NATURAL HISTORY AND PATHOGENESIS OF TAKOTSUBO CARDIOMYOPATHY

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In
Medicine
At
The University of Adelaide
(Faculty of health Sciences)

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Declaration

This thesis is the result of my own investigation, except where otherwise stated. It contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Kuljit Singh (9.2.2015)
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Statement of contribution to research

The studies were conceived and designed jointly by Professor John Horowitz and myself.

Execution

I performed all the recruitment and organization of patients into the studies, with the assistance of Ms. Jeanette Stansborough (research nurse). I collected all the clinical data with assistance from Ms. Stansborough. I performed all the data search and meta-analysis with the assistance of Ms. Kristen Carson. I analysed of the echocardiographic studies for humans and animals. Some of the immunohistochemistry studies, in particular detection of apoptosis (TUNEL staining), Poly ADP ribose (PAR Staining) were performed by me. I also performed platelet aggregometry on new patients diagnosed with TTC between 2012 and 2014. Dr. Giovanni Licari performed immunohistochemistry studies of measurement of 3-nitrotyrosine and TXNIP. I performed quantification of staining for 3-nitrotyrosine and TXNIP with the assistance of Dr. Giovanni Licari. I performed quantification of staining for apoptosis, and inter-observer analysis were performed with Dr. Betty Raman. Plasma 3-Nitrotyrosine levels were analyzed in Prof. Tsikas’s laboratory in Hannover, Germany.

Analysis

All the data were collated and analyzed by myself.
List of published studies


Table of Contents

Declaration ........................................................................................................................................... 3
Acknowledgements .......................................................................................................................... 5
Statement of contribution to research .............................................................................................. 7
List of published studies .................................................................................................................... 9
List of figures ..................................................................................................................................... 15
Abstract ........................................................................................................................................ 20

1 Chapter: Literature review .............................................................................................................. 22
  1.1 Cardiac cell death ...................................................................................................................... 23
      1.1.1 Cell death ....................................................................................................................... 23
      1.1.2 Cardiac cell death ......................................................................................................... 24
  1.2 Evolution of cardiovascular medicine: From acute myocardial infarction to Takotsubo Cardiomyopathy ...................................................................................................................... 24
  1.3 Takotsubo cardiomyopathy ...................................................................................................... 25
      1.3.1 History ........................................................................................................................... 27
          1.3.1.1 The problem of historical definition and diagnosis ............................................... 29
          1.3.1.2 Evolving views regarding diagnostic algorithms ..................................................... 30
      1.3.2 Epidemiology .................................................................................................................. 33
          1.3.2.1 Incidence and prevalence ....................................................................................... 33
          1.3.2.2 Age of onset ........................................................................................................... 34
      1.3.3 Clinical characteristics ...................................................................................................... 35
          1.3.3.1 Antecedent psychological stress in TTC ................................................................. 35
          1.3.3.2 Medical and surgical illness .................................................................................... 35
          1.3.3.3 Association of cancer with TTC .............................................................................. 36
          1.3.3.4 Symptom onset ...................................................................................................... 37
      1.3.4 Electrocardiographic, biochemical and imaging abnormalities ......................................... 37
          1.3.4.1 Electrocardiographic changes ................................................................................ 37
          1.3.4.2 Biochemical markers ............................................................................................. 38
          1.3.4.3 Echocardiographic changes ................................................................................... 45
          1.3.4.4 Cardiac magnetic resonance imaging .................................................................. 49
      1.3.5 Pathogenesis ...................................................................................................................... 52
          1.3.5.1 TTC as ischemia ...................................................................................................... 52
          1.3.5.2 Role of interaction between catecholamines and myocardium in the pathogenesis of TTC .. 56
      1.3.6 Natural history .................................................................................................................... 68
          1.3.6.1 The acute phase issues ............................................................................................ 68
          1.3.6.2 Long term outcome- incomplete recovery and problem of recurrence .................. 71

2 Chapter: Pathogenesis of Takotsubo Cardiomyopathy-Animal work ......................................... 74
  2.1 Introduction ............................................................................................................................... 75
  2.2 Methodology ............................................................................................................................. 78
      2.2.1 Development of rat model of TTC ................................................................................ 78
2.2.2 Echocardiographic analysis ..............................................................79
2.2.3 Immunohistochemical studies..........................................................81
  2.2.3.1 3Nitrotyrosine, TXNIP and PAR staining .....................................82
  2.2.3.2 TUNEL staining .........................................................................83
2.2.4 Quantification of staining ..............................................................84
  2.2.4.1 3NT and TXNIP staining quantification ......................................84
  2.2.4.2 TUNEL staining quantification ................................ ...................85
  2.2.4.3 PAR staining quantification .......................................................86
2.3 Statistical analysis ...........................................................................87
  2.3.1 Effects of Isoprenaline .................................................................87
  2.3.2 Effects of 3AB .............................................................................87
2.4 Results ............................................................................................87
  2.4.1 Induction of TTC ........................................................................88
    2.4.1.1 Echocardiographic analysis ......................................................90
    2.4.1.2 Histological/immunohistological evaluation ...........................93
  2.4.2 Modulation of TTC: effects of 3AB .............................................96
    2.4.2.1 Echocardiographic analysis .......................................................96
    2.4.2.2 Immunohistochemistry results .................................................99
  2.4.3 Discussion ...................................................................................101
3 Chapter: Pathogenesis of Takotsubo Cardiomyopathy- Human experiments ..........107
  3.1 Introduction ....................................................................................108
  3.2 Methods ........................................................................................109
    3.2.1 Plasma 3NT concentration ..........................................................110
    3.2.2 Post-mortem studies in TTC patients .........................................110
    3.2.3 Statistical methods ....................................................................111
  3.3 Results ............................................................................................111
    3.3.1 Evaluation of plasma 3-NT concentrations ..................................111
      3.3.1.1 Clinical characteristics ..........................................................111
    3.3.2 Plasma 3NT comparison .............................................................113
    3.3.3 Post mortem studies .................................................................116
  3.4 Discussion ......................................................................................120
4 Chapter: Implications of right ventricular involvement in Takotsubo cardiomyopathy....122
  4.1 Introduction ....................................................................................123
  4.2 Methods ........................................................................................125
    4.2.1 Statistical methods ....................................................................127
  4.3 Results ............................................................................................127
    4.3.1 Overall basic characteristics .....................................................128
    4.3.2 RV involvement predicts greater LV injury ..................................129
    4.3.3 RV involvement is a univariate correlate of hypotension, shock and PHS ........130
4.3.4 RV involvement predicts more extensive LV involvement, but is not a multivariate correlate of hypotension, shock or PHS ................................................................. 134

4.4 Discussion .............................................................................................................. 135

4.5 Study limitations.................................................................................................... 138

4.6 Conclusions........................................................................................................... 138

5 Chapter: Meta-Analysis and systematic review of clinical correlates of acute mortality in Takotsubo cardiomyopathy .............................................................................. 139

  5.1 Introduction ........................................................................................................... 140

  5.2 Methods ............................................................................................................... 142

      5.2.1 Study eligibility.............................................................................................. 142

      5.2.2 “Primary” vs. “secondary” TTC................................................................. 142

      5.2.3 Data Sources and search strategy ............................................................. 142

      5.2.4 Study selection and data extraction.......................................................... 143

      5.2.5 Quality assessment...................................................................................... 143

      5.2.6 Statistical analysis....................................................................................... 143

  5.3 Results .................................................................................................................. 146

      5.3.1 Literature identification............................................................................... 146

      5.3.2 Basic characteristics................................................................................... 146

      5.3.3 Mortality....................................................................................................... 147

          5.3.3.1 Overall ................................................................................................. 147

          5.3.3.2 Clinical correlates of mortality rates.................................................. 148

          5.3.3.3 “Primary” vs. “secondary” TTC......................................................... 148

          5.3.3.4 Gender differences.............................................................................. 149

          5.3.3.5 Advanced age..................................................................................... 151

          5.3.3.6 Catecholamine use............................................................................ 152

      5.3.4 Complications............................................................................................... 152

      5.3.5 Quality assessment...................................................................................... 153

  5.4 Discussion ............................................................................................................. 156

6 Chapter: A meta-analysis of recurrence of Takotsubo Cardiomyopathy .................. 167

  7.1 Introduction ........................................................................................................... 168

  7.2 Methods ............................................................................................................... 169

      7.2.1 Inclusion criteria........................................................................................... 169

      7.2.2 Search strategy............................................................................................ 169

      7.2.3 Statistical analyses...................................................................................... 170

  7.3 Results .................................................................................................................. 171

  7.4 Discussion ............................................................................................................. 177

8 Summary and future perspectives ............................................................................. 186

  8.1 Summary: major findings ................................................................................. 187

  8.2 Mechanistic issues ............................................................................................ 188

      8.2.1 Key findings............................................................................................... 188
List of figures

Figure 1: Left ventricular in Takotsubo cardiomyopathy, mimicking the appearance of an “octopus trap”.................................................................26

Figure 2: Electrocardiogram of a patient diagnosed with Takotsubo cardiomyopathy showing presence of T wave inversion in all the 12 leads.................................................................31

Figure 3: Cardiac MRI showing presence of apical oedema in a patient diagnosed with Takotsubo Cardiomyopathy.................................................................51

Figure 4: Molecular mechanisms of peroxynitrite mediate cell death. ........................................65

Figure 5: Postulated NO signaling cascade with possible sites for intervention. .......................66

Figure 6: Radial strain at different time intervals post 5 mg/Kg of Isoprenaline. .........................78

Figure 7: Analysis of rodent left ventricular apical strain post Isoprenaline ............................80

Figure 8: Reduction in left ventricular apical strain 24 hours post.........................................90

Figure 9: Apical, mid-ventricular and basal left ventricular fractional area shortening at baseline and 24 hours post-isoprenaline.........................................................92

Figure 10: Mean data for TUNEL content in the apical and basal myocardial sections from control rats (blue) and those treated with isoprenaline (red). P = NS, Bonferroni post hoc correction for apex and base.................................................................94

Figure 11: Mean data for 3 nitrotyrosine content in the apical and basal myocardial sections from control rats (red) and those treated with isoprenaline (blue). .................................................95

Figure 12: Mean data for TXNIP content in apical and basal myocardial sections from control rats and those treated with isoprenaline. Data are represented as % of the microscopic field. 96

Figure 13: Mean data for LV apical strain at baseline and after treatment with isoprenaline in rats with and without pre-treatment with 3 aminobenzamide.................................................98

Figure 14: Mean data for apical fractional area shortening at baseline and after treatment with isoprenaline in rats with and without pre-treatment with 3 aminobenzamide.................................................98

Figure 15: Mean data for 3 nitrotyrosine percentage staining at left ventricular apex and base after treatment with isoprenaline in rats with and without pre-treatment with 3 aminobenzamide. .................................................................99
Figure 16 Mean data for TXNIP percentage staining at left ventricular apex and base after treatment with isoprenaline in rats with and without pre-treatment with 3 aminobenzamide.

Figure 17: Mean data for TUNEL stain percentage (quantification of apoptosis) at left ventricular apex and base following isoprenaline treatment with and without 3 aminobenzamide pretreatment.

Figure 18: Plasma 3-NT concentrations in Takotsubo cardiomyopathy patients (TTC) and age-matched controls (C). There was no significant difference between the groups.

Figure 19: Concentration of 3NT at the LV apex and base of TTC patients and control.

Figure 27: Pooled analysis with RD and 95% CI comparing acute mortality in primary and secondary TTC groups.

Figure 28: Pooled analysis with OR and 95% CI comparing acute mortality in males and females diagnosed with TTC.

Figure 29: Pooled analysis with RD and 95% CI comparing acute mortality in males and females diagnosed with TTC.

Figure 30: Risk of bias assessment presenting review author’s judgments about each quality criterion presented as a percentage across all included studies. Criterions were based on an adaption of the categories reported in the Tooth et.al. 2005 paper ‘Quality of reporting of observational longitudinal research’.

Figure 31 Risk of bias summary with review author’s judgments about each quality criterion for each included study. Criterions were based on an adaption of the categories reported in the Tooth et.al. 2005 paper ‘Quality of reporting of observational longitudinal research’.

Figure 32: Schematic presentation of literature search and identification of studies.

Figure 33: Adjusted rate of recurrence per 100 patients per year among the studies included in the systematic review (Shown in percentage).

Figure 34: Accumulative incidence of recurrence of Takotsubo cardiomyopathy at 6-month intervals.
Figure 35: The graph showing correlation of percentage of patients discharged on β-adrenergic blockers with incidence of recurrence. Each dot represents a separate study. (R = -0.207, P = 0.28).

Figure 36: The graph showing correlation of percentage of patients discharged on angiotensin converting enzyme inhibitor and angiotensin receptor blockers (ACE/ARB) with incidence of recurrence. Each dot represents a separate study. (R = -0.448, P = 0.016).

Figure 37: Detailed risk of bias assessment showing review authors judgement about each risk of bias item for each included study (red = high risk of bias, green = low risk and yellow = not applicable or unclear risk).

Figure 38: Risk of bias graph containing review author’s judgment about each risk of bias item presented as a percentage across all included studies (red = high risk of bias, green = low risk and yellow = not applicable or unclear risk).
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
</tr>
<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>ADMA</td>
<td>Asymmetric dimethyl arginine</td>
</tr>
<tr>
<td>ARB</td>
<td>Angiotensin receptor blocker</td>
</tr>
<tr>
<td>BNP; NT-proBNP</td>
<td>B-type natriuretic peptide; amino-terminal prohormone of BNP</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CA</td>
<td>Coronary angiogram</td>
</tr>
<tr>
<td>CMR</td>
<td>Cardiac magnetic resonance imaging</td>
</tr>
<tr>
<td>CVA</td>
<td>Cerebrovascular accident</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>TOE</td>
<td>Transesophageal echocardiography</td>
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<tr>
<td>TTE</td>
<td>Transthoracic echocardiography</td>
</tr>
<tr>
<td>TTC</td>
<td>Takotsubo cardiomyopathy</td>
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<tr>
<td>LAD</td>
<td>Left anterior descending artery</td>
</tr>
<tr>
<td>LGE</td>
<td>Late gadolinium enhancement</td>
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<tr>
<td>LOS</td>
<td>Length of stay</td>
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<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>LVSV</td>
<td>Left ventricular stroke volume</td>
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<tr>
<td>LVOT</td>
<td>Left ventricular outflow tract</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>MR</td>
<td>Mitral regurgitation</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>Non-ST elevation myocardial infarction</td>
</tr>
<tr>
<td>PASP</td>
<td>Pulmonary artery systolic pressure</td>
</tr>
<tr>
<td>PAR</td>
<td>Poly (ADP) ribose</td>
</tr>
<tr>
<td>PARP</td>
<td>Poly (ADP) ribose polymerase</td>
</tr>
<tr>
<td>PCWP</td>
<td>Pulmonary capillary wedge pressure</td>
</tr>
<tr>
<td>PHS</td>
<td>Prolonged hospital stay</td>
</tr>
<tr>
<td>RV</td>
<td>Right ventricle</td>
</tr>
<tr>
<td>SAH</td>
<td>Subarachnoid hemorrhage</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST elevation myocardial infarction</td>
</tr>
<tr>
<td>TXNIP</td>
<td>Thioredoxin interacting protein</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>VT</td>
<td>Ventricular tachycardia</td>
</tr>
<tr>
<td>VF</td>
<td>Ventricular fibrillation</td>
</tr>
<tr>
<td>WMSI</td>
<td>Wall motion score index</td>
</tr>
<tr>
<td>3NT</td>
<td>3-Nitrotyrosine</td>
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<tr>
<td>3AB</td>
<td>3-Aminobenzamide</td>
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Abstract

Introduction: Takotsubo cardiomyopathy (TTC) is a transient left ventricular (LV) systolic dysfunction of uncertain pathogenesis, which occurs predominantly in ageing women. Although there is considerable uncertainty about the pathogenesis of TTC, pronounced catecholamine release and an acute inflammatory process is implicated. Furthermore, natural history of TTC is unknown and correlates of acute complications and incomplete recovery have not been evaluated.

Methods: In the 5 experimental chapters, this thesis examines aspects of (a) pathogenesis and (b) natural history of TTC. As regard the pathogenesis, we hypothesized that increased release of nitric oxide (NO) in patients with TTC potentially induces the formation of peroxynitrite (ONOO⁻) anion with associated redox stress, protein nitration and downstream activation of thioredoxin interacting protein (TXNIP). Evaluation of presence of nitrosative stress was performed in a rat model of TTC in parallel with human experiments looking at the local and systemic rise in the 3-nitrotyrosine (3-NT) as a footprint of ONOO⁻ formation. As a part of clinical investigations, we evaluated the role of RV involvement in early hemodynamic derangement. We performed a meta-analysis to assess the impact of “secondary” TTC, male gender, advancing age and catecholamine use on mortality in TTC. We used a similar approach to assess the correlates of recurrence rate of TTC.

Results:

A. Pathogenesis: In rat model of TTC, there was substantially increased myocardial 3-NT (2.9 ± 0.6 % and 0.3 ± 0.1 %; p< 0.01) and TXNIP content (16.5 ± 5.2 vs 0.5± 0.2%; p < 0.01). Furthermore, use of poly (ADP) ribose polymerase (PARP)-1 inhibitor attenuated the isoprenaline induced LV systolic dysfunction. In human experiments, plasma concentrations of 3-NT did not differ significantly between TTC (2.26 ± 0.22 nmol/L) and control subjects (2.20 ± 0.25 nmol/L). However, myocardial 3-NT and TxNIP content
were increased 4-fold and 10-fold respectively. Furthermore, myocardial content for poly (ADP) ribose (PAR) activity was increased 4 folds.

B. **Clinical investigations:** RV involvement occurred in 1/3rd of TTC patients. Hypotension was noted in 21% of TTC patients, while shock occurred in 16%. RV involvement was a univariate but not a multivariate correlate of either hypotension or shock and did not result in prolonged hospital stay. RV involvement predicted more extensive LV hypokinesis and LV systolic dysfunction.

C. **Meta-Analysis:** In-hospital mortality among patients with TTC was 4.5% (95% CI, 3.1%-6.2%). Male gender was associated with higher mortality (OR 2.6, 95% CI, 1.5-4.6, p=0.0008) so was “secondary” TTC (RD-0.11, 95%CI; -0.18 to -0.04, p=0.003). TTC had 1-2% annual recurrence rate, which was independent of clinic utilization of beta-blocker prescription, but inversely correlated (r =-0.45, p = 0.016) with ACEi/ARB prescription. Patients with severe TTC at index admission were noted to have more recurrences.

**Conclusion:**

A. TTC is associated with evidence of nitrosative stress within left ventricular myocardium.

B. RV involvement is not an independent predictor of hemodynamic derangement.

C. Male gender and “secondary” TTC are associated with higher mortality and use of ACEi might reduce recurrence rate.
1 Chapter: Literature review
1.1 Cardiac cell death

For God’s sake, let us sit upon the ground

And tell sad stories of the death of kings;

How some have been deposed; some slain in war,

Some haunted by the ghosts they have deposed

Some person’s by their wives: some sleeping killed

-Shakespeare “Life and Death of Richard the Second”.

1.1.1 Cell death

Just like death of humans, there is large debate on what precipitates cell death. Different parameters are involved in different type of cell deaths. Death of cardiac cells can be classified into actively regulated or unregulated and passive or accidental. Apoptosis is a form of a regulated cell death where apoptotic cells show cytoplasmic shrinkage and nuclear condensations. These apoptotic cells undergo phagocytosis and do not contribute to inflammation like necrotic cells. On the other hand, a significant proportion of necrotic cell death is passive and unregulated. We now know that necrotic cell death is partially regulated thus is called necroapoptosis. Autophagy, on the other hand, is different from apoptosis and necrosis and is a survival mechanism. It can be defined as a cell recycling process that results in clearance of inefficient cellular components, notable mitochondria.
1.1.2 Cardiac cell death

“Strangulation” of the heart cell with myocardial ischemia following coronary artery occlusion is one of the many ways in which cardiac cells die (1). This process is associated with rise in the level of plasma troponin or creatine kinase concentration. Both the programmed cell death “apoptosis” and unregulated cell death “necrosis” occur during heart attacks. However, heart cells die via both of these mechanisms even in the absence of ischemia. Hence, the reason for rise in the level of these cardiac biomarkers, reflecting cardiac cell death in conditions such as: renal failure, pulmonary embolism and myocarditis. While necrosis-related cell death predominates in myocardial infarction, cardiac cells do undergo apoptosis and autophagy following infarction (2). Similarly, in other cardiac conditions such as heart failure apoptosis may predominate but there is always necrotic death of cardiac cells. Autophagy, which is also related to these syndromes, is a relatively new kid on the block whose role is currently unresolved.

1.2 Evolution of cardiovascular medicine: From acute myocardial infarction to Takotsubo Cardiomyopathy

Acute myocardial infarction (AMI) remains one of the major causes of morbidity, mortality and hospital admissions worldwide despite recent advances in cardiovascular medicine. It is usually associated with a sudden complete or partial blockage of a coronary artery because of an acute atherosclerotic plaque rupture.

