acute, transient increase in LVM has previously been observed in patients with AMI (84). TTC should distinguish in this respect, in terms of preserved LVM during recovery, since there should not be any loss of muscle mass. However, this theory seems nullified in practice, presumably due to inflammation and edema, consistent with myocardial stress (84). Potential reasons could also be

prolonged acute hospitalization, and increased caution with reduced physical activity during recovery, e.g. because of anxiety as previously documented in patients with AMI (69). The overall hospitalization for the USÖ patients [median 4.0 days (range 2.0-4.0)], however, cannot be considered as long-term stay in this context. While the reported results from the stress scores (PTSS-10 and MADRS-S), as obtained in study II, at least can dismiss dominance against emotional disturbances at the time of follow-up.

In previous MRIs, patients with TTC have been found with pericardial effusion and/or markers of myocardial edema at onset (42). Acutely, none of the USÖ patients had signs of pericardial effusion (Table 1), while no myocardial edema could be demonstrated (due to no such MRI protocol was used). Taken together, however, the effect on LVM in study IV is most likely a result of transient inflammation and myocardial edema, rather than because of myocardial atrophy. Supportive, in this respect, is the profound differences in various SWT levels, along with elevated markers of myocardial damage at onset (Table 5). Besides, not all TTC cases are consistent with visible liquid formations (20).

The LVM results may have been a direct reflection of the acute increase in LV size (39), mainly depicted as the TTE measurement of LV end-diastolic volume (EDV), which was higher at onset (Table 2). The simultaneous acute increase in LVEDV and in LVM (by the TE-method), however, did not show an interaction by additional correlation analysis; neither by TTE nor when using the LVEDV measurements as obtained by MRI in study II [rs=-0.06, p=0.85 (TTE) and rs=0.63, p=0.09 (MRI)]. In the previous study by Stensaeth et al. (42), LVEDV did not change during recovery, despite an acute increase in LVM (even greater than in study IV). Further, in study I, the end-diastolic measurements of biventricular size (both length and width) remained unchanged between the two study phases. Thus, a change in LV size is probably not the main reason for the acute increase in LVM as associated with TTC.

Further, in study IV, a transient impairment of GRS was found, with acceptable correlation against the WMSI scoring. There was also a correlation between the acute impairment in apical GRS and the acute increase in LVM by the TE-method (Fig. 22). The same comparison did not, however, show any interaction by supplementary analyzes performed for each study phase separately, which is consistent with a previous study (85). This supports that the demonstrated pattern in these two parameters, from onset to recovery, is due to TTC rather than because of a specific measure of LVM. Besides apical GRS, no similar interactions were seen regarding the other GRS data (i.e. the overall average of 12 segments and separately for papillary muscle level). However, this is not surprising based on previous findings, but only confirms the general description of TTC, as being an

essentially apical disease (11, 19), which may also explain the invariable findings regarding basal wall thickness.

The apical SWT sites narrowed during recovery, both by MRI and TTE. Intertechnique correlation, however, was reached only for the septal site; probably because septum was easier to distinguish (not least regarding TTE). Differences in measuring conditions (between MRI and TTE) may also have influenced, although the septal site seems to be the most sensitive indicator, in this regard. Despite this, no interaction was found between apical SWT and TE-derived LVM, while the same trend was noted between apical measurements of septal SWT and GRS (Fig. 22). This is probably due to the typical widespread effect, beyond single coronary territories, implying that no specific segment seems relatively more sensitive at TTC. Supportive in this, is also the supplementary Kruskal-Wallis analysis that was carried out in the original study IV (see "Figure 6" in paper IV for complete accounting).

Loading conditions, such as degree of filling etc., can influence on LV geometry (39), and thus, may have had a bearing on the results in study IV. At onset, however, the majority of continuous TTE data were within expected normal limits (Table 2, USÖ part). As seen, LVEF is one exception, with importance in terms of potential influence, although the overall findings in study IV suggests that LVEF is an independent factor in this respect, which probably is secondary to the demonstrated hyper dynamics in the basal parts of the LV. No obvious interaction was found between the study specific data of GRS and LVM, by comparisons against biventricular size and functional measurements, while the same trend seemed to apply both in the presence of obesity and hypertension. Taken together, with respect to the full accounting of potential influences as shown in Table 1 (USÖ part), external loading conditions should not have had too much impact on the LVM and GRS results in study IV.

