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TAKOTSUBO STRESS CARDIOMYOPATHY AND DIFFERENT TYPES OF STRESS

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ABSTRACT

Myocardial infarction with normal coronary angiography (MINCA) is an important subgroup of myocardial infarction for which the prevalence, underlying pathophysiology, prognosis and optimal management are still largely unknown. Interest in and awareness of MINCA has increased in recent years due to the frequent use of coronary angiography, the description of Takotsubo stress cardiomyopathy (TSC) and new sensitive troponin assays. The purpose of this thesis was to investigate the prevalence and clinical characteristics of MINCA using cardiovascular magnetic resonance (CMR) and particularly to investigate the effect of stress on TSC using Doppler tissue imaging (DTI), heart rate variability (HRV), salivary cortisol (SC) and non-invasive coronary flow reserve (CFR).

In **Study I,** 176 patients with MINCA were screened at five coronary care units in the Stockholm Metropolitan Area. 152 of them were investigated using CMR which showed 67% of the patients as being normal, 19% having signs of myocardial necrosis and 7% the diagnosis was myocarditis in of the patients. The remaining patients were either diagnosed with hypertrophic cardiomyopathy or were undecided. Twenty-two percent of all MINCA with a normal CMR fulfilled the Mayo Clinic criteria for TSC.

In **Study II,** we tested the hypothesis that compared to sex- and age-matched controls TSC patients have an increased vulnerability to stress even after the acute event. Using dobutamine stress echocardiography (DSE) and DTI we investigated the TSC patients approximately 20 months (619 ± 297 days) after the acute event. At rest left ventricular myocardial performance index (LV-MPI) was significantly higher for TSC patients (p=0.01). During stress, however, there were no significant differences between the groups.

Study III was in many ways similar to Study II except that mental stress was used instead of dobutamine stress. The study was performed 28 months after the acute event. In addition to DTI, HRV and SC were also studied. During mental stress there were no significant differences between TSC patients and sex- and age-matched controls for DTI, HRV and SC. There was a trend towards less increase in SC after stress in TSC patients compared to controls. A self-estimated acute stress scale (Likert-type scale from 0–6) was 2.8 and 2.6 during mental stress for TSC patients and controls, respectively. During the acute event TSC patients retrospectively estimated their acute stress level at 4.4. In **Study IV,** dobutamine stress was used to investigate the effect of stress on non-invasive CFR. At low-dose dobutamine, CFR was significantly lower in TSC patients compared to controls (p=0.017). There were no differences in CFR at high-dose dobutamine between the groups.

Conclusion: MINCA is more common than previously thought and is associated with a normal CMR. TSC constitutes a substantial part of MINCA. Studies II and III point to a slow recovery for TSC patients measured by DTI but no sign of vulnerability was revealed by dobutamine or mental stress measured by DTI, HRV or SC. We could not confirm that the catecholamine dobutamine induced microvascular dysfunction in TSC patients. However, we found a small but significant difference in CFR at low-dose dobutamine, which implies that the role of microvascular function in TSC needs to be further explored.

Key words: Takotsubo stress cardiomyopathy, cardiovascular magnetic resonance, dobutamine stress echocardiography, mental stress, coronary flow reserve

LIST OF PUBLICATIONS

I. Collste O*, Sörensson P*, Frick M, Agewall S, Daniel M, Henareh L, Ekenbäck C, Eurenius L, Guiron C, Jernberg T, Hofman-Bang C, Malmqvist K, Nagy E, Arheden H, Tornvall P. Myocardial infarction with normal coronary arteries is common and associated with normal findings on Cardiovascular Magnetic Resonance - results from the Stockholm Myocardial Infarction with Normal Coronaries study. Journal of Internal Medicine 2013 Feb; 273(2): 189-96. *shared first author.

II. Collste O, Alam M, Sundqvist M, Olson J, Wardell J, Tornvall P, Frick M. Vulnerability to sympathetic stress does not persist in Takotsubo stress cardiomyopathy. Journal of Cardiac Failure 2014 Dec;20(12):968-72.

III. Collste O, Tornvall P, Sundin Ö, Alam M, Frick M. No myocardial vulnerability to mental stress in Takotsubo stress cardiomyopathy. PLoS One 2014 Apr 2(9);4: e93697.

IV. Collste O, Tornvall P, Alam M, Frick M. Coronary flow reserve during dobutamine stress for Takotsubo stress cardiomyopathy. Manuscript.

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LIST OF ABBREVIATIONS

|	
MINCA	Myocardial infarction with normal coronary angiography
TSC	Takotsubo stress cardiomyopathy
CFR	Coronary flow reserve
CMR	Cardiovascular magnetic resonance
ACS	Acute coronary syndrome
LVOT	Left ventricular outflow tract
LGE	Late gadolinium enhancement
DTPA	Diethylenetriaminepentaacetic acid
CAD	Coronary artery disease
NT-proBNP	N-terminal prohormone brain natriuretic peptide
MSNA	Muscle sympathetic nerve activity
ARI	Anger recall interview
MA	Mental arithmetics
DSM	Diagnostic and Statistical Manual for Mental Disorders
ACTH	Adrenocorticotropine hormone
MPI	Myocardial performance index
LV	Left ventricle
RV	Right ventricle
HRV	Heart rate variability
ECG	Electrocardiography
SDNN	Standard deviation of normal to normal intervals
ACE	Angiotensin converting enzyme
CRF	Corticotropine releasing factor
CT	Computed tomography
SSFP	Steady-state free precession
TE	Echo time
TR	Repetition time
EF	Ejection fraction
E/A	Early and atrium velocity ratio
E/E´	Early flow velocity and early tissue Doppler velocity ratio
DT	Deceleration time
DTI	Doppler tissue imaging
IVCT	Isovolumetric contraction time
IVRT	Isovolumetric relaxation time
ET	Ejection time
DSE	Dobutamine stress echocardiography
LAD	Left anterior descending
GLS	Global longitudinal strain
SF36-PCS	Short-form health survey with physical composite score

INTRODUCTION

In the last two decades the amount of acute or sub-acute coronary angiographies performed has increased dramatically [1]. Almost all large or medium-size hospitals in Sweden today have the ability to perform coronary angiography in an acute or sub-acute manner. One factor driving the increased use of acute or sub-acute coronary angiography is the existence of ever more sensitive cardiac enzymes. Recently high-sensitive troponins have come into clinical use [2]. Troponins have the ability to record even the most minutely affected myocardium although there are still only limited amounts of data with regards to high-sensitive troponins [3]. Accordingly, the amount of patients in whom the cause of chest-pain or elevated troponins is unclear has increased. The increasing number of patients without a proper diagnosis puts clinicians in a diagnostic dilemma. What are the reasons for elevated troponins in the patients when the underlying cause cannot be found on coronary angiography? What else can be done to increase the number of patients with a proper diagnosis?

MYOCARDIAL INFARCTION WITH NORMAL CORONARY ANGIOGRAPHY

During the first decades of the 20th century there were observations of acute myocardial infarction without obstructive coronary disease. In those days the diagnosis was made during necropsy [4-6]. Since the introduction of coronary angiography in the 1960s clinicians have observed an increasing number of cases of myocardial infarction with normal coronary angiography (MINCA) [7] [8]. In the 1970s several cardiac enzymes began to be measured and an even more exact diagnosis was possible. Already by the early 1970s an estimate was done that of all cases of acute myocardial infarction MINCA constituted around 4% [9]. The origins of these cases were thought to be mainly spontaneous reperfusion of a previously occluded artery but also misinterpretation of the coronary angiography, secondary myocardial infarction due to large myocardial mass or low haemoglobin or myocardial infarction due to coronary artery spasm [10].

During the late 1970s and early 1980s focus shifted towards coronary artery spasm as a common cause of MINCA [11]. However, coronary artery spasm could not be verified as a common cause in another study [12]. In the mid-1980s more cases of myocarditis were presented as possible causes of MINCA [13]. Towards the end of the 1980s several case reports of stunned myocardium with concomitant MINCA appeared [14]. In summary, a multitude of causes to MINCA has been postulated through the years although mostly through case reports of only one or a few patients (see Figure 1). There have been few researchers that have tried to incorporate all possible causes of MINCA into a single larger study. However, for most of this past century it has probably been hindered by technology rather than by will.

Figure 1. Possible causes of myocardial infarction with normal coronary angiography.
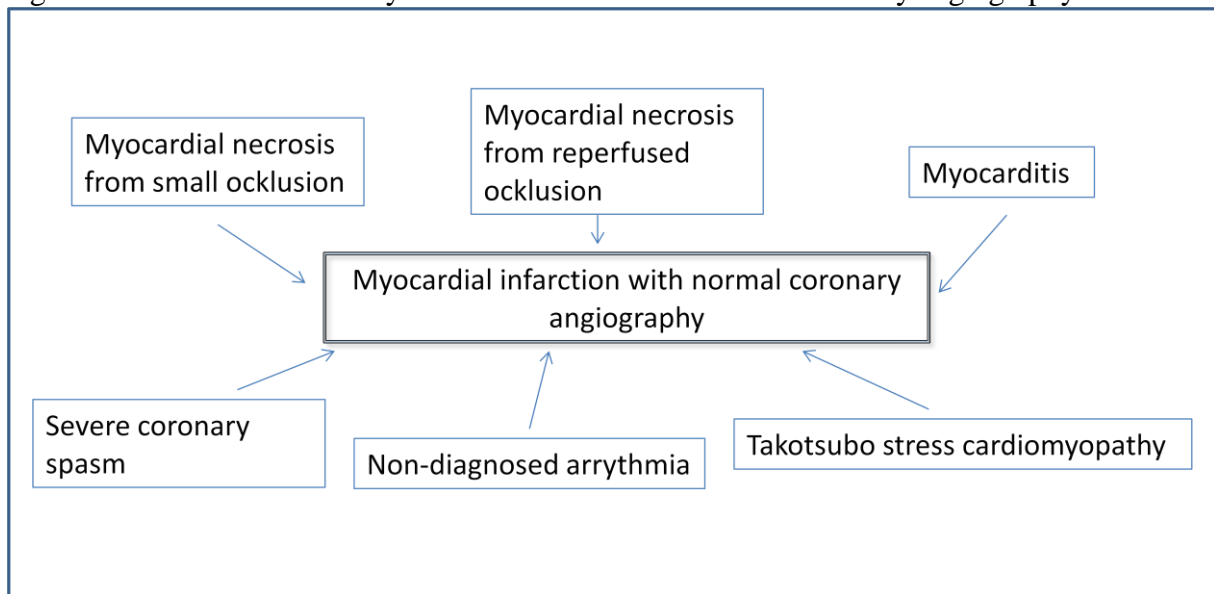
TAKOTSUBO STRESS CARDIOMYOPATHY

Takotsubo stress cardiomyopathy (TSC) is a disease where acute and high levels of psychological or physiological stress cause reversible hypokinesia of parts of the left ventricle. TSC is also known as apical ballooning syndrome, stress-induced cardiomyopathy, broken-heart syndrome, ampulla cardiomyopathy and various other names. As early as the mid-1970s two possible cases of TSC were described when Greenberg et al. and Ciraulo reported on two separate cases of MINCA with reversible mid- and apical left ventricular hypokinesia, respectively [15][16]. In 1987 Shaw et al. reported a case involving transient shock and extensive myocardial akinesia caused by a pheochromocytoma crisis [17]. In 1990 Sato et al. described and named Takotsubo stress cardiomyopathy (TSC) after a Japanese fishing pot used for trapping octopuses [18][19]. Sato et al. described multivessel spasm occurring either spontaneously or after administration of intracoronary ergonovine. In the same study they found that the spasm was resolved after intracoronary administration of nitroglycerin.

