Pathophysiology and treatment in experimental stress-induced cardiomyopathy

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ABSTRACT

Background: Stress-induced cardiomyopathy (SIC) is an acute cardiac affliction associated with significant morbidity and mortality. It is characterized by potentially reversible regional myocardial akinesia which may involve up to 70% of the heart. An episode of SIC is often preceded by a strong emotional or somatic stressor. Catecholamine is implicated in the pathogenesis of SIC but the mechanisms involved are unknown. Neither American nor European guidelines provide any treatment recommendations for SIC, and no randomized clinical studies on treatment in SIC have been performed.

Aims: The aim of my thesis was to develop a representative animal model of SIC that can be used to study the pathophysiology behind the syndrome and to test potential treatment strategies.

Methods: We used 10-week-old male Sprague Dawley rats. The SIC models presented in the thesis are based on intraperitoneal administration of a catecholamine, given as a bolus. We infused fluid and/or pharmacological agents through the right jugular vein and measured arterial blood pressure through a catheter inserted in the right common carotid artery. We used small animal echocardiography to study cardiac morphology and function and performed histological analyses of cardiac tissue to detect perturbations in lipid metabolism.

Results: In manuscript I we reproduced the clinical phenomena associated with SIC in rats by administration of catecholamine.

In manuscript II we show that perfusion defects did not appear to precede the development of SIC in our rat model.

In manuscript III we show that the development and morphological type of SIC appear to depend on hemodynamic factors rather than specific adrenoreceptor subtypes.

In manuscript IV we show that left ventricular filling pressure and cardiac output appear to be near-normal in experimental and clinical SIC despite hypotension. We also show that interventions for pharmacologically increased blood pressure increase acute mortality in the rat model.

In manuscript V we show, in two different experimental set-ups, that isoflurane anesthesia dose-dependently prevents experimental SIC.

Conclusion: Clinical SIC phenomena can be reliably reproduced in rat models. Because development of SIC appears to depend on hemodynamic factors, SIC should be considered a cardiocirculatory syndrome, in which the cardiomyopathy is one component. Isoflurane could be the anesthetic of choice in patients at increased risk of developing SIC, e.g. patients with severe somatic illness.

Key words: adrenoceptor, cardiocirculatory syndrome, catecholamine, echocardiography, isoflurane, stress-induced cardiomyopathy, Takotsubo cardiomyopathy,

Sammanfattning på svenska

Stress-inducerad kardiomyopati (SIC) är en allvarlig akut hjärtsjukdom där patienten riskerar att avlida. Vid SIC förlorar ofta stora delar av hjärtat sin pumpförmåga vilket kan leda till allvarliga komplikationer, t.ex. akut hjärtsvikt, elakartade rytmrubbningar, chock eller hjärtruptur. Om patienten överlever den akuta fasen är dock tillståndet ofta helt övergående.

SIC föregås ofta av kraftig psykisk eller fysisk (s.k. somatisk) stress. Stresshormoner kallade katekolaminer tros spela en viktig roll i sjukdomsutvecklingen men sjukdomsmekanismerna vid SIC är inte kartlagda. Varken Amerikanska eller Europeiska riktlinjer erbjuder några råd vad gäller behandling av patienter med SIC och inga randomiserade kliniska studier har genomförts avseende behandling för dessa patienter.

Syftet med mitt doktorandprojekt var att etablera representativa djurmodeller av SIC som sedermera kan användas för att studera sjukdomsmekanismerna bakom tillståndet och för att utvärdera olika behandlingsalternativ.

Genom att injicera 10 veckor gamla Sprague Dawley råttor med isoprenalin (en katekolamin) kunde vi reproducera de karakteristika som ses hos patienter med SIC. Vi undersökte hjärtfunktion med specialtillverkad ekokradiografiutrustning och mätte blodtrycket kontinuerligt genom att föra ned en kateter i arteria carotis communis. Vi studerade också med hjälp av histologi hur hjärtvävnaden påverkats av SIC avseende ämnesomsättning av fettsubstanser.

I manuskript I beskriver vi råttmodellen och hur vi lyckas reproducera de viktiga karakteristika som ses hos patienter med SIC.

I manuskript II visar vi att genomblödningen i hjärtmuskeln inte är uppenbart nedsatt i de påverkade områdena i hjärtat. Således beror funktionsnedsättningen i vår modell sannolikt inte på nedsatt genomblödning.

I manuskript III visar vi att olika katekolaminer har olika effekt på blodtrycket och är olika benägna att orsaka SIC i vår modell. Vi visar också att manipulation av blodtrycket tidigt i förloppet påverkar utvecklandet av SIC.

I manuskript IV visar vi att hemodynamiska faktorer, t.ex. blodtryck och hjärtats fyllnadstryck, skiljer sig åt mellan råttmodeller för SIC och hjärtinfarkt såväl som mellan patienter med SIC och hjärtinfarkt. Vi visar också att behandling av blodtrycket (senare i

förloppet jämfört med manuskript III), som ofta är lågt vid SIC, leder till ökad risk att råttorna avlider.

I manuskript V visar vi att behandling med narkosmedlet isofluran förhindrar att råttorna utvecklar SIC.

Sammanfattningsvis har vi etablerat en experimentell modell som kan användas vid framtida studier kring sjukdomsmekanismer och behandling av SIC. Vi har visat att SIC är ett kardiovaskulärt syndrom där hjärtsjukdomen är en komponent samt för första gången påvisat ett framgångsrikt behandlingslaternativ för patienter med SIC. Framtida studier bör bygga vidare på våra fynd avseende kärlträdets roll vid SIC. Vidare bör isofluranbehandling studeras i en randomiserad klinisk studie.

List of manuscripts

The thesis is based on the following manuscripts, referred to in the text by their Roman numerals (I-V):

I: Shao Y, Redfors B, Scharin Täng M, Möllmann H, Troidl C, Szardien S, Hamm C, Nef H, Borén J, Omerovic E.
Novel rat model reveals important roles of β-adrenoreceptors in stress-induced cardiomyopathy.
Int J Cardiol. 2013 Oct 3;168(3):1943-50.

II: Redfors B, Shao Y, Wikström J, Lyon AR, Oldfors A, Gan L, Omerovic E.
Contrast echocardiography reveals apparently normal coronary perfusion in a rat model of stress-induced (takotsubo) cardiomyopathy.
Eur Heart J Cardiovasc Imaging. 2014 Feb 15(2):152-7.

III: Redfors B, Ali A, Shao Y, Lundgren J, Gan L, Omerovic E.
Different catecholamines induce different patterns of takotsubo-like cardiac dysfunction in an apparently afterload dependent manner.
Int J Cardiol. 2014. Jun 15;174(2):330-6.

IV: Redfors B, Shao Y, Ali A, Sun B, Omerovic E.
Rat models reveal differences in cardiocirculatory profile between takotsubo syndrome and acute myocardial infarction.
J Cardiovasc Med. 2014. Aug 8. [Epub ahead of print]

V: Redfors B, Oras J, Shao Y, Seeman-Lodding H, Ricksten SE, Omerovic E.
Cardioprotective effects of isoflurane in a rat model of stress-induced cardiomyopathy (takotsubo)
Int J Cardiol. In print.

CONTENT

Abbreviations

ATP	Adenosine-triphosphate
AMP	Adenosine-monophosphate
ECG	Electrocardiogram
EDA	end-diastolic areas
EKV	ECG-gated kilohertz visualization technique
ESA	end-systolic areas
FAC	fractional area change
LAX	parasternal long axis
LV	left ventricle
SIC	Stress-induced cardiomyopathy

1. INTRODUCTION

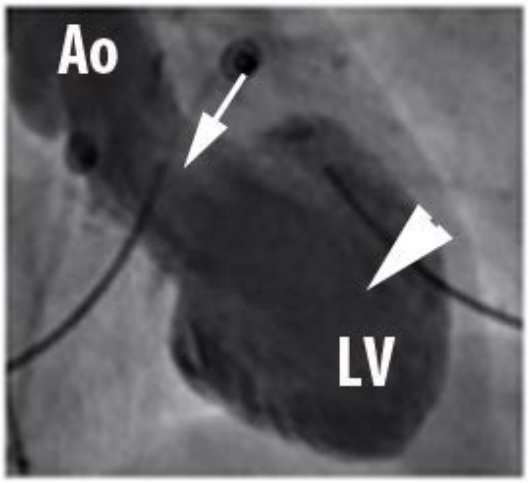

Figure 1. Ventriculogram in a 64-year-old woman with stress-induced cardiomyopathy.

End systolic image of the left ventricle. Note the narrow lumen of the normally contracting basal segments (arrow) and the dilated lumen of the akinetic apical segments (arrowhead).
Ao, aorta; LV, left ventricle.

1.1. Stress-induced cardiomyopathy in brief

Stress-induced cardiomyopathy (SIC) is an increasingly recognized acute cardiac affliction which is characterized by severe regional left ventricular dysfunction that cannot be explained by one or more occlusive culprit lesions of a coronary artery. A preceding somatic and/or emotional stressor can be identified in a majority of these patients (1, 2).

SIC may lead to lethal complications, including malignant arrhythmias, cardiogenic shock and ventricular rupture. However, organ dysfunction is typically reversible if the patient survives the acute phase. SIC is poorly understood and no consensus exists on specific diagnostic criteria, although several proposals have been published (3-9) (Table 1).

1.2 Different morphological types of stress-induced cardiomyopathy

SIC typically presents as widespread akinesia in the apical segments with hyperkinesia of basal segments, so called “apical ballooning” (Fig. 1), but other patterns of cardiac dysfunction exist and are being reported with increasing frequency (1). In this thesis, I will refer to the apical variant as typical SIC and the reverse basal variant, where the cardiac base is akinetic with preserved function in the apex, as atypical SIC. In discussions of the experimental rat models these variants will be referred to as typical SIC-like cardiac dysfunction and atypical SIC-like cardiac dysfunction respectively.

1.2 Epidemiology

In August 2011 we reviewed all published studies on SIC. We found 1042 publications from 42 different countries (1). We showed that SIC is prevalent worldwide and that it may be at least as common in the European and US populations as among Asian subjects, in whom it was first described (1, 10).

Approximately 3-4% of all patients that undergo emergent coronary angiography suffer from SIC and approximately 90% of SIC patients are women. Hence, SIC differs from ischemic heart disease, where men are more commonly afflicted than women (11-13).

1.2 Clinical features of stress-induced cardiomyopathy

SIC has emerged as a rather common entity and is an important differential diagnosis in patients with chest pain. SIC associates with ECG changes indicative of ischemia and elevated plasma levels of cardiac proteins. Although several attempts have been made to distinguish between SIC and acute myocardial infarction based on non-invasive diagnostic criteria, as of yet these two conditions can only be reliably distinguished by invasive procedures, i.e. an absence of explanatory coronary culprit lesions on the angiogram (14-18).

The prognosis in SIC is considerably better than the morphological picture at presentation would suggest. That is, patients with SIC that present with left ventricular akinesia involving >50% of the ventricle often fare well. For comparison, an acute myocardial infarction involving a similarly sized portion of the left ventricle (LV) would invariably lead to death (19). Despite this fact, in-hospital death rates are similar among patients with SIC to the rates among patients with acute myocardial infarction (20-24). Risk factors associated with a poorer prognosis in SIC include male gender, advanced age, a somatic rather than emotional triggering stressor, and low ejection fraction (25, 26).

We currently lack guidelines regarding the treatment and follow-up of SIC patients. No randomized clinical studies on treatment in SIC have been performed and neither American nor European guidelines provide any treatment recommendations for SIC. Instead, these patients are often treated as though they had suffered from an acute coronary event and heart failure, a treatment strategy that may be counterproductive and which may account for some of the deaths among patients with SIC (22).