The pathophysiology of AMI was unknown until 1880 when a German pathologist, Carl Weigert, showed a relationship between coronary artery occlusion and myocardial damage (3). Thirty years thereafter in 1910, two Russian scientists documented the clinical features of AMI in a living patient. In the 1920s various authors published clinical series of patients with AMI and it was accepted that AMI was caused by sudden blockage of coronary arteries. Even though the use of anticoagulation in AMI first occurred in 1930, it was not until 1950 when
Craven hypothesized that aspirin could be used for prevention of coronary artery thrombosis (4). However, the major breakthrough in the treatment of AMI occurred in 1980 following a paper by DeWood et al, who performed coronary angiography in live patients within 24 hours of onset of AMI (5). The demonstration in this study of coronary artery occlusion in association with AMI provided critical impetus towards the routing utility of invasive intervention in such patients.

Early intervention not only reduced mortality and morbidity but also led to new insights into acute cardiovascular conditions. In particular, it has gradually become apparent that coronary thrombosis, despite being a major cause of AMI, is not the only cause of acute myocardial injury. Thus electrocardiographic (ECG) changes and release of cardiac biomarkers such as troponin and creatine kinase can occur in non-occlusive coronary artery disease as well. Although many causes of acute myocardial injury have now been described to occur in the absence of coronary artery thrombosis, one of these originally termed Takotsubo Cardiomyopathy (TTC) represents a particularly important differential diagnosis and represents the subject matter of thesis.

1.3 Takotsubo cardiomyopathy

Takotsubo cardiomyopathy (TTC), also known as stress cardiomyopathy, apical ballooning syndrome or “broken heart syndrome” is a transient myocardial dysfunction with an unknown pathogenesis and variable natural history. It presents as an acute coronary syndrome (ACS) in the absence of an acute plaque rupture (6).

The word “Takotsubo” comes from the Japanese name for octopus trap because of similarity of the shape left ventricle (LV) takes during the initial phase of the condition. In its typical, and classical form, there is regional wall motion abnormality (RWMA) involving the apical segments of the LV. The apex of the LV becomes hypokinetic while the base is hyperkinetic giving it the appearance of an “octopus trap” (Figure 1). The RWMA can also occur in the mid part or the base of the LV giving rise to mid-ventricular and basal TTC variants.
respectively. Occasionally, there is involvement of the apex and mid LV at the same time.

The apical TTC is the most common variant occurring in 65% of cases while mid-ventricular TTC occurs in 30% and basal variant in 5% of the cases (7).

![Image of left ventricular in Takotsubo cardiomyopathy, mimicking the appearance of an “octopus trap”](image)

**Figure 1: Left ventricular in Takotsubo cardiomyopathy, mimicking the appearance of an “octopus trap”**

In approximately 30% of the cases TTC occurs in a biventricular form, also involving the right ventricle. These cases are usually the more severe end of the spectrum and are associated with more complications. Differentiation of TTC with RV involvement from anterior infarct may be diagnostically challenging.

TTC is predominantly a disease of aging women. Chest pain, dyspnea, shock, vomiting, atrial or ventricular arrhythmias etc. can all be presenting symptoms of TTC (8). However, TTC is sometimes diagnosed incidentally on the basis of an abnormal electrocardiogram (ECG) in patients admitted with non-cardiac conditions. The symptoms of TTC are preceded by emotional or physical stress in roughly 80% of the cases in the remainder it can occur without any obvious precipitant (9). The acute patient presentation is therefore similar to an ACS presentation, involving ECG changes and rise in cardiac biomarkers. ST elevation on the ECG is found in roughly 45% of cases in this circumstance diagnosis are on urgent coronary angiography (CA) in usual (10). While TTC has been so far thought to be a relatively benign disorder, acute complications and slow and incomplete recovery are common. The clinical
severity of the acute phase of TTC is variable, varying from mild asymptomatic hypotension and mild mitral regurgitation (MR) to severe left ventricular out flow tract (LVOT) obstruction, severe MR, shock and cardiac arrest. Similarly, in the “recovery” phase of TTC patients can continue to have symptoms of dyspnea, lethargy and tiredness for up to 2 years despite having a “normal” TTE (11).

In this chapter we will discuss the history, epidemiology, diagnosis, natural history and pathogenesis of TTC. In particular the natural history and pathogenesis of TTC will be reviewed in great detail as a basis for the experimental chapters.

1.3.1 History

As the name suggests TTC was first described in Japan and was named “Takotsubo” for the first time by Sato et al., in 1990 (Sato 1990). Even though Sato et al coined the name “Takotsubo”, there have been case reports about this condition even prior to 1990. In 1989, Iga et al (12) described a case report of development of reversible apical WMA in a patient with phaeochromocytoma. This case report was important because it described the relation between the TTC and rise in catecholamine levels because of phaeochromocytoma. This association of high catecholamine with TTC is the only thing that we know for sure about the pathogenesis of this condition.

Until 2000, there were only a few case reports about TTC, published mainly by Japanese investigators. However, the number of publications rose dramatically as the awareness about TTC increased in Europe and North America and since 2008 there have been nearly 300 publications each year in peer reviewed medical journals.

One of the first case series in the Western population was reported in Belgium where the author reported 13 patients who developed TTC because of emotional stress and physical illness, TTC occurred mostly but not only in women and was associated with severe complications and shock (13). This case series, despite being small, highlighted that TTC was
not as benign as once thought, that it occurred commonly in Western populations and that it could occur in critically ill patients.

By 2010 it was very clear that acute TTC episodes are associated with morbidity and mortality but knowledge related to long-term outcomes was limited. With increasing numbers of TTC cases being diagnosed many registries have been developed in Japan, USA, Australia and Europe to clarify the clinical features and natural history of the disease. Among publications evaluating natural history was that of Parodi et al (14), who followed 116 patients prospectively for 2.0 ± 1.3 years. This publication resolved many misconceptions about TTC. The investigators found that recurrences of TTC occur rarely. However, more importantly recurrences of symptoms of chest pain and dyspnea in the absence of LV abnormalities are very common and lead to frequent apparently unnecessary hospitalizations.

Also, long-term mortality in patients diagnosed with TTC is approximately three times that of general population and β blocker therapy started during hospital admission does not appear to protect against recurrence.

Despite a sudden increase in clinical knowledge of TTC, we have gained relatively little information about the pathogenesis of this condition. The only thing we know for sure is that there is an antecedent rise in catecholamine levels. How this rise in catecholamine levels affects myocardium, how it affects predominantly regions of myocardium, why it affects mainly aging women and the reasons for the variability in presentation are many of the questions which are unanswered. We now know that the previously proposed hypothesis of coronary artery vasospasm or resolved clot in a wrap around left anterior descending (LAD) coronary artery is not sustainable. Recent development of animal models may facilitate understanding of the pathogenesis of TTC.

In summary, in the last 20 years we have become more familiar with the clinical aspects of TTC. We know it can be associated with short and long-term morbidity and it is much more common than first thought. However, our understanding of pathogenesis and basis for inter-individual variability in acute severity of attacks is still minimal.
1.3.1.1 The problem of historical definition and diagnosis.

There is no universally accepted definition for TTC and the criteria for diagnosis of this condition in the presence of coronary artery disease remains controversial. A clinical definition of TTC can be based on presence of reversible segmental wall motion abnormalities of the left ventricle in the absence of fixed coronary artery disease. The most widely used diagnostic criteria for TTC are those proposed by the Mayo clinic, which require absence of epicardial coronary artery disease. However, Madhvan and Prasad made changes to these “Mayo Clinic Criteria” in 2010 and this modification has been widely used for the diagnosis of TTC since then (Table 1)(15). Essentially, “Mayo Clinic Criteria” makes the diagnosis of TTC entirely by a process of exclusion, where as more recently a number of “positive” bases for making this diagnosis have emerged. For example, TTC has specific biochemical and imaging findings that can be used to diagnose this condition: the role of coronary imaging in diagnosis is therefore subsidiary.

1.3.1.1.1 The problem of concomitant fixed coronary artery disease in TTC patient.

While there was substantial uncertainty about the pathogenesis of TTC, its diagnosis was based on phenomenology (eg. reversible abnormalities of regional ventricular systolic function), which could not be attributed to “conventional” myocardial ischemia or infarction. Despite this, TTC has at times been classified as a type of infarct and various investigators have postulated various ischemic bases, such as multi vessel coronary spasm.

Nevertheless, it has gradually emerged that TTC involves a process of intense myocardial inflammation and associated edema, as discussed extensively in section1.3.4.4.1. As this key aspect of pathogenesis has emerged, mainly from cardiac MRI findings (16) it has become self evident that there is no reason why TTC cannot coexist with previous myocardial infarction or with presence of fixed coronary artery disease.
Table 1: Mayo Clinic diagnostic criteria for Takotsubo cardiomyopathy (apical ballooning syndrome)

1. Transient hypokinesis, akinesis, or dyskinesis of the left ventricular mid segments with or without apical involvement; the regional wall motion abnormalities extend beyond a single epicardial vascular distribution; a stressful trigger is often, but not always, present*  

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

3. New electrocardiographic abnormalities (either ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin  

4. Absence of: Pheochromocytoma, myocarditis  

*There are rare exceptions to these criteria such as those patients in whom the regional wall motion abnormality is limited to a single coronary territory.  

**It is possible that a patient with obstructive coronary atherosclerosis may also develop Takotsubo cardiomyopathy. However, this is very rare in our experience as well as in the published literature, perhaps because such cases are misdiagnosed as an acute coronary syndrome.

The historic definition of TTC, by exclusion of coronary artery disease, makes it a diagnosis of exclusion. The authors of the “Mayo Clinic Criteria” accept that the TTC can occur in the presence of coronary artery disease and even in cases of acute plaque rupture (high adrenaline state), however, these cases are difficult to diagnose and are usually diagnosed as ACS (15).

The diagnosis of TTC is easy when there is no coronary artery disease, many cases of TTC will show incidental fixed coronary artery disease where the wall motion abnormality extends beyond the distribution of a single vascular territory.

1.3.1.2 Evolving views regarding diagnostic algorithms

Nearly two-thirds of TTC cases present without ST elevation on the ECG (10). These cases are usually managed initially as NSTEMI and tend to undergo semi-urgent coronary angiography within 24 to 48 hours. This window of 24 to 48 hours gives us a possible time
frame of prospective consideration of TTC before resorting to the “traditional” process of excluding an acute ischemic/infarctive process. It has emerged that ECG, imaging and biochemical parameters, which are related to the pathogenesis of TTC, can all be used to assist in the diagnosis of the condition within the first 24-48 hours.

A number of clinical/biochemical parameters are worthy of note in this regard: -

1. **Antecedent stress**: This feature is found more commonly with TTC than ACS. While stress could also theoretically precipitate ACS as well as vasospasm of the coronary arteries, the presence of an obvious stressful event especially in aging female patient should raise the possibility of TTC.

2. **ECG changes**: The presence of multiregional T wave inversion on the ECG is a characteristic feature of TTC (Figure 2). These changes usually develop 24 hours after the onset of symptoms, and represent a potential contrast to the notionally “single region” changes usually seen with ACS.

![Figure 2: Electrocardiogram of a patient diagnosed with Takotsubo cardiomyopathy showing presence of T wave inversion in all the 12 leads.](image-url)
3. **Markers of inflammation vs. necrosis**: Both TTC and AMI are associated with an element of myocardial cell death. However, irrespective of the extent of initial hemodynamic disturbance, the extent of myocardial injury in TTC is almost always small (17). Conversely, as TTC is ultimately an inflammatory condition, it is associated with more systemic evidence of inflammation than AMI.

Thus, the following biochemical changes alone or in combination are potentially useful to distinguish TTC from AMI:

a) **Markers of myocardial injury- CK and troponin**: Even though the rise of the plasma Troponin T level is similar in the two groups, the blood levels of creatine kinase does not rise as much in TTC as in the cases of ACS. The inappropriate rise in the level of CK relative to Troponin level can be helpful in early suspicion of TTC.

b) **Markers of inflammatory activation- HsCRP, BNP and NT-proBNP**: There is enough evidence that rise in plasma NT proBNP level is much higher in the TTC group than the ACS group. Even though, there can be some overlap of the NT proBNP values between the two groups, in TTC the rise of plasma NT proBNP occurs in the absence of pulmonary odema. The rise in the plasma NT proBNP without pulmonary odema, a characteristic finding of TTC, not only signifies the different mechanism of release of NT proBNP but also helps in the diagnosis of TTC.

There are now many reports in the literature suggesting that various combinations of these markers have diagnostic utility in this area.

4. **Pattern of regional systolic dysfunction**: -

   a. **Echocardiography (TTE) and cardiac MRI (CMR)**: Other than wall motion abnormalities that can not be explained by a single vascular territory, presence
of right ventricular apical ballooning on TTE and CMR can point towards the diagnosis of TTC than ACS. Similarly, absence of late gadolinium enhancement (LGE), presence of apical odema and a gradient in the odema are some of the many findings, which are more specific for TTC (16, 18).

A combination of clinical, biochemical and imaging findings can be used to diagnose cases of TTC without any need of coronary angiography.

1.3.2 Epidemiology

1.3.2.1 Incidence and prevalence

The apparent incidence of TTC has climbed because of increased awareness and evolution in the cardiac imaging in TTC. While the “true” incidence of TTC remains a subject of dispute and is potentially affected by issues such as the precise definition used, it is clear that it is not a rare condition (19). Among the whole population presenting with ACS, TTC occurs roughly in 2% of cases (20). However, the diagnosis of TTC is markedly subject to potential selection bias, because it is substantially expedited by coronary angiography/cardiac catheterization, which serves to exclude relevant myocardial ischemia and to demonstrate presence of regional left ventricular hypokinesis. This may lead to serious underestimation of the diagnosis of TTC: - in particular old women, patients in intensive care units acute post-surgical patients and patients with relative contraindications to coronary angiography will be misdiagnosed as ACS, while these groups are the ones at highest risk of developing TTC. The only group where we can be reasonably definite about the real incidence is in women presenting as STEMI in cardiac centers performing 24/7 primary angioplasty. The incidence of TTC in women presenting as STEMI can vary from 5 to 10%. While the data from USA shows that 5% of women presenting with STEMI are actually TTC(21), 4-year experience form our institution found the incidence to be 9% among such women (10).
1.3.2.2 Age of onset

It might be more appropriate to call TTC a disease of aging women rather than a disease of post-menopausal women. While it can occur in any age group, the apparent peak incidence occurs in mid-sixties. There have been case reports of occurrence of TTC in patients as young as 4 years (22). However, there are substantial caveats: - It is difficult to calculate the risk in the very old because of potential bias against diagnostic coronary angiography, and/or increased pre-hospital mortality. The mean age of onset of TTC might also vary depending on whether it is precipitated by an emotional stress or a physical illness. Reports of TTC in younger individuals tend to emphasize emergence secondary to severe physical illness. Of course it is possible that this simply reflects a higher threshold of catecholamine release to precipitate TTC in young.

1.3.2.3 Gender

Initially it was thought that TTC only affects women. However, nearly 10% of reported TTC cases are in males (23). Among data from western centers between 90 to 100% of cases are in women (24), while a large data set from Korea show only 70% of the patients with TTC are women (25, 26). While this “East West” gender heterogeneity is startling no basis for it has been proposed to date.

The pathophysiological basis of female propensity to TTC is unknown. Some authors have postulated that TTC is a disease of the post-menopausal women, which occurs after the protective effects of estrogen are lost (27). However, experimental evidence to support this correlation is minimal. Ueyama et al suggested that estrogen were protective in the rat model of TTC. On the other hand, epidemiological data is hardly supportive: TTC does not occur in pre-pubertal girls, and there is no evidence that post-menopausal estrogen therapy is protective. Finally, the “estrogen protection” theory would suggest that TTC should occur mainly in men (28).
1.3.3 Clinical characteristics

1.3.3.1 Antecedent psychological stress in TTC

The idea that emotional stress can trigger the emergence of cardiovascular disease is not new. Increase in plasma catecholamines following sudden emotional stress can lead to myocardial injury through atherosclerotic plaque rupture, coronary artery vasospasm or TTC. Epidemiologically, it has been reported that “heart attacks” occur concurrently with events which may have an effect on catecholamine release in large segment of the population. Such reports include days of Scud missile attacks in Israel (29), the most recent Los Angeles earthquake(30) and days of German participation in the last World Soccer cup (31)! In none of the above cases was the proportion of TTC events determined. However, the relationship between TTC and emotional/psychological stress may have been over-estimated. Less than 70% of all the cases of TTC appear to be precipitated by psychological stress. The relationship between psychological stress and TTC has been over exaggerated in some post hoc analyses and by sensationalized interviews post TTC. Nearly 20 to 30% of the cases of TTC do not have emotional or psychological stress (11).

1.3.3.2 Medical and surgical illness

Any non-cardiac medical illness and elective or urgent surgical procedure can precipitate TTC. Two of the most common causes of TTC that have been known for many years are CVA (cerebrovascular accident) and phaeochromocytoma. It is well known that intracranial hemorrhage (ICH) and ischemic stroke are associated with ST elevation in the absence of any coronary artery disease. We are aware of these findings even before the term “Takotsubo cardiomyopathy” was coined. These changes of ECG in the past were thought to be the false changes because of stroke. We now know that these changes post- ICH is most likely because of TTC. Epileptic seizures are also well known to induce TTC.
There have been number of case reports depicting the relation between the catecholamine producing phaeochromocytoma and TTC. It is especially important to rule out phaeochromocytoma in patients presenting with recurrent TTC.

Another important subset of patients who are very prone to develop TTC is critically ill patients in the intensive care units (32). It is easily to miss TTC in such patients and the incidence of TTC in intensive care is probably underestimated. Most of the patients with TTC in intensive care unit are treated as ACS because of relative contraindication to coronary angiography and CMR imaging. It is important to keep TTC in mind while looking after a troponin positive ICU patient. The diagnosis of TTC can be suspected from the rise in NT Pro BNP, typical ECG features of TTC and TTE changes even in the absence of CA and CMR.

Not only the medical illness but also treatment of a medical condition can predispose to development of TTC. There have been case reports of TTC occurrence following administration of adrenaline for anaphylaxis, post electroconvulsive therapy in a depressed patient, treatment of depression with drugs such as Venlafaxine, following use of Isoprenaline in cardiac electrophysiology study, treatment for shock with inotropic medications and post-influenza vaccination (33).

1.3.3.3 Association of cancer with TTC

The association for cancer with TTC is common, however, the plausible explanation for the phenomenon is not known. Burgdorf et al evaluated 50 TTC patients for possible malignancies and compared with and 50 age and gender matched anterior myocardial infarction controls (34). There was significant association of TTC with old and new diagnosis of malignancy in TTC patients when compared to age matched AMI patients (p=0.01, odds ratio 16.95, 95% confidence interval [CI] 1.93-304.60). Author speculated the role of paraneoplastic syndrome as the possible cause of TTC in patients with malignancies. Furthermore, treatment of cancer patients with chemotherapy/radiotherapy has shown to
stimulate TTC (35, 36). Whether this occurrence of TTC with malignancies is just because of physical illness or the two conditions have some underlying molecular similarities is something worth exploring.

1.3.3.4 Symptom onset

The mode of presentation can vary in patients with TTC. Most of the patients present as an ACS with chest pain and positive cardiac biomarkers. In some the symptoms of onset can be just dyspnea in a small proportion the diagnosis of TTC is made incidentally on an abnormal ECG. While the earlier cases of TTC were described in women presenting with STEMI, as more cases are being diagnosed we are noticing that the cases of TTC presenting as NSTEMI have increased.

While the acute symptoms are chest pain and dyspnea, chronically patients with TTC complain of fatigue, dyspnea and tiredness, which can last for months to year. This occurs despite the apparent “normalization” of the TTE changes of wall motion abnormalities.

1.3.4 Electrocardiographic, biochemical and imaging abnormalities

1.3.4.1 Electrocardiographic changes

The ECG feature of TTC can be divided in two groups depending upon the type of presentation. Nearly 40% of cases of TTC present as STEMI and undergo urgent coronary angiography (37). When ST elevation is the mode of TTC presentation, it is hard to differentiate it from AMI on the initial ECG (38). However, TTC should be suspected when the initial ECG shows “global” ST elevation or there is ST elevation without any reciprocal changes. On the other hand, gradual development of global T wave inversion can be an important diagnostic clue of TTC and can be found in nearly two-thirds of cases of TTC (39).

These changes can be variable depending up on the underlying variant of TTC but in most of the cases there is multi regional T wave inversion 48 hours post development of TTC (40).
Along with global T wave inversion, there is gradual prolongation of the QTc interval reaching its peak after 72 hours (41).

The prolongation of QTc interval has been associated with development of *torsade de pointes* during the acute period of the illness (42). TTC has also been found to be associated complete atrio-ventricular block, which could be the cause of torsade de pointes in some cases (43). There have been association between the frequency of arrhythmias and RR interval variability, which is a sign of increased vagal tone (44). During the acute phase of TTC non-lethal arrhythmias in particular atrial fibrillation and short runs of ventricular tachycardia are quite common. However, not all cases of TTC related cardiac arrest are associated with long QTc particularly if the mode of TTC presentation is cardiac arrest, then the QTc interval has been found to be normal (8).

### 1.3.4.2 Biochemical markers

TTC is associated with number of markers of (extensive) inflammation: Indeed, as will be discussed in Section 1.3.5, this inflammation appears to play a central role in the pathophysiology of the disorder. Evidence for inflammation includes, CMR imaging and direct biopsy data (45, 46). Some systemic biomarkers in TTC can related to the myocardial inflammation (CRP, NT proBNP), non-specific apoptosis and necrosis of the cardiac cells (Troponin, creatine kinase) or because of underlying non-cardiac pathology (phaeochromocytoma related catecholamine release).