Figure 2. Possibly one of the first reported cases of Takotsubo stress cardiomyopathy in 1975[16].
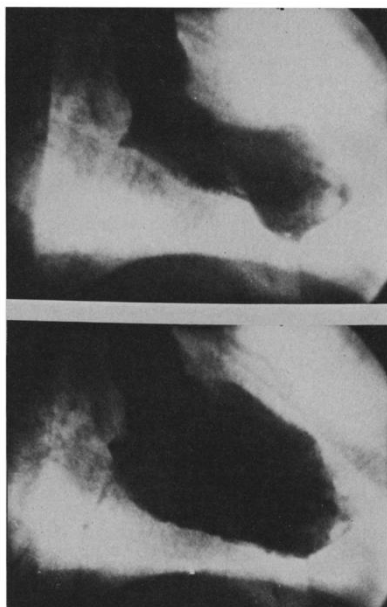
During the 1990s a number of case reports of TSC were described, mainly by Japanese authors [20-22]. TSC subsequently became associated with other diseases that is, development of TSC after debut of subarachnoid hemorrhage and more examples of TSC during a pheochromocytoma crisis [23-25]. This more firmly associated TSC with high levels of catecholamines as seen during a pheochromocytoma crisis. In a larger study Tsuchihashi et al. retrospectively described 88 cases of TSC and their clinical features, types of triggering stress and laboratory analysis findings [26]. In other series of TSC patients Bybee et

al. studied the clinical manifestations of TSC in an American population, while Desmet et al. described what they thought were the first cases of TSC in a white population [27][28]. Altogether these series of case reports led Bybee et al. to propose the Mayo Clinic criteria for TSC (see Table 1) [29]. The Mayo Clinic criteria, however, have recently been questioned mainly because of more evidence for non-typical forms of TSC [30].

Table 1. Mayo Criteria for the clinical diagnosis of the transient left ventricular apical ballooning syndrome [29].

1. Transient akinesis or dyskinesis of the left ventricular apical and mid-ventricular segments with regional wall-motion abnormalities extending beyond a single epicardial vascular distribution

2. Absence of obstructive coronary disease or angiographic evidence of acute plaque rupture

3. New electrocardiographic abnormalities (either ST-segment elevation or T-wave inversion)

4. Absence of
    Recent significant head trauma
    Intracranial bleeding
    Pheochromocytoma
    Obstructive epicardial coronary artery disease
    Myocarditis
    Hypertrophic cardiomyopathy

* All 4 criteria must be met.

After the renewed interest in TSC started in the early 2000s, Owa et al. studied four TSC patients, assessing myocardial perfusion using ^{201}Tallium, fatty acid metabolism using ^{123}I-15-(p-iodophenyl)-3-R,S-methyl-pentadecanoic acid (^{123}I-BMIPP) and sympathetic nerve function using ^{123}I-metaiodobenzyl-guanidine (^{123}I-MIBG). They found impairment of all types of scintigraphy in the acute phase [31]. Akashi et al. also studied sympathetic nerve function using ^{123}I-MIBG in 8 patients with TSC and found an impaired function [32]. The report on a case in which both scintigraphy and coronary flow reserve (CFR) had been performed showed severely reduced myocardial perfusion and reduced CFR in the acute phase and only mild improvement after an additional assessment of perfusion at 15 days, indicating both abnormal perfusion and microcirculation in the acute phase [33]. In a study by Ito et al. seven patients were examined using both echocardiography and scintigraphy. Wall motion score, myocardial perfusion, fatty acid metabolism and sympathetic nerve function were studied. The examinations showed impaired wall motion score, reduced myocardial perfusion (^{99m}Tc-Tetrofosmin), impaired fatty acid metabolism and impaired sympathetic nerve function [34]. In conclusion, scintigraphic studies to date have shown impaired perfusion, fatty acid metabolism and nerve function in the acute phase, with slow recovery over the subsequent months.

Up to the mid-2000s only the most typical shapes of TSC had been described. From then on a variety of TSC shapes were described, including reverse TSC with basal hypokinesia [35] and right ventricular hypokinesia [36]. A larger study involving 107 North American TSC patients found the classical shape in 54%, mid-ventricular hypokinesia in 29% and basal hypokinesia in 1% [37]. Other variants including regional hypokinetic patterns constituted the rest. It became evident that not only classically shaped Takotsubo-like hypokinetic patterns appear in this disease but that rather a multitude of shapes are possible. There is some evidence to support the idea that sympathetic nerve distribution contributes to the individual shape of the hypokinesia

seen [38]. We should keep in mind, though, that no cardiovascular magnetic resonance (CMR) examination was performed in the above-mentioned studies and CMR can thus result in modification of the initial diagnosis, especially in the case of regional hypokinetic patterns, to myocarditis or myocardial necrosis (see section on CMR, below). In addition, assessment of ventricular function in TSC is possibly also a matter of timing [39]. Regional remission of hypokinesia or other diseases, such as myocarditis, could be the simple answer to some of the regional hypokinetic patterns that have been described in the literature.

The classical triggering factor of TSC is an emotional upsetting event. In a large study on 136 TSC patients by Sharkey et al. 47% had an emotional trigger while a physiological stressor could be identified in 42% [40]. In an even larger meta-analysis Pilgrim et al. identified 28 case series comprising a total of 563 patients. They identified an emotional trigger in 44% and a physiological stressor in 36% [41]. Physiological stressors were most often an exacerbation of medical disease. In fact, even myocardial infarctions caused by an acute coronary syndrome (ACS) or spontaneous coronary dissection have been shown to trigger TSC [42,43].

Some researchers have suggested that TSC and left ventricular outflow tract (LVOT) obstruction appear in 20–25% of TSC cases [44,45]. However, right ventricular TSC cannot be explained by LVOT obstruction and neither can basal or mid-ventricular variants [36]. It could be that LVOT obstruction is just an effect of TSC in some patients, not an underlying cause, with the hyperdynamic basal myocardium producing a more pronounced LVOT obstruction than would otherwise exist.

The typical TSC patient is a postmenopausal woman. There is some evidence that point to gender differences in sympathetic nerve regulation and others to gender differences in endothelial function [46,47], while in an animal model, oestrogen has attenuated TSC [48]. However, no complete explanation of this difference in gender incidence has this far been postulated. When it comes to other baseline characteristics of patients with TSC, these have been most thoroughly described by Pilgrim et al. In this large meta-analysis, 90% of TSC patients were women of a mean age range from 62 to 76 years, hypertension was present in 49% and smoking was present in 21% [41]. In the same meta-analysis troponin elevation was also presented. In thirteen case series troponin levels were measured and found to be elevated in 85% of TSC cases. In other words, negative troponin does not exclude the occurrence of TSC.

Endomyocardial biopsies have been performed in subsets of a few case series [22,49-52]. Typical histological findings were contraction band necrosis, but also interstitial fibrosis, mild cell infiltration and focal myocardial depletion. No histological evidence of myocarditis was found and viral antibody titres were negative.

In recent years some researchers have linked TSC to capture myopathy, which for several decades has been known to exist in the animal kingdom [53,54]. However, what most researchers describe when examining the dead animals is rhabdomyolys, rather than just cardiomyopathy. Extensive rhabdomyolysis with or without cardiomyopathy is different from the clinical picture of TSC, in which no skeletal rhabdomyolysis and only light or moderate elevation of cardiac troponins are seen. Accordingly, the connection or association between capture myopathy and TSC has yet to be scientifically proven.

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*Pathophysiology of Takotsubo stress cardiomyopathy*

Recently there have been attempts to more accurately describe the pathophysiological pathways of TSC [55-60]. However, in the majority of cases these studies have been performed in animals and not in humans. Studies on mouse or rat models have so far shown that TSC is a result of the direct effects of high levels of epinephrine on the ventricular myocardium and that at high levels epinephrine is negatively inotropic with a switch in β2-adrenoceptor coupling in ventricular cardiomyocytes, from the Gs protein signalling pathway to the Gi protein signaling pathway. Animal studies have also shown that the density of β-adrenoceptors is greatest at the apical myocardium of the mammalian heart, which explains the regional nature of the stunning in response to high levels of circulating epinephrine after stressful stimuli. Furthermore, there is some evidence that cardiac lipotoxicity is associated with TSC.

CARDIOVASCULAR MAGNETIC RESONANCE

Magnetic resonance imaging is a technique that uses strong magnetic fields and radio frequency signals to form images of the body. By both releasing and detecting radio frequency signals while using oscillating magnetic fields at specific resonance frequencies, computers can translate data to form images of the investigated region of the body. Gradients in the magnetic field control the orientation of the image while contrast between different tissues is determined by the rate at which excited atoms return to their equilibrium state [61]. Early work on magnetic resonance imaging began in the 1950s when Herman Carr produced the first one-dimensional image [62]. In the 1970s Lauterburn and Mansfield developed the technique further using magnetic gradients and faster mathematical techniques to produce the first two-dimensional images [63] [64]. Work on CMR was already being performed in the 1980s but initial attempts to visualize the heart were distorted by respiratory and cardiac motion. These problems were at least partially resolved by the introduction of electrocardiographic gating [65]. The advent of this technique, combined with breath-holding techniques and faster scanning techniques, made it possible for the field expand to include cine imaging and techniques to characterize the heart muscle [66-68].

In the field of MINCA, CMR has the potential to play a significant role. It is known that CMR can readily identify both myocardial necrosis caused by an occlusive coronary event and myocarditis, using gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) contrast [69] [70]. Late gadolinium enhancement (LGE) is a finding according to which gadolinium contrast is enhanced in the myocardium (5-20 minutes after injection) and delineates necrotic tissue[71]. The transmural extent of LGE has also been linked to myocardial viability; therefore, the technique is increasingly used in the investigation of coronary artery disease (CAD) [72]. See Figure 3 for examples of LGE in CMR.