Clinical implications

Biventricular systolic long-axis function

The improvement in LV systolic long-axis shortening (MAM, total of four annulus sites), as demonstrated in study I, is in line with the improvement of LVEF obtained by the biplane Simpson's method, and these simultaneous changes did also show a tendency to interact, although only moderately [r=0.55, p=0.05], but still implying that measurement of MAM (tentatively as derived from four sites) is a good clinical option regarding systolic LV function assessment, in terms of poor image quality, in the cases of suspected TTC. The same assumption should be applicable also regarding measurements of s', primarily as obtained by PW-DTI,

with respect to the combined results from study I and III; an acute decrease in s' was demonstrated, in each of the study-specific populations and regardless of the number of mitral annulus sites included in the calculation. And in this case, averaging of two s' sites (septal and lateral) seems clinically good enough, in line with the recommended (35).

The measurement of TAM, in study I, yielded values within normal at the acute phase (for the USÖ population), while the same was documented in study III (i.e. applicable also for the U.S. population). This might imply that TAM is a less sensitive variable in suspected TTC cases. However, as an acute decrease actually was demonstrated in study I, the RV systolic function is most probably affected (at least to some extent). Supportive in this, is one of the few previous studies with a focus on the RV involvement at TTC (86); acute RV impact was demonstrated and described to follow a similar WMA pattern as does the LV in this disease, and further, the RV involvement (when applicable) was consistent with a longer and more critical hospitalization course (as compared with patients with isolated LV involvement).

Taken together, the use of TAM, in terms of being a highly available clinical tool, should be considered as appropriate in the cases of suspected TTC. And further, the lack of sensitivity in DTI-derived s' measurements of the RV, as taken together with the findings on the LV, in overall speaks in favor of the use of M-mode measurements of MAM and TAM, in this regard.

Markers of emotional and cardiac stress

In study II, the SAECG variable of fQRS was the only physiological marker with a demonstrated interaction against emotional findings (that is, the acute scores of PTSS-10). However, late potentials as assessed by SAECG, is primarily seen as a tool for detection of structural abnormalities within the cardiac tissue, rather than as a measurement of acute autonomic disturbances (30). Besides, the clinical interpretation of the complete SAECG analyzes (i.e. when all three variables are taken into account), were not significantly different between the two phases (Fig. 17), while the isolated finding regarding fQRS duration (within normal limits at both phases) cannot rule out, for example, an AMI (30). Further, none of the HRV variables correlated against acute PTSS-10 scores, while the scores itself, nor supports the fact that psychological stress has a say in causing TTC (i.e. in terms of a typical pathological pattern unlike other heart disease). Taken together, these different findings make it difficult to draw a firm conclusion on the acute fQRS and SDNN/-SDANN reductions in study II; they might be a product of temporary heart failure or caused by emotional stress or even a blend of the two.

In contrast to the expected, acute levels of stress hormones were not elevated in study II, thus surmising a lack of clinical use, while making the catecholamine hypothesis look less likely at the same time. However, the small sub-set with complete data, as well as the methodological issues (i.e. the use of diazepam etc.), probably had a bearing in this regard. Thus, levels of stress hormones might as well have been increased in the acute phase; in line with the majority of literature. Supportingly, such indications could still be found (in study II) but in terms of other methods as used in addition to the biochemical screening, not least regarding the HRV results (75). Further, typical look as in the onset of TTC (i.e. apical ballooning) has been recognized both in patients with pheochromocytoma (87), as well as in cases of subarachnoid hemorrhage (88).

Thus, the findings in study II cannot allow rejecting the catecholamine hypothesis, while the overall conclusion had probably been quite similar (regardless of the outcome from the catecholamine samples). Patients undergoing an episode of TTC show signs of alterations in the autonomic cardiac tone, along with markers of acute heart failure as well as a tendency to posttraumatic stress. However, these phenomena seem to occur independently of each other, while no isolated property is unique for TTC; perhaps expressions like "stress-induced" should be avoided, in favor of such as "transient LV dysfunction with normal coronary findings"? (Which is also in line with the aforementioned criteria as proposed for TTC (11); emotional aspects are stated as "a common additional finding", however, should not be considered as a definitive criterion.) In the USÖ cohort, barely half of the subjects were recognized with some kind of trauma as preceding the onset (Table 1). Taken together, stress-induced influences cannot be totally excluded, in terms of differential diagnostic patterns; the overall indicia in study II, however, suggested another direction in the pursuit of a common denominator.

Diastolic heart function

Despite a tended acute decrease in e' velocity in study I (PW-DTI, total of four annulus sites), no similar trend was demonstrated when averaging was done based on two sites (study III). The latter procedure, i.e. solely using septal and lateral sites, is in line with the way that e' is suggested as sufficiently, when it comes to the use in the clinical routine (35). The discrepancy in this respect, between study I and III, leads one to surmise a lack of sensitivity for e' (in terms of being an appropriate variable in the clinic). Due to the increase in LVEF and in MAM, a shift in the other conventional DF variables could be expected as well. Although greater differences in LVEF, in terms of lower values acutely as well as higher at the recovery (compared to the USÖ data) have previously been demonstrated in TTC, and despite this, no changes in diastolic variables were demonstrated (89).