#### 1.3.4.2.1 BNP/NT-proBNP

B-type natriuretic peptide (BNP) is a neurohormone that is secreted from the ventricular muscle; NT proBNP is a 76 amino acid inactive product of the cleavage of the pro hormone proBNP. It was initially thought that the release of BNP in TTC reflected volume or pressure overload of the ventricles. However, it is now clear that ischemia and inflammation can also stimulate BNP release (47). There is no storage mechanism for BNP in cardiomyocytes and it
is secreted in bursts after cleavage of its pro-hormones. Inside the heart cells, pro BNP 1-108 is split into its active (BNP 77-108, BNP 1-32) and inactive (NT pro BNP 1-76) fragments and is released in the blood. While the BNP has a short half-life of 22 minutes, the NT proBNP has a longer half-life of few hours and this long half-life of NT proBNP makes it useful for diagnostic purposes.

BNP exerts multiple physiological effects by binding to the natriuretic peptide receptor and stimulating increased intracellular cyclic GMP production (48). The main effects of BNP include vasodilatation, diuresis, and inhibition of renin and aldosterone production and hypertrophy of cardiac myocytes. Recent data suggest that BNP may also suppress release of superoxide from neutrophils. However, Chan et al (2012) have demonstrated that BNP and other cGMP-releasing hormones may also stimulate catecholamine secretion. Although these findings have not yet been placed in full clinical context, it is certainly possible on this basis that BNP release might tend to perpetuate the catecholamine release state which triggers attacks of TTC (49).

Historically the main clinical utility of BNP and NT proBNP has been to exclude the diagnosis of acute heart failure in the emergency department. NT proBNP elevation during the acute episode of the TTC has been reported widely in the literature (50, 51). When compared with age matched AMI patients, there is much higher rise in the NT pro BNP in the TTC group.

The usual stimulus of BNP release in the heart failure is pressure overload because of rise in the filling pressure of the left ventricle. However, the LV filling pressure in TTC is not usually elevated and severe elevation of pulmonary capillary wedge pressure (PCWP) is extremely rare despite common occurrence of shock (52, 53). This raises the suspicion of a different mode of release of BNP in cases of TTC. It is quite possible that this release of BNP reflects inflamed myocardium. Therefore, not only is the rise of BNP and NT pro BNP important for the diagnosis of TTC, it also reflects a role in the pathogenesis of the condition. The early rise in NT pro BNP can induce hypotension that occurs commonly in the acute
phase of TTC. It remains uncertain whether the “BNP” detected in the commercially available assays is real BNP or whether it might contain inactive moieties such as pro BNP (54). However, any active released BNP might contribute to the hypotension, which occurs commonly in the acute stages of TTC.

1.3.4.2.2 Other inflammatory markers

It would not be incorrect to regard TTC as a type of myocarditis where inflammation is induced by sudden rise in catecholamine levels. The interaction between the catecholamines and myocardium has been tested by us and will be discussed in a later chapter of this thesis.

The evidence of inflammation of the myocardium in TTC has been confirmed by the histological heart samples and by usual biochemical markers of inflammation. Nef et al, performed biopsies from the inter-ventricular septum during the acute and recovery phase of TTC and found changes of inflammation (55). Inflammatory changes including infiltration of the myocardium by macrophages have been shown by other authors even when the common causes of myocarditis such as infection has been ruled out (56).

Furthermore, a correlation between the rise in the CRP and reduction of the LV ejection fraction has been found thus strengthening the theory that TTC is an inflammatory disorder:- the more the inflammation the “sicker” the LV (57). There is also strong correlation between the CRP and BNP levels in TTC suggesting that in TTC both of these markers highlight inflammation.

1.3.4.2.3 Catecholamine elevation: the pivotal event?

As will be summarized in section 1.3.5.2, there is substantial evidence that either increased catecholamine secretion (in majority of cases) or catecholamine administration, together with increased myocardial “susceptibility” associated with the aging female heart, represents a central aspect of the pathogenesis of TTC. Indeed, while most of the post-receptor events
inducing inflammation of myocardium remain uncertain at this stage, there is little controversy regarding the role of catecholamines (58).

That being the case, the physiological roles and signal transduction cascade for catecholamine should be reviewed in some detail.

1.3.4.2.4 Catecholamine synthesis

Catecholamines are secreted from brainstem, adrenal medulla and from the postganglionic fibers of the sympathetic nervous system. They occur physiologically in 3 main forms: adrenaline, noradrenaline and dopamine. All of these hormones are produced from the amino acids phenylalanine and tyrosine. Tyrosine is converted to L-3,4 dihydroxyphenylalanine (L DOPA), which then is converted to dopamine. Further metabolic changes in the dopamine lead to the production of adrenaline and noradrenaline. Every step of formation of catecholamine, from tyrosine to epinephrine, requires a catalyst. Tyrosine hydroxylase, which is a catalyst, required for the conversion of tyrosine to L-DOPA, is the rate limiting step in the catecholamine synthesis.

Catecholamines are stored in synaptic vesicles to protect the hormone from degradation by other enzymes within the nerve terminal. This “packaging” also provides a method for release of a programmed amount of catecholamine when a nerve impulse stimulates one or more vesicles to release its contents. Similar synthesis of catecholamines occurs in chromaffin cells of adrenal medulla, which are modified postganglionic cells.

1.3.4.2.4.1 Catecholamine release

Adrenaline is released mainly from the adrenal medulla in response to sympathetic stimulation. Noradrenaline on the other hand, is released mainly from sympathetic nerve endings. Plasma concentration of noradrenaline are determined by the balance between this release and a re-uptake process: the resultant “spill over” of noradrenaline, especially in the heart, is increased in many forms of heart failure (Mesler 1983)
One recent interesting finding by Chan et al. (49), regarding the mechanism of release of catecholamine could be one of the links in the pathogenesis of TTC. Chan et al. (49), found that perfusion of heart cells with natriuretic peptide lead to 3-fold increase in the release of noradrenaline. Despite the traditional views, we therefore now know that cyclic GMP and BNP can stimulate the release of catecholamines in the cardiac cells: - BNP is not always “cardio-protective”.

1.3.4.2.4.2 Adrenergic receptors

The adrenergic receptors are types of G protein coupled receptors that are found in many body organs and cells. The adrenergic receptors can be divided in two groups: Alpha (α) and Beta (β) receptors with further 2 subtypes for the α and 3 subtypes of the β receptors. The physiological effects of catecholamines such as: tachycardia, positive inotropy, lusitropy, vasoconstriction, gluconeogenesis etc. are induced by the stimulation of these receptors by adrenaline and noradrenaline. Even though, these receptors are found in all the organs, the discussion in this thesis will be limited to the cardiovascular system.

Ahlquist described β- adrenergic receptors for the first time in 1948 (59). At the moment we know about 3 subtypes of β-adrenergic receptors and a 4th type of receptors has been proposed (60). In a healthy heart of a young adult 80% of the β-receptors are of β2 receptor subtype and 20% correspond to the β1 subtype. In elderly or failing hearts the distribution and relative affinities of β-adrenergic receptors remain uncertain.

As already mentioned, β-adrenergic receptors belong to a family of G-protein coupled receptors that act by coupling with guanine nucleotide binding proteins (61). The β1 receptor is coupled to stimulatory G protein (Gs) made up of 3 subunits. On stimulation of the β1 receptor by an agonist there is dissociation of the 3 subunits of Gs protein that leads to activation of adenylyl cyclase (AC). Activation of AC catalyzes the change of ATP to cAMP
and induces the activation of cAMP dependent protein kinase A (PKA) which further phosphorylates many regulatory proteins including β adrenergic receptors themselves. This stimulation of the β adrenergic receptors leads to cardiac muscle excitation and contraction. There are other important functions, which are modified by the stimulation of β adrenergic receptors, such as: gene transcription and growth and can induce death. Some of these effects occur because of activation of mitogenic-activate protein kinase (MAPK) by the GPCR. On the other hand, β2 adrenergic receptors exhibit dual coupling to both Gs and Gi proteins. **Therefore, other than increasing the c-AMP and PKA activity, the effects of β2 adrenergic receptor coupling to Gs protein are not similar to the β1 adrenergic receptor. The β2 adrenergic receptor Gi coupling activation inhibits cAMP synthesis and reduces activation of PKA.**

### 1.3.4.2.4.3 Age induced β adrenergic receptor desensitization

Age induced β adrenergic receptor desensitization can be defined as the age related reduction in the response of β adrenergic receptors because of molecular changes. This phenomenon has also been well described in heart failure as well (61). The effects of this desensitization can be found in many clinical scenarios for example elderly individuals have lesser degree of tachycardia but greater rise in blood pressure with exercise (62) and during exercise the increase in the stroke volume in elderly occurs via cardiac dilation while in the young this increase occurs in the absence of any increase in diastolic dimensions. The decrease in the β-adrenergic receptor responsiveness could be due to changes in the G protein and kinase activity. Bohm et al, found no difference in the number of β-adrenergic receptor between young and old rats but found increased Gi content (63). Indeed, evidence regarding the reduction in β-adrenergic receptor numbers is inconsistent. Xiao et al (64), found clear evidence of reduction in the number of both β1 and β2 adrenergic receptors with age in various strains of rats. They did not find any change in the activity of the Gi proteins and claimed that
most of the reduction in the function of adrenergic receptors was because of reduced density. This results are in clear contrast with the findings of Ferrera et al (62).

Whether it is because of reduction in the number or because of impaired function of receptors, overall β-adrenergic receptor desensitization occurs with age. Also, there is substantial evidence of the same phenomenon in other conditions including heart failure, which involve increased catecholamines secretion. Acute sudden stress or chronic heart failure or aging lead to increases rise in catecholamine levels inducing desensitization of β-adrenergic receptors: this may represent important link to the pathogenesis of the TTC.

1.3.4.2.4.4 Beta-3 adrenergic receptor

Beta-3 (β3) receptors, just like other β-adrenergic receptors are G protein-coupled, which are found in many organs of humans and other animals. The gene for β3 adrenergic receptor was coded in 1989 (65). One major feature that differentiates β3 adrenergic receptors from other adrenergic receptors is that stimulation of this receptor induces a negative inotropic response. Another important distinguishing feature of the β3 adrenergic receptors is their lack of desensitization with chronic increase in catecholamines and with aging (66). Furthermore, the β3 adrenergic receptor requires much higher catecholamine concentrations for its activation than the other 2 types of β adrenergic receptors (67). All this evidence suggests that following sudden or prolonged activation by the catecholamines the function of β3 adrenergic receptors will be preserved while that of the other 2 types of β adrenergic receptors will exhibit partial desensitization and will lead to net negative inotropy.

While the function and regulation of β1 and β2 adrenergic receptors reduce with age, there is evidence that the function of β3 adrenergic receptors may gradually increase with age (68). Birenbaum et al (69), demonstrated impaired inotropic effect of isoproterenol in senescent hearts in vivo strengthening the changes in the β adrenergic receptor function with over expression of β3 receptor function.
The negative inotropic function of the \( \beta_3 \) adrenergic receptors is linked to the release of nitric oxide (NO). Initially it was thought that this release of NO related to \( \beta_3 \) adrenergic receptor occurs because of eNOS dependent production of NO in the human ventricle. However, new evidence suggests that \( \beta_3 \) adrenergic receptor modulation under pathological conditions can also lead to production of NO through nNOS and iNOS (70, 71).

1.3.4.2.4.5 Catecholamine elevation in TTC

In most studies patients with TTC have relatively higher levels of plasma metanephrine and normetanephrine when compared to those with conventional ACS. Akashi et al, in 2002 showed the connection between the rise in the catecholamine levels and TTC in a case report (72). Wittstein et al, in in a small series of TTC patients confirmed the earlier finding of raised catecholamine levels and noticed that the level of plasma catecholamines in the acute stages of TTC were 2 to 3 times that of age matched AMI and 7 to 34 times that of normal values (73). The levels of the plasma catecholamine decreased progressively in the TTC group over the first week but still were elevated compared with ACS group. Akashi et al noted similar findings. He also obtained evidence of cardiac adrenergic nervous dysfunction localized to the apex during the acute phase of TTC and gradual improvement of this by 3 months (74). It should be noted, however, that a few studies have failed to document catecholamine elevation in TTC. It is not clear whether this reflects late sampling or selection of relatively mild cases.

1.3.4.3 Echocardiographic changes

Since its inception transthoracic echocardiography (TTE) has always been an integral part of the diagnosis of cardiac conditions. Indeed this has evolved into the pivotal methodology for screening patients for TTC.
1.3.4.3.1 Standard techniques (2-D echocardiography and Doppler)

The segmental wall motion abnormalities of TTC on 2-D TTE can be easily differentiated from other cardiac conditions. It is also easy to differentiate the main variants of TTC on TTE. Apical TTC, which is the most common variant of TTC, can be seen on TTE as LV apical dyskinesis along with compensatory hyperkinesis of the basal LV segments. Because of this appearance of the LV the word “apical ballooning” is commonly used for the TTC related apical wall motion abnormalities. In conjunction with the clinical data and biochemical findings this appearance of the apical ballooning, which is not limited to a single coronary vascular territory, is enough to virtually confirm the diagnosis of TTC. The other feature, which is helpful to confirm the diagnosis and to differentiate TTC from LAD infarct, is the involvement of the right ventricle (RV) apex (75). RV involvement can occur in roughly one third of the cases of TTC and is a mirror image of the LV involvement. This is specific for TTC.

TTC induces transient systolic dysfunction. In a number of studies has been defined as a reduction in the LV ejection fraction (LVEF) that resolves with time. However, in most cases, LV systolic dysfunction is regional only, and it is common for other regions of the LV to exhibit hyperkinesis. Overall, in many cases, LV ejection fraction is therefore with in normal limits, even acutely. On the other hand, regional LV systolic dysfunction is by definition impaired. The majority of recent studies have utilized measures of regional hypokinesis, such as wall motion scores, as measures of extent of impairment of regional contractility.

As mentioned earlier, one third of the case of TTC have RV involvement. This involvement of the RV ventricle can contribute to the dynamics of the LV. So far no study has evaluated the importance of RV systolic dysfunction in the TTC and its impact on hemodynamics.

The utility of echocardiography in the acute stages of TTC may extend beyond diagnosis, to indexation of risk of hemodynamic compromise (although this is somewhat controversial)(76, 77). More importantly, echocardiography can be utilized to detect rare complications of TTC.
such as severe mitral regurgitation. It might be argued that evaluation of the extent of apical wall motion impairment might assist in predicting risk of mural thrombosis and thus need for anticoagulation, but this possibility has never been fully tested. Occasional patients with TTC display evidence of mid-ventricular obstruction on echocardiography. However, at present this finding has no therapeutic implications.

In theory, echocardiography could also be utilized to quantitate any improvement of diastolic function in TTC: there are a number of well-validated indices of LV diastolic function. Furthermore, on theoretical grounds, there are increasing reasons to expect that diastolic dysfunction will be present. For example, preliminary data suggest the presence of energetic impairment, which should lead to impaired LV relaxation. On the other hand, in practice, few investigators have performed detailed evaluation in this area. Furthermore, data from right heart studies also suggest that severe diastolic dysfunction is rare in TTC.

The conventional wisdom has been that rapid recovery of LV wall motion post-TTC is universal. However, more recently this has been shown not to be absolutely true, in the sense that both symptoms and subtle abnormalities on echocardiography may persist for months after acute episodes (16, 53, 78). Interestingly, what is currently lacking is detailed literature on recovery post TTC as evaluated by stress, rather than resting TTE.

1.3.4.3.2 New techniques of TTE

Echocardiographic images should in theory provide a basis for reproducible and accurate quantification of cardiac function. However, even with records of good technical quality, reproducibility of echo-based pressure and wall motion data is usually mediocre. Additionally many patients’ present difficulties regarding acquisition of good quality echo data, due to factors such as obesity, emphysema and atrial fibrillation.
Improved definition of myocardial borders and hence of wall motion can be achieved with myocardial contrast echocardiography (79). However, this is an expensive technique and the use of contrast involves a small risk of adverse reactions.

A number of new techniques have avoided the use of contrast but have concentrated on quantification of regional motion of identified “targets” on the myocardial surface.

Speckle tracking echocardiography (STE) is a relatively new imaging method, which is based on an analysis of the spatial dislocation of the “speckles” on 2D TTE. STE by tracking the movement of speckles during a cardiac cycle permits evaluation of myocardial distortion in 3 spatial directions: longitudinal, radial and circumferential. Furthermore, the use of STE is not only limited to the LV: - it can be applied to other cardiac chambers. The semi-automated nature of this technique reduces inter-observer variation of the measured values. To acquire good results with the STE good 2D TTE images are required so that ideal definition of the endocardial border can be obtained (80).

The main components of STE are “strain”, “strain rate”, “longitudinal strain”, “radial strain”, “circumferential strain”, “twisting” and “torsion”. “Strain” is myocardial deformation of an analyzed segment in relation to its initial location, while “strain rate” represents myocardial deformation rate velocity and is expressed in seconds$^{-1}$. Similarly, “longitudinal” and “radial” strain represent base to apex and radially centered myocardial deformation respectively. “Twisting” is defined as the net difference between means LV apical and basal rotation during the systole and “torsion” is twisting normalized with base to apex distance.

Using the above-mentioned modalities of speckle tracking assessment of cardiac conditions with TTE has become much more accurate. The dysfunction of the LV found with speckle tracking correlates well with the drop in the LVEF in the cases of LV systolic dysfunction such in the cases of dilated cardiomyopathy.
However, the real advantage of speckle tracking is in cases where LV dysfunction is subtle and cannot be quantified or even detected up by the standard TTE techniques. For example, LV strain impairment occurs much earlier than the decline in the EF in cases of cardiac amyloidosis. This particular property of strain helps to assess LV function much more accurately than the LVEF or wall motion score that have been used till now. It is also true in the cases of ischemia, strain impairment occurs in the initial stages of ischemia even before the wall motion abnormalities can be seen. This ability of speckle tracking to delineate the subtle degrees of dysfunction has given us more insight in LV involvement in TTC.

As expected, the long axis LV function is impaired during the acute phase of TTC (Burri et al., 2008, Heggemann et al., 2009b, Mansencal and Dubourg, 2009, Meimoun et al., 2011, Loiske et al., 2011). However, Burri et al, noticed an interesting finding using speckle tracking, that LV dysf

function in acute TTC is not limited to the apex but is also found in the base of the LV which until then was generally though to be spared. Also, these investigators found that the diastolic dysfunction occurs as commonly as systolic dysfunction in the acute phase of TTC. This might therefore represent an assessment tool to assess the recovery of LV function in TTC.

1.3.4.4 Cardiac magnetic resonance imaging

Although the diagnosis of TTC was essentially made via coronary angiography/ventriculography when the disorder was first described, greater appreciation of its pathogenesis has led to change in diagnostic methodology. At present, cardiac magnetic resonance imaging (CMR) has a special role in the diagnosis and pathogenesis of TTC (81). In the last 10 yeas there has been a significant growth in the use and sophistication of CMR, which offers greater insight into cardiac structural change than echocardiography.

Not only CMR gives better endocardial definition and hence quantitation of wall motion abnormality and volumetric quantification of cardiac chambers, it also has a distinctive
capability to add information regarding tissue composition. Delayed enhanced CMR facilitates detection of myocardial fibrosis or scar. Delayed imaging obtained 10 to 20 minutes after administration of Gadolinium contrast agent with an inversion pulse to suppress the normal myocardial tissues is used to detect late gadolinium enhancement (LGE). This enhancement of the myocardial tissues helps in identification of the presence of sub-endocardial or transmural myocardial infarction. This imaging sequence is commonly used in TTC to rule out myocardial infarction. However, it should be remembered that there could be presence of fibrosis in TTC and other “non Ischemic” cardiomyopathies as well in the absence of myocardial infarction (82, 83). The pattern of delayed enhancement in these cardiomyopathies is different form the myocardial infarction in two particular characteristics (84). The presence of scar in these conditions follows a “non-coronary artery distribution”: - a single vascular territory cannot explain its presence. Secondly, the LGE, as in the case of dilated cardiomyopathy or myocarditis, usually occurs towards the mid wall or towards the epicardium, while ischemia causes sub-endocardial LGE.

1.3.4.4.1 Ventricular wall edema

The most characteristic finding on CMR of TTC is presence of transmural edema involving mainly the apex and mid ventricular region. The predominant sites of edema correspond to the wall motion abnormalities found on CMR cine images. Similarly, this edema does not follow a single vascular territory: - this can also be used to distinguish TTC from myocardial infarction, where transmural edema corresponds to a vascular territory. Acute infective myocarditis can also have a similar clinical presentation and can show high signal intensity on T2 sequence. However, the distribution of edema is more heterogeneous and frequently has sub-epicardial location.
The other important thing about the T2 signal in TTC is that in most of the cases these changes are much more evident when the CMR is performed in the acute phase of the disease. When performed a few weeks after the diagnosis, the intensity of the T2 signal has decreased and it becomes more difficult to distinguish it from the normal myocardium while the duration of the high signal is much longer in cases of myocardial infarction. However, it should be recognized that in most cases of TTC abnormalities in T2 signal can be detected by sensitive measures at least for 3 months post onset of illness.