Figure 3. Myocardial necrosis (left) and myocarditis (right) on CMR.
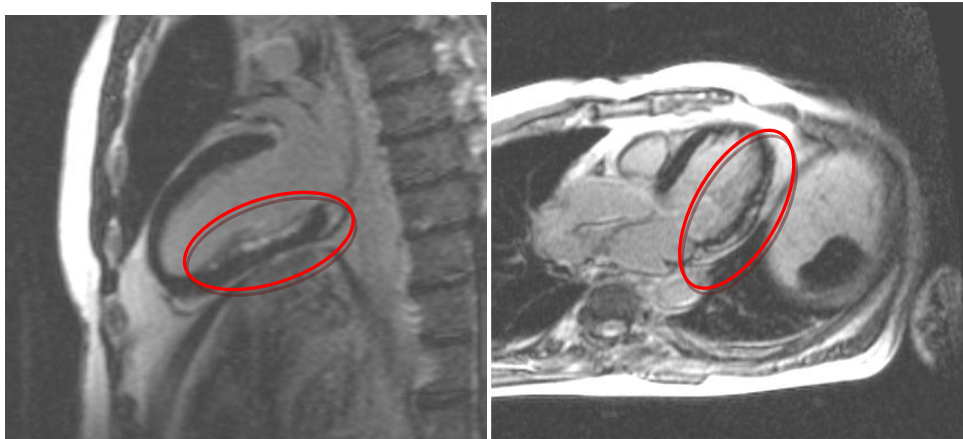
In 2007, for the first time, Assomull et al. studied the role of CMR in MINCA [73]. By using CMR they found an identifiable basis for troponin elevation in 65% of the patients. Myocarditis was found in 50% of patients, myocardial infarction (myocardial necrosis) in 12% and cardiomyopathy in 3%. Following Assomull et al., Laraudogoitia Zaldumbide et al., Gerbaud et al., Chopard et al. and Leurent et al. also investigated MINCA with CMR [74-77]. However, these studies included MINCA patients in different ways. For example, Assomull et al. prospectively included patients referred for CMR from 18 hospitals while Laraudogoitia Zaldumbide et al. excluded patients with signs of anterior myocardial infarction. No data are available on how many of the included patients underwent chest computed tomography examination. Thus, several questions remain that these studies have not yet answered. How common is MINCA in an ACS clientele? What is the true incidence of TSC? Are the clinical characteristics found in previous studies representative for an emergency department setting?

There are also studies involving CMR that have focused entirely on TSC. In 2009 Abdel-Aty et al. were the first to demonstrate a left ventricular T2-weighted oedema on CMR matching the extent of hypokinesia [78]. One year later Eitel et al. investigated 37 patients with suspected TSC and found LGE consistent with myocardial necrosis in 19% and with myocarditis in 11% [79]. Seventy percent had no signs of LGE; instead, they had an elevated T2-weighted oedema ratio and global relative enhancement consistent with TSC. The first study, which included both controls and a follow-up CMR after three months, was performed by Neil et al. [80]. The regional extent of myocardial oedema correlated inversely with myocardial strain (except at the apex). There was also a direct correlation of the extent of myocardial oedema with peak levels of N-terminal prohormone of B-type natriuretic peptide (NT-proBNP). Iacucci et al. studied 17 patients with TSC (without controls) in the acute phase and after four months [81]. T2-weighted images showed oedema matching the extent of regional dysfunction. No cases of LGE were observed. In five patients who underwent endomyocardial biopsy, histology confirmed the massive interstitial oedema associated with typical contraction-band necrosis. In conclusion, oedema visualized by CMR is a characteristic feature of TSC, a circumstance that can be used to obtain a more accurate diagnose of TSC in the clinical setting.

STRESS TESTS

Dobutamine stress echocardiography

Catecholamines have a strong connection to the development of TSC [41]. Hence, a direct way to induce stress is by infusion of a catecholamine. The most commonly used catecholamine for stress tests is dobutamine ((RS)-4-(2-{[4-(4-hydroxyphenyl) butan-2-yl]amino}ethyl)benzene-1,2-diol). Its primary mechanism is direct stimulation of β_1-receptors, but also weak β_2-receptor stimulation and selective α_1 activity. It increases heart rate, myocardial contractility and cardiac output, and thus increases myocardial oxygen demand. Dobutamine was developed in the 1970s as a structural analogue of isoprenaline [82].

The standard dobutamine stress protocol consists of continuous intravenous infusion of dobutamine in 3-minute increments, starting with 5 µg/kg/min and increasing to 10, 20, 30 and 40 µg/kg/min. If >85% of the calculated maximum heart rate is not reached, an additional dose of 0.25 mg Atropine (up to a maximum of 1 mg Atropine) is added to the 40 µg/kg/min dobutamine infusion [83].

Mental stress test

Since TSC has a strong connection to mental stress, and not a lot is known about the mechanism behind this connection, there is reason to devote further study to the connection between TSC and mental stress [41]. In 1935 Stroop studied the effect of the interference of verbal reactions, a work that was subsequently developed into the Stroop colour word test [84,85]. This was based on findings published in 1929 in Germany [86]. The Stroop colour word test has mainly been used to measure selective attention, cognitive flexibility and processing speed but has also been used to evoke overall mental stress with measurable effects on arterial blood pressure, muscle sympathetic nerve activity (MSNA), heart rate and perceived stress. In 1992 Callister et al. used both the Stroop colour word test and mental arithmetics (MA) to study the effect of stress [87]. Both types of stress evoked an increase in arterial blood pressure, MSNA, heart rate and perceived stress, despite heart rate increases of only 15% and 11%, respectively, in the two tests. Mental stress has been studied using MA and been known to increase arterial blood pressure, cardiac output and heart rate [88]. The anger recall interview (ARI) was used to measure cardiovascular reactivity in women in a study by Anderson et al [89]. Mental stress has also been studied in patients with CAD in whom an ARI and MA induced a blunted salivary cortisol response compared to controls, despite a heart rate increase of only around 7% during stress [90].

SELF-ESTIMATED ACUTE STRESS

In stress research self-estimation of stress has previously been applied to long-term stress or to medium to long-term effects of acute stress estimated in a post-stress environment. In 1983 a perceived stress scale was introduced in an effort to measure long-term stress [91]. Since the mid-1990s Acute Stress Disorder (ASD) has been present in the Diagnostic and Statistical Manual for Mental Disorders (4th ed.; DSM–IV) [92]. The accompanying interview (ASDI) is a 19-item, dichotomously scored interview schedule that is based on criteria from the DSM (4th edition) [93]. This diagnosis was introduced to describe posttraumatic stress reactions that occur between two days and four weeks following a trauma. Towards the end of the 1990s Kudielka et al. measured heart rate, adrenocorticotropin (ACTH), free salivary cortisol, total plasma

cortisol and heart rates and found significant responses to a stress test [94], and a visual analogue scale confirmed that the same amount of stress was experienced by young and elderly subjects. In a study by Lesage et al. the visual analogue scale was studied and found to be at least as discriminating as a questionnaire when it came to highlighting differences in stress levels between two groups [95]. The visual analogue scale is a continuous scale, which has been shown to be the preferred scale type for estimating subjective psychometric response (e.g., while the patient is experiencing the pain) [96]. However, for collecting and analysing perceived stress in a post-stress environment, a Likert or discrete type of scale can be preferred [97]. If one assumes equal distances between values in a Likert-type scale, it can be interpreted or approximated as an interval scale even though in reality it is ordinal. With an interval scale one can calculate mean values and standard deviations [98].

For Studies II-III of this thesis a Lickert-type 7-grade discrete scale was used. On this scale 0 means no self-perceived acute stress while 6 means maximum self-perceived acute stress, and the distances between each value are assumed to be equal.

MYOCARDIAL PERFORMANCE INDEX

Myocardial performance index (MPI), derived from different time-phases of the cardiac cycle, gives an estimate of the overall systolic and diastolic ventricular function [99]. MPI can be derived from both Doppler flow and tissue Doppler measurements, the former being the original version used by Tei et al. [100-102]. Tissue Doppler derived MPI has been shown to correlate better with left ventricular ejection fraction and functional capacity compared to pulsed-wave Doppler [103] (figure 4). MPI has been shown to be a sensitive marker for ventricular function [99] and in recent years has also been shown to be associated with heart failure as well as to be a predictor of cardiovascular events in patients with known left ventricular dysfunction [104-106].

There are previous case reports involving TSC in which not only the left ventricle but also the right ventricle has been affected. There is one case report in which an isolated right ventricular involvement is described. Furthermore, in a recent study right ventricular MPI (RV-MPI) was shown to be helpful in differentiating obstructive coronary disease from TSC. For this reason it would be of interest to study not only left ventricular MPI (LV-MPI) but also RV-MPI [36][107-109].

Figure 4. Measuring myocardial performance index
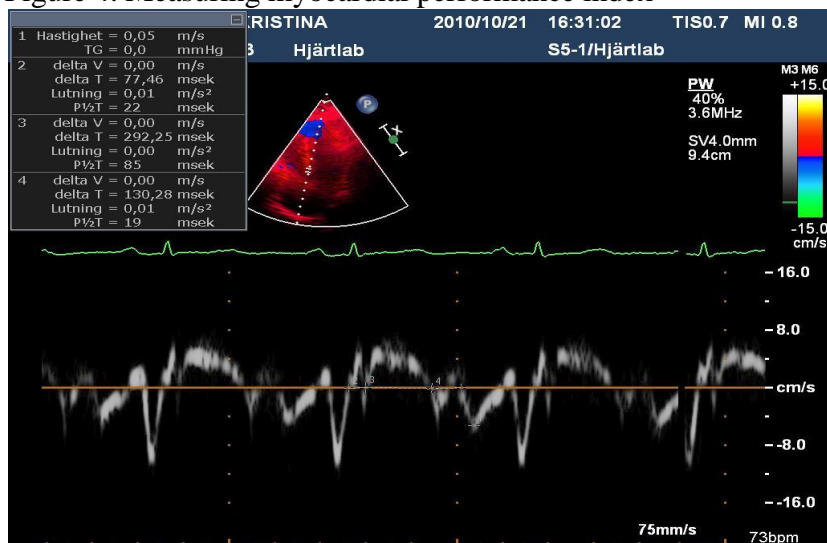
HEART RATE VARIABILITY

Heart rate variability (HRV) is the physiological phenomenon of variation in the time interval between heart beats (figure 5). In 1733 Rev. Stephen Hales became the first person to describe the beat-to-beat variation during respiration [110]. In 1895 Willem Einthoven, using galvanometers, became the first person to continuously record the electrical activity of the heart [111]. In the early 1960s Norman Holter developed a small portable recording device for collecting ambulatory ECGs [112]. This invention sparked further interest in understanding the relationship between beat-to-beat variation of heart intervals and heart disease. With the introduction of modern signal processing techniques it became possible to analyse more subtle variations in beat-to-beat intervals [113].

There are primarily two approaches to analysing HRV: time-domain methods and frequency-domain methods. Time-domain methods are usually easier to calculate, while frequency-domain methods can yield more information. Only the normal beats, originating from the sino-atrial node, are included in the calculation – hence the term 'standard deviation of normal-to-normal' (SDNN) intervals. Accordingly, atrial and ventricular arrhythmias are excluded. From this it is also possible to calculate mean NN interval, mean heart rate and range (longest NN minus shortest NN). Due to the ease of calculating SDNN, it is the most widely used time-domain method of HRV [114]. SDNN measures the overall variability from periodic and random sources.

Several factors affect HRV: cigarette smoking has been linked to low HRV [115], beta-blockers and ACE-inhibitors increase HRV [116],[117] and, diabetes mellitus and hypertension have been shown to decrease HRV [118],[119]. In addition, low SDNN has previously been linked to increased mortality after acute myocardial infarction [114] and increased mortality in the elderly [120]. Recently HRV in a TSC population was studied and compared with controls [121]. Using a Holter 24-hour ECG recording HRV was measured on admission to hospital. SDNN was significantly

lower in the TSC group compared with controls, although it was normalized a few weeks after admission to hospital on a follow-up 24-hour ECG.