SIC is also a common complication in the critically ill and has been reported to occur in up to ¼ of all patients hospitalized in intensive care units (24, 27-30). Among these patients SIC associates with particularly high mortality (28, 30). SIC is common (33%) in subarachnoid hemorrhage (5, 31, 32) and in patients who suffer brain-death.

Gothenburg criteria
● Potentially reversible hypokinesis, akinesis or dyskinesis in left ventricular segments and frequently, but not always, a stressful trigger (psychological or physical)
● The absence of other pathological conditions (e.g. ischemia, myocarditis, toxic damage, tachycardia etc.) that more credibly explain the regional dysfunction
● No elevation or modest elevation in cardiac troponin (i.e. disparity between the troponin level and the amount of dysfunctional myocardium)
● Normal, or near normal, left ventricular filling pressure*
● Low, or near normal, peripheral vascular resistance and normal, or near-normal, cardiac output*
* Optional diagnostic criteria that are not mandatory, but when positive increase the likelihood of tako-tsubo syndrome diagnosis

Table 1. Gothenburg criteria for diagnosing stress-induced cardiomyopathy.

The criteria are partly based on experimental and clinical data presented in this thesis.

1.4 The autonomic nervous system, sympaticoadrenal overdrive and SIC

The different factors that may trigger SIC appear to have in common activation of the sympaticoadrenal system. Although the many triggering events described to date are diverse, they can be arbitrarily divided into two categories, namely emotional and somatic stressors. These categories can be considered different parts of a continuous spectrum, linked through the interface of neurology and psychiatry (5) (Fig. 2).

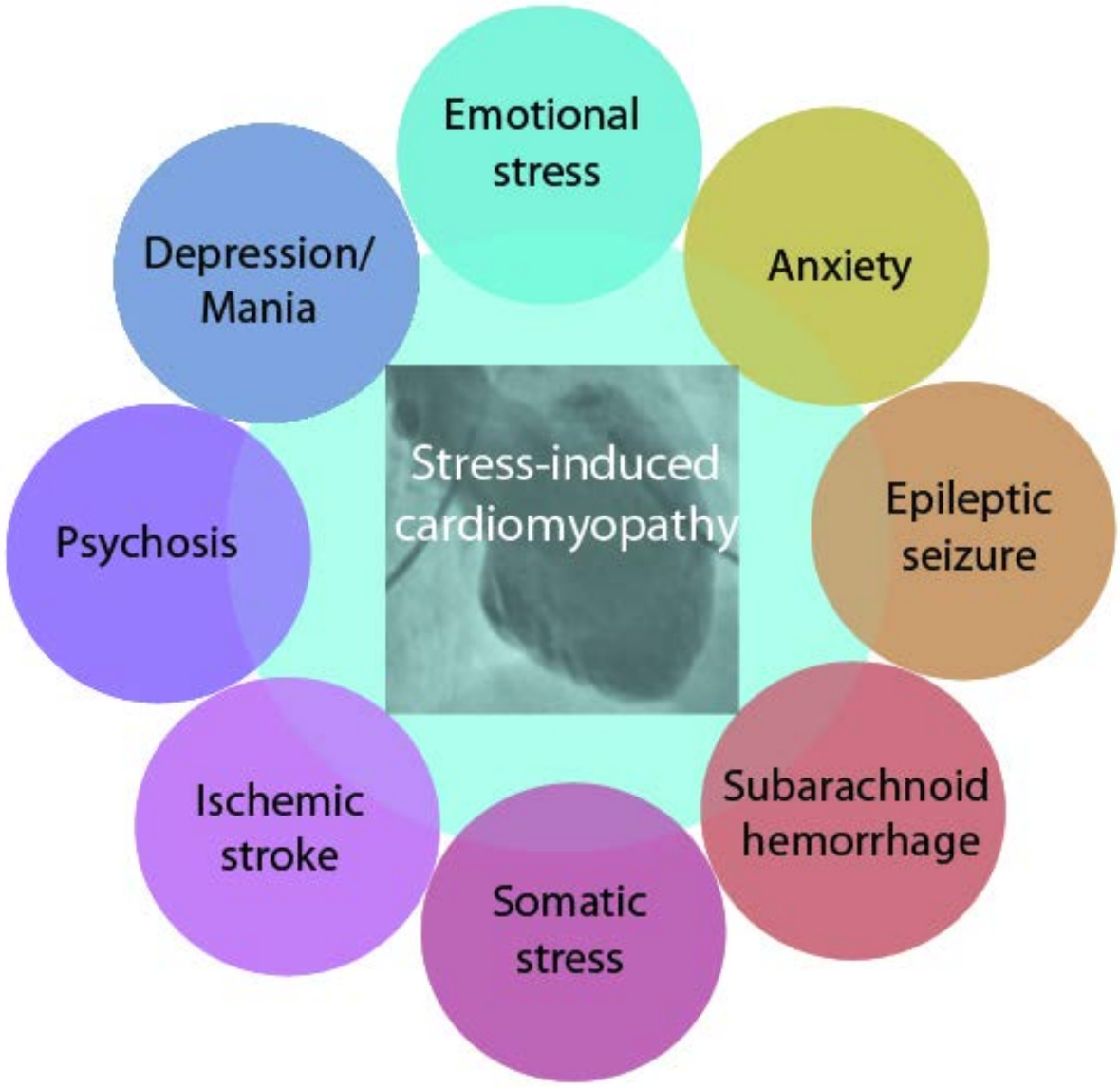

Figure 2. *The various emotional and somatic conditions known to trigger stress-induced cardiomyopathy all associate with overactivity of the sympaticoadrenal system.*

Cardiac protein release and reversible left ventricular dysfunction in the setting of elevated plasma catecholamine levels is common in patients with an acute intracranial episode (33-35).

Several recent case reports have highlighted the connection between subarachnoid hemorrhage and SIC (36, 37).

Similar degenerative post mortem histological findings were seen in the hearts of patients with subarachnoid hemorrhage as in patients with SIC and pheochromocytoma (38, 39). Consistent with observations in the general SIC cohort, among patients that presented with subarachnoid hemorrhage, release of cardiospecific proteins was much more common in women. Furthermore, in a multivariate analysis which also included ejection fraction, cardiac protein release was associated with lower systolic blood pressure (33).

Much like subarachnoid hemorrhage, ischemic stroke appears to be intimately linked to SIC (40, 41). Elevated plasma levels of cardiac proteins are present in almost 20% of these patients and cardiac protein release is associated with a poor prognosis (42).

The third group of intracranial events that has been documented to trigger SIC is epileptic seizures. These patients also display the SIC phenotype with left ventricular apical akinesia and typical patchy myocardial lesions (43). Similar to the situation in patients with subarachnoid hemorrhage and ischemic stroke, as well as in the general SIC cohort, women are overrepresented among epileptic patients that develop SIC (44). The finding that SIC secondary to epilepsy appears to be associated with more severe complications has led some authors to speculate that SIC may explain some cases of sudden unexpected death in epilepsy (44).

It should not be considered a great leap from the intimate relationship between SIC and acute lesions of higher cerebral centra, including the insular area (40), to the relationship between SIC and emotional stressors. There are reports that exacerbations of psychiatric illnesses have in themselves acutely triggered SIC (45). That acute, severe emotional stress can trigger SIC has been known since the syndrome was described, and this observation led to coining the terms “stress-induced cardiomyopathy” and “broken heart syndrome”.

1.5 Catecholamine in stress-induced cardiomyopathy

The etiology, epidemiology and pathophysiology of SIC remain largely elusive, but catecholamines are believed to play a pivotal role. First, the intimate relationship described above between the sympaticoadrenal system and SIC implies a role for catecholamine in SIC. Other evidence in favor of an important role of catecholamine in the pathogenesis includes the finding that plasma catecholamine levels are severely elevated in patients with SIC, up to three times higher than in patients presenting with acute myocardial infarction and Killip class III (39). SIC is also common in patients with pheochromocytoma (39, 46), and iatrogenously administered beta adrenoreceptor agonists have been documented to trigger episodes (39, 47, 48).

However, beyond adrenergic overstimulation, the pathophysiology behind SIC and the mechanisms underlying the remarkable functional recovery are unknown.

1.6 Role of age, gender and hormonal status

The high incidence of SIC among post-menopausal women, in whom the ovarian follicles have disappeared and no longer produce estrogen or progesterone, begs the question whether a connection exists between female hormone status and susceptibility to develop SIC (49). Studies in an early rat model suggested that estrogen provides some degree of protection against SIC (50, 51). The rat model discussed in this thesis could be used to address these issues, but the question is beyond the scope of this thesis.

1.7 Current hypotheses regarding the pathophysiology in stress-induced cardiomyopathy

Four main hypotheses regarding the pathogenesis of SIC have been proposed. Sato et al., who in 1990 described the first case of SIC, proposed that microvascular dysfunction and coronary artery spasm cause an ischemic insult on downstream cardiomyocytes (10). This hypothesis is supported by findings in a few case series (52).

Others have proposed that a spontaneously dissolved occlusive coronary artery thrombus in one of the main coronary arteries explains SIC (53). The different patterns of SIC are explained by differences in the culprit vessel and by heterogeneities in the coronary vasculature. In SIC, the affected myocardium often extends beyond the territory traditionally considered to be supplied by one single main coronary artery. The current hypothesis explains this finding either by locating the thrombus to the left main coronary artery, by the presence of a vascular anatomical variant called wrap-around left anterior descending coronary artery which supplies the entire left ventricular apex, or by postulating that simultaneous thrombi occur in more than one main coronary artery (53). A causative regional supply defect is assumed by both these hypotheses (4, 52, 53).

The third hypothesis postulates that excess regional cardiac catecholamine release overloads adrenergic receptor signaling systems in certain cardiac regions (54). Local catecholamine is released not only from sympathetic nerves but also from intra-cardiac stores (55, 56). Regional variations within the LV in catecholamine production and/or release are postulated to explain why some areas are more susceptible to local surges in catecholamine.

Finally, the hypothesis that has gained the most support of late suggests that SIC-like cardiodepression is caused by direct effects mediated by epinephrine on the β2-adrenoreceptor (57). Norepinephrine, which does not act on the β2-adrenoreceptor is postulated not to cause SIC. According to the hypothesis of these authors, epinephrine excess activates the β2-adrenoreceptor, which in turn couples to inhibitory G-proteins and shuts down the contractile apparatus (57). The apical cardiac dysfunction that is observed in most patients with SIC is explained by a postulated base-to-apex β2-adrenoreceptor gradient.

1.8 Rationale for using rat models to study stress-induced cardiomyopathy

Experimentation on animals is ethically debatable and, even if one accepts the concept of human exceptionalism, upon which all reasonable arguments favoring animal experiments rests, important concerns exist that require careful contemplation (58). Animal models of clinical disease are typically exaggerated and sometimes sub-optimally represent the desired patient category. Only a minority of all discoveries made in animal models have been successfully translated to clinical praxis (59, 60). Therefore, all animal experimentalists are obligated to carefully weigh the expected scientific value of experiments against the suffering of the study subjects. Only studies that are likely to yield important insights into disease and/or therapeutic mechanisms and that cannot be performed in human subjects or *in vitro* should be considered (61).

However, clinical studies suffer from important limitations, and not all scientific questions can be addressed in *in vitro* models. For example, causative mechanisms are difficult to address once a disease has already been diagnosed. SIC is a dynamic syndrome and it is important to recognize that parameters recorded at the time of presentation to the emergency department may be poor surrogates for the same parameters at the time of symptom onset. We therefore consider animal models to be required, as a complement to clinical studies, in our quest to decipher the pathophysiology behind this enigmatic syndrome.