Further, all the diastolic variables of the RV remained unchanged during recovery, while previous accounts are sparse in this respect; taken together, with the aforementioned findings in study I regarding long-axis function, TTC seemed as a primarily systolic disease, at this point.

In study III, a relatively higher degree of acute diastolic impact was demonstrated, by adding more subjects into the analysis, although most DF variables could still be considered as being less sensitive and consistent with lack of feasibility. Pulmonary vein velocities as an example, are known as proper indicators of DF (in the absence of arrhythmias), and these velocities could not be measured sufficiently to provide any added value; a greater feasibility might have yielded another outcome in study III. As mentioned, alterations in other DF variables could probably have been found with an even larger sample, as also supported by the aforementioned, more recent TTC compilation (on 227 subjects); slightly greater alterations in conventional DF variables were documented, including some that seemed unaffected in study III (37). The demonstrated findings in study III, with respect to other reports and thus including the previous results in study I, still implies that the cooperative approach (in study III) did pay off in this regard.

Although 2D strain seems to be a useful tool at TTC, in line with study IV (regarding the GRS analyzes) and as previously reported (90), this technique still has some limitations in terms of generalizability (mainly due to manufacture-specific algorithms), which can be consistent with difficulties, e.g. in terms of clinical interpretation and comparability. The latter issue is less desirable in collaborative studies, not least when the involved centres do not have the possibility to use similar equipment (as in study III). The aforementioned study by Meimoun et al. (90), among other things, demonstrated a correlation between LV untwisting rate and mean e', where the former variable is considered as a global index of DF (assessed by 2D strain). Taken together, conventional techniques and variables of DF, such as e', still plays a role in terms of generalizability. Not least in TTC, many times requiring cooperative studies between centres (not certainly using the same equipment) in order to obtain statistically adequate population size; with respect to its elusive nature and relative rarity. Strain techniques, by own experience, are still not routine in the general clinic at TTE, and yet TTC is relatively unknown among sonographers (from a broad perspective). There is a potential risk to miss the diagnosis at onset, and since recovery is relatively rapid, it is of clinical benefit that the natural course of TTC, including DF, can be recognized by conventional techniques as frequently used in the clinic (as focused on in study I and III). Supportive in this regard is also the above mentioned compilation by Citro et al. (37), where several conventional DF parameters (e.g. E/A ratio and

mean e'), could be identified as acute markers of increased risk at TTC; regarding major adverse events at short-term follow-up.

It is important to recall, however, that the population size affects the degree of significance. But since all DF variables as obtained in study III, except from e', did not interact with systolic function measurements, a higher number of individual alterations, in this respect, had not certainly given added value to the context of study III. To sum up, mean e' (septal-lateral sites by PW-DTI) was the only individual DF variable with a demonstrated impact acutely, as well as the only diastolic indicator proven to interact with the systolic recovery; the clinical usefulness should thus be considered as appropriate in the cases of suspected TTC. Despite that the loading conditions of the LV were quite similar at both study phases, while a shift to better DF stages was demonstrated, changes in filling degree including a more distinct recovery in LV systolic function may have a direct bearing on the DF impact. Conclusions about DF, in patients with suspected TTC, should thus be carefully considered based on the results from study III.

Effect on LVM and geometry

Diagnostic evaluating of LV geometry is of general importance within cardiac care (39, 40), thus including in TTC, which seems both relevant and possible by TTE based on the results from study IV. The acute increase in LVM is relatively small (Table 2), but significance was still found while the LVH geometries remained unchanged during recovery, underlining the importance of an actual estimation in this respect. Relying solely on the LVM impact is not enough, however, since this is not unique for TTC (84). SWT as measured in apical septum seems as an isolated variable of importance, the overall findings in study IV, however, indicates that SWT measurements are less useful in terms of geometric patterns for diagnosis purposes. In this respect, RS seems more reasonable and in particular regarding apical assessment, as demonstrated by the interaction against LVM. Although RS is considered as the relatively least robust 2D-derived strain technique (27), its clinical benefit has previously been declared in TTC, as mentioned (90), with similar feasibility numbers as in study IV. Supportingly, in this respect, is also the demonstrated interaction regarding GRS and WMSI scoring, that is, the effect on LV concentric wall motion is objectively consistent with the subjective impression. Although LVEF is emphasized at TTC (19, 20), the overall results from study IV, including indicates that RS seems more sensitive to the geometrical impact of the LV, and may thus contribute in terms of additional diagnostic value.