Figure 5. R-wave intervals.
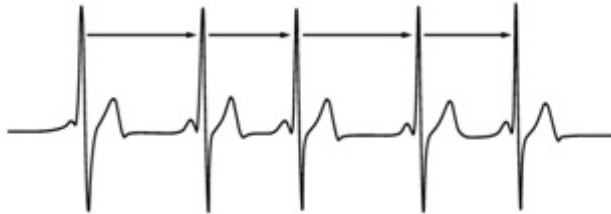
SALIVARY CORTISOL

Cortisol, or hydrocortisone, is a steroid hormone or, more specifically, a glucocorticoid, produced by the zona fasciculata in the adrenal cortex [122]. In response to stress and low levels of glucose cortisol is released. Its primary functions are to increase blood sugar through gluconeogenesis, suppress the immune system, and aid in fat, protein and carbohydrate metabolism. It also decreases bone formation. The hypothalamus secretes corticotropin-releasing factor (CRF) which stimulates the production of ACTH by the anterior pituitary gland. ACTH stimulates production of cortisol by the adrenal cortex.

Figure 6. Feedback mechanism for cortisol regulation.
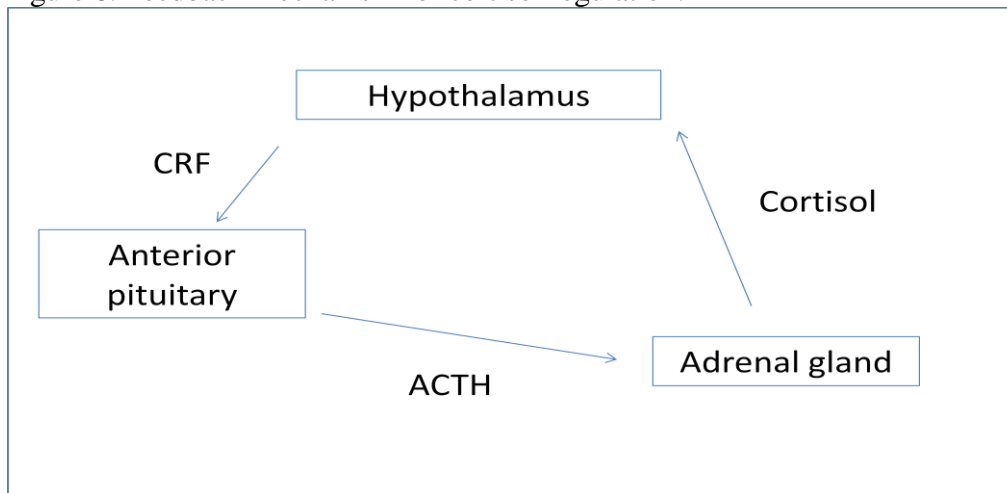
Salivary cortisol reflects levels of free cortisol in blood [123]. Since many forms of stress are known to increase free levels of cortisol, salivary cortisol can be used in stress research. In fact, it is thought of by some researchers as the method of choice for stress research because of the ease by which it can be collected and handled. However, the method has known caveats. For example, around one third of salivary cortisol is known to be enzymatically converted to cortisone in saliva [123]. In a recent study Kastaun et al. were able to show that salivary cortisol

stress response was blunted in 19 TSC patients compared to 20 healthy controls [124]. Apart from the study by Kastaun et al. there are no previous studies on salivary cortisol and TSC.

CORONARY FLOW RESERVE

Early experiments involving assessment of coronary flow and coronary flow reserve (CFR) began in the 1970s. CFR is an estimate of the remaining ability to increase flow in a vessel after taking into account stenosis, microcirculation and collateral flow. Initially experiments were carried out in anaesthetized dogs using contrast medium to induce hyperemic flow [125]. During the second half of the 1970s Cole et al. developed an instrument to measure intracoronary flow using a Doppler catheter[126]. In the 1980s digital arteriographic methods were developed to assess the CFR during invasive coronary angiography more indirectly [127]. In the two last-mentioned studies contrast medium was used to induce coronary hyperemia. The use of better pharmacologic substances was subsequently introduced, enabling Serruys et al. to compare digital subtraction angiography with intracoronary Doppler flow measurements before and after coronary angioplasty using papaverine [128] [129]. They found that the same quantity of CFR that can be recruited by transluminal coronary occlusion can also be recruited pharmacologically.

The first attempts to visualize the coronary arteries by using transthoracic Doppler echocardiography were made in 1987 [130]. However, it lasted until 1998 when for the first time Hozumi et al. calculated CFR using transthoracic Doppler echocardiography with hyperemia from intravenous adenosine [131]. CFR using dobutamine was also studied although via transoesophageal Doppler echocardiography that actually has to be considered a semi-invasive technique [132]. They could show that transoesophageal Doppler echocardiography using dobutamine was similar in accuracy to assessments using wall motion abnormalities to detect ischemia. In a study by Petropoulakis et al. dobutamine and adenosine were compared by using two concomitant intracoronary Doppler flow-wires [133]. They found that dobutamine was better suited for detecting ischemia while adenosine was preferred for assessing myocardial perfusion. They also showed that after 20 µg/kg/min a further increase in dobutamine did not yield any additional information regarding ischemia in stenotic vessels (figure 7).

Figure 7. Assessing the flow-velocity curve in the left anterior descending artery.
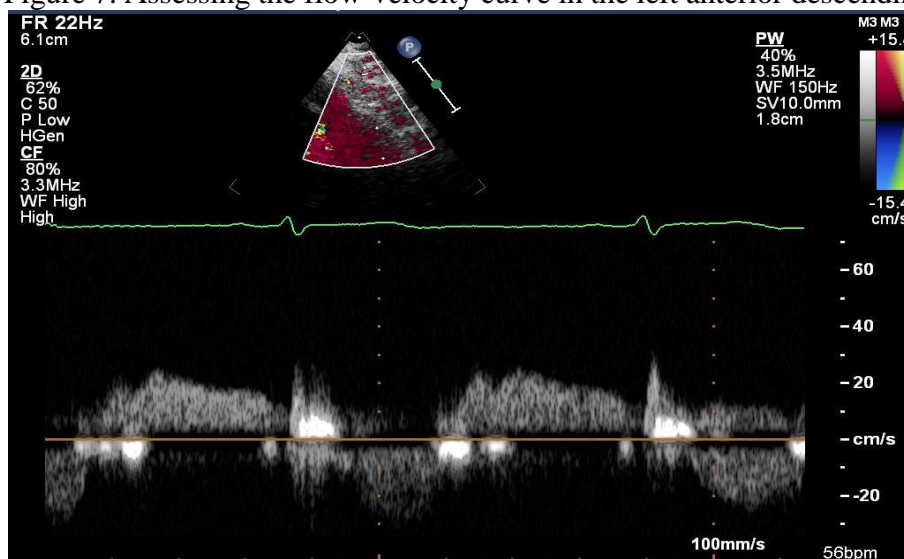
Coronary flow reserve in Takotsubo stress cardiomyopathy

One possible explanation for TSC could be microvascular dysfunction. Previous studies have shown decreased coronary flow reserve (CFR) during the acute phase of TSC [134] [135]. CFR is thought to give an estimate of the maximal remaining flow reserve in a vessel, taking into account stenosis in the vessel, microvascular circulation and collateral flow [136]. Several studies have shown that CFR may be estimated by non-invasively assessing the intracoronary flow velocity [131] [136]. Previously, the effects of dipyridamole, adenosine and cold pressor test on invasive and non-invasive CFR have been studied [134] [137] [138]. However dobutamine, a catecholamine that closely resembles adrenaline and therefore mimics, possibly more accurately, an acute TSC-like event, has not been studied regarding its effect on CFR in TSC. However, in a study on CAD patients after revascularization by coronary angioplasty, CFR using dobutamine was found to correlate negatively with the wall motion score index and positively with tissue Doppler imaging peak velocity despite the absence of stenosis [139].

AIMS

The hypothesis of this thesis was that:

1. Cardiovascular magnetic resonance imaging facilitates the diagnosis of MINCA.
2. TSC patients have an increased vulnerability to sympathetic stress measured by MPI.
3. TSC patients have an increased vulnerability to mental stress measured by MPI, HRV and salivary cortisol.
4. CFR is reduced during dobutamine stress in patients with a previous episode of TSC.
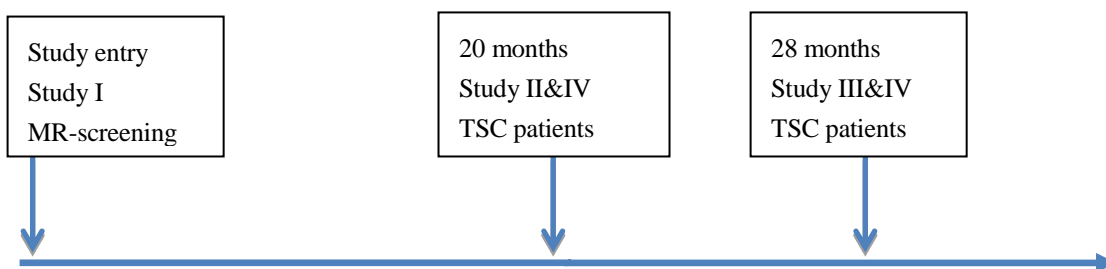
METHODS

STUDY I

Study group

In Study I, a total of 176 patients were screened from June 2007 until May 2011 at five coronary care units in the Stockholm Metropolitan Area. The patients, who were between 35 and 70 years of age, had chest pain typical for myocardial infarction, ECG showing sinus rhythm on admission, elevated troponins and coronary angiography with no or minimal signs of atheromatosis. Minimal atheromatosis was defined as irregularities in the vessel wall not giving rise to any stenosis >30%.

Myocarditis was excluded by CMR imaging; some patients with classical features of myocarditis or perimyocarditis were not investigated with coronary angiography and were therefore not eligible for the study. The first 100 screened patients were investigated with chest computed tomography (CT) to exclude pulmonary embolism. Because all chest CTs were normal in these patients the protocol was changed to D-dimer and chest CT was only performed upon suspicion of pulmonary embolism.

Figure 8. Time-chart for the different studies in this thesis.