The myocardial oedema in TTC on CMR is a sign of underlying inflammation. The extent of LV oedema increases in proportion to rise in the plasma catecholamine levels, and also with rise in the CRP and NT-pro BNP. Detection of myocardial oedema on CMR can therefore be helpful to quantify the injury similar to rise in the plasma NT pro BNP level. While distribution of oedema reveals the presence of an apex-base gradient in cases of TTC the base of the LV is not completely spared, as was been reported in the literature initially (81). The presence of oedema in the base indicates that TTC is a disorder of the whole LV myocardium, albeit one, which displays asymmetry.
1.3.5 Pathogenesis

1.3.5.1 TTC as ischemia

Perhaps due to the fact that most cases of TTC present somewhat like AMI, many early investigators assumed that TTC represented a form of ischemic myocardial injury (85). The idea persisted and (indeed still persists in some quarters (86) despite obvious problems such as lack of coronary distributional correlates (87). Nevertheless it is appropriate to review the full range of hypotheses thus far advanced, with evidence “for and against” each of these.

1.3.5.1.1 Is there ischemia?

In the first case series of TTC the finding was ascribed to multi-vessel coronary artery spasm (Sato et al, 1990). Since then there has been debate as to whether TTC is ischemic stunning of LV following either coronary plaque rupture or from coronary artery spasm. While some authors are still reporting cases of TTC induced by spasm (88), none of the studies using myocardial perfusion gated single photon emission computed tomography (SPECT) have shown ischemia in the affected segments of LV in TTC (89).

At the same time, biopsy studies from patients diagnosed with TTC have shown features including interstitial infiltrates consisting primarily of mononuclear lymphocytes, leukocytes and macrophages and contraction bands. Presence of inflammatory infiltrates and contraction bands in TTC myocardial samples differentiate it from the coagulation necrosis that is found in cases of ACS occurring after coronary artery blockage.

1.3.5.1.2 Might there be large vessel dysfunction?

Abnormal myocardial perfusion because of spasm of the epicardial coronary artery can lead to regional stunning of the myocardium. In cases of TTC this could theoretically occur via either coronary plaque rupture or coronary artery spasm.
a) **Coronary artery spasm**: Sato et al, in 1990 while reporting the first case of TTC speculated that ischemia caused by multi-vessel spasm is the cause of transient heart failure (Sato et al, 1990). There are also a few case reports reporting the presence of LAD spasm (90, 91). However, no obvious coronary artery spasm is noted on coronary angiography in most cases of TTC. Furthermore, one obvious argument against this theory is that LV dysfunction in TTC is not limited to one vascular territory. Furthermore, induction of coronary spasm (for example with acetylcholine injection) does not usually induce substantial LV systolic dysfunction in the relative region of myocardium.

b) **Coronary plaque rupture**: It was suggested that coronary artery plaque rupture induced coronary artery ischemia leads to myocardial stunning. Since the left anterior descending artery (LAD) supplies the anterior wall of the LV and type II LAD wraps around the LV apex, a perfusion defect in the LAD was assumed to be the cause of the stunned LV apex (92). The investigators advocating the theory of LAD plaque rupture performed intravascular ultrasound study (IVUS) in 5 patients diagnosed with typical TTC and demonstrated presence of atherosclerotic plaque rupture in mid LAD (92, 93). However, none of these IVUS findings have been replicated. On the contrary, Haghi et al performed IVUS studies on 10 TTC patients and demonstrated absence of plaque rupture, positive remodeling or presumed coronary thrombus in all of the patients (87). Another theoretical problem with the hypothesis that plaque rupture initiates TTC episodes is the implication that catecholamine exposure should cause not only an initial event, but also a propensity towards recurrence of this event in the same blood vessel: - this seems highly improbable.

c) **Other limitations of “large vessel dysfunction” theory**: What ever the mechanism of large vessel dysfunction be, this theory has a number of limitations. Multi-vessel coronary artery spasm or a plaque rupture in the type II LAD would not be able to explain the discrepancy between the severe apical dysfunction and mild rise in the cardiac biomarkers. Furthermore, the ECG findings of TTC differ significantly from those of a single coronary vessel plaque
rupture. While in the case of TTC the ECG shows “global” change in all the leads in LAD plaque rupture the ECG abnormalities are usually limited to the anterior leads (94). It will also be difficult to explain the non-apical variants of TTC and recurrence of TTC in the same patient by the epicardial coronary plaque rupture. Finally, the histological changes found in cases of TTC are somewhat different from those of ischemic stunning (95). Finally the idea that TTC is engendered by a single episode of regional ischemia with consequent “stunning” of myocardium is consistent with recovery of myocardial function with in several days. In fact, there is increasing evidence that full recovery from TTC episodes takes greater than 3 months in most of the cases (see section 1.3.6). Again, this finding seems inconsistent with a pathogenesis involving plaque rupture.

1.3.5.1.3 Might there be small vessel dysfunction?

Given the paucity of evidence to support a pivotal role of either large vessel coronary vasospasm or of acute plaque rupture in the pathogenesis of TTC, a number of investigators have suggested that “microvascular dysfunction (MVD)” might be important. The implication is there fore either that

- Microvessels constrict inappropriately and/or
- There is attenuation of the vasodilator component of coronary autoregulation at the arteriolar level.

In fact, there are several case reports that in the presence of beta adrenoceptor blockade unopposed alpha adrenoceptor stimulation may affect coronary tone and/or autoregulation. Specifically, Robertson et al in 1980 reported precipitation of variant angina attacks with beta adrenoceptor antagonists (96). Analogously, Midge and coworkers showed that cold exposure triggered a reduction in coronary blood flow in patients with coronary artery disease, which was exacerbated by pretreatment with propranolol and blockade by phentolamine (97).
On the other hand, it is extremely difficult in practice to evaluate microvascular reactivity per se in the scenario of an acute attack of TTC, given the potential occurrence of LV diastolic dysfunction and associated extramural compression of coronary arterioles. It is also problematic to determine changes in myocardial oxygen demand in TTC.

The available data entirely derived from vascular reactivity studies in situ, rather than on isolated perfused coronary micro vessels. These can be summarized as follows:

1. Kume et al (98), measured coronary flow reserve volume with Doppler wire in 8 patients diagnosed with TTC during the acute period and 3 weeks later. They found the coronary flow reserve volume to be decreased in the acute phase and it returned back to normal at 3 weeks.
2. Elesber et al proposed similar theory of microvascular dysfunction as the underlying cause of TTC (99) finding that the coronary perfusion in TTC, measured by TIMI flow grade, to be impaired and correlated with the severity of myocardial dysfunction.
3. Another author reported severe myocardial metabolic abnormality and impaired coronary perfusion at rest in TTC (100).

Despite all these studies reporting microvascular dysfunction in TTC, none proves this to be the cause of TTC. *The microvascular dysfunction is most likely an effect rather than the cause of TTC.* It has been well proven in the animal and human models that myocarditis is associated with reduced microvessel reserve. Klein et al (101), showed that patients with biopsy-proven inflammatory myocarditis have a diminished coronary reserve due to reduced coronary vasodilator capacity. This is because of the involvement of the intramural coronary vasculature in inflammatory heart disease. Furthermore, the clinical presentation of TTC involves ST elevation, chest pain, dyspnea and occasional ventricular fibrillation, which is very similar to myocarditis presentation. The microvascular dysfunction in TTC is an effect of the myocarditis cause by the sudden rise in the catecholamine level rather than the cause.
1.3.5.1.4 LVOT obstruction

The apical form of TTC by definition includes both hyperkinetic and hypokinetic regions of myocardium, often with relatively sharp demarcation between these regions. This transition may be associated with an intra-ventricular pressure gradient, similar to that present in “obstructive” hypertrophic cardiomyopathy (HCM). The presence of outflow obstruction gradient has been claimed in 10 to 25% of cases of TTC (102) although there is considerable doubt as to the impact of this “obstruction” on stroke volume. It has been proposed that outflow tract “obstruction” is central to the pathogenesis of TTC (103). Additionally it has been proposed as a basis for hypotension in some patients soon after onset of TTC.

There is little support of the former hypothesis, especially given the occurrence of LVOT obstruction in only a minority of cases. An alternative is that variable existence of pressure gradient reflects heterogeneity of TTC severity with in the LV and that this is essentially a variable consequence rather than the cause of the disease.

1.3.5.2 Role of interaction between catecholamines and myocardium in the pathogenesis of TTC

There is substantial evidence that either increased catecholamine secretion (in majority of cases) or catecholamine administration, together with increased myocardial “susceptibility” associated with the aging female heart, represents a central aspect of the pathogenesis of TTC. A causative role for catecholamine effects can be supported both by the animal and human data. There are multiple examples where external administration of adrenaline or drugs leading to catecholamine “surge” induced TTC. Abraham et al. reported 9 cases of TTC following administration of adrenaline or dobutamine (104). Furthermore, cases of TTC have been reported as a result of the Irunkandi Jellyfish poisoning which is postulated to stimulate catecholamine release (105, 106). Furthermore, reports of increased prevalence of TTC among patients treated with those antidepressants, which inhibit the reuptake of, released
catecholamine support a primary role of catecholamine as a precipitant of TTC (107). Intracranial hemorrhage and phaeochromocytoma are well-known causes of TTC related to endogenous secretion of catecholamine.

Epidemiologically, TTC often occurs as a complication of serious medical illnesses associated with catecholamine release, for example, there are several case reports of association of TTC with phaeochromocytoma, even in the absence of other (symptomatic) complications of phaeochromocytoma. Similarly, a common cause of “secondary” TTC is stroke, whether ischemic or hemorrhagic. Intracranial hemorrhage (ICH) is a recognized cause of catecholamine surge that can last longer than a week (108). Furthermore, rats with induced subarachnoid hemorrhage showed resistance to the development of TTC after undergoing pharmacological or surgical sympathectomy (109). The ECG changes often associated with intracranial hemorrhage including widespread ST elevation or T wave inversion are also consistent with underlying TTC. The evidence of catecholamine surge as a cause of the TTC is strong and compelling.

Analogously, there has been development of animal models of TTC with the use of Isoprenaline and other catecholamines. For example, Shao et al, used 50 mg/Kg doses of Isoprenaline to induce TTC-like changes in the LV of Sprague Dawley rats (109). They demonstrated the development of TTC like changes using transthoracic echocardiography. Paur et al, using adrenaline instead of Isoprenaline, produced similar changes in Sprague Dawley rats (110).

While from the above examples the role of catecholamines in inducing TTC seems definite, the precise catecholamine involved endogenously and the associated receptor-systems are less clear. This is relevant because the effects of adrenaline and noradrenaline on vasculature and their affinity for the various β receptors differ. While adrenaline secretion occurs predominantly from the adrenal gland, noradrenaline is secreted largely from sympathetic nerve terminals. The frequent finding of higher noradrenaline levels in patients with TTC
and the existence of cardiac sympathetic hyperactivity on the (123) I-metaiodobenzylguanidine ((123) I-MIBG) scan suggests noradrenaline being the primarily responsible catecholamine (73, 74), whereas reports of TTC provoked by EpiPen or in association with phaeochromocytoma support a role for adrenaline.

Potentially more important is the precise pattern of receptor activation by released catecholamines, and possible variability in post-receptor signaling in the induction of negative inotropic (and hence superficially “paradoxical”) effects of catecholamine stimulation. As discussed previously, data from animal experiments have tended to support a central role for $\beta_2$ – adrenoceptor activation, but have not completely clarified the mechanisms whereby this stimulus leads to myocardial depression, nor the mechanisms whereby this stimulus leads to myocardial depression nor the mechanisms whereby these changes may be of relatively prolonged duration.

1.3.5.2.1 Catecholamine/myocardial interaction

The exact mechanism underlying the relationship between sympathetic spur and myocardial stunning is undetermined in TTC. The possibility of epicardial coronary artery spasm because of increased sympathetic tone has already been discussed in the section 1.3.5.1.1 and the limitations of this theory have been explained. Similarly, the related postulate of microvascular spasm because of catecholamine release can be completely excluded.

Another possible mechanism of myocardial “stunning” in TTC could be “direct” myocardial injury induced by the catecholamine surge. Previous studies have shown that prolonged exposure to catecholamines induces redox stress with in the myocardium. Catecholamine-induced changes on the myocardium involving free radicals were demonstrated in the context of forms of myocardial injury associated with conditions such as prolonged tachycardia. Singal et al, found that isoprenaline treatment in rats lead to myocardial fibrosis and reduction in ATP and treatment with antioxidants prevented such changes (111). Furthermore, free
radicals can interfere with sodium and calcium transporters. This can lead to increase in the trans-sarcolemmal calcium influx and overload of calcium that leads to myocyte dysfunction. Mann et al, demonstrated that increased catecholamine levels reduce the viability of cardiac cells through cyclic AMP mediated calcium burden (112). Of course, none of the animal studies in this era were intended to recapitulate TTC, and it remains to be determined whether the findings can be extrapolated.

The similarity between the pathogenesis of TTC and catecholamine induced injury are further supported by histology studies that have been performed in patients with TTC. In 2005, Wittstein et al described the myocardial biopsy features of 5 patients with TTC. On histology, significant intestinal infiltrates consisting primarily of mononuclear lymphocytes and macrophages and contraction bands without myocardial necrosis were found (73), similar to the changes found in rats with Isoprenaline induced cardiomyopathy.

Before we propose precise mechanisms for pathogenesis of TTC that will form the basis for the animal experiments, it is important to review a range of mechanisms that had been proposed in the last 30 years to explain the morphological and functional damage induced by catecholamines.

1.3.5.2.2 Fatty acid oxygenation

Nearly 50 years ago, Maling et al noticed typical feature of fatty degeneration in myocardial lesions induced by high levels of catecholamines (113). This theory was further supported by the findings of other authors who found that fatty acids are cytotoxic to cardiac cells and can induce heart failure in dogs (114, 115). Catecholamines increase plasma free fatty acid levels by encouraging the release of lipids from the adipose tissues. Advocates of this theory proposed that increased free fatty acids might cause myocardial damage by uncoupling of oxidative phosphorylation and by modifying the lipid bilayer of the sarcolemmal membrane. However, it was noticed that the high concentrations of fatty acids essential to induce
myocardial injury are never found in either the experimental or naturally occurring catecholamine cardiomyopathy.

1.3.5.2.3 Mitochondrial signal transducer effector pathway

Sustained catecholamine exposure can lead to calcium (Ca\(^{2+}\)) overload in the cytosol and mitochondria. Following Isoprenaline injection, plasma levels of Ca\(^{2+}\) decrease because of uptake of Ca\(^{2+}\) to organs such as heart, skeletal muscle and mononuclear cells. In the heart cells, this rise in the Ca\(^{2+}\) in the mitochondria induces mitochondrial cell death, a phenomenon that was explained in 1974 for the first time (116). It is suggested that Ca\(^{2+}\) overload in the mitochondria leads to oxidative stress and mitochondrial permeability transition leads to cell death.

This pathway has been given a new name of mitochondrial signal transducer effector pathway (MSTE), which explains the non-ischemic cardiomyocyte necrosis. A catecholamine surge leads to the movement of the Ca\(^{2+}\) from the plasma to inside the cells leading to cytosolic and mitochondrial Ca\(^{2+}\) overload. This signal of Ca\(^{2+}\) overloads leads to the induction of oxidative stress (transducer) by these organelles and subsequent opening of the mitochondrial membrane permeability transition pore (mPTP). The opening of the pore, constitutes the effector signal of the pathway, leading to solute entry, osmotic swelling and structural degradation of the organelles (117). This programmed sequence of events leads to necrosis of the heart cells with leakage of the intracellular contents that lead to rise in plasma troponin.

1.3.5.2.4 Oxidative stress

The pathophysiological events triggered by the rise in intracellular Ca\(^{2+}\) are further intensified by the oxidative stress that develops upon sustained exposure of catecholamines. Activation of the alpha-adrenoreceptors by noradrenaline leads to activation of NADPH oxidase, with ensuing generation of superoxide anion radical in cardiomyocytes (118). Furthermore,
monoamine oxidase (MAO) reliant oxidative deamination of catecholamines forms hydrogen peroxide (H2O2), which may be converted to highly reactive hydroxide radical through metal catalysis. Last and most importantly, catecholamines are readily oxidized into toxic compounds called “aminochromes”. This process occurs instinctively at a low rate but is noticeably augmented in the company of oxidants and free radicals such as superoxide, redox metals and by enzymatic catalysis.

When the formation of free radicals and oxidants over power the antioxidant capacity a state of oxidative stress develops with profound cytotoxic consequences related to oxidative damage in lipids proteins and nucleic acids.

1.3.5.2.5 Other potential biochemical effectors-Nitric oxide, Peroxynitrite and TXNIP

Before describing the relationship of nitric oxide (NO) with TTC, attention should be drawn to findings that not only is NO release partly under adrenergic control (119) but also, at the same time NO, via cGMP generation, (49) is able to potentiate noradrenaline release constituting a potential “vicious cycle”.

1.3.5.2.5.1 Nitric oxide (NO)

NO is an omnipresent messenger which is enzymatically formed from L-arginine by three isoforms of nitric oxide synthetase (NOS): endothelial type NOS (eNOS), neuronal type NOS (nNOS) and cytokine inducible NOS (iNOS). In the cardiovascular system endothelial NOS (eNOS) leads to the synthesis of NO from L-arginine, while in the nervous system this is achieved by nNOS (120). However, NO may also be generated by reduction of nitrite, especially under hypoxic conditions (121). There are several biochemical processes underlying the effects of NO. NO may be taken up by red blood cells and released locally under hypoxic conditions but its biochemical effects either result from soluble guanylate cyclase activation or via protein S-nitrosylation. In general, NO acts via the stimulation of guanylate cyclase, which is a heterodimeric enzyme with subsequent formation of cyclic-
GMP. This production of NO by eNOS is dependent on Ca\(^{2+}\) regulation. On the other hand, iNOS is developed by variety of cells in response to inflammatory stimulus and production of NO by iNOS is not dependent on Ca\(^{2+}\) regulation. The condition where the role of iNOS has been studied extensively is “septic shock” where sepsis induced increased expression of iNOS leads to vascular dysfunction.

Furthermore, NO derived from the nNOS and eNOS has an important role in control of cardiac contractility (122). The effects of NO on cardiac contractility are variable, on one hand NO inhibits L type Ca\(^{2+}\) channels, while at the same time it stimulates sarcoplasmic reticulum-related Ca\(^{2+}\) release (123-125). The NO-dependent contraction is significantly altered in the chronic heart failure. It has been speculated that eNOS related excessive release of NO in chronic heart failure leads to cardiac depression. Hare et al, proved that use of a NOS inhibitor use in patients with heart failure leads to potentiation of beta-adrenergic inotropic response (126). However, the underlying mechanism of tis response and involvement of different isoforms of NOS in chronic heart failure remains unclear.

The release of NO, irrespective of mechanism, is likely to have substantially different physiological effects depending upon the presence or absence of homeostatic disorders including anoxia or redox stress. Under anoxia, No release from nitrite is potentiated, leading to increased effects in hypoxic vascular beds. Under redox stress, “uncoupling” of NOS leads to concomitant production of NO and superoxide potentially inducing peroxynitrite (ONOO\(^{-}\)) formation, with substantially different biological effects from those of NO.

1.3.5.2.5.2 Potential for pathogenic effects of peroxynitrite

Although NO is often described as toxic, it is neither toxic and nor it directly kills organisms or tumor cells. Furthermore, neither superoxide nor NO are generally toxic in the tissues because of effective means in the human body to minimize their accumulation. While NO is rapidly removed by its diffusion through tissues into red blood cells, superoxide is removed
by the scavenging enzymes called super oxide dismutases. However, potential toxic effects of NO emerge when it combines with superoxide to form a powerful oxidant called peroxynitrite (ONOO'). The kinetics of the NO-superoxide interaction are sufficiently rapid to permit some formation of ONOO' despite rapid clearance of superoxide by SOD. No enzyme is required for the formation of ONOO' and it is not even required that NO and superoxide are produced in the same cells. The diffusion of NO over short distances is so fast that it can effectively combine with superoxide in adjacent cells. Every time NO and superoxide collide they form ONOO'.

Under normal conditions the production of ONOO' resulting in little oxidative stress and contributing more to physiological effects, such as vasodilatation. However, under some disease conditions, the production of ONOO' increases because of increased production of NO and for superoxide. A rise in the level of ONOO' (reflecting in part the greater stability of ONOO') leads to dysfunction of critical cell processes and potential induction or apoptosis and necrosis.

ONOO' is not a free radical in nature but still it is much more reactive than its parent molecules superoxide and NO. The half-life of ONOO' is relatively short but it is sufficient to cross and to traverse greater distances that either NO or superoxide biological membranes between cells. Two mechanisms have been proposed to explain oxidation of target molecules by ONOO':

1. ONOO' and its protonated form peroxynitrous acid can cause direct oxidative modifications through electron oxidation processes (127). However, a few chemical groups directly react with ONOO' such as thiols, Zn and iron centers.