Rat models have played an important role in the advancement of heart failure therapy. Studies in the rat laid the groundwork for the introduction of the concept of angiotensin converting enzyme inhibition in heart failure and have helped to elucidate the mechanisms of action of modern pharmacological agents which are used in the treatment of heart failure, including beta-blockers (62-64).

2. AIM

The general aim of this thesis was to establish a reliable animal model of SIC-like cardiac dysfunction, begin to decipher the pathophysiology behind the disease and identify treatment strategies that could be beneficial for patients with SIC.

The specific aims for each individual manuscript were as follows:

I: To establish a reliable animal model that reproduces clinical phenomena observed in SIC and that can serve as an experimental platform for studying the pathophysiology of SIC and the effect of various treatment strategies

II: To decipher whether perfusion defects are the underlying mechanism behind isoprenaline-induced regional SIC-like apical dysfunction in rats

III: To study which catecholamine induces SIC-like cardiac dysfunction in rats and how these catecholamines affect hemodynamic and cardiac functional parameters; to draw inferences regarding the importance of adrenoceptor subtype and/or hemodynamic factors in the pathophysiology of the SIC

IV: To study the differences in cardiocirculatory status, i.e. hemodynamic parameters, between rat models of SIC-like cardiac dysfunction and acute myocardial infarction; to assess the same parameters in the respective patient cohorts in an effort to validate the experimental models; to evaluate the effect of adherence to clinical praxis, i.e. intervention against hypotension, in the experimental SIC model

V: To study the effect of parenteral and gaseous anesthetic agents in the rat model of SIC-like cardiac dysfunction; to identify target treatment strategies that have the potential to be translated to clinical medicine

3. METHODOLOGICAL CONSIDERATIONS

Detailed descriptions of the methods used are presented in the respective manuscripts at the end of the thesis. Only a brief discussion of the most essential methods is included here.

Animals

10-week-old Male Sprague Dawley rats were used in all studies. We chose to use male rats based on preliminary experiments that showed less data variance in groups of male rats. Less variance in the data increases statistical power and allows for the use of smaller groups, which is preferable both from financial, and more importantly, ethical standpoints. Because SIC-like cardiac dysfunction could be reproducibly induced in male rats, we believe that pathophysiological processes can be adequately addressed in these models. However, the fact that 90% of SIC patients are female needs to be acknowledged and kept in mind.

Arterial and venous canulation and assessment of hemodynamic parameters

The right carotid artery and jugular vein were dissected free and cannulas were inserted for continuous monitoring of arterial pressure and delivery of intravenous infusions, respectively. Different approaches were used to acquire data on invasive hemodynamics in the different manuscripts, which should be taken into consideration when extrapolating and comparing data across the manuscripts.

Small animal echocardiography

Echocardiography was performed on anesthetized rats. Left ventricular SIC-like cardiac dysfunction as well as cardiac function were assessed in cine loops acquired in the parasternal long axis projection by an ECG-gated acquisition technique. Degree of SIC-like cardiac dysfunction was traced along the endocardial border and expressed as percentage of total LV endocardial length. A parasternal long axis projection was chosen because it is the most

reproducible projection in small animals (65). Consistent positioning of the probe can be verified by inclusion of the aortic valve, the mitral valve and the maximal left ventricular lumen area. See manuscripts for further detail.

With regard to contrast echocardiography, we initially also desired to estimate absolute perfusion of the left ventricular apex (dysfunctional) and base (functional) at each time point. We aimed to achieve this by infusing a known volume of contrast at a constant rate at pre-defined time intervals before each assessment. However, pilot experiments revealed the difficulty of assuring an equal volume of contrast at each infusion. That is, the contrast agent precipitated in the syringe and therefore required stirring between each assessment. For practical reasons, we therefore decided to settle for only comparative perfusion assessment between apical and basal regions. Importantly, only assessment of relative perfusion has been validated in small animal contrast echocardiography, a fact that supported that decision (66). See manuscript II for details on how contrast echocardiography was performed.

See the respective manuscripts for details on other methods and statistical analyses.

4. RESULTS AND CONCLUSIONS

The main results and conclusions of the respective manuscripts are summarized below. For more detail, see the respective manuscripts at the end of the thesis.

Manuscript I:

Novel rat model reveals important roles of β-adrenoreceptors in stress-induced cardiomyopathy

This manuscript introduces a rat model of SIC. We show that intraperitoneally administered isoprenaline induces SIC-like cardiac dysfunction in Sprague-Dawley rats. We show that this model reproduces the clinical phenomena observed in patients with SIC, including characteristic morphology, electrocardiographic findings, histological findings and adverse events. Most importantly, we show that cardiac dysfunction recovers after five to seven days, an important characteristic of clinical SIC.

We conclude that isoprenaline may induce Takotsubo-like cardiac dysfunction in rats. Because isoprenaline is a β-adrenoceptor agonist, this finding indicates that β-adrenoceptors play a role in Takotsubo.

Manuscript II:

Contrast echocardiography reveals apparently normal coronary perfusion in a rat model of stress-induced (Takotsubo) cardiomyopathy

We show that coronary perfusion, as assessed by small animal contrast echocardiography, appears to be similar between apical (dysfunctional) and basal (normally functioning) myocardial segments. The ratio of estimated apical:basal perfusion remained near 1.0 at all time points (i.e. 5, 10, 20, 30 ,40 50, 60, 70, 80 and 90 minutes post catecholamine) and 95% confidence intervals never dropped below 0.75%. These findings indicate that apical

perfusion, compared to basal perfusion, is not severely impaired at least until 90 minutes post catecholamine. Because apical dysfunction developed approximately 40 minutes post catecholamine, impaired apical perfusion defects were unlikely to explain SIC-like dysfunction in this model.

We conclude that perfusion defects appear not to precede, and therefore do not explain, isoprenaline-induced SIC-like cardiac dysfunction in our rat model.

Manuscript III:

Different catecholamines induce different patterns of takotsubo-like cardiac dysfunction in an apparently afterload dependent manner

We show that, when tested at six different doses in the dose range 1% - 100% of the maximum tolerated dose, the catecholamines isoprenaline, epinephrine, norepinephrine, phenylephrine and dopamine were all associated with SIC-like cardiac dysfunction in the rat. All catecholamines except isoprenaline caused an increase in blood pressure and were associated with atypical SIC-like dysfunction (i.e. basal akinesia with preserved apical function). Isoprenaline lowered blood pressure and was associated with typical SIC (apical akinesia with preserved function in basal segments).

By lowering the blood pressure to below 120 mmHg by non-catecholamine vasodilators in the hypertensive models, we shifted the phenotype to typical apical akinesia with preserved basal function. Increasing blood pressure to above 120 mmHg in the isoprenaline model attenuated SIC-like apical dysfunction but did not result in basal dysfunction.

We conclude that, in these catecholamine-based rat models of Takotsubo-like cardiac dysfunction, high blood pressure associates with basal cardiac dysfunction whereas low blood pressure associates with apical dysfunction. We propose that hemodynamic factors

may be important in SIC and that SIC should be considered a cardiocirculatory syndrome rather than a mere cardiomyopathy.

Manuscript IV:

Rat models reveal differences in cardiocirculatory profile between takotsubo syndrome and acute myocardial infarction

We show that left ventricular filling pressure is increased and cardiac output decreased in rat models of acute myocardial infarction whereas isoprenaline-induced SIC-like cardiac dysfunction in rats is associated with near-normal left ventricular filling pressure and preserved or increased cardiac output. We also show that, similar to the situation in rats, left ventricular filling pressure is near-normal in SIC patients.

We go on to show that adherence to current clinical praxis and intervention against hypotension in the rat model of SIC caused increased acute mortality.

On the basis of these observations we conclude that SIC differs hemodynamically from acute myocardial infarction, i.e. the two syndromes differ in their respective cardiocirculatory profiles. We conclude that, in the rat model, the animals are hemodynamically compensated despite low blood pressure and that our attempts to increase blood pressure resulted in increased mortality.

We interpret these findings to further support the concept of a SIC syndrome that may require specifically tailored therapy, rather than a mere cardiomyopathy.

Manuscript V:

Isoflurane anesthesia preserves left ventricular regional deformation patterns in a rat model of stress-induced cardiomyopathy (Takotsubo)

Inhalation of isoflurane protected rats against isoprenaline-induced SIC-like cardiac dysfunction. A statistically significant protective effect of isoflurane was consistently observed in two consecutive experimental set-ups. Isoflurane's protective effect persisted if mitochondrial ATP-sensitive potassium channels were blocked by glibenclamide, an intervention that blunts isoflurane's protective effect against ischemia-reperfusion injury. Furthermore, multivariate models indicate that isoflurane's protective effect cannot be completely explained by its effects on hemodynamic parameters.

We conclude that isoflurane is protective against isoprenaline-induced SIC in rats. We propose that isoflurane may be the anesthetic of choice when anesthetizing in-hospital patients at increased risk of developing SIC.

5. DISCUSSION

5.1. The pathophysiology behind stress-induced cardiomyopathy

Our experiments have led to several new insights and hypotheses regarding the pathophysiology behind SIC. In **manuscript I** we show that an intraperitoneally delivered isoprenaline bolus reproduces the clinical phenomena of SIC (67). Our findings were recently reproduced by an independent group (68). In **manuscript II** we show that isoprenaline-induced SIC-like cardiac dysfunction in our model does not associate with significant perfusion defects. Isoprenaline, a β-adrenorecetor selective catecholamine, is associated with positive inotropic and chronotropic effects as well as with peripheral vasodilation. At high doses, isoprenaline increases systemic metabolism and significantly raises body temperature.

Epinephrine, norepinephrine, phenylephrine and dopamine on the other hand also act on alpha adrenergic receptors and cause peripheral vasoconstriction rather than vasodilation, thus increasing blood pressure. Consistent with previous reports, in **manuscript III** we observed significantly increased blood pressure in rats that were administered these agents. Contrary to isoprenaline, these drugs preferentially caused atypical forms of SIC-like dysfunction where the dysfunctional region is located in the basal regions of the heart. The positive inotropic effects of these drugs on the heart could be expected to be particularly strong in the rat heart, in which alpha receptor subtypes make up a greater portion of adrenergic receptors compared to humans.

Intriguingly, interventions to lower blood pressure in these models resulted in a shift towards the apical variants of SIC. Equally intriguing was the finding that interventions aimed at increasing blood pressure in the isoprenaline model prevented the development of SIC-like apical dysfunction. These findings indicate that alterations in the characteristics of the peripheral vasculature and their effect on left ventricular afterload play an important role in SIC.