Study entry	20 months	28 months
Study I	Study II&IV	Study III&IV
MR-screening	TSC patients	TSC patients

CMR imaging protocol

A standard CMR protocol included standard steady-state free precession (SSFP) cine imaging and T2-weighted oedema imaging, and late gadolinium enhancement (LGE) for fibrosis detection was used. The CMR protocol differed slightly between the different sites. The investigation was performed with the patient in the supine position using a cardiac coil by means of one of three 1.5 T system models (General Electric Healthcare, Signa Excite TwinSpeed, Waukesha, Wisconsin, USA; Siemens Sonata, Erlangen, Germany; or Philips Intera CV, Best, Netherlands) during vector-ECG monitoring. The image protocol included scout images, localization of the short axis and then covering of the whole left ventricle (LV) with retrospectively gated cine SSFP images. The following parameters were typically used: SSFP (echo time [TE] 1.6-3.3 ms, repetition time [TR] 2.8-3.6 ms, flip angle 60º, 25 phases, 8 mm slice, no gap, matrix , 160-226x141-226). T2-weighted images (triple inversion recovery; TE: 60-80 ms, TR: 2 R–R interval, TI: 150-170 ms, slice thickness 8 or 14 [14 mm with GE TwinSpeed] mm, gap 8 mm, flip angle: 90-180 degrees, matrix 226-256x226-256) were acquired in the same long- and short-axis planes. LGE images were acquired 15-20 min after contrast injection of intravenous gadolinium-DTPA (0.2 mmol/kg) using a 2D or 3D (3D with

Philips Intera CV) inversion recovery gradient echo sequence 2D or 3D (TE 1.1-3.3 ms, TR 3.8-7.0 ms, inversion time 180-300 ms to null the myocardium, 8 mm slice, no gap, matrix 240-256x180-192) in the same slice orientation as cine SSFP images. Each slice was obtained during end-expiratory breath-holding. Two-, three- and four-chamber views were also obtained to confirm the findings.

CMR analysis

All CMR images were analysed offline, using freely available segmentation software (Segment V.1.8 R1405; http://segment.heiberg.se/). Two independent experts with vast CMR experience carried out the interpretation of the CMR examinations. All clinical data were blinded and the interpretation was done without prior knowledge of the CMR report from the investigating hospital, thereby minimizing inter-hospital variation. In case of disagreement, a third CMR specialist was consulted to obtain a consensus.

End-diastolic and end-systolic volumes were measured in the phases with the largest and smallest LV volumes, respectively. LVEF, stroke volume and LV mass were calculated on cine SSFP sequences using manual delineation of the endocardial and epicardial borders including papillary muscles and trabeculations when contiguous with the left ventricle. LV mass was calculated by multiplying the myocardial volume by the density of myocardial tissue (1.05 g/ml). All volumes were indexed to body surface area.

T2-weighted images were visually interpreted to detect areas of high signal compatible with oedema. LGE images were assessed for subendocardial enhancement in the distribution of a coronary artery suggesting myocardial infarction or midwall/subepicardial enhancement suggesting myocarditis. Patients with patchy involvement on LGE (intramyocardial, including both subepicardial and subendocardial) were considered to have myocarditis. Examinations with normal volumes and function and with no LGE or T2-weighted abnormalities were considered normal CMR examinations.

STUDY II

Study group

Twenty-two patients with a previous episode of TSC and 22 sex- and age-matched controls were investigated using DSE. The TSC patients and controls were recruited from the SMINC (Stockholm Myocardial Infarction with Normal Coronaries) study. All TSC patients had a previous normal CMR. The controls were not investigated using coronary angiography prior to their inclusion in the study but had no signs or symptoms of coronary artery disease and a previous normal exercise stress test. Study II was performed a mean 20 months after the acute event (see Figure 8).

Echocardiography

A Philips (Amsterdam, Netherlands) iE33 was used for echocardiography. First, the left ventricular ejection fraction (EF) and function of the mitral, tricuspid and aortic valves were recorded. Transmitral flow was recorded by pulsed-wave Doppler placed between the mitral leaflet tips in an apical four-chamber view. Early (E) and late (A) transmitral flow velocities were recorded. The ratio of early-to-late transmitral peak velocities (E/A) and the deceleration time (DT) were obtained and calculated. The pulsed-wave Doppler tissue imaging (DTI) was performed by activating the DTI function. Images were acquired by using a variable-frequency

phased array transducer. The filter settings were kept low (50 Hz) and gains were adjusted at the lowest possible level to minimize noise and eliminate the signals produced by the transmitral flow. A 4.0 mm sample volume was used. Four sites at the LV were selected. A Doppler velocity range of -15 to 15 cm/s was selected. In the apical four-chamber view, the DTI cursor was placed near the septal and the lateral sites of the mitral annulus. In a similar way, the velocities at the anterior and inferior sites of the LV were recorded from the apical two-chamber view. A mean value was calculated from the above four sites and used to assess the LV-MPI and left ventricular filling pressure (E/E´ -ratio of transmitral early wave and DTI-derived early diastolic velocity). The tricuspid annular velocities were also measured similarly by placing the sampling volume near the tricuspid ring of the RV free wall in the apical four-chamber view. In this way the RV-MPI was calculated. MPI was calculated by measuring the isovolumetric contraction time (IVCT), isovolumetric relaxation time (IVRT) and ejection time (ET) and derived from (IVCT+IVRT)/ET. An example of how MPI was measured can be seen in Figure 1. The images obtained with echocardiography were analysed in Syngo Dynamics software (Siemens Healthcare, Erlangen, Germany). A mean of three consecutive cycles was used to calculate all echo-Doppler parameters.

Dobutamine stress echocardiography
Dobutamine stress echocardiography (DSE) was performed according to the consensus statement of the European Association of Echocardiography [83]. An increasing dose of dobutamine was administered (5, 10, 20, 30 and 40 ug/kg/min) until the heart rate reached >85% of the calculated maximum heart rate. If necessary, at the highest dose of dobutamine, an additional dose of atropine was administered to reach the calculated maximum heart rate. At the highest dose of dobutamine E/A, E/E´, LV-MPI and RV-MPI were assessed.

STUDY III

Study group
The TSC patients (n=22) and controls (n=22) in Study III were investigated by applying mental stress, and the study group was almost identical to the study groups in Study II (see Study III for a complete description of the study groups). For the HRV data 18 TSC patients and 20 controls were investigated because that part of the study was started after the overall study. Study III was performed a mean two years and four months after the acute event (see Figure 8).

Mental stress
The mental stress test consisted of two parts. The first part was an anger recall interview [140 141] in which the patient and control were asked to recall an upsetting situation in the few months prior to the investigation and then speak about that situation for a few minutes. A few follow-up questions were asked by the interviewer about this upsetting situation. The second part of the mental stress was a mental arithmetic task [140 142] that was performed directly after the anger recall interview. The patient or control was asked to subtract 7 from 200 in continuing steps as fast as possible. This test was done with increasing stress by the interviewer.

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Self-estimate acute stress

In a post-stress environment the TSC patient and the control were asked to estimate their level of recently experienced mental stress on a Likert-type discrete 7-grade scale (0-6). The TSC patients were also asked to estimate (on the same Likert-type scale) the level of acute stress they experienced during the acute event.

STUDY IV

Study group

The study groups consisted of 22 patients with a previous episode of TSC and 22 sex- and age-matched controls. The two independent groups in this study were investigated using CFR (see CFR section below) during dobutamine and mental stress, and the study groups were similar to those in Studies II and III, depending on the type of stress used.

Coronary flow reserve

All patients and controls were investigated using dobutamine and mental stress. A Philips (Amsterdam, Netherlands) iE33 was used to obtain echocardiograms. First, the left ventricular (LV) ejection fraction (EF) and function of the mitral, tricuspid and aortic valves were recorded. For the flow-velocity recordings a 3.5 Mhz high-frequency probe was used. The filter settings were kept at 150 Hz and gains were adjusted at the lowest possible level to minimize noise. A 10 mm sample volume was used. A Doppler velocity range of -15 to 15 cm/s was selected. At rest the flow-velocity curve of the left anterior descending (LAD) artery was assessed from an apical two-chamber view. If necessary, SonoVue (Bracco, Milano, Italy) contrast was used to enhance the flow-velocity signal. Non-invasive CFR was calculated from the peak diastolic velocity in LAD at rest, low-dose and high-dose dobutamine, where CFR is the velocity during stress divided by velocity at rest. A mean of three consecutive cycles was used to calculate all parameters.

In the manuscript (Study IV), only the results of dobutamine stress were published.

RESULTS AND DISCUSSION

STUDY I: MYOCARDIAL INFARCTION WITH NORMAL CORONARY ARTERIES IS COMMON AND IS ASSOCIATED WITH NORMAL FINDINGS ON CARDIOVASCULAR MAGNETIC RESONANCE - RESULTS FROM THE STOCKHOLM MYOCARDIAL INFARCTION WITH NORMAL CORONARIES STUDY.

The baseline characteristics for Study I can be seen in Table 2.

Table 2. Baseline characteristics for Study 1.

	I All screened **n=176**	**II** All CMR **n=152**	**III** Myocarditis on CMR **n=11**	**IV** AMI on CMR **n=29**	**V** Normal CMR **n=102**	Comparison between groups III, IV and V **(p-value)**
Age, years	58.5	58.0	48.0*	57.0$^\#$	59.0	**0.04**
Female	112 (63)	98 (64)	6 (55)	12 (43)	73 (72)	**<0.01**
Current smoking	27 (15)	25 (17)	0 (0.0)	3 (11)	19 (18)	**0.21**
Prior smoking	66 (37)	56 (37)	3 (27)	15 (50)	35 (34)	**0.33**
Family history of CAD	51 (29)	43 (28)	4 (36)	3 (11)	34 (33)	**0.04**
Diabetes mellitus	7 (4)	6 (4)	0 (0.0)	2 (7)	3 (2)	**0.17**
Treated hypertension	58 (33)	52 (34)	2 (18)	9 (32)	35 (35)	**0.63**
Treated hyperlipidemia	22 (13)	19 (13)	3 (27)	2 (7)	11 (11)	**0.17**
Normal ECG	94 (53)	81 (53)	8 (73)	16 (54)	53 (52)	**0.47**

Maximum troponin level (times above normal)	25.5	25.7	30.7	60.5[#]	16.6	<0.01

Values are in number of patients (or percentage). Maximum troponin level (times above normal) and age are median values. CMR: cardiovascular magnetic resonance; AMI: acute myocardial infarction; CAD: coronary artery disease; ECG: electrocardiography. Initially all groups were compared in order to detect any significant differences. [*]p = 0.02 vs. group V, [#]p < 0.01 vs. group V.

According to RIKS-HIA, the total number of patients in the Stockholm Metropolitan Area with ACS from June 2007 to May 2011 was 4412. Of these 277 had MINCA, according to SCAAR[1]. Thus, during the inclusion period we screened in total 64% of all of the eligible MINCA patients. The study flow of Study I can be seen in Figure 9.