2. Or ONOO' decomposes into reactive radical to cause oxidation indirectly (128).
1.3.5.2.5.2.1 Molecular mechanisms of peroxynitrite mediated cell death

Peroxynitrite dependent cytotoxicity is mediated by its innumerable effects; lipid peroxidation, protein nitration and oxidation, DNA oxidative damage, activation of matrix metalloproteinases (MMP) and inactivation of a series of enzymes. Mitochondrial enzymes are most vulnerable to the damaging effects of ONOO$^-$ leading to reduction in the ATP formation and induction of mitochondrial permeability transition by opening of the permeability transition pore. These events lead to the reduction in the ATP formation, mitochondrial damage by mitochondrial swelling and permeabilisation of the outer mitochondrial membrane.

This leads to efflux of several apoptosis inducing molecules including cytochrome C and apoptosis inducing factor (AIF). In addition to its effects on the mitochondria, ONOO$^-$ induces more severe oxidative injury to the DNA resulting in DNA strand breakage. This leads to the activation of nuclear enzyme called poly ADP-ribose polymerase (PARP). Activated PARP consumes NAD to build up poly ADP ribose polymers (PAR), which are rapidly metabolized by poly ADP-ribose glycohydrolase (PARG). Some free PAR may exit the nucleus and travel to mitochondria where they amplify the mitochondrial efflux of AIF.

Once excessive oxidative and nitrosative stress induces DNA damage, for example in various forms of reperfusion injuries and other pathophysiological conditions the cell may undergo apoptosis by in case of moderate PTP opening and PARP activation with preservation of cellular ATP, or necrosis in the case of widespread PTP opening and PARP over-activation, leading to massive NAD consumption and collapse of cellular ATP (Figure 4).
1.3.5.2.2 Possible role of ONOO⁻ in TTC

As discussed in the above sections, TTC patients exhibit augmented NO signaling even during recovery. TTC patients exhibit greater platelet responsiveness to anti-aggregatory effects of NO, and secondly, plasma concentrations of ADMA are lower in patients with TTC than in normal controls. Since catecholamines stimulate β adrenoceptors, some of which are coupled to NOS, the potential for a marked increase in NO release/effects is particularly relevant in TTC. Furthermore, catecholamines can induce oxidative stress (including
superoxide release) by NOS “uncoupling”. Thus TTC are both chronically predisposed to, and actually imposed with, for production of ONOO$, which can lead to downstream activation of PARP, ATP depletion and apoptosis. Interestingly, some recent findings pointed for interactions between ONOO$ and the thiredoxin system. It has been shown that one of the actions of ONOO$ is activation of thioredoxin interacting protein (TXNIP) (Figure 5), an inflammatory activator that has been found in multiple diabetic and cardiac conditions. Conversely, TXNIP also potentially interacts with nitrosative stress, as shown below.

1.3.5.2.6 Thioredoxin (TRX) thioredoxin interacting protein (TXNIP)

There are effective means in the human body to reduce or minimize the accumulation and effect of reactive oxygen species to reduce oxidative stress. One such anti-oxidant molecule is thioredoxin (TRX), which is found in two forms TRX1 and TRX2. TRX is essential for human life because it keeps a check on the redox stress (129). The functions of TRX differ in the various cell compartments. In the nucleus it regulates the cell growth and angiogenesis while in the cytosol it leads to inhibition of apoptosis signal regulator kinase 1(130).
Thioredoxin interacting protein (TXNIP) inhibits the effect of TRX in a redox dependent way. TXNIP acting as a competitive inhibitor forms a bond with the TRX and removes it from the proteins whose function is inhibited by TRX. Thus by removing the “anti-oxidant” TRX, TXNIP shifts the balance towards excessive reactive oxygen species generation. At the same time TXNIP also exerts redox-independent functions such as: induction of apoptosis and induction of TXNIP expression by glucose.

TXNIP plays a major role in various disease states and its function is studied extensively in diabetes. It has been found that beta cell TXNIP is up regulated in diabetes. TXNIP leads to beta cell apoptosis and TXNIP deficiency protects against diabetes (131). Most of the literature on involvement of TXNIP in cardiac function has been related to two cardiac conditions 1) Ischemia-reperfusion injury and 2) Conditions causing cardiac pressure overload. In ischemia reperfusion injury models the main role of TXNIP is induction of oxidative state by competitive inhibition of TRX (132). This also constitutes part of the basis for use of Calcium channel blockers and ACE inhibitors post MI since Ca++ blockers and ACE inhibitors reduce the expression of TXNIP. In response to cardiac pressure overload induction of TXNIP occurs, the extent of which has positive correlation with cardiomyocyte apoptosis (133). Furthermore, Yoshioka et al found that in TXNIP knock out mice pressure overload leads to less cardiac muscle hypertrophy (134).

There have been recent studies showing some association of nitrosative stress with TXNIP (135). TRX has been shown to mediate protein denitrosylation and hence reducing the nitrosative stress (136). Forrester et al recently demonstrated that endogenously synthesized NO suppresses TXNIP expression, thereby facilitating TRX mediated denitrosylation and excessive suppression of TXNIP will augment nitrosative stress (137).

In the animal work on the pathogenesis of TTC we have tried to evaluate the TXNIP expression in the setting of nitrosative stress.
1.3.6 Natural history

The critically important aspects of the natural history of TTC are:

- a) **The acute phase**: a main issue is of potential for hemodynamic decompression and/or arrhythmias.
- b) **The “recovery” phase**: the main issue is the time course of return to normal physiology: early data suggested that recovery was generally rapid.
- c) **The potential for recurrence**

In all of these aspects, information has been fragmentary until very recently, and remains subject to substantial selection bias (based, for example, on diagnostic criteria for TTC, philosophy regarding investigation of very elderly patients, and physician awareness of the diagnosis).

In all cases, the available literature tends to be fragmentary: prospective cohort studies are very rare.

1.3.6.1 The acute phase issues

1.3.6.1.1 Mortality

Although, there is no detailed prospective study evaluating the acute complications and mortality of TTC, the acute course of TTC has been historically thought to be generally favorable (138). However, recent evidence shows that 30-day mortality in TTC is similar to that of anterior MI (139). When reporting risk of acute death in patients diagnosed with TTC it should be borne in mind that concomitant potentially fatal non-cardiac conditions such as: subarachnoid hemorrhage can contribute to short-term mortality. For this particular reason, in this thesis we have defined “secondary TTC” as development of TTC in patients already suffering from potentially life-threatening medical or surgical conditions and “primary TTC” as
that which developed in the absence of such critical illness. We performed a systematic review and meta-analysis to assess the impact of “secondary TTC” on acute complications and mortality.

At the same time, the risk of acute death can be “under-called” because not all the cases of TTC are diagnosed. Elderly patients who have higher risk of complications can be misdiagnosed as myocardial ischemia because of relative contraindication to invasive cardiac procedures and reluctance to utilize cardiac MRI imaging. At the moment there is also limited knowledge about the outcomes in very elderly patients diagnosed with TTC. Overall our knowledge of increased risk of mortality and complications comes from two small retrospective studies that suggested increase in rate of complications with advancing age (140, 141). We have tried to assess the risk and clinical correlates of acute death in patients diagnosed with TTC, which will be discussed in detail in Chapter 5 of this thesis.

Potential causes of mortality in the absence of pre-existent extra-cardiac disease include

a) Shock status, which occur in up to 10% of cases despite rarity of pulmonary odema. This complication is discussed extensively in Chapter 4. However, it is important to appreciate that it rarely reflects pulmonary odema and may result from a combination of inotropic impairment and inappropriate vasodilation.

b) Tachyarrhythmias: especially torsade de pointes, occurring mainly in the first 24 hours.

c) Ventricular mural thrombosis and subsequent embolism, which is relatively rare.

1.3.6.1.2 Hemodynamic variability

While the majority of patients diagnosed with TTC are normotensive on presentation nearly 15 to 25% develop hypotension (142). The severity of hypotension varies and not uncommonly it can be associated with shock that contributes to short-term morbidity and mortality (143, 144). Both the pathogenesis and treatment of shock and hypotension represent
a considerable dilemma. The pathogenesis behind shock is not simple because during acute onset of TTC various factors control blood pressure. While the sudden rush of catecholamines would tend to raise the blood pressure, the development of LV systolic dysfunction along with vasodilatation caused by BNP leads to net hypotension. It also has been suggested that LVOT obstruction and acute mitral regurgitation may occur in acute cases of TTC, inducing hypotension (145). However, LVOT obstruction tends to be rare and mitral regurgitation is usually mild in most of the cases. A current finding by Chong et al that systolic blood pressure does not correlate with LV systolic function demonstrates that the cause of hypotension is multifactorial and not entirely due to the drop in cardiac output (142). Furthermore, pulmonary capillary wedge pressure in patients diagnosed with TTC is usually normal or mildly elevated, making cardiogenic cause of shock less likely. Also, the evidence is accumulating that TTC is associated with increase in NO signaling and high release of BNP. Both of these agents are strong vasodilators making vasodilatation a contributor to the hypotension, although paradoxically BNP can also stimulate incremental catecholamine release (49).

Acute management of shock is as challenging both in theory and practice. Most of the inotropic agents used for hypotension are catecholamines that can potentially aggravate the condition and systolic dysfunction and should be avoided. In the absence of clear evidence, insertion of intra aortic balloon pump (IABP) in patients with severe shock has become the default management option. However, some authors advise against the use of IABP in shock because of the belief that negative aortic pressure caused by the deflation of balloon accelerates flow through LVOT and aggravates obstruction (146). Santoro et al provided preliminary data for efficacy of use of Levosimendan in the treatment of hypotension in a small set of TTC patients (147).

Since RV involvement occurs in nearly two-thirds of cases of TTC it could be a potential contributor to the development of shock/hypotension similar the RV infarct. Furthermore, RV
involvement has been associated with frequent complication in the acute stage of TTC (148, 149). However, remains to be determined whether RV involvement is primarily a marker of more severe TTC or whether it contributes to overall hemodynamic impairment. In chapter 4 we have tried to evaluate the significance of the presence or absence of RV involvement in TTC.

1.3.6.1.3 Other acute complications

Other non-hemodynamic complications include atrial and ventricular arrhythmias. Overall atrial fibrillation is the most common arrhythmia and life threatening arrhythmias such as \textit{torsades de pointes} occur occasionally. It is not clear which subset of patients are more prone to arrhythmias. Maximum QT prolongation occurs between 48-72 hours and while it theoretically increases the risk of \textit{torsades de pointes}, only 3% of patients develop cardiac arrest (150). Furthermore, patients presenting with cardiac arrest, who are found to have TTC on angiography, usually have normal QTc interval at the time of cardiac arrest (8). Whatever the cause of ventricular arrhythmia may be, it is one of the most important causes of TTC related mortality requiring close monitoring of TTC patients in the first 3 days.

Thromboembolism is a common complication of TTC and its exact frequency is not known. A recent study from a small data set showed the frequency to be around 14% (151). The major cause of embolic phenomenon in TTC is development of mural thrombus. At the same time, some of the embolic episodes occur because of new onset of atrial fibrillation post TTC. The other life threatening complications of TTC such as pericardial effusion and LV wall rupture are relatively rare.

1.3.6.2 Long term outcome- incomplete recovery and problem of recurrence

Given the progressive expansion of databases on this subject over the past 3 years, most of the literature evaluating the chronic course of TTC patients is very recent. The long-term survival of patients diagnosed with TTC in the available large series is similar to that in the general
community with the exception of a small mortality rate following acute episodes (152).

Despite having a low risk of mortality during follow-up, symptoms of chest pain, dyspnea and fatigue persist in some patients for 2 years (11). Earlier studies reported “recovery” of TTC as resolution of wall motion abnormalities and normalization of LV ejection fraction (153). It has also been found that BNP levels do not completely normalize even after 3 months (53): whether this is because of slowly resolving inflammation and/or development of fibrosis is unclear. Indeed, recent CMR studies have shown presence of fibrosis on follow up scans in some of the patients suggesting that these changes may have permanent sequelae.

It is possible that patients’ symptoms of fatigue and dyspnea are also related to unresolved inflammation. A key question, therefore, relates to heterogeneity of recovery rates and extent of eventual recovery. This has simply not been studied to date: the realization that recovery us often slow and sometimes incomplete is a very new concept in TTC. Therefore, the idea of planning therapeutic intervention to accelerate recovery is also a very new one. Furthermore, recent preliminary studies with magnetic resonance spectroscopy (MRS) have demonstrated acute impairment of myocardial energetics in TTC: the process of recovery in this regard is also very relevant. The use of MRS and other modalities evaluating myocardial energetics may be able to provide data regarding recovery parameters in future.

As follow up of TTC is increasingly extensive, it is becoming obvious that problem of recurrence is substantial. While there are multiple case reports on recurrence only 1 retrospective and 1 prospective study have tried to evaluate the risk of recurrence and those have produced very different results. While the Mayo clinic data revealed 4-year recurrence rate to be approximately 12%, the data by Parodi et al showed recurrence rate to be as low as nearly 1% per annum (11, 152). Variability in the recurrence rate is likely secondary to selection bias. Furthermore, no clinical correlates have been found to predict recurrence of TTC. Given the relatively fragmentary nature of the available data on recurrence, it is important to deduce whether the available literature provides any degree of reliable
information. We therefore performed a meta-analysis and systematic review on this that will be discussed in chapter 6 of this thesis.
Chapter: Pathogenesis of Takotsubo Cardiomyopathy-Animal work
2.1 Introduction

A large number of publications in the last 5 years have suggested TTC to be of non-ischemic origin but have not provided any clear pathological mechanism of catecholamine-myocardial interaction in TTC. Given the difficulties in delineating the pathogenesis of TTC from human data a number of investigators have attempted to develop an animal model of TTC. Ueyama et al performed the first of these studies in 2007, where he utilized immobilization stress to induce apical hypokinesis in rats to evaluate the role of sex hormones in the pathogenesis of TTC (154). Since then multiple investigators have used direct or indirect methods to increase catecholamine mediated effects on myocardium in rodents to produce TTC-like changes.

Ellison et al, were the first to use the β-adrenoceptor agonist isoprenaline to induce TTC-like changes in male Wistar rats and confirmed development of such changes with echocardiography using fractional area shortening (FAS). Even though the experiments were performed in male rats the results gave some incremental insight: at least β-adrenoceptor stimulation was finally implicated (155). Furthermore, this rat TTC model shared many features of TTC in humans. Nearly 20% of the rats died following a small subcutaneous dose (5 mg/kg) of isoprenaline because of ventricular arrhythmias; there was transient reduction in the LVEF along with FAS which improved by day 3 and blood pressure of isoprenaline treated rats was lower than that of controls on day 1, returning to normal by day 6. These investigators proposed that myocyte damage in TTC resulted from increases in sarcolemmal calcium in the myocytes. Catecholamine-induced leakage of Ca$^{2+}$ from sarcoplasmic reticulum through a dysfunctional RYR2 leads to myocyte damage, inflammation, necrosis and apoptosis. While various animal and human biopsy studies have supported the finding of inflammation in TTC, the extent of associated necrosis in TTC is clearly limited (156), and the discrepancy between marked inflammation and limited necrosis therefore of great interest.
Paur et al. described a rat model of TTC using high dose epinephrine in male Sprague Dawley rats (110). They proposed biased agonism of epinephrine for β2 receptors by showing stimulation of the Gs subunit β2 adrenoceptors by epinephrine at low concentrations and Gi subunit at higher concentrations. This shift of net β2 receptor signaling from stimulatory to inhibitory subunit with high dose of epinephrine was proposed as the underlying cause for the negative inotropy and cardio-depressant properties of TTC. However, these investigators did not evaluate the basis for associated myocardial inflammation. Similarly, a Swedish group produced an animal model of TTC with the use of a higher dose of isoprenaline in male Sprague Dawley rats (109).

TTC is a predominantly a disorder of elderly women and using male young rats may not entirely represent the pathogenesis of the disease. The authors in these previous publications have not provided any published basis for their choice, but we believe high mortality with isoprenaline in “elderly” female rats could be the reason for choosing younger and male rats for experiments. Despite the fact that recent studies in rodent models of TTC have added to our understanding of the basis for impairment of myocardial contractility in the acute phase of the disorder. These findings do not address the issue of presence of myocardial inflammation following the rise of catecholamines, nor do they consider the possible role of energetic impairment. The coupling of β2 adrenoceptors to NOS plus the recently published evidence of augmented NO signaling in TTC raises the possibility that β2 adrenoceptor stimulation may induce inflammation via nitrosative stress (formation of peroxynitrite). As regards the issue of energetic impairment in catecholamine–induced acute cardiac damage, Desrois et al, while intending to mimic acute myocardial infarction with isoprenaline administration in rats, actually produced TTC-like apical hypokinesis with depletion of high energy phosphates demonstrated via magnetic resonance spectroscopy and also biochemically (119).

**To test the hypothesis that increased release of NO potentially induces the formation of ONOO⁻ anion with associated redox stress, protein nitration and downstream activation**
of the α-arrestin TxNIP we created a rat model of TTC. We divided the studies in three main components:

1. Development of rat model of TTC: In the preliminary experiments we developed a rat model of TTC using isoprenaline in 5-month-old female Sprague Dawley rats. We confirmed the development of TTC using speckle tracking on TTE and fractional area shortening (FAS).

2. Evaluation of biochemical correlates of TTC: We wished first to test the hypothesis that Isoprenaline induces nitrosative stress in this model. Hence we evaluated induction of tyrosine nitration on proteins via quantitation of 3-nitrotyrosine (3NT).

Furthermore, TXNIP synthesis is stimulated by tissue anoxia and by ROS production and results in incremental inflammatory activation and exacerbation of stress responses in mitochondria and endoplasmic reticulum. We therefore sought to determine whether TXNIP production might also be increased in this model.

The studies reported in this thesis relate to immunohistochemical evaluations of 3-NT, poly ADP ribose (PAR) and TXNIP.

3. Intervention with PARP inhibitor: In the third part of experiments we assessed the impact of the PARP-1 inhibitor 3-aminobenzamide (3AB) on the LV mechanics and nitrosative stress in the rat model of TTC.
2.2 Methodology

2.2.1 Development of rat model of TTC

In view of the predominant occurrence of TTC in aging women we sought to develop an animal model of TTC in aging female rats. The Institutional Ethics Committee approved the animal study of TTC. Initially, 50-mg/kg Isoprenaline, as used in the previous animal experiments of TTC (157), was injected intra-peritoneally in female Sprague Dawley rats aged 9 months. This induced TTC but led to an unacceptably high mortality of 80%. The dose of isoprenaline was therefore reduced to 15 mg/Kg and then 5 mg/Kg to reduce the mortality. At the same time, we evaluated the impact of Isoprenaline on different age groups. Five mg/Kg of intra-peritoneal isoprenaline injection in 4 to 5 months old Sprague Dawley rats induced TTC with a mortality of approximately 25%, in all cases occurring within 2 hours of isoprenaline injection.

In one rat, serial changes in apical radial strain were quantified over 96 hours post isoprenaline injection, as shown in Figure 6. These data suggested that peak effects occurred

![Graph showing radial strain over time](image)

**Figure 6: Radial strain at different time intervals post 5 mg/Kg of Isoprenaline.**
around 24 hours, with subsequent partial resolution by 96 hours.

Therefore, rats were injected with 5mg/Kg Isoprenaline and echocardiography was performed at baseline and 24 hours. Additionally 4 untreated rats were used as controls, largely for biochemical comparisons.

The subsequent interventional studies were performed sequentially.

1. Initially, the effect of isoprenaline alone was evaluated, with echocardiographic and biochemical assessment 24 hours thereafter. Untreated rats were utilized as biochemical controls.

2. Subsequently (once we had established the parameters of isoprenaline effect) we tested the hypothesis that 3 aminobenzamide (3AB), a selective PARP-1 inhibitor, limited isoprenaline-induced impairment of contractility. In this case, rats treated 3AB alone were utilized as a reference group to exclude intrinsic inotropic effect of 3AB, but the primary comparison was between rats treated with isoprenaline/3AB vs. isoprenaline alone.

2.2.2 Echocardiographic analysis

Effects of Isoprenaline on LV regional and global function were evaluated by 2-dimensional TTE. All the imaging studies on rodents were performed under anesthesia using 2% isoflurane. Animals were secured on a warming pad and limb-lead electrocardiograms recorded throughout echocardiography. A GE 10s sector array ultrasonic probe with frequency range of 4.0-10.5 MHz (10 sector, mode 2298589, general Electronics Healthcare, US) was utilized for acquisition of echocardiographic views. Imaging was performed in the shallow left decubitus position. For analysis of images, parasternal long axis and short axis images of the LV were utilized. Because of tachycardia it was difficult to reliably quantitate changes in LV wall motion and hence speckle tracking and fractional area shortening were
used to analyze segmental systolic function (FAS). Parasternal long axis images were used for FAS and short axis were used for radial strain measurement.

a) **Strain analysis:** Radial strain was measured from the short axis view at the level of the apex. Image depth was set at 2 – 2.5 cm with a frame rate of 240 -260 frames per second. At least 14 frames were collected during each heartbeat. Echocardiographic images were transferred to an echo PAC PC workstation where data was analyzed using a speckle-tracking algorithm software incorporated into the PC workstation (Q analysis software) (GE version 11.1.1). This is a validated software that detects and tracks the ultrasonic interference pattern called “speckle” inherent to 2D TTE after segmenting the ventricular silhouette into six segments. “Speckles” are discrete echogenic areas triggered by meddling pattern of myocardial tissue inconsistencies. Because of changing tissue orientation during a cardiac cycle “speckle” appear and disappear. To track the “speckles” LV apex endocardial border was drawn in the end-systolic frame of the cardiac cycle. Depending upon the image quality upto three consecutive cardiac cycles were selected for analysis. Semi-automated tracing of

![Image of echocardiogram analysis](image)

**Figure 7: Analysis of rodent left ventricular apical strain post Isoprenaline**
the endocardial and epicardial borders of the LV apex were performed and verified over 3 cardiac cycles. The wall motion was corrected if needed to achieve the best quality tracking through the cine loop. The segment of the LV analyzed was divided into 6 standard anatomical segments and strain/strain rate of each individual segment was obtained. Apical strain was the average of the 6-segment strain (Figure 7). We repeated this procedure three times to obtain an average of three values.