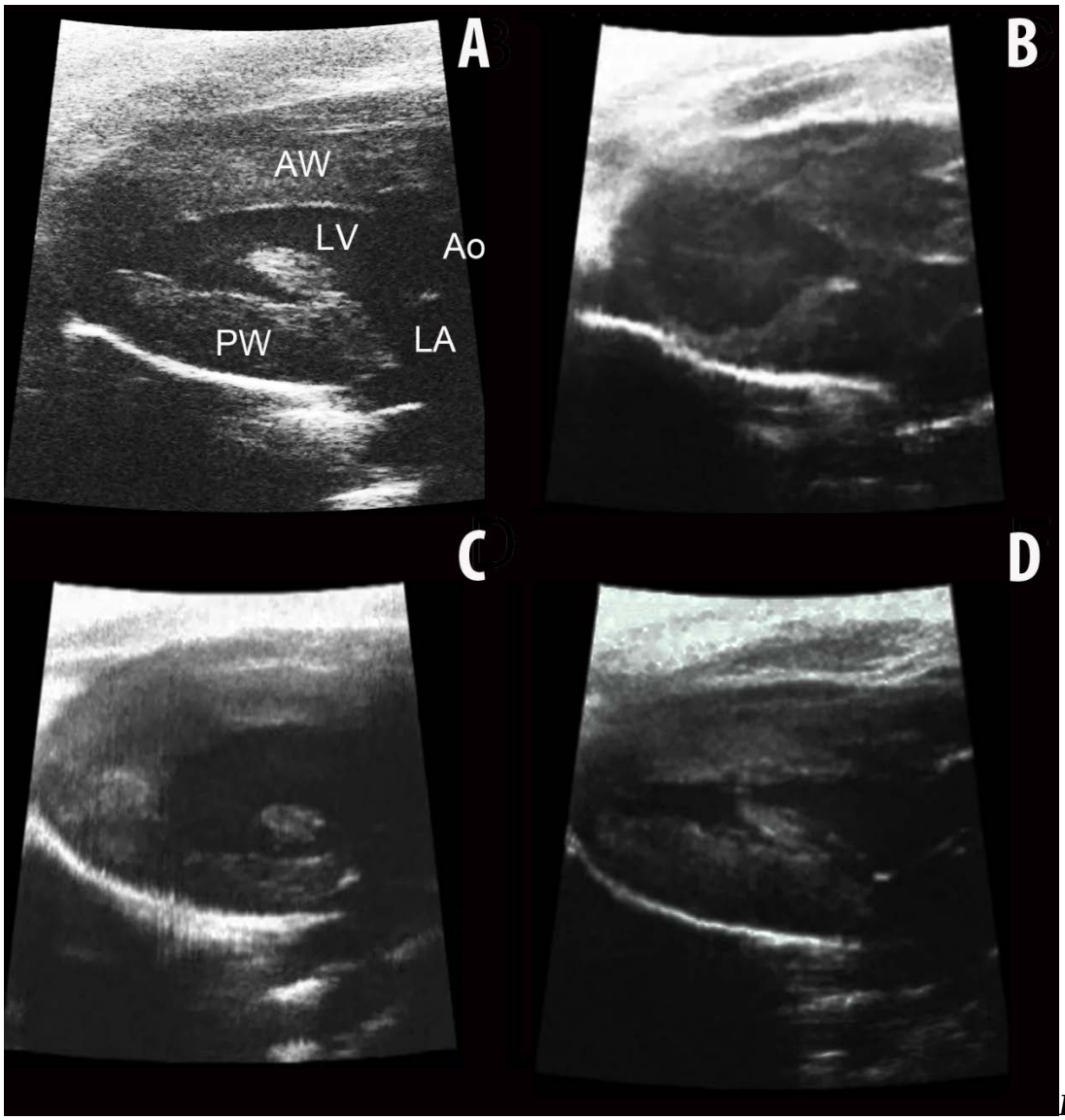

Figure 3.

End-systolic images of SIC-like cardiac dysfunction in rats.

Parasternal long axis view. Under normal conditions, the entire LV deforms uniformly (B). Catecholamine-administration in rats may induce SIC-like apical (C) or basal (B) dysfunction. Cardiac function was normalized seven days post catecholamine administration (E).

Ao, aorta; AW, anterior wall; DOP, dopamine; EPI, epinephrine; ISO, isoprenaline; i.p., intra-peritoneal; i.v., intravenous; LA, left atrium; LV, left ventricle; NOR, norepinephrine; SIC, stress-induced cardiomyopathy; PHE, phenylephrine; PW, posterior wall.

Our data presented in **manuscript IV** indicate that a significant outflow tract obstruction may occur in the isoprenaline-based rat model. Hyperthermia, increased inotropy, tachycardia and left ventricular outflow tract obstruction could all serve to increase myocardial oxygen demand, particularly in the apical regions of the heart. That is, if significant outflow obstruction is present, one would expect the luminal volume to be greatest near the apex.

We propose that, because of differences in arterial blood pressure profiles in the different models as well as potential differences in conduction and regional contractile patterns, the distribution of regional wall tension within the left ventricle favors the development of different patterns of SIC-like cardiac dysfunction. Low arterial blood pressure and strong inotropic drive may cause a near obliteration of the left ventricular lumen during systole, which would be expected to significantly afterload apical regions in the heart. High arterial blood pressure on the other hand counteracts the strong inotropic drive, resulting in less pronounced cardiomyocyte shortening and different patterns of regional wall stress distribution. The adrenergic system also affects the spatiotemporal pattern of cardiac contraction. Differences in regional activation patterns could also be of importance in distributing systolic wall stress among LV regions (Fig. 1)(69).

5.1.1 A new pathophysiological hypothesis

We propose that regional mechanical overload of given parts of the heart play an important role in SIC (70). Cardiomyocytes within these regions may be rendered “metabolically insufficient”. In other words, these cardiomyocytes experience a demand:supply mismatch on the basis of excessive metabolic demand. SIC may in essence be a protective organ response, i.e. protective metabolic shutdown or acute down regulation of non-vital cellular functions, which serves to protect the affected regions from necrosis. This would explain the apparently complete recovery observed in SIC.

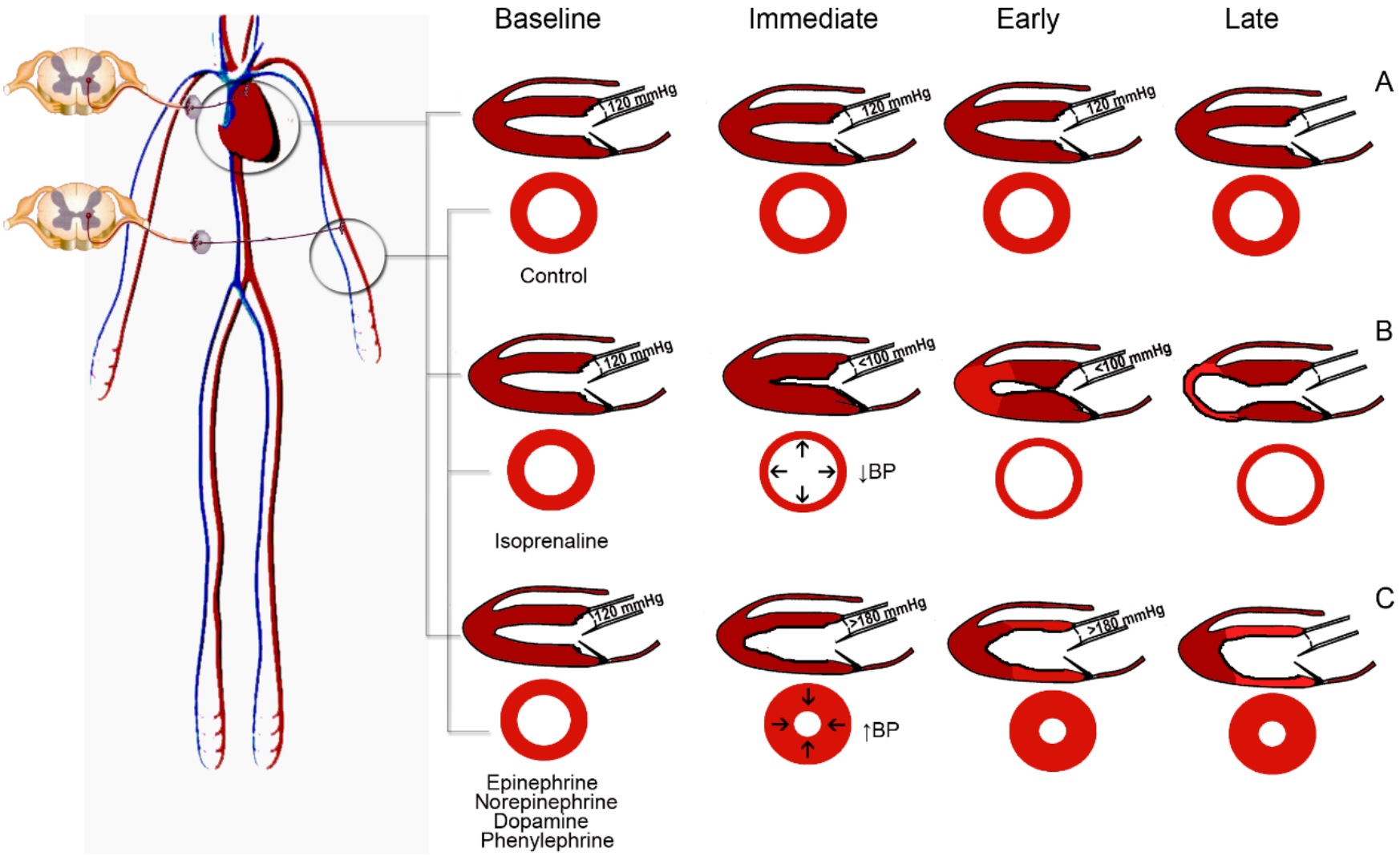
Baseline
Immediate
Early
Late
A
120 mmHg
Control
B
<100 mmHg
↓BP
Isoprenaline
C
>180 mmHg
↑BP
Epinephrine
Norepinephrine
Dopamine
Phenylephrine

Figure 4. Proposed mechanisms behind apical and basal SIC-like cardiac dysfunction.

*End-systolic illustrations of the heart and muscular arteries during a normal cardiac cycle (A), post administration of isoprenaline (B) or other catecholamines (C). A. During a normal cardiac cycle, the rat left ventricle contracts uniformly. Neural reflex arcs ensure appropriate coupling between the heart and the arterial system by adaptions in blood vessel tone and/or force of cardiac contractions. B. After an isoprenaline challenge, left ventricular inotropy increases, making systolic contractions more forceful. At the same time, peripheral vasodilation causes a drop in arterial pressure and reduces left ventricular afterload. These strong vasodilator and inotropic stimuli override autonomic reflex arches. Thus increased contractility and reduced afterload cause the ejection fraction to increase, with near obliteration of the left ventricular lumen during systole (immediate). After some time, apical akinesia develops (late). We propose that transient left ventricular outflow tract obstruction, which develops when the ventricle contracts too forcefully, creates a pressure gradient between the apex and the arterial system, raising wall tension in regions located more apical than the obstruction (early). According to Laplace's law (**T**=**P**x**r**/**h**), high pressure in combination with greater volume in apical regions may overload these cardiomyocytes and lead to loss of function. C. After a challenge with the other catecholamines, all of which were associated with high blood pressure, basal akinesia with preserved function in the apical area was observed. Again, the strong adrenergic stimulus may override compensatory autonomic reflex arches. If peripheral vascular resistance is high, then global left ventricular afterload, which also affects the cardiac base, develops. The left ventricle needs to develop more tension before the afterload is overcome, ejection fraction decreases rather than increases despite increased contractility, and luminal obliteration does not occur. Because the heart is constructed to optimize fluid dynamics, i.e. a current from apex to base, we propose that in the setting of high afterload more*

early shortening takes place in apical regions and it may instead be basal regions that experience higher wall stress.

BP, blood pressure; h, regional wall thickness; P, luminal pressure; r, regional luminal radius; T, wall tension;

5.1.2. Ischemic stunning and stress-induced cardiomyopathy

An episode of sub-lethal ischemia causes ischemic stunning, i.e. loss of contractile function, as well as preconditioning. These two phenomena confer protection against subsequent episodes of ischemia. If an acute coronary syndrome follows, the final infarct size will be smaller than it had been in the absence of the preceding ischemic episode (71). Because the majority of ATP is consumed by the contractile apparatus under normal conditions, down regulating or shutting down the contractile apparatus can preserve energy metabolites that can instead be used for maintenance of vital cellular functions. Therefore, myocardial stunning is most likely an important protective mechanism. Such metabolic down regulation can be expected to be even more effective in SIC, where coronary perfusion is not severely impaired (72). The cell is instead exposed to too high wall stress, which poses high demands on the contractile apparatus and increases the energy demand. By shutting down contractile activities, the heart can completely escape this threat. In fact, biopsies obtained from patients with SIC, similar to ischemically preconditioned tissue, show evidence of activated cell survival signalling (73). SIC may therefore be a form of protective ischemic stunning that serves to protect the heart against excessive demand. Alternatively, SIC may be an inappropriately triggered stunning response to perceived ischemia (71, 74). That is, the cell may misinterpret excessive metabolic demand as insufficient nutrient supply.