Figure 9. Study flow of Stockholm Myocardial Infarction with Normal Coronaries study
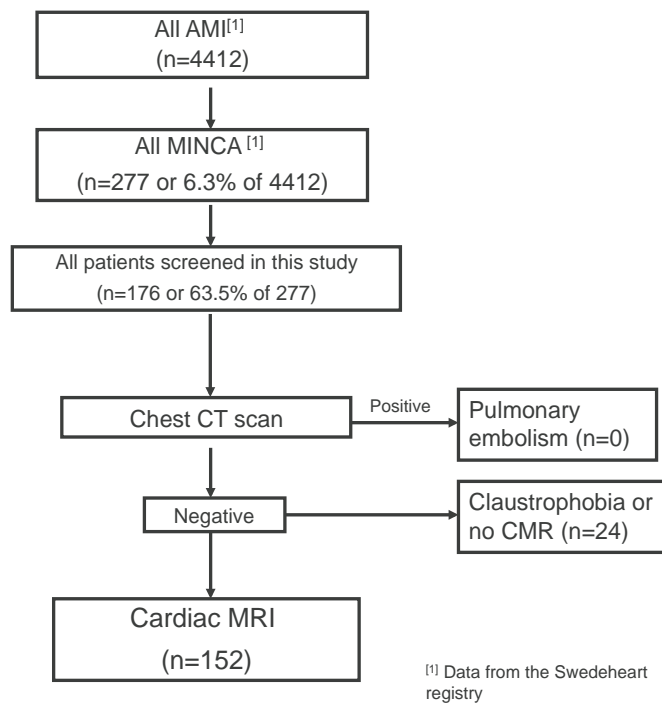
Results of cardiovascular magnetic resonance
In 102 patients CMR was normal. Twenty-nine patients had signs of myocardial necrosis and 11 patients had signs of myocarditis. With 10 patients, either they had hypertrophic cardiomyopathy or their CMR diagnosis was not conclusive. The CMR results can be seen in Figure 10. Twenty-two percent of all patients screened with CMR fulfilled the Mayo Clinic criteria for TSC[29].

Figure 10. Pie chart of cardiovascular magnetic resonance results.
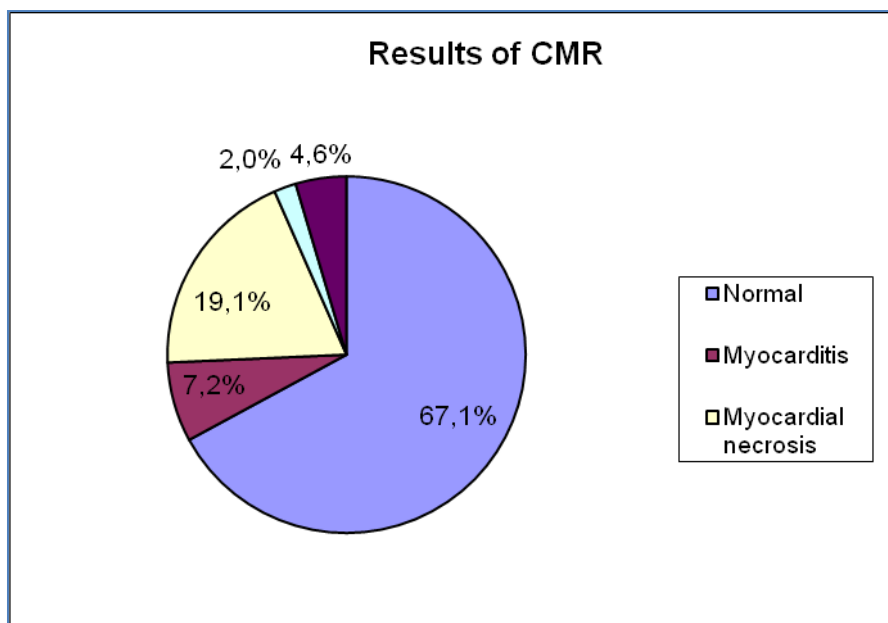
The diagnosis of TSC demands further comments. The reversible hypokinesia found in TSC is time-dependent and can sometimes be difficult to assess. In Study I we have no exact data as to the extent to which left ventricular angiography or echocardiography were used. Nor is the extent to which these investigations were used evident in previous studies involving CMR in MINCA patients. [73-76 143 144]. If left ventricular angiography and echocardiography are performed soon after admission to hospital the incidence of TSC would most probably be higher since it would enhance the possibility of demonstrating the myocardial dysfunction typical of TSC.

One important finding of Study I is that CMR increases the number of patients who receive a proper diagnosis. By using CMR we found that around half of all MINCA patients received a correct diagnosis, while more than half of the patients reported having received a different diagnosis after CMR that what they had at discharge. This finding has important implications for the prognosis and medical treatment of MINCA patients.

As already mentioned in the Introduction section above, there are previous studies that have used CMR on a MINCA population [73-76 143 144]. What separates Study I from the other studies is that all of the patients in the Stockholm Metropolitan Area who were between 35 and 70 years of age were eligible for our study, while in the other studies there was a younger clientele and higher incidence of myocarditis. We chose to exclude patients who were younger than 35 in Study I, so that coronary angiography would be clinically motivated. The CMR in our study was performed at a median of 12 days after admission to hospital. In previous studies the time from admission to CMR varies substantially. In the study by Stensaeth et al. the median time to CMR was only 20 hours [143]. In a larger study by Gerbaud et al. time to CMR was a median 6.2 days [75]. In two other studies this information is not available [74 144], while in a study by Chopard et al. it was 10 days [76]. The time to CMR has important clinical implications for the diagnosis of

TSC since the systolic dysfunction often disappears within one week. It also has implications on the extent to which myocardial oedema can be assessed in TSC patients.

STUDY II: VULNERABILITY TO SYMPATHETIC STRESS DOES NOT PERSIST IN TAKOTSUBO STRESS CARDIOMYOPATHY

The mean age of both groups was 62.9 years. In both groups, all but one individual were female. Current smoking and treatment with beta-blockers were more common in the TSC group. Baseline characteristics are shown in Table 3.

Table 3. Baseline characteristics

	Takotsubo	Control	p-value
Number	22	22	n.a.
Age (years, mean value)	62.9	62.9	n.a.
Gender (male/female)	1/21	1/21	n.a.
Currently a smoker	18%	0%	0.04
Diabetes mellitus	4.5%	0%	0.33
Hypertension	45.5%	22.7%	0.12
Beta-blocker	50%	13.6%	0.01
Calcium blocker	18.2%	0%	0.04
ACE inhibitor	45.5%	13.6%	0.02

ECG: electrocardiography; ACE: angiotensin converting enzyme; n.a.: not applicable

At rest there were no significant differences in conventional DTI parameters between the groups, whereas LV-MPI differed significantly between the groups (see Table 4).

Table 4. Baseline echocardiographic data.

	Takotsubo	Control	p-value
E/A	0.90	1.01	0.26
DT	180	187	0.58
E/E´	8.20	7.90	0.48
LV-MPI	0.59	0.53	0.01
RV-MPI	0.47	0.38	0.08

EF: ejection fraction; E/A: early and atrial transmitral flow fraction; DT: deceleration time; E/e´: early transmitral flow and early tissue Doppler velocity fraction; LV-MPI: left ventricular myocardial performance index; RV-MPI: right ventricular MPI.

During DSE there were no significant differences between the groups in standard diastolic variables, LV-MPI (0.80 and 0.91, p=0.43) or RV-MPI (0.69 and 0.81, p=0.32).

Data from registries and retrospective studies show that recurrence of TSC is rare [41 145 146]. There have been some reports of episodes of TSC being triggered by performing a DSE [147 148]. Previously no one has investigated TSC patients using catecholamine stress. Accordingly, the results of Study II are of interest. Of 22 TSC patients we could not precipitate a second TSC event in any of them. In fact we could not even distinguish the TSC group from controls when using sensitive DTI. The DTI technique used was MPI. MPI has been shown to sensitively assess LV function and functional capacity, and it correlates with mortality and an increased risk of developing heart failure [103 104].

Our conclusion from Study II is that it seems as if the previous vulnerability to catecholamine stress does not persist after the acute event in TSC patients. However, one possible explanation for the lack of negative inotropic response to dobutamine stress is the fact that dobutamine only has a weak beta2 effect. In animal studies the negative inotropic effect at high catecholamine doses is mediated via the beta2-receptors [57 59]. Shao et al. induced a TSC-like pattern in rats using isoprenaline (a non-selective beta-adrenergic agonist[149]) and Paur et al. produced apical depression of myocardial function using epinephrine (non-selective alpha and beta adrenergic agonist[150]). The apical depression in the latter study could be prevented by pertussion toxin infusion and G(i) inactivation. Hence, dobutamine, despite being a catecholamine, could possibly be insufficient to precipitate a TSC event even at high doses. Thereby, the exact mechanism for precipitating TSC and the rarity of persisting vulnerability to stress in TSC patients still needs to be explored further.

STUDY III: NO MYOCARDIAL VULNERABILITY TO MENTAL STRESS IN TAKOTSUBO STRESS CARDIOMYOPATHY

Twenty-two TSC patients and 22 sex- and age-matched controls were investigated with DTI. The mean ages of the groups were 63.2 and 63.6 years, respectively. Current smoking and beta-blockers were more common in the TSC group. See Study III for a complete description of the baseline characteristics of both groups.

Mental stress test
All TSC patients and controls completed the mental stress test. At rest the heart rate for the TSC patients was 58 beats per minute (bpm), and for the controls, 60 bpm. During peak stress they reached 89 and 94 bpm, respectively (p=0.28).

In this study we used ARI and MA as our mental stress tests [88] [89]. That both types of stress can induce a cardiovascular reaction was validated previously. We could possibly have induced a greater amount of stress in the TSC patients and controls using the Stroop colour word test or other mental stress test [84]. However, since we had already achieved 53% and 57% increases in heart rate for TSC patients and controls, respectively, the additional effect of, for example, a Stroop colour word test would have been limited. Furthermore, there are ethical reasons for limiting the amount of stress we induce on patients or controls.

Myocardial performance index
LV- and RV-MPI did not differ significantly between TSC patients and controls at rest or during mental stress.

Our conclusion from Study III is that there is no remaining vulnerability to mental stress in TSC patients measured by sensitive tissue Doppler (MPI). To our knowledge, there are no previous studies on LV- or RV-MPI and mental stress performed in the non-acute phase. However, in one previous study Bhat et al. compared RV-MPI assessed during the acute phase in 17 patients with TSC and 46 patients with obstructive LAD disease [109]. They found that RV-MPI was significantly higher in TSC patients compared to in patients with obstructive LAD disease (1.03 versus 0.44, p<0.001). Hence, there are indications that MPI is pathological in the acute phase while no significant difference in RV- or LV-MPI can be seen in TSC patients compared to controls during mental stress more than six months after the acute event.

Heart rate variability
For the HRV data, 20 TSC patients and 18 controls were studied. The HRV part of the study started after the DTI and SC parts – hence the lower number of TSC patients and controls examined with HRV. The duration of Holter-ECG was 32 minutes and 30 minutes for the TSC patients and controls, respectively. Overall SDNN was 61 and 76 ms for TSC patients and controls, respectively (p=0.21) while SDANN was 41 and 47 ms, respectively (p=0.19). SDANN pre-stress was 11 ms and 16 ms for TSC patients and controls, respectively (p=0.11). Both groups increased significantly in SDNN from pre-stress to during stress (p=0.002). Excluding beta-blocker treated TSC patients or controls, there was a trend towards a greater increase in SDNN from pre-stress to during stress in the TSC group (p=0.055). There was no significant difference between the groups post-stress.

Salivary cortisol

The second basal SC measurement, taken 30 minutes after waking-up, was 12.4 and 17.3 nmol/L in TSC patients and controls, respectively (p=0.25). The difference in SC before and after mental stress was -0.6 and 0.6 for TSC patients and controls, respectively (p=0.099). In all other measurements no significant differences could be seen between the groups.