Experienced investigators blinded to the treatment status and phase of treatment performed all the echocardiographic analysis involving strain measurement. Control rats were also included in the blinding process. Inter-observer coefficient of variability (COV) for measurement of apical strain was 13%.

b) Fractional area shortening: FAS was performed by M mode echocardiography in the parasternal long axis view at three predefined positions in the left ventricle. Basal LV measurements were performed 3 mm below the mitral annulus. Mid ventricular FAS was recorded at the level of the papillary muscle and apical measurements were done 3 mm above the LV apex. Along with FAS, measurements of the LV ejection fraction and wall thickness were performed. All the measurements were done from leading to edge to leading edge. All the measurements were performed before and after Isoprenaline injection. The thickness of the intraventricular septum and posterior LV wall was measured.

2.2.3 Immunohistochemical studies

Post echocardiography rats were humanely killed under anesthesia. Rat hearts were excised and divided into apical and basal LV sections, which were preserved for analysis by western blot and immunohistochemistry. For immunohistochemical studies tissue sections were fixed in 9% formaldehyde and paraffin embedded. Immunohistochemical studies for evaluation of regional left ventricular content of 3NT, TXNIP and PAR were performed in a similar manner. However, for quantification of apoptosis, Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining protocol was used.
2.2.3.1 3Nitrotyrosine, TXNIP and PAR staining

Immunohistochemical staining for evaluation of regional LV content of 3NT, TXNIP and PAR was performed in a similar manner using the same protocol but different antibodies (ABCAM for 3NT, MBL International Co., Wodburn [MA] for TXNIP and Tavigen for PAR) and different antibody concentrations. The general protocol used for the analysis of the staining is as follows:

Tissue sectioning and rehydration

Paraffin sections of 4 µm were cut and positioned on Superfrost Plus charged microscope slides for drying. They were heated for 45 minutes at 60°C. This was followed by Xylene wash for 5 minutes, which was repeated 3 times. Immersing the slides in reducing concentrations of ethanol performed rehydration of the slides.

Antigen retrieval

Slides were then boiled in 0.01-mol/L citrate buffers at pH 6 for 20 minutes in microwave oven and allowed to cool for 30 minutes in a water bath at 23°C. The slides were then washed with PBS.

Immunostaining

Sections were then incubated in a humidity chamber with blocking buffer (2% serum, 5% bovine serum albumin) for 45 minutes and then patted dry. Primary antibody was applied in a dilution of 1:300 for 3NT, 1: 100 for PAR and 1:500 for TXNIP and incubated overnight at 4°C. This was followed by repeated PBS washes. Sections were incubated for 30 minutes with 3% hydrogen peroxide to block endogenous peroxidases and then washed again with PBS. Secondary antibody was then applied (100 µL per slide) and incubated for 60 minutes followed by repeat PBS wash.

Dehydrate and mount slides
Nuclei were counter stained with Gill’s haematoxylin and slides were mounted in DePex sections were visualized utilizing the Nova Red Kit (Vector Laboratories, Burlingame, CA)

2.2.3.2 TUNEL staining

TUNEL staining was performed in addition to the biochemical investigations in order to quantitate apoptotic response. Paraffin sections of 4 microns were cut and positioned on Superfrost Plus slides for drying. They were then heated to 45° C to remove air bubbles, cooled to room temperature. We initially used fluorescent staining for detection of apoptosis. However, because of difficulty in quantification of fluorescent staining we chose another system (DeadEnd™ Colorimetric TUNEL System), results of which were more reproducible. DeadEnd™ Colorimetric TUNEL System is a non-radioactive system that provided accurate detection of apoptotic cells. Deparaffinization of the tissue sections was achieved by immersing the slides in the Xylene solution for 5 minutes two times. This was followed by twice washing of the slides in 100% Ethanol for 5 minutes. Tissue sections were rehydrated by immersing them in graded Ethanol solutions (95%, 90%, 70% and 50%) for 3 minutes each at room temperature. Slides were then washed by dipping for 5 minutes in 0.85% NaCl and then for 5 minutes in phosphate buffer solution (PBS).

Tissue sections were fixed by immersing the slides in 4% formaldehyde solution for 15 minutes. We then marked the slides with PAP pen. This was followed by wash in the PBS solution for 5 minutes twice. Protinase K 20µg/ml solution was prepared from 10 mg/ml Protinase K stock solution by diluting 1:500 in PBS. 100 µl of this solution was added to the tissue on each slide and incubated it for 15 minutes at room temperature followed by PBS wash for 5 minutes. Tissue sections were refixed with second in 4% formaldehyde solution followed by 5-minute PBS wash. After removing the excess fluid from the slides, tissue sections were covered with 100 µl of Equilibration buffer for 10 minutes.
While the sections were equilibrating we prepared rTDT reaction mix from the available kit. Following equilibration of the tissue sections 100 µl of rTDT solution was added to the sections and sections were covered with cover slips. This was followed by incubation for 60 minutes at 37°C. Reaction was finished by immersing the slides in the SSC solution for 15 minutes. Slides were then washed with PBS twice for 5 minutes. Immersing the slides in 0.3% hydrogen peroxide solution in PBS for 5 minutes at room temperature blocked endogenous peroxidases. Slides were once again washed with PBS for 5 minutes. 100 µl of diluted Streptavidin HRP was added to the tissues sections and slides were incubated for 30 minutes at room temperature. Slides were washed with PBS solution and 100 µl of DAB solution was added to the slides and slides were incubated for 6 minutes at room temperature. This was followed by thorough wash with deionized water. Slides were rehydrated using different concentrations of ethanol. Permanent mounting medium was used to mount the slides and slides were analyzed under Nikon microscope.

2.2.4 Quantification of staining

2.2.4.1 3NT and TXNIP staining quantification

Investigators blinded to treatment status using Image J software performed quantification of the staining on immunohistochemistry sections. Image J is a java based software program developed by National Institutes of Health (NIH), which can be used to quantitate staining intensity. While there are number of ways to express the data using Image J, we chose to quantitate data as % stained area. To reduce the inter-observer variability we were very consistent while taking the microscopic pictures of the slides and used same microscope (NIKON) for all the pictures. Furthermore, the same settings of lighting and magnifications parameters were used for all the images.

All the slides were analyzed on a Nikon optical microscope using 4, 10 and 20 X magnifications. However, for staining quantification we stored images at 4 X magnifications.
Initially, 4 random samples on each slide were used for stain quantification, but because of high inter-observer variability (COV= 30%) a minimum of 8 regions of each tissue section were analyzed. This reduced the inter-observer COV to 10%. Eight sections of each slide were stored separately in TIFF format. All the images were then transferred to a computer and analyzed using same setting on Image J software.

The image to be analyzed was opened in Image J. Threshold section of image J was opened (ctrl + shift +T) and all the parameters (Hue, Saturation and Brightness) were set to zero. Thresholding method was kept as default and threshold color: red. To measure the total area Hue was adjusted to a value of 0 -255 and Saturation to a value of 35 -255 and Brightness bar was brought to a point at which all cells/tissue was selected. While changing the Brightness bar we made sure none of the background which was also stained at time was selected and that only tissues was chosen. Now that all the tissue was covered ctrl + M was used to measure the total area which was recorded in an Excel sheet. Now to measure the stained area we decreased Hue bar to a value of 0- 13 without making any change to the Brightness and Saturation values. Adjustment in the Saturation and Brightness was only made to fine-tune the results if required. The results of stained area were also copied in Excel sheet that along with the total area was used to calculate the % of staining per section. An average of 8 sections were used to obtain the final result.

2.2.4.2 TUNEL staining quantification

Detection of apoptosis was performed using TUNEL staining. Image J software was used to analyze the apoptosis content of 8 regions of each tissue section at 10 X magnification. Mean percentage content of apoptosis for each rat was calculated by getting an average of the 8 region results.

Automated counting of single color image was done using Image J without the need of any additional plugins. The image to be analyzed was saved in a JPEG format from NIKON.
microscope at 10 X magnifications. Saved image was opened in Image J software. All the images were converted to greyscale before starting the analysis. If the image was an RGB color image then it was converted to grey scale clicking on EDIT → OPTIONS →CONVERSIONS to “scale when converting”. This was followed by IMAGE → TYPE → 8 or 16 bit to convert to greyscale. Following the conversion of the image to greyscale, we clicked on Image to Adjust the Threshold. The sliders in the Threshold sections were changed to cover all the section we wanted to count. If some of the cells/nucleus to be counted were touching each other then by clicking on Process background noise was subtracted. After confirming that the area needed is highlighted, Apply button was clicked which created binary vision with only 2 pixel intensities, Black and White. If there were still particles touching each other then modifications were made by clicking on Process → Binary → Watershed. This clarified the edges of the cells/nucleus to be counted.

To count the number of cells click on Analyze → Analyze particles. To count the entire slide cells we left the Size of the Pixels from 0 to infinity. Now to calculate the stained cells (apoptosis) setting was changed from 0- infinity to 150 – 500. A copy of the image was made and all counted cells were shown as numbered outlines. Before clicking for the results we made sure that summarize section was turned on. This was followed by click on the results that showed the number the calculated cells and stained proportion of the area in a new Window. This information was saved in an Excel sheet.

2.2.4.3 PAR staining quantification

PAR staining, limited to the nuclei of the cells, utilized % area stained and quantified it using Image J.

The staining quantification was performed using 10 X magnifications. The image was changed to black and white by clicking on edit and changing the image to 8 or 16 bit. This was followed by change in the threshold setting so that the entire stained nucleus were
highlighted. Then this was converted to binary image by changing threshold from red to black and white. By clicking on the analyze particles % stained area was calculated.

2.3 Statistical analysis

2.3.1 Effects of isoprenaline

Analyses of left ventricular structure and function before and 24 hours after isoprenaline injection were performed utilizing Student’s paired t test. Comparison of myocardial 3NT and TXNIP content between treated and control rats were made utilizing non-paired t tests, after evaluation for normal distribution of data, while evaluation of effects on expression and regional heterogeneity of 3NT, TXNIP and TUNEL stain content was performed by 2-way ANOVA with a Bonferroni test for post hoc comparisons. All data are expressed as mean ± SEM unless otherwise stated.

2.3.2 Effects of 3AB

As previously stated, this evaluation was performed after completion of comparison of control and isoprenaline-treated rats.

Sample size calculations have been performed on the basis of isoprenaline data in part A. On that basis that strain decreases by approximately 14 ± 4 (SD)% at 24 hours, a study utilizing 20 rats has approximately 87% power to detect a 4% difference between groups at p < 0.05: that is, to detect potential 30% limitation of negative inotropic change.

The primary comparison for this component of the study was between apical strain in rats treated with 3AB/Isoprenaline vs. those treated with Isoprenaline alone. Treatments with 3AB alone were utilized in 2 rats to demonstrate absence of intrinsic inotropic effects.

2.4 Results
2.4.1 Induction of TTC

Only 15 rats out of 21 survived post isoprenaline injection. Six rats died within 2 hours of the isoprenaline injection. This appeared to occur suddenly, consistent with an arrhythmic basis. Echocardiographic and immunohistochemistry data are compared for the remaining 15 rats at baseline and 24 hours with control rats.

<table>
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<tr>
<th>Rat No.</th>
<th>Baseline</th>
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<th>24-hr post-isoprenaline injection</th>
</tr>
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</tr>
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Table 2: Left ventricular apical strain in 15 surviving rats at baseline and 24-hours post-isoprenaline injection.

*P<0.00001 vs baseline
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<th>Rat No.</th>
<th>IVSD Baseline</th>
<th>IVSD 24-Hr</th>
<th>LV wall Baseline</th>
<th>LV wall 24-Hr</th>
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<td>4.4</td>
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</table>

Table 3: Apical intraventricular septal diameter (IVSD), left ventricular (LV) wall thickness and left ventricular ejection fraction pre and post Isoprenaline treatment.
2.4.1.1 Echocardiographic analysis

Most of the rats had gross wall motion abnormalities 24 hours post isoprenaline injection. Even though it was difficult to calculate an accurate wall motion score it was obvious that most predominant hypokinesis occurred at the LV apex.

As shown in Table 2 and Figure 5, there was substantial decrease in the apical strain in most of the rats with mean reduction of apical strain of approximately 35% from baseline value of

Figure 8: Reduction in left ventricular apical strain 24 hours post Isoprenaline treatment.
34.5 (± 6.4) to 21.9 (± .7) post-isoprenaline treatment (p <0.00001). Individual strain values of the 15 rats are shown in Table 2. On the other hand, there was no significant change post isoprenaline in the apical, mid-ventricular and basal left ventricular FAS (Figure 9). While there was a trend towards increased thickness of IVSD and LV wall diameter this was not statistically significant. Similarly, the fall in the LV ejection fraction post isoprenaline treatment was not statistically significant (Table 3).
Figure 9: Apical, mid-ventricular and basal left ventricular fractional area shortening at baseline and 24 hours post-isoprenaline.
Histological/immunohistological evaluation

Hematoxylin/eosin staining of the left ventricular sections revealed patchy infiltration of myocardium with macrophages and neutrophils. Inter individual coefficient of variability for estimates of 3NT and TXNIP were 13% and 9% respectively (n=5), in isoprenaline treated rat hearts.

2.4.1.2.1 3-Nitrotyrosine content

Inter-observer coefficient of variability for myocardial 3NT content was 13% (n=5), in isoprenaline treated hearts. Tissue staining for 3NT was heterogeneous, with predominant perivascular localization. Initial comparisons between isoprenaline-treated and control rat hearts were made for the proportion of myocardial staining percentage for 3NT. 3NT immunostaining was increased approximately 10 times in the apical myocardium of isoprenaline treated rats (p=0.0012). However, there was no statistical difference between the basal level of 3NT in the isoprenaline treated and control rats (Figure 11). Similarly, we did not find any statistically significant difference in 3NT concentration between the apex and base of the isoprenaline treated rat hearts.

2.4.1.2.2 TXNIP

TXNIP content was increased approximately 20 fold in the apical and basal myocardium of isoprenaline-treated rats (p=0.01). Tissue (cytoplasmic) staining for TXNIP content was homogeneous and there was no significant difference between apical and basal TXNIP content (Figure 12).

2.4.1.2.3 TUNEL
TUNEL staining was used to quantitate apoptosis. Isoprenaline-treated rat heart sections showed minimal apoptosis that was mainly found in the apical sub-endocardial tissue. Apoptosis in the apical section of the LV of isoprenaline-treated rat was nearly 4 times that of LV base (Mean ± SD) (1.4% ± 0.74 vs. 0.4% ± 0.5). The apical content of apoptosis was nearly 10 times that of apex of control rat hearts (1.4% ± 0.74 vs. 0.16 ± 0.1) (Figure 10).

Figure 10: Mean data for TUNEL content in the apical and basal myocardial sections from control rats (blue) and those treated with isoprenaline (red). P = NS, Bonferroni post hoc correction for apex and base.
Figure 11: Mean data for 3 nitrotyrosine content in the apical and basal myocardial sections from control rats (red) and those treated with isoprenaline (blue).

ANOVA
Isoprenaline F=8.8, p=0.006
Site F=0.05, p=0.8
Interaction F=0.03, p=0.9
Figure 12: Mean data for TXNIP content in apical and basal myocardial sections from control rats and those treated with isoprenaline. Data are represented as % of the microscopic field.

2.4.2 Modulation of TTC: effects of 3AB

In accordance with the central hypothesis that inflammation and regional negative inotropic changes in TTC are engendered by nitrosative/oxidative stress, 3 aminobenzamide (3AB) a PARP-1 inhibitor was used as an intervention. The purpose was to determine if pretreatment with 3AB before injection of Isoprenaline preserves LV function.

2.4.2.1 Echocardiographic analysis

There was no change in the apical strain and FAS at 24 hours in the 2 rats injected with 3AB alone (50 mg/Kg). Furthermore, injection with 3AB alone did not lead to any mortality.
Among the 19 rats injected with 3AB half an hour before the Isoprenaline injection, 6 died following the isoprenaline treatment. In the remaining 13 rats echocardiographic analysis apical strain and FAS was performed as shown in Figure 13 and Figure 14.

A 2-way analysis of variance evaluated changes in apical strain of rats treated with, isoprenaline alone and rats who had pretreatment with 3AB before isoprenaline injection. There was a significant decrease in the apical strain over a 24-hour period within the total group (F = 34, p < 0.0001). Rats who were pretreated with 3AB showed a better-preserved apical strain compared to rats treated with Isoprenaline alone (F = 8.36, p = 0.005). However, the interaction between the time period and pretreatment with 3AB was not significant (F = 0.9, p = 0.3).

On 2-way analysis of variance of FAS, there was significant decrease in FAS over 24 hour period after isoprenaline treatment (F = 5.2, p = 0.03) but no significant preservation of FAS with the use of 3AB pretreatment (F = 0.09, p = 0.7). Similarly, the interaction of two groups was not significant (F = 0.05, p = 0.8).
Figure 13: Mean data for LV apical strain at baseline and after treatment with isoprenaline in rats with and without pre-treatment with 3 aminobenzamide.

ANOVA
Period F=34, p < 0.0001
Treatment F=8.36, p=0.005
Interaction F=0.9, p=0.3

Figure 14: Mean data for apical fractional area shortening at baseline and after treatment with isoprenaline in rats with and without pre-treatment with 3 aminobenzamide.

ANOVA
Period F=5.2, p =0.03
Treatment F=0.09, p=0.7
Interaction F=0.05, p=0.8
2.4.2.2 Immunohistochemistry results

2.4.2.2.1 3-Nitrotyrosine and TXNIP percentage

Data for regional 3NT content in left ventricles of rats treated with isoprenaline ± 3AB are described in Figure 15. Overall, there was no significant heterogeneity, either as regards apical vs. basal 3NT content, nor regarding effects of 3AB. However, it was noted (surprisingly) that there was a trend (p = 0.07) towards increased 3NT content in 3AB-treated rat hearts.

Analogously, TXNIP content varied neither regionally nor with treatment modality in hearts from isoprenaline versus isoprenaline/3AB-treated rats (Figure 16).

![Figure 15: Mean data for 3 nitrotyrosine percentage staining at left ventricular apex and base after treatment with isoprenaline in rats with and without pre-treatment with 3 aminobenzamide.](image)

**ANOVA**

Apex vs. Base $F= 0.7, p = 0.4$

Inhibitor $F= 3.5, p= 0.07$

Interaction $F=1.3, p = 0.2$
2.4.2.2.2 TUNEL stain content

There was no significant difference in TUNEL staining between the isoprenaline-treated and isoprenaline/3AB-treated rats.
2.4.3 Discussion

In the current study, performed in a rat model of TTC, we sought first to test the hypothesis that TTC is associated with the development of nitrosative stress, signifying increased generation of ONOO\(^-\) within the myocardium. The first step was to induce a state of myocardial contractile impairment consistent with the clinical features of TTC, and associated (as with TTC) with underlying inflammatory activation. Dose ranging led to utilization of a regimen that was associated with approximately 25% (presumably arrhythmic) mortality and with some degree of LV apical hypokinesis. These wall motion changes were consistent with those seen in other rodent models of TTC. Specifically, there was no consistent reduction in apical fractional shortening, but a fairly consistent and highly significant reduction in apical...
strain. A trend was noted towards increased wall thickness: it is possible that this reflects development of (inflammatory) oedema. In one rat, prolonged follow-up established that changes tended to resolve rather than increase beyond 24 hours.

As regards the biochemical hypothesis underlying the isoprenaline experiments, it was expected that an increase in myocardial ONOO’ would lead to protein nitration and activation of PARP-1, which leads to cardiac energetic impairment and resulting in myocardial contractile impairment and increased rates of cell death (as revealed in Chapter 1). We utilized the generation of 3-NT as a marker of ONOO’ effect (158). The results of the investigation indicate that 3-NT formation is increased in rat left ventricular myocardium 24-hours post isoprenaline treatment as compared to control rats. However, there was no LV apex to base gradient in 3NT concentration. Furthermore, the myocardial 3NT distribution was patchy and predominantly, but not entirely, perivascular. This finding raises the possibility that the NO and/or superoxide which combine to generate ONOO’ are predominantly formed in vascular endothelium or smooth muscle. Interestingly, it has previously been postulated that material(s) released from endocardial endothelium may account for the negative inotropic changes in TTC (159).