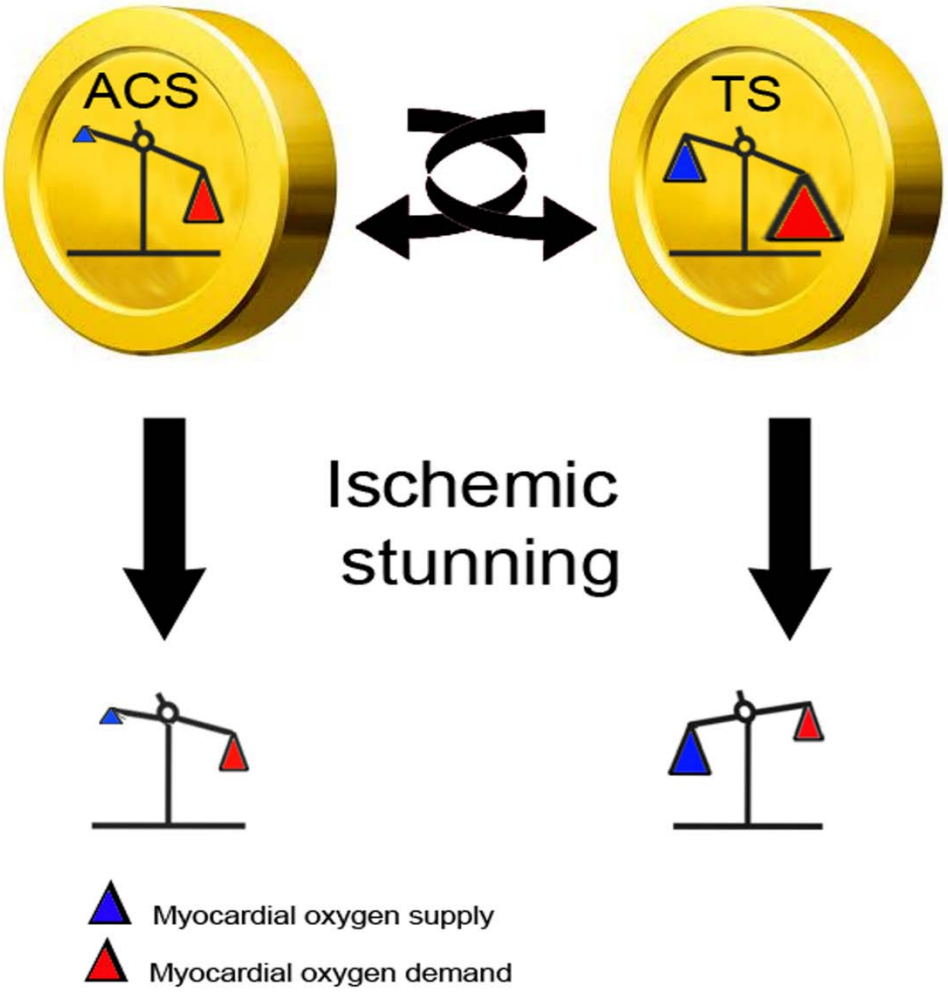

Figure 5. The link between SIC and acute coronary syndromes.

We propose that SIC is a form of ischemic stunning. Similar to ischemic stunning in the setting of acute coronary syndromes, the trigger may be a sense of ischemia, i.e. a shortage of oxygen supply relative to oxygen demand. However, in SIC, the basis for this imbalance is excessive oxygen demand rather than a shortage of oxygen supply secondary to occlusion of a coronary artery. Because oxygen supply remains adequate for maintenance of basal cellular functions, by shutting down contractile activities, ischemic stunning effectively corrects the supply:demand mismatch in SIC.

ACS, acute coronary syndromes; SIC, stress-induced cardiomyopathy.

5.1.3 Cardiac dynamics: Conduction and contraction

Cardiac myofiber architecture and the spatiotemporal sequence of regional electrical activation during systole and diastole determine the pattern of myocardial deformation during the cardiac cycle and assure an equal distribution of regional wall stress. For a more detailed summary of the dynamics of cardiac deformation, the reader is referred to an excellent review by Sengupta et al. (75).

Redistribution of wall stress plays an important role in cardiac disease, including chronic ischemic heart disease. A reduction in wall thickness can be observed in early activated regions during chronic asynchronous electrical activation (76). Eccentric and concentric hypertrophy are adjustments made by the LV to restore wall tension. Although the LV can compensate for these "milder" forms of persistent wall stress heterogeneities in the chronic setting, this may not be the case in acute SIC. In this setting, the regional increase in wall stress may occur so fast and may be of sufficient magnitude that the LV does not have time to adapt.

In fact, a recent report on eight female patients with SIC found significantly increased LV end-systolic pressure in the acute phase (77). Because adult females on average have lower normalized LV volume and mass than men and LV mass is known to decrease with advanced age, elderly females may be at increased risk of developing high LV wall stress (78). This could explain the predominance of elderly females among patients with SIC.

Left ventricular outflow tract obstruction, which significantly increases the wall tension of apical regions within the LV, is common in patients with SIC (79). Importantly, LV outflow obstruction is a dynamic condition and may have resolved once the patient undergoes echocardiographic examination. Hence, an absence of LV outflow tract obstruction on echocardiography does not exclude its presence in the acute phase, i.e. as a causative factor for SIC.

5.1.4. Role of catecholamine in stress-induced cardiomyopathy revisited

The role of catecholamine in SIC may in fact be predominantly “mechanical”. High levels of catecholamine may induce high wall stress and increase oxygen demand in selected areas in the left ventricle through its effects on contractile patterns and afterload. However, even if mechanical overload and excess metabolic demand in selected parts of the LV would be a prerequisite for SIC, direct effects of catecholamine on the cardiomyocytes may still contribute in the pathogenesis. That is, individual cardiomyocytes may respond differently to excessive demand in the absence of adrenergic input. Catecholamine plays a role in ischemic preconditioning and could do so also in SIC (Fig. 4) (71, 74).

5.2. Competing hypotheses revisited

As discussed in the introduction, three main hypotheses regarding the pathogenesis of SIC have been proposed previously, two of which include causative perfusion defects (52, 53).

5.2.1. Causative perfusion defects

Reversible perfusion defects in the left ventricular apex in patients presenting with SIC have been documented. However, these perfusion defects were detected well after cardiac dysfunction had developed (80, 81). Hence, these observations do not tell us whether or not perfusion defects were present before and/or during the development of cardiac dysfunction. In fact, since approximately 80% of myocardial energy is consumed by the contractile apparatus, one can expect myocardial energy demand to decrease significantly in the akinetic areas in SIC (82). Not surprisingly, myocardial fatty acid metabolism is decreased in acontractile regions of the heart in patients with SIC (81). Because autoregulatory mechanisms ensure a close correlation between myocardial energy requirements and regional perfusion, decreased perfusion in these regions is an expected consequence of an episode of SIC (74, 83). In **manuscript II** we show that, at least in our rat model, no detectable regional perfusion defects

precede the development of SIC-like cardiac dysfunction. Because cause should precede outcome, we can conclude that catecholamine-induced SIC-like cardiac dysfunction may occur in rats in the absence of causative regional perfusion defects.

In clinical SIC, cardiac dysfunction typically extends beyond the vascular territory of a single main coronary artery (5, 70). This observation refutes the hypothesis of an isolated occlusive thrombus. For the hypothesis stipulating that coronary vasospasm causes SIC to hold, vasospasm would have to occur in both major coronary arteries simultaneously.

The focus of cardiovascular researchers has recently partly been shifted from the macrocirculation, i.e. large epicardial conductance vessels, to the arterioles and capillaries in the microcirculation (84-87). A limited ability of the microvasculature to compensate for significant stenoses in upstream conductance vessels has been shown to be associated with symptomatic angina pectoris and adverse cardiovascular events. Intriguingly, recent studies report an increased risk of a cardiovascular event also in patients without macroscopic coronary artery disease but in whom the coronary microvascular response to vasodilators is impaired (84).

Coronary microvascular function has been demonstrated to be diffusely impaired in patients with the SIC syndrome shortly after presentation (88-90). Again, microvascular dysfunction that is documented after cardiac dysfunction has already developed may represent the consequence rather than the cause of SIC-like cardiac dysfunction (83, 91, 92). For example, myocardial edema is known to be present in akinetic regions and may impair microvascular function (83, 91).

As we have previously proposed, however, perfusion defects may play a permissive or facilitator role in SIC (70). That is, diffusely impaired microvascular or macrovascular function, i.e. impaired perfusion reserve, may render the myocardium more sensitive to acute increases in afterload. In other words, SIC may still be an ischemic

cardiomyopathy in the sense that the metabolic demand placed on cardiomyocytes in the affected region exceeds the capacity of the vascular system to supply the cells with sufficient oxygen and/or nutrients (Fig. 4). Although the main message in this thesis is that a demand:supply mismatch may occur despite normal coronary perfusion, we believe that there may still be a role for impaired perfusion, i.e. supply defects, in some cases of SIC. The threshold for induction of SIC-like cardiac dysfunction may be decreased in regions with impaired coronary flow. In manuscript II we show that perfusion of the apical segments was not significantly decreased compared to perfusion of basal segments. However, we did not measure perfusion in absolute terms and cannot exclude that perfusion defects were present in both regions (72).

Perfusion defects may also cause regional ischemia of remote areas during the early phase of an acute increase in afterload. This may result in slowed or altered conduction in these areas, which in turn may translate to dyssynchronous cardiac contractions and redistribution of wall stress to other regions. It could then be the cardiomyocytes in these regions rather than in the remote, mildly ischemic region that are exposed to extraordinarily high wall tension and are rendered metabolically insufficient (93).

5.2.2. Excess regional cardiac catecholamine release

During stress, catecholamine reaches the heart through three distinct sources, namely from sympathetic nerve terminals, from the bloodstream and from local stores within the heart itself (54). Studies suggest that norepinephrine is predominantly released from sympathetic neurons whereas less than half of the epinephrine is thought be neuronal in origin (94-96). Sympathetic nerve terminal density has been shown to be higher in the cardiac base than in mid or apical regions (97, 98).

However, heterogeneities in the regional release of catecholamine do not appear to be necessary for the development of experimental SIC. In our rat models, systemically

administered catecholamine reproducibly induces SIC. In these models, the majority of catecholamine reaches the heart from the bloodstream and would be expected to distribute uniformly to different regions of the LV. Hence, local surges of catecholamine are unlikely to explain SIC-like cardiac dysfunction in our model. Furthermore, clinical SIC may also occur after systemically administered catecholamine and may occur in the absence of an intact autonomic nervous system (47, 48).

It should be mentioned that regional variations in catecholamine synthesis and/or release may help to explain why some regions of LV are affected in SIC whereas other regions are not, also in the absence of regional differences in catecholamine concentrations during the acute phase. Numerous studies have shown that catecholamine exposure influences the expression and functional characteristics of adrenergic receptors (99-101). Hence, certain regions may be more sensitive to excess catecholamine than others at the time of the event. Although more research is needed, heterogeneities in regional catecholamine release and/or receptor function could play a role in the metabolic shutdown which we propose occurs secondary to too high afterload. Alternatively, these regional heterogeneities play a key role in the development of SIC through mechanisms other than those proposed in this thesis.