Some cases of TTC have been demonstrated with LGE uptake at MRI (91), implying that it is beneficial if TTC might be recognized irrespective of MRI, or at

least that there should be alternatives in cases where MRI is not a possible option. Besides, it is unlikely that an AMI, affecting the whole apex, would be consistent with an overall concentric recovery at three months follow-up (at least not in terms of a transmural impact). Taken together, a transient impact on LV geometry, as assessed by repetitive measurements of both LVM and RS, preferably by the methods described in study IV, may help in the differentiation of patients with suspected TTC.

CONCLUSIONS

- Besides an increase in LVEF (by Simpson's method) our demonstrated recovery, in biventricular systolic long-axis function, indicates that also the longitudinal fibers are affected at TTC; preferably assessed by measuring MAM and TAM. Despite acute impairment in LV diastolic tissue velocities (by PW-DTI), most of the conventional diastolic LV variables, together with the diastolic indices of the RV as well as the biventricular length, remained unaffected. Thus, TTC would primarily be considered as a state of systolic deterioration, with effect on both the ventricles.

- In addition to clinical and laboratory indices of heart failure, patients with TTC have altered cardiac autonomic function, and prevalence of borderline or definite posttraumatic stress syndrome. But this is in line with findings in myocardial infarction, and does not allow conclusions in terms of a differential diagnostic pattern at TTC. Perhaps terms like "stress-induced" or "broken heart syndrome" would be toned down in this respect, in favour of such as "a transient LV dysfunctional state".

- TTC associates with a transient diastolic LV dysfunction, similarly as and also with tendency to occur in parallel with the systolic impact. This can be assessed by the use of conventional Doppler techniques, including measurement of individual variables of diastolic heart function, e.g. E/A ratio and preferably mean of septal and lateral e' (derived by PW-DTI).

- At onset, TTC is associated with increased LV mass, which primarily seems to be an apical effect with tendency to parallel the alterations in concentric wall movement. In TTC, conventional TTE assessment of LV geometry shows adequate consistency against MRI, primarily for the 2D-derived method by truncated ellipsoid formula regarding LV mass.

Taken together, this thesis has shown that TTC mainly associates with transient impact on the LV, in terms of geometry and function, where the latter includes both systolic and diastolic indices. This applies irrespectively of emotional impact, and can preferably be recognized by conventional TTE measurements which are often used in clinical practice, and as it seems even without the requirement of MRI as a diagnostic tool, by additional strain analysis of concentric wall motion.

Future aspects

No common diagnostic pattern could be outlined in study II. However, the combined use of both emotional as well as physiological stress markers, still yielded some indications towards an emotional denominator, and thus, this approach seems clinically relevant and should inspire further studies with similar design regarding TTC. Thus, a potential thought would be to repeat a broad approach as in study II, but with better focus on emotional aspects, by using a more thoroughly qualitative method (e.g. interviews and subsequent thematic analysis), provided that such data still can be linked with physiological aspects, preferably including estrogen-based elements (with well-known protective effect in this regard, as mentioned). The results could help to better understand the onset, and thus be of importance regarding prevention of this disease. A thoughtful and interdisciplinary design, however, is a prerequisite in this regard (as was the case in study II).

Typically in TTC, a transient dysfunction of the LV is seen. The RV function, however, is equally important. In terms of mortality, a failing RV is consistent with relatively worse prognosis (92). A clinical dilemma at TTE, regarding the RV assessment, is its relatively complex geometry and anatomical location (in contrast to the LV), thus, there is a need to further study the diagnostic utility for variables as reflecting the RV condition. By 2D strain, more sensitive regions of the heart tissue can be depicted (as mentioned), where technical solutions allow for assessment even at more difficult conditions regarding the insonation; 2D strain has previously been recognized with good utility for segmental imaging of the RV free wall (93). The amplitude of TAM remains as an important indicator of the RV function. Admittedly, the overall data of TAM, in this thesis, were within the normal (implying non-significant impact, globally seen), nevertheless, an acute reduction in TAM was observed (for the USÖ part); likely due to a local effect, according to the previous reflection regarding the results of study I. (Supportingly, is also a 4-chamber image as obtained by MRI, shown in Fig. 2 (image E); the apical ballooning, typically seen at onset, definitely seems to involve the RV.)

Thus, a future reality (in terms of TTE) is to further investigate the natural course of the RV at TTC, focusing on segmental function, by 2D strain. Based on previous data regarding the global RV impairment at onset (4, 9), and the expected loss of measurable wall segments (93), an appropriate population should correspond to about 25 subjects. The idea is to perform a retrospective analysis of the collected image sets used in this thesis (for the USÖ patients), as compiled with additional data by external collaboration (to achieve the desired sample size); all necessary preparations for this project have already been completed, which most likely will be the undersigned's continuation in the field of TTE/-TTC...

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