**TXNIP content** was nearly 20-fold greater in the rats 24 hours post isoprenaline treatment compared to controls. As for 3NT there was no apex base gradient. However, unlike 3NT, TXNIP spread was homogeneous. TXNIP is an α-arrestin that exerts a pivotal role as an inflammatory activator (160). While it has previously been shown that TXNIP expression may be increased by redox dependent and independent methods in conditions such as ischemia reperfusion injury, hyperglycaemia or non-laminar vascular flow (161), it has recently emerged that ONOO’ (via PARP) is also a modulator of TXNIP activation (162). The current data therefore demonstrate that TXNIP expression is increased in this model but does not permit us to define the mechanism(s) underlying this change of TTC.
There was detectable but only minor apoptosis following isoprenaline treatment. TUNEL staining for apoptosis showed patchy distribution predominantly in the apical sub-endocardium 24-hour post Isoprenaline. No apoptosis was noted in the control rat heart cells. Our findings are similar to those in a recent study where no myocardial apoptosis was detectable following 7 days isoprenaline treatment in male Wistar rats (119). At the same time there is substantial data documenting rise in troponin as a marker of cardiac cell death following isoprenaline treatment in human and animal models (163). It is possible that some of the cellular damage induced by Isoprenaline is necrotic rather than apoptotic, but overall these findings are consistent with minimal levels of cell death in TTC clinically.

In accordance with the second hypothesis that inflammation and regional negative inotropic changes in TTC are engendered by nitrosative/oxidative stress, in the second part of our experiments we used a single dose of 3AB (PARP-1 inhibitor) as an intervention to reduce PARP mediated downstream activation of inflammation and that induces systolic dysfunction following isoprenaline treatment.

There was improvement in the LV systolic function as measured by LV apical strain in rats receiving 3AB pre-treatment before isoprenaline vs. isoprenaline alone. On the other hand there was no significant difference in biochemical markers of nitrosative stress between 2 groups. There was a trend towards an increase in the apical 3NT concentration in the 3AB/Isoprenaline group. There was also no change in the TXNIP concentration. Similarly, there was no difference in apoptosis between isoprenaline and 3AB/isoprenaline group. However, it would not be expected that a PARP inhibitor would modify 3-NT or TXNIP generation. More likely, there would be less formation of PAR, the product of PARP-1 activity: this was not measured.

The observations which led to our initiating the current investigation included the findings that TTC is associated with intense and prolonged inflammatory activation, with myocardial
edema involving both apex and base of the LV and persisting for at least 12 weeks after onset of symptoms, together with extensive release of BNP, NT-proBNP and CRP as systemic manifestations of inflammatory activation (57, 78). Furthermore, patients with TTC exhibit evidence of increased platelet responsiveness to NO and also have lower ADMA concentrations than age-matched female controls (164), raising the possibility of enhanced NO signaling.

We therefore postulated that TTC might be associated with catecholamine-induced stimulation of myocardial β2 adrenoceptors, as demonstrated in recent studies in rats and mice (157, 165), and that the coupling of these receptors to NO synthase (166) might lead to increased generation of ONOO⁻, particularly within apical myocardium. Given that catecholamine stimulation equally might activate β3 adrenoceptors, which are also coupled to NO synthase (167), this effect might also contribute to ONOO⁻ generation. Furthermore, given that TXNIP activation is partially dependent on ONOO⁻-mediated activation of the peroxynitrite/Poly (ADP-Ribose) Polymerase [PARP] pathway (162), we chose to evaluate myocardial content not only of 3-NT, a marker of nitrosative stress, but also of TXNIP, a pivotal activator of the inflammasome.

The studies performed in rats extend previous observations that TTC-like changes can be induced by exposure to large doses of catecholamines, provided sufficient β-adrenoceptor stimulation is engendered (157, 165). A number of previous studies have induced such changes with isoprenaline, but none have evaluated responses in ageing female animals. In our pilot experiments, such rats were particularly sensitive to isoprenaline, with mortality rates of 80%, while mortality rates in 4-5 month old female rats were of the order of 30%. While there was substantial heterogeneity of changes in fractional shortening, the measurement of LV apical strain revealed consistently reduced following isoproterenol administration.
Peroxynitrite also may stimulate soluble guanylate cyclase, releasing cyclic GMP, which may contribute to further release of catecholamines (49). Nitrosative stress is associated with PARP- mediated energetic impairment (168), which may well contribute, together with inflammatory activation, to the observed reduction in strain. Hence, on the intervention studies using PARP-1 inhibitor 3AB (169) we noticed less deterioration of LV systolic function following isoprenaline treatment. These effects may occur through improvement of myocardial energetics (170), however in future with the MR spectroscopy this can be clarified further.

**Limitations:** The current study had a number of limitations. As regards the initial experiments with isoprenaline, these did not delineate the bases for increased TXNIP expression, nor indeed whether peroxynitrite is pivotally important in the pathogenesis inflammation.

The intervention with 3AB was partially successful in limiting the negative inotropic effects following isoprenaline. However, PAR formation was not assessed, nor was putative PARP-1 activation. Only immunohistochemical quantitation was used; addition of immunoblotting would have provided additional confirmation of the results. Furthermore, only one dose of 3AB was utilized: although this corresponds well with previous regimens in rats, it may not have produced maximal effects. PARP-1 activation contributes to redox stress, but also to energetic impairment, and this was not assessed. Finally, PARP-1 is not the only downstream mediator of ONOOʰ biological effect: hence PARP-1 inhibition may have not affected many of the pre-oxidant effects of ONOOʰ. All of these possibilities remain to be assessed.

To date, no effective treatment options for TTC have emerged. In particular, β-adrenoceptor antagonists appear to be ineffective (171) while use of ACE inhibitor tended to reduce the episodes of recurrence in a recent meta-analysis. The current findings raise the potential for interventions to reduce myocardial inflammation in TTC, with possible consequences of
limiting hemodynamic disturbances in the early stage of the disease and accelerating recovery.
3 Chapter: Pathogenesis of Takotsubo Cardiomyopathy- Human experiments
3.1 Introduction

While recent developments in the rodent model of TTC in the last 5 years have facilitated understanding of the pathogenesis of TTC, human data on pathogenesis of TTC have been scarce because of difficulties in obtaining myocardial samples during acute episodes of TTC. There have also been limited human biopsy studies in TTC patients (156). In most of these studies myocardial biopsy samples were obtained from the intraventricular septum rather than periaipical region.

In the planned experiments, we sought to test the hypothesis that in the clinical context, (as was evidently the case in the animal model-see chapter 2) TTC was associated with evidence on increasing nitrosative stress. We tested this hypothesis by:

1. Measuring systemic (plasma) concentrations of 3NT
2. Measuring myocardial expression of 3NT in LV samples from patients dying of TTC.

In both cases, patients of similar age dying without cardiac disease were utilized as control.

As already discussed in the previous chapters we recently evaluated the role of NO signaling in patients diagnosed with TTC. We performed a case-control study against an age-matched normal female population. This revealed that TTC was associated with substantially greater tissue responsiveness to NO than occurred in controls, together with lower plasma levels of the NO synthase inhibitor asymmetric dimethylarginine (ADMA): these differences were seen both acutely and after 3 months’ follow-up. These data therefore suggest a “paradoxical” increase in NO formation and effect in TTC patients.

Furthermore, there is strong evidence that catecholamine surges can lead to β2-adrenoceptor mediated signaling, which is coupled to NOS activation and therefore may contribute to ONOO- formation. Our data from the imaging and biochemical studies have revealed the presence of LV myocardial inflammation during acute episodes of TTC slowly resolving over
3 months (16). **However, a question that has been unanswered so far is how catecholamine surges lead to myocardial inflammation.**

We tried to answer this utilizing rat model of TTC in the Chapter 2 and describing the increased myocardial nitrosative stress and increased expression of TXNIP following isoprenaline administration. Analogously, we now hypothesized that in patients diagnosed with TTC, increased release of NO potentially induces the formation of ONOO\(^{-}\) anion with associated redox stress, protein nitration and downstream activation of TXNIP. We quantitated 3-nitrotyrosine (3NT) in myocardial biopsy samples of patients who died during the acute episodes of TTC. We also measured the plasma 3NT concentration following acute diagnosis of TTC.

### 3.2 Methods

The Institutional Ethics of Human Research Committee approved the protocol and informed consent was obtained in all cases. All the patients diagnosed as TTC presented as an acute coronary syndrome (ACS) and in general underwent coronary angiography within 48 hours. Patients presenting as STEMI underwent urgent coronary angiography while coronary angiography was performed within 48 hours in patients presenting as NSTEMI. All the patients underwent coronary angiography followed by cardiac magnetic resonance imaging (CMR) to establish the diagnosis of TTC. All the patients included in this study fulfilled the Mayo Clinic Criteria for the diagnosis TTC.

Transthoracic echocardiography (TTE) was performed using General Electric Vivid 7 echocardiography system (GE Medical Systems, Milwaukee, WI), within 48 hours of admission either before or after coronary angiography. LV Systolic dysfunction during the acute episode of TTC was evaluated using LV wall motion scoring index (WMSI) and by measurement of LVEF. LV wall motion score was obtained utilizing a 17-segment model on TTE (172). Extent of LV hypokinesis was expressed as LV WMSI (173). LVEF was obtained
using Simpson’s method by calculating the average of apical 4-chamber and 2-chamber view volumes. Left ventricular stroke volume (LVSV) was calculated by LVOT velocity time integral (VTI) and LVOT diameter.

Serial blood tests were performed during the acute admission of the patient and peak values of Troponin T, creatine kinase (CK), metanephrine, normetanephrine and NT-pro BNP were noted. Patients were monitored closely during the first 24 hours post diagnosis and heart rate and blood pressure were noted hourly for the first 6 hours followed by 4 hourly admission thereafter.

### 3.2.1 Plasma 3NT concentration

In order to determine whether there might be systemic circulatory evidence of nitrosative stress in patients with TTC, blood samples were taken by venesection from patients within 48 hours of onset of symptoms of TTC ($n = 23$) and from 16 age-matched healthy women. Venous blood was collected into tubes containing potassium ethylene diamine tetra acetic acid (EDTA) and centrifuged at 3000 rpm for 10 minutes at 2°C. Plasma was stored at -80°C until analysis. Determination of plasma 3-NT was performed by tandem mass spectrometry coupled with gas chromatography (174): this assay was performed in the laboratory of Professor Tsikas in Hannover, Germany.

### 3.2.2 Post-mortem studies in TTC patients

Over a period of 3 years, there were 8 in-hospital deaths of patients after diagnosis of an acute episode of TTC. In 5 of these cases, permission was obtained from patients’ families for performance of limited autopsy to evaluate changes within the myocardium. Control myocardial samples were also obtained from the left ventricles of 2 female patients aged > 50 years who had died of non-cardiac causes.

In patients dying with TTC biopsy samples were obtained from the apex and base of the left ventricle. For Immunohistochemical studies tissue section were fixed in 9% formaldehyde
and paraffin embedded. Immunohistochemical studies for evaluation of regional left ventricular content of 3NT were performed in a similar manner. The immunohistochemistry staining protocol for 3NT was similar to the rat staining protocol (Section 2.2.3). Analysis of the stain was performed using Image J NIH software as described before in section 2.2.4.

### 3.2.3 Statistical methods

Graph Pad Prism (Version 6) and SPSS Version 17 (SPSS, Inc., Chicago, IL) were the statistical software packages were used. Comparison between normally distributed data were performed via non-paired t-tests, while Wilcoxon tests were used for skewed data and Chi Square tests for proportional data. Data are expressed as mean ± SD unless otherwise mentioned.

### 3.3 Results

#### 3.3.1 Evaluation of plasma 3-NT concentrations

##### 3.3.1.1 Clinical characteristics

Basic characteristics of 23 TTC patients are summarized in Table 4.
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<tr>
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<tr>
<td>“NSTEMI” presentation</td>
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<td>In-hospital death</td>
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<tr>
<td>Post discharge death</td>
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<td>Cardiac arrest</td>
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<td>Shock</td>
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<td>Admission BP (mm Hg)(mean ± SD)</td>
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<tr>
<td>Troponin I ng/L (median ± SEM)</td>
<td>512 ± 92</td>
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<tr>
<td>C Reactive Protein (median ± SEM)</td>
<td>56 ± 28</td>
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<tr>
<td>Creatine Kinase ng/L (median ± SEM)</td>
<td>246 ± 50</td>
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<td>NT-BNP ng/L (median ± SEM)</td>
<td>7330 ± 2400</td>
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<tr>
<td>LVEF (%)</td>
<td>45 ± 11</td>
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<tr>
<td>WMSI</td>
<td>1.9 ± 0.2</td>
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</tbody>
</table>

Table 4: Basic characteristics of 23 TTC patients who underwent measurement of plasma 3-nitrotyrosine levels.

As expected all the patients diagnosed with TTC were females with mean age of 67 years.

On coronary angiography, no significant coronary artery disease was found in any of the 23 patients. The majority of the patients had typical peri-apical ballooning pattern of TTC. While all the patients had ECG abnormalities, less than 40% presented as STEMI (n = 8 [33%]). Markers of cardiac cell damage were mildly elevated: in particular there Troponin-I and creatine kinase levels were only marginally above population norms. Blood pressure at presentation was relatively low and signs of peripheral hypoperfusion/hypoxia were present in
3 patients. In-hospital stay was complicated by cardiac arrest in 1 patient and one patient died during the admission. Overall, LV systolic function was mildly impaired and WMSI normalized at 3 months follow-up. Despite considerable elevation of NT pro BNP levels, none of the patients had pulmonary odema.

### 3.3.2 Plasma 3NT comparison

Plasma 3 NT levels of 23 TTC patients were compared with age and gender-matched 16 controls. Note that TTC patients had somewhat greater BMI values than control (Table 5).

<table>
<thead>
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<th>Controls (n = 16)</th>
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<td>Age (yrs.)</td>
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<td>LVEF %</td>
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<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>6</td>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>BMI</td>
<td>30 ± 6</td>
<td>26 ± 4</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 5: Age, gender, echocardiographic features and cardiac risk factors of TTC group and controls.

Data regarding the plasma 3-NT concentration determinations are shown in Figure 18. There were no significant differences between patients (2.26 ± 0.22 nmol/L) and control subjects (2.20 ±0.25 nmol/L). Plasma 3NT values of individual TTC patients and controls are shown in Table 6.
Figure 18: Plasma 3-NT concentrations in Takotsubo cardiomyopathy patients (TTC) and age-matched controls (C). There was no significant difference between the groups.
<table>
<thead>
<tr>
<th>Number</th>
<th>TTC</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.82</td>
<td>2.89</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>0.63</td>
</tr>
<tr>
<td>3</td>
<td>2.89</td>
<td>0.94</td>
</tr>
<tr>
<td>4</td>
<td>3.39</td>
<td>3.38</td>
</tr>
<tr>
<td>5</td>
<td>4.31</td>
<td>1.79</td>
</tr>
<tr>
<td>6</td>
<td>1.71</td>
<td>2.53</td>
</tr>
<tr>
<td>7</td>
<td>2.24</td>
<td>1.18</td>
</tr>
<tr>
<td>8</td>
<td>1.47</td>
<td>2.78</td>
</tr>
<tr>
<td>9</td>
<td>2.13</td>
<td>1.90</td>
</tr>
<tr>
<td>10</td>
<td>1.86</td>
<td>2.03</td>
</tr>
<tr>
<td>11</td>
<td>2.14</td>
<td>1.15</td>
</tr>
<tr>
<td>12</td>
<td>2.51</td>
<td>2.17</td>
</tr>
<tr>
<td>13</td>
<td>1.73</td>
<td>2.32</td>
</tr>
<tr>
<td>14</td>
<td>4.87</td>
<td>2.88</td>
</tr>
<tr>
<td>15</td>
<td>1.88</td>
<td>2.04</td>
</tr>
<tr>
<td>16</td>
<td>1.73</td>
<td>4.61</td>
</tr>
<tr>
<td>17</td>
<td>2.58</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1.95</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>1.63</td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Plasma 3NT values in nmol/L for 23 patients and 16 controls
3.3.3 Post mortem studies

Data for the 5 TTC patients whose hearts were available for study are summarized in Table 7. All the patients underwent coronary angiography and were found to have no significant coronary artery disease. The mode of presentation in most of the patients was ACS while one patient presented as out of hospital cardiac arrest (ventricular arrhythmia). The patients who presented as cardiac arrest, despite successful resuscitation, died of hypoxic brain injury 3 days after the diagnosis of TTC. Sub-arachnoid hemorrhage was the cause of death in 2 other patients. The cause of death in other 2 patients was shock and both of these patients died with in 24 hours of diagnosis of TTC.
<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>86</td>
<td>61</td>
<td>47</td>
<td>73</td>
<td>67</td>
</tr>
<tr>
<td>Presentation</td>
<td>STEMI</td>
<td>NSTEMI</td>
<td>Cardiac arrest</td>
<td>STEMI</td>
<td>STEMI</td>
</tr>
<tr>
<td>Time between diagnosis and death</td>
<td>10 hours</td>
<td>24 hours</td>
<td>3 days</td>
<td>5 days</td>
<td>12</td>
</tr>
<tr>
<td>NT-pro BNP (ng/L)</td>
<td>13600</td>
<td>6530</td>
<td>1300</td>
<td>13800</td>
<td>8452</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>40%</td>
<td>45%</td>
<td>45%</td>
<td>40%</td>
<td>40%</td>
</tr>
<tr>
<td>Predominant Hypokineses</td>
<td>Apical</td>
<td>Apical</td>
<td>Mid Ventricle</td>
<td>Mid ventricle</td>
<td>Apical</td>
</tr>
<tr>
<td>Peak Troponin T (Ng/L)</td>
<td>652</td>
<td>157</td>
<td>555</td>
<td>524</td>
<td>494</td>
</tr>
<tr>
<td>Peak CK (units/L)</td>
<td>180</td>
<td>81</td>
<td>100</td>
<td>200</td>
<td>684</td>
</tr>
<tr>
<td>Plasma Metanephrine (Pmol/L)</td>
<td>1280</td>
<td>350</td>
<td>230</td>
<td>5610</td>
<td>NA</td>
</tr>
<tr>
<td>Plasma Normetanephrine (Pmol/L)</td>
<td>5500</td>
<td>1060</td>
<td>370</td>
<td>3730</td>
<td>NA</td>
</tr>
<tr>
<td>Cause of death</td>
<td>Shock</td>
<td>Shock</td>
<td>Cardiac arrest</td>
<td>SAH</td>
<td>SAH</td>
</tr>
</tbody>
</table>

Table 7: Clinical characteristics and biochemical parameters of 5 patients who underwent autopsy after dying during an acute attack of TTC

In all cases, autopsy was performed at least 24 hours post patients’ death. Control autopsy data were obtained from 4 women of similar ages (mean 59.5 ± 0.5 years).

Left ventricular apical myocardium from TTC patients exhibited approximately 6-fold elevation of 3-NT content relative to controls (means 24.1 ± 0.9% vs. 0.4 ± 0.3%) (Figure 19).
Similar to the animal staining results, human tissue staining for 3NT was heterogeneous (Figure 20, Figure 21), with predominant perivascular localization (Figure 22).

Figure 19: Concentration of 3NT at the LV apex and base of TTC patients and control.
Figure 20: A tissue section of left ventricular apex of a patient diagnosed with TTC showing presence of 3NT staining.

Figure 21: Left ventricular tissue section from control stained for 3NT.

Figure 22: Left ventricular apical tissue section of a patient diagnosed with TTC stained for 3 NT. Predominance of 3NT staining can be noted around the vessel.
3.4 Discussion

In parallel with the studies on the animal model of TTC, in the current investigations we sought to confirm the hypothesis that TTC in humans is associated with evidence of nitrosative stress systemically (plasma) and locally (myocardium). We used 3-NT as a marker of nitrosative stress. We analyzed plasma levels of 3-NT within 24 hours of diagnosis of TTC in women and healthy aged matched controls. The results revealed that there was no increase in the plasma 3-NT concentration in patients with TTC in general, suggesting that there is no systemic evidence of nitrosative stress in most TTC patients. Whether 3NT penetration in the circulation is detectable in coronary sinus blood in TTC patients remains to be determined.

On the other hand, autopsy data from the patients who died during the acute episode of TTC revealed changes analogues to those found in the rat myocardium (Chapter 2). The results of the investigations indicate that 3NT formation is increased in the LV myocardium in patients with TTC as compared to controls. However, we did not find any apex to base gradient of 3NT content. Furthermore, as found in the rat myocardial tissue, the 3NT distribution was patchy and predominantly perivascular. These findings therefore confirm the presence of nitrosative stress in the human myocardium as in the rat model of TTC. Once again, however, these data do not themselves indicate a major pathophysiological role for nitrosative stress.

To evaluate the potential nexus between the ONOO⁻ and TXNIP, we stained the human myocardial sample for poly ADP ribose (PAR), which is synthesized after activation of the nuclear DNA repair enzyme PARP. In pilot studies, we found PAR staining in the patients with TTC was extensive and limited to the nuclei of the cells, as expected. The patients with TTC have nearly 4-fold greater myocardial staining for PAR activity than controls (p=0.012). The importance of PAR staining is that it implicates PARP-1 activation, and consequent energetic impairment.
The studies of human myocardium were performed after autopsy examination 48 hours post patients’ death and therefore may be subject to artefactual changes. However, these human data were similar to those seen in the rat model. It is possible that demonstration of increased 3-NT in human myocardium, represents the result of a relatively transient process (as with nitrosative stress in other models) and therefore the examination of myocardium in patients who died early in the course of the disease may have facilitated detection of 3-NT content.