5.2.3. β2-adrenoceptor/Gi-depedent cardiodepression

Another hypothesis regarding the pathophysiology behind the SIC syndrome attempts to explain the affliction by β2-adrenoceptor mediated inhibition of the contractile apparatus (57). Catecholamine exerts its effects through adrenoceptors. At the level of the heart, catecholamine typically increases inotropy and lusitropy through activation of β-adrenoceptors. At physiological levels of the endogenous catecholamines, β1 and β2-adrenoceptors both couple to the Gs protein family, thereby increasing levels of cyclic AMP through the activation of enzyme adenylate cyclase (102, 103). Increased levels of cyclic AMP translates to increased

contractile activities through activation of protein kinase A, which phosphorylates several downstream intracellular targets (104).

However, at supraphysiological concentrations of epinephrine, the β2-adrenoceptor has been demonstrated to instead couple to the Gi protein family (105, 106). Activation of this protein family has the opposite effect on cyclic AMP concentrations and leads to depressed contractile activities, i.e. negative inotropism (105). The authors propose that excess catecholamine in SIC causes acute cardiodepression through β2-adrenoceptor mediated activation of Gi proteins (57). The selective apical dysfunction observed in typical SIC cases is explained by a postulated apicobasal gradient in adrenoreceptor subtypes, with a predominance of β2-adrenoceptors in the apex (107).

These authors postulated that, because epinephrine acts at β2-adrenoceptors whereas norepinephrine does not, SIC-like cardiac dysfunction can be induced by the former but not by the latter (107, 108). They tested a single dose of each catecholamine and showed that fractional shortening in the apical region decreased after intravenous bolus infusion of epinephrine but not after norepinephrine (107). This decrease in fractional shortening could be prevented by treating the rats with pertussis toxin, an inhibitor of the Gi protein family (107). On the basis of the findings presented in their paper, which include complementary molecular analyses but which rest heavily on the *in vivo* findings described above, the authors considered their hypothesis corroborated (107). Thereafter their hypothesis has emerged as the leading hypothesis regarding the pathophysiology behind SIC.

Fractional shortening is a one-dimensional M-mode echocardiographic measure cardiac wall motion (109). Fractional shortening in the apical region was only decreased in the authors' model, not absent or reversed, which would be expected if true akinesia or dyskinesia was present(107). Thus the authors were not able to induce the regional akinesia that is typical for clinical SIC. At best, they induced hypokinesia of the apical segments. However, the

apparent lack of detectable apical hypokinesia in their supplementary magnetic resonance imaging video sequence, which is supposed to show a representative example, begs the question of whether SIC-like cardiac dysfunction was at all present in their model (107).

In **manuscript III**, we tested several doses of epinephrine and norepinephrine and show that they both may cause clearly detectable SIC-like cardiac dysfunction. Although our observations were made in rat models, so were the observations that led the authors to consider their hypothesis corroborated (107). If one accepts the premises that norepinephrine does not act through the β2-adrenoceptor/Gi-pathway, which is the premise on which the authors' study design and conclusion depend, our findings effectively refute their hypothesis (110).

In a recent review, the authors argue that the observations from two separate groups, theirs and ours (**manuscript III**), that interfering with the β2-adrenoceptor/Gi-pathway ameliorates SIC-like cardiac dysfunction in rats, support the "β2-adrenoceptor/Gi-pathway hypothesis" (67, 107, 111). While it is true that we observed less extensive SIC-like left ventricular dysfunction when we pretreated rats with the Gi-pathway blocker pertussis toxin, we also observed less pronounced dysfunction when we pre-treated the rats with the substance ICI118,551, which is considered to activate the β2-adrenoceptor/Gi-pathway (57, 67, 112). It is unclear in both cases whether the consequences of the intervention can be ascribed effects at the level of the heart or if extra-cardiac effects, including effects on the vasculature, explain the lesser degree of SIC-like cardiac dysfunction in these rats (110). Administration of pertussis toxin, which is a lethal substance, causes significant systemic effects, many of which do not involve interactions with the Gi-pathway in cardiomyocytes, and which in turn set into motion a myriad of secondary processes and reflex arcs(113).

In theory, the β2-adrenoceptor/Gi-pathway hypothesis could fit well into the hypothesis presented in this thesis. That is, the β2-adrenoceptor and Gi pathway could mediate the metabolic shutdown that occurs in response to excessive afterload. Unfortunately, although

the β2-adrenoceptor/Gi-pathway hypothesis appears sophisticated and theoretically appealing, it is currently inadequately supported.

5.3. Treatment of patients with stress-induced cardiomyopathy

5.3.1. Preventing development of stress-induced cardiomyopathy

In the pre-hospital setting, we are unable to identify patients at increased risk of developing SIC. If these patients could be identified, preventive measures could be undertaken to minimize the risk of SIC. On the basis of current knowledge, these patients could be started on β blocker therapy.

In-hospital SIC, however, which is frequently encountered in the critically ill, may be easier to predict. Cardiac dysfunction is associated with a particularly poor prognosis in these patients (30). In these patients, adequate cardiac output is often achieved by balancing inotropic and vasoactive support. Despite the fact that avoiding "inotropic overload" may protect against SIC, this may be a difficult task (27). In manuscript V, we show that isoflurane prevents isoprenaline-induced SIC-like cardiac dysfunction in rats. If these findings can be reproduced in clinical SIC, isoflurane could be an alternative anesthetic regimen for patients with severe somatic disease. In fact, volatile isoflurane sedation in intensive care units has recently been introduced off-label in mechanically ventilated patients with subarachnoid hemorrhage (114, 115).

5.3.2. Treatment in the acute phase

No internationally recognized treatment guidelines exist for SIC. Because these patients appear similar to patients with acute coronary syndromes, treatment guidelines for the latter should be followed until the diagnosis of SIC is firmly established (116, 117). Negative inotropes should probably be avoided in the acute phase as they may attenuate the compensatory hyperkinesia that typically develops in unaffected myocardial segments. In **manuscript IV** we show that SIC associates with a different cardiocirculatory profile than AMI in both rats and in patients.

These findings have been corroborated in a recent report from Australia that reported low pulmonary capillary wedge pressures despite low blood pressures in patients with SIC (118). These findings indicate that SIC patients may be hemodynamically well compensated despite apparent hypotension. On the other hand, it should be mentioned that another recent study reported increased filling pressures in patients with SIC (119). Regardless, we also show that, in rat models, acute mortality increases significantly if interventions are undertaken to maintain blood pressure above 90 mmHg. Therefore, until convincing clinical data are available, we argue for strict adherence to the "*primum nil nocere*" principle when treating patients with SIC. Because untreated SIC is associated with complete clinical recovery in the majority of patients, interventions should only be undertaken if they are deemed absolutely necessary.

6. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

We have reproduced the clinical phenomena associated with SIC in rats by administration of catecholamine.

No regional perfusion defects precede development of SIC in our rat model.

The development and morphological type of SIC appear to depend on hemodynamic factors, indicating that it may be more appropriate to consider SIC a cardiocirculatory syndrome of which the cardiomyopathy is one component.

Left ventricular filling pressure and cardiac output appear to be near-normal in experimental and clinical SIC despite hypotension. Interventions to pharmacologically increased blood pressure increase acute mortality in the rat model.

Isoflurane anesthesia prevents experimental SIC and could be beneficial in patients at increased risk of developing SIC, e.g. patients with severe somatic illness.

Future studies should address which hemodynamic factors play a role in the pathophysiology of SIC and whether or not catecholamine is necessary for developing the cardiomyopathy.

Promising treatment alternatives should be studied in the rat models and, if protective in the models, should thereafter be tested in clinical studies. Isoflurane’s protective effects need to be reproduced in clinical SIC.

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References:

1. Shao Y, Redfors B, Lyon AR, Rosengren A, Swedberg K, Omerovic E. Trends in publications on stress-induced cardiomyopathy. International journal of cardiology. 2012;157(3):435-6.
2. Deshmukh A, Kumar G, Pant S, Rihal C, Murugiah K, Mehta JL. Prevalence of Takotsubo cardiomyopathy in the United States. Am Heart J. 2012;164(1):66-71 e1.
3. Prasad A, Lerman A, Rihal CS. Apical ballooning syndrome (Tako-Tsubo or stress cardiomyopathy): a mimic of acute myocardial infarction. Am Heart J. 2008;155(3):408-17.
4. Wittstein IS. Stress cardiomyopathy: a syndrome of catecholamine-mediated myocardial stunning? Cellular and molecular neurobiology. 2012;32(5):847-57.
5. Redfors B, Shao Y, Omerovic E. Stress-induced cardiomyopathy (Takotsubo)--broken heart and mind? Vascular health and risk management. 2013;9:149-54.
6. Madias JE. Why the current diagnostic criteria of Takotsubo syndrome are outmoded: A proposal for new criteria. International journal of cardiology. 2014.
7. Parodi G, Citro R, Bellandi B, Provenza G, Marrani M, Bossone E. Revised clinical diagnostic criteria for Tako-tsubo syndrome: the Tako-tsubo Italian Network proposal. International journal of cardiology. 2014;172(1):282-3.
8. Kawai S, Kitabatake A, Tomoike H. Guidelines for diagnosis of takotsubo (ampulla) cardiomyopathy. Circulation journal : official journal of the Japanese Circulation Society. 2007;71(6):990-2.
9. S YH. Too many cooks spoil the broth: the currently existing diagnostic criteria for Takotsubo syndrome. International journal of cardiology. 2014;173(3):568-70.
10. Dote K, Sato H, Tateishi H, Uchida T, Ishihara M. [Myocardial stunning due to simultaneous multivessel coronary spasms: a review of 5 cases]. J Cardiol. 1991;21(2):203-14.
11. Tillmanns H, Waas W, Voss R, Grempels E, Holschermann H, Haberbosch W, et al. Gender differences in the outcome of cardiac interventions. Herz. 2005;30(5):375-89.
12. Hemingway H, Langenberg C, Damant J, Frost C, Pyorala K, Barrett-Connor E. Prevalence of angina in women versus men: a systematic review and meta-analysis of international variations across 31 countries. Circulation. 2008;117(12):1526-36.
13. Shin JY, Martin R, Suls J. Meta-analytic evaluation of gender differences and symptom measurement strategies in acute coronary syndromes. Heart & lung : the journal of critical care. 2010;39(4):283-95.
14. Johnson NP, Chavez JF, Mosley WJ, 2nd, Flaherty JD, Fox JM. Performance of electrocardiographic criteria to differentiate Takotsubo cardiomyopathy from acute anterior ST elevation myocardial infarction. International journal of cardiology. 2011.
15. Ahmed KA, Madhavan M, Prasad A. Brain natriuretic peptide in apical ballooning syndrome (Takotsubo/stress cardiomyopathy): comparison with acute myocardial infarction. Coron Artery Dis. 2012;23(4):259-64.
16. Parkkonen O, Allonen J, Vaara S, Viitasalo M, Nieminen MS, Sinisalo J. Differences in ST-elevation and T-wave amplitudes do not reliably differentiate takotsubo cardiomyopathy from acute anterior myocardial infarction. Journal of electrocardiology. 2014.
17. Madias JE. Biomarkers for differentiating Takotsubo syndrome from acute ST elevation myocardial infarction. The American journal of cardiology. 2014;114(2):327.
18. Nascimento FO, Yang S, Larrauri-Reyes M, Pineda AM, Cornielle V, Santana O, et al. Usefulness of the troponin-ejection fraction product to differentiate stress cardiomyopathy from ST-segment elevation myocardial infarction. The American journal of cardiology. 2014;113(3):429-33.
19. Caulfield JB, Leinbach R, Gold H. The relationship of myocardial infarct size and prognosis. Circulation. 1976;53(3 Suppl):I141-4.