Both HRV and SC responses were normal for the TSC group. One could speculate as to whether a larger study sample would have been able to show a difference in HRV or SC. In our study there was a tendency toward less of an increase in SC both after waking up and after a stressful event. A recent study that compared 19 TSC patients, 20 patients with non-ST elevation myocardial infarction and 20 healthy controls found a trend towards a blunted salivary cortisol response in the TSC patients compared to healthy controls after correction for differences between both groups [124]. That study was performed a mean 18 months after the index event and the result was achieved despite a heart-rate increase of only 6%. In our study (Study III), we also noted a trend towards a blunted SC response in TSC patients while increasing the heart rate by 53% during mental stress. One explanation for the non-significant results could be that salivary cortisol measurements have high intrinsic variability, which inhibits our ability to effectively assess differences in salivary cortisol response between TSC patients and controls [123].

STUDY IV: CORONARY FLOW RESERVE DURING DOBUTAMINE STRESS FOR TAKOTSUBO STRESS CARDIOMYOPATHY

Baseline characteristics of Study IV were similar to those of Studies II and III, depending on the type of stress used when assessing CFR (see Study IV for a complete description of baseline characteristics).

Coronary flow reserve

There were no significant differences in heart rate between the groups at any point. Resting heart rate was 68 beats per minute (bpm) and 68 bpm for the TSC patients and controls, respectively, while heart rate at low-dose dobutamine was 99 bpm and 94 bpm, respectively. Maximum heart rate at high-dose dobutamine was 131 bpm and 134 bpm for TSC patients and controls, respectively.

CFR at low-dose dobutamine was significantly lower in the TSC group compared to controls: 1.51 and 1.72, respectively (p=0.017). At high-dose dobutamine CFR was 1.95 and 2.21 in the TSC group and controls, respectively (p=0.098).

We also studied CFR during mental stress. These data were not included in Study IV but are shown below (see Table 5). During both ARI and MA there were no significant differences between the groups.

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Table 5. Coronary flow reserve during mental stress.

	Takotsubo	Control	p-value
CFR ARI	1.51 [±0.30]	1.43 [±0.24]	0.35
CFR MA	1.49 [±0.35]	1.53 [±0.25]	0.67

CFR: coronary flow reserve; ARI: anger recall interview; MA: mental arithmetics.

Several studies have investigated CFR using adenosine or dipyridamole [134] [137] [138]. In conclusion, these studies show that CFR measured using adenosine or dipyridamole is pathologic in the acute or sub-acute phase but normalized after some weeks or a few months. There have been no previous studies on TSC patients with CFR using dobutamine stress. In study IV, which involved the use of dobutamine stress, at one year and eight months after the acute event CFR was similar to controls at high-dose dobutamine but still significantly lower compared to controls at low-dose dobutamine. There are three possible explanations for this: firstly, we induced a mild microvascular dysfunction in TSC patients using dobutamine stress that is only significant compared to controls at low-dose dobutamine. The possible explanation for a non-significant result during high-dose dobutamine is the challenging assessment of coronary flow. Secondly, CFR is pathological after the acute event (and only assessable when using dobutamine). The differences between points 1 and 2 would be that microvascular dysfunction is either induced during stress or is chronically impaired. Thirdly, baseline characteristics are the explanation for pathologic CFR during low-dose dobutamine stress and not the fact that these are patients with a previous episode of TSC. In all three instances a more challenging assessment of peak coronary flow velocity could have influenced the results at high-dose dobutamine. In other words, a large variation in means at high-dose dobutamine might have obscured a true significant difference between TSC patients and controls.

GENERAL DISCUSSION

Baseline characteristics of MINCA

In order to compare our group of MINCA patients with previous studies of MINCA, using CMR, the baseline characteristics with and without our patients in study I are shown in Table 6.

Table 6. Meta-analysis of baseline characteristics in studies on MINCA with CMR. Column A: Study I; Column B: patients from study [72-76]; Column C: patients from study [72-76] + Study I.

Total number of patients	A n=152	B n=464	C n=616
Mean age	58.0 (years)	49.0 (years)	51.2 (years)
Male sex	35	60	54
Family history of CAD[#]	28	27[#]	28[##]
Smoking[#]	37	38[#]	37[##]
Hypertension[#]	33	33[#]	33[##]
Diabetes mellitus[#]	4	7[#]	6[##]
Myocarditis on CMR	7	44	35
Acute myocardial infarction on CMR	19	21	20
Takotsubo stress cardiomyopathy	22	14	16

Percentage; CAD: coronary artery disease; CMR: cardiovascular magnetic resonance; [#/##]For these characteristics, [#]n=277 and [##]n=429, respectively.

This meta-analysis shows that MINCA patients are not very different in baseline characteristics from patients with known CAD with smoking and hypertension being more prevalent in comparison to controls [151]. In comparison to previous studies [72-76] our MINCA group showed an increase in age, decrease in male sex and, most evidently, a drastic decrease in myocarditis. Previous studies included younger patients, whereas in Study I we restricted inclusion to patients between 35-70 years of age. The reason for this was to eliminate young patients with myocarditis; moreover, TSC is rare in patients <35 years of age. However, remaining baseline characteristics in our study, such as family history of CAD, smoking, hypertension and diabetes mellitus, seem to correspond with earlier studies.

A recent meta-analysis by Pasupathy et al. found that the prevalence of MINCA was 6% [152]. This analysis was done using 28 previous studies. Median age was 55 years but only 40% were women. In this study Pasupathy et al. also performed an analysis of all-cause mortality and found that it was 4.7% in MINCA compared to 6.7% in myocardial infarction with obstructive coronary artery disease. Although we did not perform an analysis of mortality in Study I it is interesting to compare clinical characteristics in column C (Table 6) with the meta-analysis by Pasupathy et al. It is quite obvious that most of the clinical characteristics are similar. Also the prevalence of MINCA calculated in Study I was similar to the one calculated by Pasupathy et al. (6.3 versus 6%).

Regression of systolic and diastolic dysfunction in TSC patients

When combining the results of Studies II-III one could draw the conclusion that vulnerability to catecholamine and mental stress for TSC patients does not persist or is rare at least. Furthermore, Studies II-III could also imply that sensitive DTI can assess a noticeable decrease in combined systolic and diastolic function that persists for many months after the acute event. Since all but one TSC patient or control in each group were the same in both Study II and Study III the median value of MPI at rest should be reliable. Furthermore, this could correspond to TSC patients' experience, seen in clinical practice, with a raised level of fatigue and reduced level of physical activity long after the acute event [153]. Decreased systolic and diastolic function as well as heart failure symptoms also correspond to data showing elevated levels of N-terminal pro-hormone brain natriuretic peptide (NT-proBNP) in TSC patients three months after the acute event [154]. A recent study by Neil et al. found that an impaired global longitudinal strain (GLS) at three months was associated with both persistent NT-proBNP elevation and a reduced Short-Form Health Survey Physical Composite Score (SF36-PCS) at three months [153]. In conclusion, there is some evidence for a persistent reduction and a slow long-term regression of left ventricular function in TSC patients. However, the exact mechanism by which a TSC episode regresses over time needs to be further explored. See Figure 10 for a time-chart of the regression of systolic and diastolic dysfunction in TSC.

Figure 10. Regression of systolic and diastolic dysfunction in TSC.
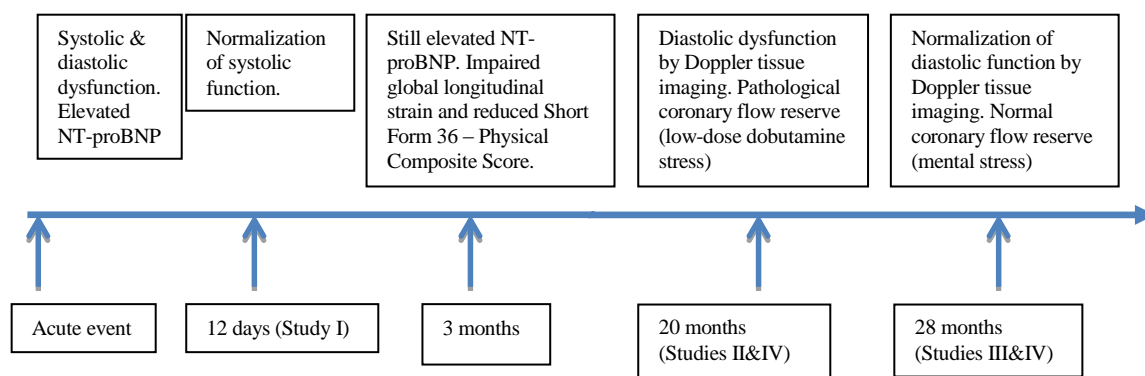
Our findings of a normalization of systolic and diastolic ventricular function as well as normal HRV and SC, at rest and during stress, indicate and strengthen an excellent prognosis for patients with TSC. This may bring long-term therapy using beta blockers or other medications in TSC patients into question.

Pathophysiology of TSC

What do Studies II-IV add to our current knowledge about the pathophysiology of TSC? Dobutamine and mental stress did not induce any wall-motion abnormalities measured using sensitive DTI, nor could we induce any microvascular dysfunction at high-dose dobutamine. Consequently, we can conclude that the vulnerability of ventricular function (or microvascular function) to the level of stress we did achieve did not persist in our group of

TSC patients. However, we found a small but significant difference in CFR at low-dose dobutamine. This could imply that microvascular function has a role in the pathophysiology of TSC. Further studies in this area are needed.

In a recent meta-analysis with the aim of identifying predisposing factors of TSC, Pelliccia et al. found that the most common comorbidities of TSC were psychological disorders (24%), pulmonary disease (15%) and malignancies (10%) [155]. Furthermore, hypertension was present in 54%, dyslipidemia in 32%, diabetes mellitus in 17% and smoking in 22%. This meta-analysis was done using 19 studies and 1,109 patients with a mean age spanning from 59 to 76 years. Compared to our small sample in Studies II-IV these clinical characteristics are similar except for diabetes mellitus which was much less common among our TSC patients. Comorbidities were not analysed in our sample for Studies II-IV, but psychological disorders, chronic obstructive pulmonary disease and malignancies were identified among the larger group of TSC in Study I (in manuscript).

Mortality among TSC patients have been disputed in the past (see table 7 below). In order to get an overview of previous studies on mortality in TSC patients I compiled mortality-data from previous studies in table 7.