20. Sharkey SW, Windenburg DC, Lesser JR, Maron MS, Hauser RG, Lesser JN, et al. Natural History and Expansive Clinical Profile of Stress (Tako-Tsubo) Cardiomyopathy. J Am Coll Cardiol. 2010;55(4):333-41.
21. Lee PH, Song JK, Sun BJ, Choi HO, Seo JS, Na JO, et al. Outcomes of Patients with Stress-Induced Cardiomyopathy Diagnosed by Echocardiography in a Tertiary Referral Hospital. J Am Soc Echocardiog. 2010;23(7):766-71.
22. Burgdorf C, Kurowski V, Bonnemeier H, Schunkert H, Radke PW. Long-term prognosis of the transient left ventricular dysfunction syndrome (Tako-Tsubo cardiomyopathy): Focus on malignancies. Eur J Heart Fail. 2008;10(10):1015-9.
23. Schultz T, Shao Y, Redfors B, Bergmann Sverrisdottir Y, Ramunddal T, Albertsson P, et al. Stress-induced cardiomyopathy in sweden: evidence for different ethnic predisposition and altered cardio-circulatory status. Cardiology. 2012;122(3):180-6.
24. Brinjikji W, El-Sayed AM, Salka S. In-hospital mortality among patients with takotsubo cardiomyopathy: a study of the National Inpatient Sample 2008 to 2009. Am Heart J. 2012;164(2):215-21.
25. Singh K, Carson K, Shah R, Sawhney G, Singh B, Parsaik A, et al. Meta-analysis of clinical correlates of acute mortality in takotsubo cardiomyopathy. The American journal of cardiology. 2014;113(8):1420-8.
26. Madhavan M, Rihal CS, Lerman A, Prasad A. Acute heart failure in apical ballooning syndrome (TakoTsubo/stress cardiomyopathy): clinical correlates and Mayo Clinic risk score. J Am Coll Cardiol. 2011;57(12):1400-1.
27. Redfors B, Shao Y, Omerovic E. Stress-induced cardiomyopathy in the critically ill - why inotropes fail to improve outcome. International journal of cardiology. 2013;168(4):4489-90.
28. Park JH, Kang SJ, Song JK, Kim HK, Lim CM, Kang DH, et al. Left ventricular apical ballooning due to severe physical stress in patients admitted to the medical ICU. Chest. 2005;128(1):296-302.
29. Kosuge M, Ebina T, Hibi K, Tsukahara K, Iwahashi N, Gohbara M, et al. Differences in negative T waves among acute coronary syndrome, acute pulmonary embolism, and Takotsubo cardiomyopathy. Eur Heart J Acute Cardiovasc Care. 2012;1(4):349-57.
30. Redfors B, Shao Y, Omerovic E. Is stress-induced cardiomyopathy (takotsubo) the cause of elevated cardiac troponins in a subset of septic patients? Intensive care medicine. 2014.
31. Lee VH, Connolly HM, Fulgham JR, Manno EM, Brown RD, Wijdicks EFM. Tako-tsubo cardiomyopathy in aneurysmal subarachnoid hemorrhage: an underappreciated ventricular dysfunction. J Neurosurg. 2006;105(2):264-70.
32. Mayer SA, Limandri G, Sherman D, Lennihan L, Fink ME, Solomon RA, et al. Electrocardiographic Markers of Abnormal Left-Ventricular Wall-Motion in Acute Subarachnoid Hemorrhage. J Neurosurg. 1995;83(5):889-96.
33. Tung P, Kopelnik A, Banki N, Ong K, Ko N, Lawton MT, et al. Predictors of neurocardiogenic injury after subarachnoid hemorrhage. Stroke. 2004;35(2):548-51.
34. Kono T, Morita H, Kuroiwa T, Onaka K, Takatsuka H, Fujiwara A. Left-Ventricular Wall-Motion Abnormalities in Patients with Subarachnoid Hemorrhage - Neurogenic Stunned Myocardium. J Am Coll Cardiol. 1994;24(3):636-40.
35. Simon RP, Aminoff MJ, Benowitz NL. Changes in plasma catecholamines after tonic-clonic seizures. Neurology. 1984;34(2):255-7.
36. Ennezat PV, Pesenti-Rossi D, Aubert JM, Rachenne V, Bauchart JJ, Auffray JL, et al. Transient left ventricular basal dysfunction without coronary stenosis in acute cerebral disorders: A novel heart syndrome (inverted takotsubo). Echocardiogr-J Card. 2005;22(7):599-602.
37. Das M, Gonsalves S, Saha A, Ross S, Williams G. Acute subarachnoid haemorrhage as a precipitant for takotsubo cardiomyopathy: A case report and discussion. International journal of cardiology. 2009;132(2):283-5.
38. Doshi R, Neildwyer G. Hypothalamic and Myocardial Lesions after Subarachnoid Hemorrhage. J Neurol Neurosur Ps. 1977;40(8):821-6.

39. Wittstein IS, Thiemann DR, Lima JAC, Baughman KL, Schulman SP, Gerstenblith G, et al. Neurohumoral features of myocardial stunning due to sudden emotional stress. New Engl J Med. 2005;352(6):539-48.
40. Yoshimura S, Toyoda K, Ohara T, Nagasawa H, Ohtani N, Kuwashiro T, et al. Takotsubo cardiomyopathy in acute ischemic stroke. Ann Neurol. 2008;64(5):547-54.
41. Murthy SB, Shah S, Venkatasubba Rao CP, Suarez JI, Bershad EM. Clinical characteristics of myocardial stunning in acute stroke. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2014;21(8):1279-82.
42. Kerr G, Ray G, Wu O, Stott DJ, Langhorne P. Elevated troponin after stroke: a systematic review. Cerebrovasc Dis. 2009;28(3):220-6.
43. Shimizu M, Kagawa A, Takano T, Masai H, Miwa Y. Neurogenic stunned myocardium associated with status epileptics and postictal catecholamine surge. Intern Med. 2008;47(4):269-73.
44. Stollberger C, Wegner C, Finsterer J. Seizure-associated Takotsubo cardiomyopathy. Epilepsia. 2011;52(11):E160-E7.
45. Corrigan FE, 3rd, Kimmel MC, Jayaram G. Four cases of takotsubo cardiomyopathy linked with exacerbations of psychiatric illness. Innovations in clinical neuroscience. 2011;8(7):50-3.
46. Park JH, Kim KS, Sul JY, Shin SK, Kim JH, Lee JH, et al. Prevalence and patterns of left ventricular dysfunction in patients with pheochromocytoma. J Cardiovasc Ultrasound. 2011;19(2):76-82.
47. Abraham J, Mudd JO, Kapur NK, Klein K, Champion HC, Wittstein IS. Stress cardiomyopathy after intravenous administration of catecholamines and beta-receptor agonists. J Am Coll Cardiol. 2009;53(15):1320-5.
48. Redfors B, Shao Y, Omerovic E. Stress-induced cardiomyopathy in a patient with chronic spinal cord transection at the level of C5: endocrinologically mediated catecholamine toxicity. International journal of cardiology. 2012;159(3):e61-2.
49. Col NF, Fairfield KM, Ewan-Whyte C, Miller H. In the clinic. Menopause. Annals of internal medicine. 2009;150(7):ITC4-1-15; quiz ITC4-6.
50. Ueyama T, Kasamatsu K, Hano T, Yamamoto K, Tsuruo Y, Nishio I. Emotional stress induces transient left ventricular hypocontraction in the rat via activation of cardiac adrenoceptors: a possible animal model of 'tako-tsubo' cardiomyopathy. Circulation journal : official journal of the Japanese Circulation Society. 2002;66(7):712-3.
51. Ueyama T, Kasamatsu K, Hano T, Tsuruo Y, Ishikura F. Catecholamines and estrogen are involved in the pathogenesis of emotional stress-induced acute heart attack. Annals of the New York Academy of Sciences. 2008;1148:479-85.
52. Martin EA, Prasad A, Rihal CS, Lerman LO, Lerman A. Endothelial Function and Vascular Response to Mental Stress Are Impaired in Patients With Apical Ballooning Syndrome. J Am Coll Cardiol. 2010;56(22):1840-6.
53. Ibanez B, Benezet-Mazuecos J, Navarro F, Farre J. Takotsubo syndrome: a Bayesian approach to interpreting its pathogenesis. Mayo Clinic proceedings. 2006;81(6):732-5.
54. Kume T, Kawamoto T, Okura H, Toyota E, Neishi Y, Watanabe N, et al. Local release of catecholamines from the hearts of patients with tako-tsubo-like left ventricular dysfunction. Circulation journal : official journal of the Japanese Circulation Society. 2008;72(1):106-8.
55. Osuala K, Telusma K, Khan SM, Wu S, Shah M, Baker C, et al. Distinctive left-sided distribution of adrenergic-derived cells in the adult mouse heart. PloS one. 2011;6(7):e22811.
56. Huang MH, Friend DS, Sunday ME, Singh K, Haley K, Austen KF, et al. An intrinsic adrenergic system in mammalian heart. The Journal of clinical investigation. 1996;98(6):1298-303.
57. Lyon AR, Rees PS, Prasad S, Poole-Wilson PA, Harding SE. Stress (Takotsubo) cardiomyopathy--a novel pathophysiological hypothesis to explain catecholamine-induced acute myocardial stunning. Nature clinical practice Cardiovascular medicine. 2008;5(1):22-9.
58. Singer P. New attitudes needed on animal testing. New scientist. 1990;127(1729):16.
59. van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, et al. Can Animal Models of Disease Reliably Inform Human Studies? Plos Med. 2010;7(3).

60. Perel P, Roberts I, Sena E, Wheble P, Briscoe C, Sandercock P, et al. Comparison of treatment effects between animal experiments and clinical trials: systematic review. Brit Med J. 2007;334(7586):197-200.
61. Russell WMSBRL. The principles of humane experimental technique London: Methuen; 1959. Available from: http://catalog.hathitrust.org/api/volumes/oclc/1486173.html.
62. Pfeffer JM. Progressive ventricular dilation in experimental myocardial infarction and its attenuation by angiotensin-converting enzyme inhibition. The American journal of cardiology. 1991;68(14):17D-25D.
63. Omerovic E, Bollano E, Mobini R, Madhu B, Kujacic V, Soussi B, et al. Selective beta(1)-blockade improves cardiac bioenergetics and function and decreases neuroendocrine activation in rats during early postinfarct remodeling. Biochemical and biophysical research communications. 2001;281(2):491-8.
64. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). The CONSENSUS Trial Study Group. The New England journal of medicine. 1987;316(23):1429-35.
65. Tournoux F, Petersen B, Thibault H, Zou L, Raher MJ, Kurtz B, et al. Validation of noninvasive measurements of cardiac output in mice using echocardiography. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2011;24(4):465-70.
66. Thibault H, Lafitte S, Timperley J, Tariosse L, Becher H, Roudaut R, et al. Quantitative analysis of myocardial perfusion in rats by contrast echocardiography. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2005;18(12):1321-8.
67. Shao Y, Redfors B, Scharin Tang M, Mollmann H, Troidl C, Szardien S, et al. Novel rat model reveals important roles of beta-adrenoreceptors in stress-induced cardiomyopathy. International journal of cardiology. 2013.
68. Sachdeva J, Dai W, Kloner RA. Functional and histological assessment of an experimental model of Takotsubo's cardiomyopathy. Journal of the American Heart Association. 2014;3(3):e000921.
69. Strum DP, Pinsky MR. Modeling of asynchronous myocardial contraction by effective stroke volume analysis. Anesthesia and analgesia. 2000;90(2):243-51.
70. Redfors B, Shao Y, Ali A, Omerovic E. Are the different patterns of stress-induced (Takotsubo) cardiomyopathy explained by regional mechanical overload and demand: supply mismatch in selected ventricular regions? Medical hypotheses. 2013;81(5):954-60.
71. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986;74(5):1124-36.
72. Redfors B, Shao Y, Wikstrom J, Lyon AR, Oldfors A, Gan LM, et al. Contrast echocardiography reveals apparently normal coronary perfusion in a rat model of stress-induced (Takotsubo) cardiomyopathy. European heart journal cardiovascular Imaging. 2014;15(2):152-7.
73. Nef HM, Mollmann H, Hilpert P, Troidl C, Voss S, Rolf A, et al. Activated cell survival cascade protects cardiomyocytes from cell death in Tako-Tsubo cardiomyopathy. Eur J Heart Fail. 2009;11(8):758-64.
74. Redfors B, Shao Y, Ali A, Omerovic E. Are ischemic stunning, conditioning, and "takotsubo" different sides to the same coin? International journal of cardiology. 2014;172(2):490-1.
75. Sengupta PP, Krishnamoorthy VK, Korinek J, Narula J, Vannan MA, Lester SJ, et al. Left ventricular form and function revisited: applied translational science to cardiovascular ultrasound imaging. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2007;20(5):539-51.
76. Prinzen FW, Cheriex EC, Delhaas T, van Oosterhout MF, Arts T, Wellens HJ, et al. Asymmetric thickness of the left ventricular wall resulting from asynchronous electric activation: a study in dogs with ventricular pacing and in patients with left bundle branch block. Am Heart J. 1995;130(5):1045-53.