Table 7. Mortality in Takotsubo stress cardiomyopathy

Author	Year	N=	CMR	Mortality (%)				
				In-hospital	30-day	1-year	Other	Comment
Pilgrim et al.[41]	2008	563	No	1.7				Meta-analysis
Buja P et al.[156]	2008	28	No				10.7 (2 years)	
Dib C et al.[157]	2009	105	No				12-14 (5 years)	
Sharkey W et al.[40]	2010	136	Yes			6.8		95/136 performed CMR
Song BG et al.[158]	2010	87	No	9			23 (42 months)	0% cardiac
Schultz T et al.[159]	2012	115	No	6			10 (3 years)	
Brinjikji W et al.[160]	2012	24701	No	4.2				81% had critical illness
Kwon SW et al.[161]	2013	208	No	8.7				
Weihs V et al.[162]	2013	179	No	0.6	1.3		6.7 (2 years)	10/13 non-cardiac
Prasad et al.[163]	2014	12	No				0 (2 years)	HORIZONS-AMI substudy
Citro R et al.[164]	2014	227	No	2.6				
Reglat C et al.[165]	2014	70	No				8 (4.8 years)	
Singh K et al.[146]	2014	2120	No	4.5				Meta-analysis 62% non-cardiac
Isogai T et al.[166]	2014	3719	No	5.4 and 17.9				Out-of-hospital and in-hospital TSC
Nunez Gil IJ et al.[167]	2014	202	No	2.4				
Bennett J et al.[168]	2014	139	No	6.9		12.6		
Redfors B et al.[169]	2015	302	No		4.1			SWEDEHEART

Percentage. N; number of patients, CMR; cardiovascular magnetic resonance, TSC; Takotsubo stress cardiomyopathy

Of all studies performed on TSC and mortality since 2008 only one used CMR. In other words, the quality of the TSC diagnosis should be improved in future studies. In several studies non-cardiac death is surprisingly high. Hence, the exact mechanism by which TSC patients die requires further investigation. Furthermore, one can also question the connection between mortality and a TSC event in many instances. Critical illness, malignancy or other severe co-morbidities could be the more accurate reason why a patient does not survive the first year after a TSC event. However, several studies indicate an increased mortality during the first one to two years after a TSC event and this can probably not be entirely explained by co-morbidities. Further studies in this field are needed.

Self-estimated acute stress
Directly after dobutamine and mental stress were performed the patients and controls were asked to estimate their level of acute stress during each type of stress. The TSC patients were also asked to retrospectively estimate their acute level of stress during the TSC episode itself. Self-estimated acute stress level (Likert-type scale 0–6) during mental stress was 2.7 and 2.6 for TSC patients and controls, respectively (p=0.81). During dobutamine stress the self-estimated acute stress was 3.0 and 4.0 in TSC patients and controls, respectively (p=0.05). TSC patients estimated that their stress level was 4.4 on the same Likert-type scale during the acute event.

There could be several explanations for this result. Firstly, the TSC patients could be used to high levels of stress, a hospital environment and investigations and therefore not find the dobutamine stress so stressful. Secondly, the TSC patients could have become less vulnerable to catecholamine stress after the real TSC event. In other words, a more direct biological effect can explain the difference in perceived acute stress. Mental stress was estimated in a similar way between both groups (2.7 and 2.6, respectively; p=0.81). Consequently, mental stress achieved a significantly lower level of stress than did both dobutamine stress and the acute event (for the TSC patients) and did not differ between controls and TSC patients. Even though the acute event for the TSC patients was estimated more than one year later, the results give us an indication that both the heart rate and general stress level achieved during dobutamine stress in the controls was not very far from what the TSC patients experienced during the acute event.

As already mentioned in the Introduction section, no previous study has been performed where a Likert-type scale of self-estimated acute stress has been assessed. Several studies have assessed perceived chronic or semi-acute stress (both assessing stress over weeks to months) using multiple variables [91-93] and recently some studies have used a visual analogue scale to measure the effect of a stress test [94][95]. These latter studies are regrettably not directly comparable to the results of this thesis although they give us an indication that a visual analogue scale is at least as discriminating as a questionnaire when it comes to highlighting differences in stress levels between two groups. However, a self-estimated acute stress scale could be the subject of further studies that could deepen our understanding of the level of acute stress experienced by TSC patients.

Mental stress and stress hormones in Takotsubo stress cardiomyopathy
In Study III we found no difference between TSC patients and controls with regards to salivary cortisol. Furthermore, the TSC patients experienced lower self-estimated acute stress

compared to controls during mental stress. However, in a recent study by Smeijers et al. they found that TSC was associated with higher norepinephrine and dopamine levels during mental stress (anger recall interview and mental arithmetics) [170]. TSC patients also had lower emotional arousal compared to controls while no evidence was found for dysregulated cortisol response. This study confirms our findings from Study III that TSC patients have a lower emotional arousal and normal cortisol response compared to controls while introducing the hypothesis that catecholamine hyper-reactivity and not emotional hyper-reactivity to stress is likely to play a role in myocardial vulnerability in TSC.

STRENGTHS AND LIMITATIONS

One strength of Study I is that 64% of eligible MINCA patients in the Stockholm Metropolitan Area were included in the study. In other studies using CMR on MINCA only a selected patient clientele were included or we have no data as to the extent to which these studies covered the MINCA population during the inclusion period [73-76]. One strength of Studies II-IV is the selection of both TSC patients and controls. The TSC patients were investigated with CMR prior to inclusion in these studies, thus minimizing the amount of patients whereas in reality a TSC diagnosis could be something different. Another strength of Studies II-IV is the selection of the controls. The controls were sex- and age-matched volunteers recruited from the general population often born on the same day as the TSC patients, thereby ensuring a good match, not just in sex, but also age.

One limitation of Study I is that we only included patients between 35 and 70 years of age. The reason for this was that we wanted only to include patients who had a clear indication for coronary angiography. However, we know from previous studies that a significant proportion of TSC patients are older than 70 years of age [155]. Accordingly, it seems reasonable to perform CMR even on patients older than 70 years of age. One limitation of Studies II-IV was the small sample size. Whether larger sample groups could have been able to show differences in DTI, HRV, SC or CFR between the groups remains to be determined. A second limitation was the accuracy of measuring CFR during high-dose dobutamine stress. A relatively high intra- and inter-observer variability during high-dose dobutamine limited our ability to accurately assess CFR. A possible difference in CFR at high-dose dobutamine remains to be proven. Thirdly, in this study we chose to use dobutamine instead of adrenaline, for example, for ethical and safety reasons. One possible explanation for the lack of significant results at high-dose dobutamine stress is the fact that dobutamine only has a weak beta2 effect. In animal studies the negative inotropic effect at high catecholamine doses in a TSC event is mediated via the beta2-receptors [55 57]. Therefore, dobutamine, despite being a catecholamine and reports of cases in which it has precipitated a TSC event [147 148], could possibly be inadequate to precipitate microvascular dysfunction even at high doses.

FUTURE STUDIES

In order to more accurately diagnose different types of MINCA, CMR should be performed at an early stage. If CMR were performed on day 2-4 after admission to hospital, it would be possible to asses not only LGE and wall-motion abnormalities but also the extent of oedema could (using CMR with T1- or T2-weighted protocols) [79][143]. This would give us the opportunity to make the diagnosis TSC not only from absence of LGE and presence of reversible left- or right- ventricular dysfunction but also by directly assessing the circumferential oedema typical of TSC. By using early CMR the percentage of patients with a reliable diagnosis would probably increase from the current half, approximately, of all MINCA patients, to 70% or more.

Up to now the Mayo Clinic criteria for TSC have often been used [29]. These criteria have been questioned, however, mostly because of new evidence supporting non-typical forms of TSC [30]. However, the diagnosis of TSC could be improved by using CMR criteria. Possible diagnostic criteria could include: the presence of transient myocardial oedema and/or wall-motion abnormalities with circumferential distribution, the absence of LGE corresponding to circumferential oedema and/or wall-motion abnormalities and the absence of coronary artery occlusion or stenosis, corresponding to circumferential oedema and/or wall-motion abnormalities. Similar CMR criteria were proposed by Eitel et al. in 2011, as follows: (1) severe LV dysfunction in a non-coronary regional distribution pattern; (2) myocardial oedema in the same location as the regional wall-motion abnormality; (3) the absence of high signal areas in LGE images (a cut-off value of >5 standard deviations should be used to define significance); (4) increased early myocardial gadolinium uptake. The diagnosis is confirmed if after >4 weeks all diagnostic have been completely or almost completely resolved.

By using CMR as diagnostic criteria one could reveal cases of TSC precipitated by myocarditis or obstructive coronary artery disease, as has been noted by several researchers [171][172]. Hence, the stunning (or parts of it) seen in ACS could in reality be TSC precipitated by the pain and stress experienced during an ACS and not by ischemia. Furthermore, diagnostic criteria may even include incomplete circumferential oedema or wall-motion abnormalities in which only parts of the myocardium are affected and there is no corresponding LGE or coronary artery disease. However, in cases of small oedema or wall-motion abnormalities reliable diagnostic criteria for both TSC and myocarditis probably remain difficult to establish. In such cases, a second CMR two months or more after the acute event could be helpful. A second CMR could potentially more clearly visualize LGE compared to a CMR performed in the sub-acute phase and denote the regression of myocardial oedema over time. A second CMR in combination with echocardiography, clinical findings and laboratory chemistry analysis could thus improve the number of MINCA patients with a reliable diagnosis even in the most diagnostically challenging patients.

Evidence for guidelines regarding treatment strategies for MINCA patients as well as TSC patients is still lacking. However, a few retrospective and non-randomized studies have been performed. Palla et al. noticed that pre-treatment with beta-blockers did not affect the severity of a TSC episode and Sharkey et al. found that beta-blockers were not absolutely protective against the development of a TSC event [40][173]. In a recent systematic review Singh et al. found

an annual recurrence rate of 1.5% in TSC. In the same study the recurrence rate was independent of clinic utilization of beta-blockers prescription, but inversely correlated with ACE-inhibitor and angiotensin-receptor blocker prescription [146]. However, prospective and randomized studies are needed in this field. One option for performing randomized studies could be to use the SWEDEHEART registry, which provides the opportunity to perform a prospective randomized registry clinical trial (R-RCT) [174]. One attractive study might be to evaluate the effect of beta-blocker treatment or no beta-blocker treatment in TSC patients. The SWEDEHEART registry, with its relatively large population, also has the benefit of a large amount of patient data included in the registry.

CLINICAL IMPLICATIONS

The clinical implication from Study I is that CMR increases the proportion of MINCA patients who receive a reliable diagnosis. According to our calculations approximately 50% of MINCA patients receive a reliable diagnosis after CMR and more than half of MINCA patients receive a different diagnosis after CMR compared to what they have upon their discharge from the hospital. From Studies II and III we have learned that vulnerability to catecholamine and mental stress does not persist in TSC patients. Recurrence of TSC is possible but relatively rare, and Studies II and III strengthen this hypothesis. Furthermore, after the recovery of systolic function there seems to be a mild myocardial dysfunction with slow regression and normalization after more than a year. If those of us in clinical practice acknowledge the fact that TSC patients have a slow recovery of ventricular function, our TSC patients will experience a much greater understanding of their disease and their situation.

CONCLUSIONS

From the studies in this thesis we can draw the following conclusions:

1. MINCA is more common than previously shown and is associated with a normal CMR. TSC constitutes a substantial part of MINCA.
2. Vulnerability to catecholamine stress, measured by sensitive MPI, does not persist in TSC patients.
3. Mental stress does not induce a pathological response in TSC patients measured by DTI, HRV or salivary cortisol 28 months after the acute event. Studies II-III indicate a slow recovery for TSC patients measured by DTI.
4. We could not confirm that the catecholamine dobutamine induced microvascular dysfunction in TSC patients. However, we found a small but significant difference in CFR at low-dose dobutamine which implies that the role of microvascular function in TSC needs to be further explored.

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