77. Alter P, Figiel JH, Rominger MB. Increased ventricular wall stress and late gadolinium enhancement in Takotsubo cardiomyopathy. International journal of cardiology. 2014;172(1):e184-6.
78. Cain PA, Ahl R, Hedstrom E, Ugander M, Allansdotter-Johnsson A, Friberg P, et al. Age and gender specific normal values of left ventricular mass, volume and function for gradient echo magnetic resonance imaging: a cross sectional study. BMC medical imaging. 2009;9:2.
79. El Mahmoud R, Mansencal N, Pilliere R, Leyer F, Abbou N, Michaud P, et al. Prevalence and characteristics of left ventricular outflow tract obstruction in Tako-Tsubo syndrome. Am Heart J. 2008;156(3):543-8.
80. Abe Y, Kondo M, Matsuoka R, Araki M, Dohyama K, Tanio H. Assessment of clinical features in transient left ventricular apical ballooning. J Am Coll Cardiol. 2003;41(5):737-42.
81. Kurisu S, Inoue I, Kawagoe T, Ishihara M, Shimatani Y, Nishioka K, et al. Myocardial perfusion and fatty acid metabolism in patients with tako-tsubo-like left ventricular dysfunction. J Am Coll Cardiol. 2003;41(5):743-8.
82. Braunwald E. Control of myocardial oxygen consumption: physiologic and clinical considerations. The American journal of cardiology. 1971;27(4):416-32.
83. Redfors B, Shao Y, Oldfors A, Omerovic E. Normal apical myocardial perfusion in the rat model with Takotsubo syndrome: is subsequent microvascular dysfunction and hypoperfusion an epiphenomenon? Reply. European heart journal cardiovascular Imaging. 2014;15(1):110-1.
84. Pepine CJ, Anderson RD, Sharaf BL, Reis SE, Smith KM, Handberg EM, et al. Coronary Microvascular Reactivity to Adenosine Predicts Adverse Outcome in Women Evaluated for Suspected Ischemia: Results From the National Heart, Lung and Blood Institute WISE (Women's Ischemia Syndrome Evaluation) Study. J Am Coll Cardiol. 2010;55(25):2825-32.
85. Bottcher M, Botker HE, Sonne H, Nielsen TT, Czernin J. Endothelium-dependent and -independent perfusion reserve and the effect of L-arginine on myocardial perfusion in patients with syndrome X. Circulation. 1999;99(14):1795-801.
86. Chan NN, Colhoun HM, Vallance P. Cardiovascular risk factors as determinants of endothelium-dependent and endothelium-independent vascular reactivity in the general population. J Am Coll Cardiol. 2001;38(7):1814-20.
87. von Mering GO, Arant CB, Wessel TR, McGorray SP, Bairey Merz CN, Sharaf BL, et al. Abnormal coronary vasomotion as a prognostic indicator of cardiovascular events in women: results from the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). Circulation. 2004;109(6):722-5.
88. Bybee KA, Prasad A, Barsness GW, Lerman A, Jaffe AS, Murphy JG, et al. Clinical characteristics and thrombolysis in myocardial infarction frame counts in women with transient left ventricular apical ballooning syndrome. The American journal of cardiology. 2004;94(3):343-6.
89. Ako J, Takenaka K, Uno K, Nakamura F, Shoji T, Iijima K, et al. Reversible left ventricular systolic dysfunction--reversibility of coronary microvascular abnormality. Japanese heart journal. 2001;42(3):355-63.
90. Yanagi S, Nagae K, Yoshida K, Matsumura Y, Nagashima E, Okada M, et al. [Evaluation of coronary flow reserve using Doppler guide wire in patients with ampulla cardiomyopathy: three case reports]. J Cardiol. 2002;39(6):305-12.
91. Madias JE. Is coronary vasospasm really the cause of Takotsubo syndrome? Proceedings (Baylor University Medical Center). 2014;27(3):288.
92. Madias JE. Coronary vasospasm is an unlikely cause of Takotsubo syndrome, although we should keep an open mind. International journal of cardiology. 2014.
93. Mor-Avi V, Shargorodsky B, Abboud S, Laniado S, Akselrod S. Effects of coronary occlusion on high-frequency content of the epicardial electrogram and body surface electrocardiogram. Circulation. 1987;76(1):237-43.
94. Spurgeon HA, Priola DV, Montoya P, Weiss GK, Alter WA, 3rd. Catecholamines associated with conductile and contractile myocardium of normal and denervated dog hearts. The Journal of pharmacology and experimental therapeutics. 1974;190(3):466-71.

95. Dail WG, Jr., Palmer GC. Localization and correltion of catecholamine-containing cells with adenyl cyclase nd phosphodiesterase activities in the human fetal heart. The Anatomical record. 1973;177(2):265-87.
96. Forsgren S, Moravec M, Moravec J. Catecholamine-synthesizing enzymes and neuropeptides in rat heart epicardial ganglia; an immunohistochemical study. The Histochemical journal. 1990;22(12):667-76.
97. Pierpont GL, DeMaster EG, Cohn JN. Regional differences in adrenergic function within the left ventricle. The American journal of physiology. 1984;246(6 Pt 2):H824-9.
98. Angelakos ET. REGIONAL DISTRIBUTION OF CATECHOLAMINES IN THE DOG HEART. Circulation research. 1965;16:39-44.
99. Habecker BA, Malec NM, Landis SC. Differential regulation of adrenergic receptor development by sympathetic innervation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1996;16(1):229-37.
100. Lau C, Burke SP, Slotkin TA. Maturation of sympathetic neurotransmission in the rat heart. IX. Development of transsynaptic regulation of cardiac adrenergic sensitivity. The Journal of pharmacology and experimental therapeutics. 1982;223(3):675-80.
101. Clarke GL, Bhattacherjee A, Tague SE, Hasan W, Smith PG. ss-adrenoceptor blockers increase cardiac sympathetic innervation by inhibiting autoreceptor suppression of axon growth. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(37):12446-54.
102. Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. Nature. 2009;459(7245):356-63.
103. Evans BA, Sato M, Sarwar M, Hutchinson DS, Summers RJ. Ligand-directed signalling at beta-adrenoceptors. British journal of pharmacology. 2010;159(5):1022-38.
104. Woo AY, Xiao RP. beta-Adrenergic receptor subtype signaling in heart: from bench to bedside. Acta pharmacologica Sinica. 2012;33(3):335-41.
105. Hasseldine AR, Harper EA, Black JW. Cardiac-specific overexpression of human beta2 adrenoceptors in mice exposes coupling to both Gs and Gi proteins. British journal of pharmacology. 2003;138(7):1358-66.
106. Daaka Y, Luttrell LM, Lefkowitz RJ. Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. Nature. 1997;390(6655):88-91.
107. Paur H, Wright PT, Sikkel MB, Tranter MH, Mansfield C, O'Gara P, et al. High levels of circulating epinephrine trigger apical cardiodepression in a beta2-adrenergic receptor/Gi-dependent manner: a new model of Takotsubo cardiomyopathy. Circulation. 2012;126(6):697-706.
108. Heubach JF, Ravens U, Kaumann AJ. Epinephrine activates both Gs and Gi pathways, but norepinephrine activates only the Gs pathway through human beta2-adrenoceptors overexpressed in mouse heart. Molecular pharmacology. 2004;65(5):1313-22.
109. Bhan A, Sirker A, Zhang J, Protti A, Catibog N, Driver W, et al. High-frequency speckle tracking echocardiography in the assessment of left ventricular function and remodeling after murine myocardial infarction. American journal of physiology Heart and circulatory physiology. 2014;306(9):H1371-83.
110. Redfors B, Ali A, Shao Y, Lundgren J, Gan LM, Omerovic E. Different catecholamines induce different patterns of takotsubo-like cardiac dysfunction in an apparently afterload dependent manner. International journal of cardiology. 2014;174(2):330-6.
111. Wright PT, Tranter MH, Morley-Smith AC, Lyon AR. Pathophysiology of takotsubo syndrome. Circulation journal : official journal of the Japanese Circulation Society. 2014;78(7):1550-8.
112. Gong H, Sun H, Koch WJ, Rau T, Eschenhagen T, Ravens U, et al. Specific beta(2)AR blocker ICI 118,551 actively decreases contraction through a G(i)-coupled form of the beta(2)AR in myocytes from failing human heart. Circulation. 2002;105(21):2497-503.
113. Mangmool S, Kurose H. G(i/o) protein-dependent and -independent actions of Pertussis Toxin (PTX). Toxins. 2011;3(7):884-99.

114. Villa F, Iacca C, Molinari AF, Giussani C, Aletti G, Pesenti A, et al. Inhalation versus endovenous sedation in subarachnoid hemorrhage patients: effects on regional cerebral blood flow. Critical care medicine. 2012;40(10):2797-804.
115. Bosel J, Purrucker JC, Nowak F, Renzland J, Schiller P, Perez EB, et al. Volatile isoflurane sedation in cerebrovascular intensive care patients using AnaConDa((R)): effects on cerebral oxygenation, circulation, and pressure. Intensive care medicine. 2012;38(12):1955-64.
116. Levine GN, Bates ER, Blankenship JC, Bailey SR, Bittl JA, Cercek B, et al. 2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. Circulation. 2011;124(23):2574-609.
117. Task Force on Myocardial Revascularization of the European Society of C, the European Association for Cardio-Thoracic S, European Association for Percutaneous Cardiovascular I, Wijns W, Kolh P, Danchin N, et al. Guidelines on myocardial revascularization. European heart journal. 2010;31(20):2501-55.
118. Singh K, Neil CJ, Nguyen TH, Stansborough J, Chong CR, Dawson D, et al. Dissociation of Early Shock in Takotsubo Cardiomyopathy from either Right or Left Ventricular Systolic Dysfunction. Heart, lung & circulation. 2014.
119. Medeiros K, O'Connor MJ, Baicu CF, Fitzgibbons TP, Shaw P, Tighe DA, et al. Systolic and diastolic mechanics in stress cardiomyopathy. Circulation. 2014;129(16):1659-67.