There has been some recent evidence of protective effects of ACE inhibitors in TTC. Since, ACE inhibitors have well known anti-inflammatory effects and attenuate nitrosative stress(175, 176), their use in TTC can be beneficial to fasten the recovery and limit myocardial damage. One emerging finding of our study is that PAR activity may be increased in the human myocardium of TTC patients. This provides additional support for the concept of treating TTC with PARP inhibitors.

At this particular moment there is no specific treatment for either TTC related complications or the prevention of recurrence. Our experiments form a basis for pharmacological strategies that might be used to interrupt the postulated signaling cascade that culminates in prolonged myocardial inflammation in TTC. Although, beta blockers may theoretically reduce inflammation, their use have been found to be associated with increased mortality in the rat model of TTC (165). A randomized study evaluating the use of ACE inhibitors in TTC, in order to preserve myocardial energetics and thus minimize myocardial damage and accelerate recovery is an increasingly attractive strategy.
4 Chapter: Implications of right ventricular involvement in Takotsubo cardiomyopathy
4.1 Introduction

Right ventricular (RV) function is an important determinant of early morbidity and long-term prognosis in cardiovascular diseases predominantly affecting the left ventricle. Sir William Harvery was the first person to describe the importance of right ventricle in 1616 (177). Previously the importance of RV involvement had been underestimated because studies performed 30 years ago were performed in the open pericardium dog model that failed to take into account the complex interaction between the left and right ventricle (177). However, many studies in the recent years have demonstrated the prognostic value of RV function in the acute and chronic stages of left sided heart failure, congenital heart disease and pulmonary hypertension (178, 179). Furthermore, impact of RV involvement in myocardial infarction is well known since 1930, when Saunders first described RV infarction as triad of clear lung fields, hypotension and elevated jugular venous pressure (180).

With progress in cardiac imaging visualization and understanding of right ventricular function in the cardiac diseases has improved. One of the important physiological concepts that we now know is ventricular interdependence where size, shape and compliance of one ventricle may affect the pressure volume relation ship of the second ventricle through direct mechanical interactions (181). Although ventricular dependence is always present its affect becomes more predominant in loading conditions of the heart and it contributes a lot to the pathophysiology of RV dysfunction. This phenomenon where dilation of the RV shifts the intraventricular septum to left because of pericardial constrains leading to decreased LV preload and increased LV end diastolic pressure is called, diastolic ventricular interaction, which has been clinically proved in various studies (181, 182).

As already discussed, TTC has a relatively better long-term prognosis than AMI but TTC related in-patient complications are common and occasionally life threatening (139). Kurowski et al (139) found 30-day mortality very similar between TTC and pair matched patients with anterior STEMI. Acute episodes of TTC can be complicated by hypotension,
shock, cardiac arrhythmias, thromboembolic complications and heart failure (183). These factors and underlying non-cardiac issues can increase morbidity and mortality in the acute phase of TTC. Hypotension and shock is the most common complication in the acute phase of the disease with high heterogeneity in its presentation (142). Furthermore, the treatment of hypotension can be difficult.

The derangement of hemodynamics in the acute stages of TTC has been discussed in section 1.3.6.1.2. In particular, patients admitted with TTC tend to become hypotensive and in roughly one third of cases episodes of hypotension will be associated with shock (transient hypoxic hepatic and renal injury) (142). Many of these patients will require insertion of intra aortic balloon pump for transient hemodynamic support (184). In the past, fluctuations in the hemodynamics were presumed to be related to LVOT obstruction, MR or because of pump failure. In theory, the development of hypotension in the acute period of TTC is likely to be multi-factorial in origin. For example, precipitation of TTC is closely related to a surge of catecholamine release/administration (50) in both animal models (109) and some human examples (107). Furthermore, in the cases of TTC there is remarkable release of the vasodilator BNP (53). Thus, fluctuation in blood pressure in the acute stages of the attack may reflect not only impaired inotropic state of one or both ventricles, but also changes in vasomotor tone, which in theory might either increase or decrease.

RV involvement in TTC occurs in 30% of the cases and is associated with more frequent complications (149, 185). However, the mechanism and the reasons behind RV related complications have not been investigated. As in the case of acute RV infarct it might be speculated that RV involvement in TTC could be the major contributor of hypotension, shock and acute hemodynamic instability.
We therefore hypothesized that RV involvement in acute cases of TTC contributes to the risk of hypotension. We also considered that RV involvement might induce hypotension via associations with reduction in left ventricular ejection fraction (LVEF), and in left ventricle stroke volume (LVSV), and that these factors might lead to increased length of stay (LOS) in hospital. The second aim of the study was to elucidate the predictors of shock [which was defined as systolic blood pressure (SBP) \( \leq 90 \text{ mm Hg} \) along with tissue hypo-perfusion] and prolonged LOS in TTC.

Figure 23: Right ventricular involvement in Takotsubo cardiomyopathy

4.2 Methods
The institutional Ethics of Human Research Committee approved the investigation and informed consent was obtained prior to study entry. All patients presented as ACS and in general underwent (within 48 hours) coronary angiography and contrast left ventriculogram. Patients presenting as STEMI underwent emergency coronary angiography while coronary angiography in patients presenting as NSTEMI was performed within 48 hours. In frail patients with relative contra-indications to coronary angiography, the diagnosis of TTC required (1) exclusion of myocardial infarction and demonstration of global myocardial edema with cardiac magnetic resonance imaging (CMR) (16), (2) demonstration of characteristic LV wall motion abnormalities. Transthoracic echocardiography (TTE) was performed using General Electric Vivid 7 echocardiography system (GE Medical Systems, Milwaukee, WI), within 48 hours of admission either before or after coronary angiography.

Two operators, blinded to all biochemical and hemodynamic results, analyzed the remaining 102 TTE images. RV involvement (RV⁺) of TTC was defined categorically as the presence of apical or mid RV wall motion abnormalities. Furthermore, wall motion scoring was used to quantitate the severity of RV involvement. The lateral wall of the RV was divided into three segments on apical 4-Chamber view (Figure 23). A wall motion score was given to each segment (1=Normal, 2=Hypokinesia, 3=Akinesia and 4=Dyskinesia) and a total RV Score was obtained by addition of the wall motion score of the three segments of the RV on Apical 4-Chamber view. It was therefore theoretically possible for RV Score to range from 3 to 12, with 3 implying normality and 12 severe involvement of the RV.

LV wall motion score was obtained utilizing a 17-segment model on TTE (172). To standardize the severity of LV involvement we used LV wall motion score index (WMSI) by dividing the LV wall motion score with number of segments (173). LVEF was obtained using Simpson’s method by calculating the average of apical 4-chamber and 2-chamber view volumes. Left ventricular stroke volume (LVSV) was calculated by LVOT velocity time integral (VTI) and LVOT diameter.
SBP was recorded hourly for the first 6 hours of admission, representing the usual time of onset of shock in TTC: the lowest SBP reading within this period was utilized for purposes of data analysis.

A detailed clinical and past medical history was obtained to calculate Charlson Co-morbidity score (186). Serial blood tests were performed during the acute admission of the patient and peak values of Troponin T, creatine kinase (CK), metanephrine, normetanephrine and NT-pro BNP were obtained as previously described (53, 187).

4.2.1 Statistical methods

Graph Pad Prism (Version 6) and SPSS Version 17 (SPSS, Inc., Chicago, IL) were the statistical software packages were used. Comparison between normally distributed data were performed via non-paired t–tests, while Wilcoxon tests were used for skewed data and Chi Square tests for proportional data. Linear regression was used for univariate comparisons; p < 0.20 was utilized for inclusion of parameters into multivariate modeling via backwards stepwise multiple regression. Furthermore, in order to identify correlates of the development of shock, multivariate analysis was performed utilizing RV involvement, LVEF, NT pro BNP level and plasma metanephrine as potential dependent variables. Relationships between age and admission heart rate for normotensive and hypotensive individuals were compared utilizing ANCOVA. Data were expressed as mean ± standard deviation (SD) unless otherwise stated. Reproducibility of estimates of RV score was determined on the basis of coefficients of variability between observers with parallel construction of Bland-Altman plots to estimate potential variability with greater degree of RV dysfunction.

4.3 Results
### 4.3.1 Overall basic characteristics

A total of 130 consecutive patients with TTC were evaluated. Of these, 108 underwent acute coronary angiography and in the remaining 22 CMR data were used as the primary method of exclusion of myocardial infarction and establishment of diagnosis of TTC. In 28 cases,

<table>
<thead>
<tr>
<th>Group</th>
<th>Overall</th>
<th>RV−</th>
<th>RV+</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>102</td>
<td>68</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Gender (% women)</td>
<td>100 (98%)</td>
<td>66 (97%)</td>
<td>34 (100%)</td>
<td></td>
</tr>
<tr>
<td>Age (±SD), years</td>
<td>67 (±15)</td>
<td>68 (±14)</td>
<td>67 (±14)</td>
<td>NS</td>
</tr>
<tr>
<td>Peak CK (Interquartile range) Units/L</td>
<td>198 (122-307)</td>
<td>179(121-268)</td>
<td>232 (122-432)</td>
<td>NS</td>
</tr>
<tr>
<td>Peak Troponin T (Interquartile range) Ng/L</td>
<td>390 (228-585)</td>
<td>320 (220-542)</td>
<td>560 (245-1060)</td>
<td>0.01</td>
</tr>
<tr>
<td>STEMI</td>
<td>33</td>
<td>17</td>
<td>16</td>
<td>0.04</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>69</td>
<td>51</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>LV WMSI (±SD)</td>
<td>1.8 (±0.45)</td>
<td>1.78 (±0.30)</td>
<td>1.81 (±0.7)</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF (±SD) %</td>
<td>47 (±11)</td>
<td>50 (±11)</td>
<td>40 (±12)</td>
<td>NS</td>
</tr>
<tr>
<td>LVSV (±SD) ml</td>
<td>54 (±19)</td>
<td>61 (±18)</td>
<td>40 (±12)</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic PA pressure (±SD) mm Hg</td>
<td>34.9 (±12.6)</td>
<td>33.5 (±12.1)</td>
<td>37.8 (±13.5)</td>
<td>NS</td>
</tr>
<tr>
<td>LVOT obstruction</td>
<td>11</td>
<td>4</td>
<td>7</td>
<td>0.02</td>
</tr>
<tr>
<td>Severe MR</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>RV score (±SD)</td>
<td>3.9 (±1.6)</td>
<td>3</td>
<td>5.89 (±1.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Heart Rate (±SD) beats/min</td>
<td>79 (±20)</td>
<td>78 (±16)</td>
<td>86 (±20)</td>
<td>0.3</td>
</tr>
<tr>
<td>NT-pro BNP (Interquartile range) Ng/L</td>
<td>6060 (2780-10420)</td>
<td>4860 (2560-8520)</td>
<td>7860 (4450-21000)</td>
<td>0.006</td>
</tr>
<tr>
<td>Normetanephrine (Interquartile range)</td>
<td>1050 (750-2320)</td>
<td>970 (735-1670)</td>
<td>1700 (965-2600)</td>
<td>0.04</td>
</tr>
<tr>
<td>Metanephrine (Interquartile range)</td>
<td>251 (200-367)</td>
<td>245 (200-345)</td>
<td>290 (200-500)</td>
<td>NS</td>
</tr>
<tr>
<td>Weighted Index of Co-morbidity (±SD)</td>
<td>1.3 (±1.8)</td>
<td>0.95 (±1.5)</td>
<td>2.14 (±2.1)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
echocardiographic images of the RV were of inadequate quality to characterize fully the presence and/or extent of RV involvement, and hence data from these patients were not analyzed further.

Clinical characteristics of the remaining 102 patients are summarized in Table 8 in relation to echocardiography, ECG and biochemical findings. As expected, nearly all TTC patients were women aged >50 years. Nearly 33% of cases of TTC presented as an ST elevation myocardial infarction. Consistent with previous published series, myocardial injury as measured by CK or troponin T release, was limited (17, 73), but there was extensive release of NT pro BNP with mean peak levels approximately 50 times age-matched normal values (187). All the patients were found to have LV wall motion abnormality of the apical or mid wall extending beyond a single coronary artery distribution, although overall LVEF was only mildly reduced. Only 4 patients were found to have severe mitral regurgitation (MR). Eleven patients were noted to have LVOT obstruction. Similar to the finding by Rodrigues AC et al, we noticed the regional involvement of RV wall motion abnormalities mimicked the LV region (188).

The coefficient of variability of RV score was 6%. Bland Altman plots revealed no disparity of scoring with increasing RV score. Thirty-four patients had hypokinesia, akinesia and dyskinesia of the apical and mid wall of the RV. The RV score of the 34 patients varied between 4 to 9 (median in the presence of RV involvement was 6).

4.3.2 RV involvement predicts greater LV injury

On univariate comparisons (Table 8), patients with RV+ status had evidence of greater myocardial injury, as shown by peak troponin T concentrations: furthermore NT pro BNP

| Combined age and condition related score (±SD) | 3.8 (±2.4) | 3.36 (±2.2) | 4.79 (±2.8) | 0.02 |

Table 8: Clinical characteristics, biochemical parameters and extent of echocardiographic LV and RV dysfunction in (a) the total cohort (b) patients with and without RV motion abnormalities. For data with Non-Gaussian distributions, median and interquartile values are shown.
release was also greater in RV$^+$ patients. Nor-metanephrine release was also greater in the presence of RV$^+$ status, and RV$^+$ patients had significant greater co-morbidities contributing to the Charlson index. However, there was no significant difference between RV$^+$ and RV$^-$ patients in heart rate, LVSV, LVEF or pulmonary artery (PA) systolic pressure.

### 4.3.3 RV involvement is a univariate correlate of hypotension, shock and PHS

The average SBP in the whole cohort was 106 mm Hg (± 22 mm Hg). RV$^+$ status was also associated with significantly lower SBP (96 ±27 mm Hg) than in patients with no RV involvement (RV$^-$) (111 ±18 mm Hg) ($p = 0.006$). (Table 9)

Shock (SBP ≤ 90 mm Hg) and signs of peripheral organ hypo-perfusion) was present in 17 patients. Of these, 14 patients were RV$^+$. Thus RV$^+$ was a strong univariate predictor of shock. Despite attempted resuscitation, one patient with shock died in-hospital. On the other hand, no parameter of LV function or of hormonal release was correlated with development of shock (Table 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Overall N=102</th>
<th>RV$^-$ N=68</th>
<th>RV$^+$ N=34</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (±SD) (mmHg)</td>
<td>106 (±22)</td>
<td>111 (±18)</td>
<td>96 (±27)</td>
<td>0.006</td>
</tr>
<tr>
<td>Shock (Number of patients)</td>
<td>17</td>
<td>3</td>
<td>14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LOS (±SD) days</td>
<td>5.5 (±3.8)</td>
<td>4.5(±3.1)</td>
<td>7.5 (±4.4)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 9: Univariate correlates of RV$^+$ status.
The average LOS was 5.5 days (± 3.8 days) and was markedly prolonged (p = 0.016) in patients with RV involvement. Univariate comparison of patients with and without PHS (Table 11) showed that impaired LV function (Low LVEF and high WMSI), higher NT proBNP and increased plasma metanephrine concentration predicted PHS. There was no relation of PHS with Charlson score depicting that the PHS was not related to age or other comorbidities of the patients.

<table>
<thead>
<tr>
<th>Echocardiographic Parameters</th>
<th>Shock N=17</th>
<th>No Shock N=85</th>
<th>P value (Significant &lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular wall motion score index (± SD)</td>
<td>1.8 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%) (± SD)</td>
<td>47% ± 11%</td>
<td>49% ± 11%</td>
<td>NS</td>
</tr>
<tr>
<td>LVOT obstruction (N)</td>
<td>3</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>RV⁺ status (N)</td>
<td>14</td>
<td>3</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Plasma metanephrine Ng/L #</th>
<th>Plasma Normetanephrine Ng/L #</th>
<th>N terminal brain natriuretic peptide Ng/L #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shock N=17</td>
<td>315 (250 - 494)</td>
<td>1400 (830-1930)</td>
<td>6494 (3900-9900)</td>
</tr>
<tr>
<td>No Shock N=85</td>
<td>251 (200 - 364)</td>
<td>1100 (765-2415)</td>
<td>6346 (3100-11000)</td>
</tr>
<tr>
<td>P value (Significant &lt; 0.05)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 10: Echocardiographic and biochemical univariate correlates of shock.
# = Median/interquartile range
Skewed data described in median + interquartile range
As regards the impact of extent of RV involvement, this was inversely correlated with LVSV (Figure 24) and tended to predict PHS (p=0.07).

<table>
<thead>
<tr>
<th>Echocardiographic parameters</th>
<th>Length of stay ≤ 6 days</th>
<th>Length of stay &gt; 6 days</th>
<th>P value (Significant &lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular wall motion score index (± SD)</td>
<td>1.8 (± 0.3)</td>
<td>2 (± 0.3)</td>
<td>0.009</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%) (± SD)</td>
<td>49 (± 12)</td>
<td>43 (± 9)</td>
<td>0.02</td>
</tr>
<tr>
<td>RV+ status (N)</td>
<td>20</td>
<td>14</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Plasma metanephrine Ng/L#</th>
<th>Plasma normetanephrine Ng/L#</th>
<th>P terminal brain natriuretic peptide Ng/L#</th>
<th>Combined age and condition related score (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250(200 - 360)</td>
<td>970 (730-1570)</td>
<td>4900 (2600-8100)</td>
<td>3.5(± 0.3)</td>
</tr>
<tr>
<td></td>
<td>290 (220 - 440)</td>
<td>2538 (1460-2720)</td>
<td>12000 (7000-26000)</td>
<td>4.4 (± 0.4)</td>
</tr>
</tbody>
</table>

Table 11: Univariate correlates of patients with greater or less than median length of stay
# Median/interquartile range
Skewed data described in median + interquartile range

Figure 24A and 24B: Univariate correlates of extent of RV involvement (RV score).
4.3.4 RV involvement predicts more extensive LV involvement, but is not a multivariate correlate of hypotension, shock or PHS

Finally we performed multivariate analyses of determinants of SBP, shock, LV systolic function and LOS to elucidate the overall impact of RV\(^+\) status. The results of the multivariate analyses are summarized in Table 12.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Multivariate Predictors</th>
<th>β Value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>LVEF</td>
<td>0.32</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Metanephrine</td>
<td>0.24</td>
<td>0.02</td>
</tr>
<tr>
<td>Shock</td>
<td>LVOT</td>
<td>0.03</td>
<td>0.729</td>
</tr>
<tr>
<td></td>
<td>RV score</td>
<td>-0.38</td>
<td>0.06</td>
</tr>
<tr>
<td>LVSV</td>
<td>LVEF</td>
<td>0.49</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Troponin T</td>
<td>0.22</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>CK</td>
<td>-0.14</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>RV involvement</td>
<td>-0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>LVEF</td>
<td>RV involvement</td>
<td>-0.28</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Metanephrine</td>
<td>-0.27</td>
<td>0.006</td>
</tr>
<tr>
<td>LOS</td>
<td>NT- pro BNP</td>
<td>0.26</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 12: Major results for multivariate analysis: correlate of systolic BP, LV stroke volume (LVSV), LV ejection fraction (LVEF) and length of stay (LOS).

First, determinants of systemic blood pressure were sought: significant correlates were limited to LVEF and plasma metanephrine concentrations. On the other hand, there were no independent predictors of shock including, LVOT obstructions and RV\(^+\) status on multivariate analysis.
We then sought to examine determinants of PHS: neither Charlson comorbidity score nor any of the echocardiographic parameters were determinants of PHS. However, NT pro BNP release was a direct correlate of PHS.

Finally, we sought to evaluate whether RV$^+$ status affected either LVEF or LVSV. Indeed, RV$^+$ status was a significant correlate of both these parameters.

There was no significant multivariate correlate of extent of RV involvement. A further issue for exploration was the possibility that inadequate chronotropic response to low cardiac output might have contributed to hypotension. On ANCOVA, heart rate did not vary significantly with age ($F = 0.62, P = 0.44$) and there was no significant tachycardia in hypotensive patients ($F = 0.92, P = 0.61$).

### 4.4 Discussion

Although the condition of Tako-Tsubo Cardiomyopathy (TTC) has only recently been identified (189), it is increasingly appreciated that TTC is a cause of sustained morbidity and occasional mortality, especially in aging women (52). Much of the mortality of TTC is engendered by frequent development of severe hypotension and shock during the first 24 hours post presentation and indeed in this cohort, approximately 20% of patients were initially severely hypotensive (190, 191). We have recently shown that there is no nexus between the severity of TTC (as measured by LV wall motion score or extent of myocardial edema of CMR/ T2 imaging) and occurrence of early hypotension (192). This dissociation may in part reflect the multiple autocaloidal inputs modulating systemic vascular resistance changes (e.g. release of catecholamines and BNP) superimposed on impaired LV systolic function.

In the current study we have focused on the potential impact of RV involvement as a modulator of short-term hemodynamic impairment in TTC, and potentially as a determinant of duration of hospital stay. There were a number of theoretical reasons for this. First, a
number of previous investigators have noted variable RV (apical) hypokinesis in TTC, but not fully explored its implications (193-195). Second, we have observed that, irrespective of hypotension, patients with TTC generally do not develop acute pulmonary edema(53): by analogy with the condition of RV infarction, acute RV failure might explain this and indeed LV hypovolemia via RV failure might theoretically contribute to hypotension. However, in the current study, echocardiographically estimated PA systolic pressures were generally normal or mildly elevated.

The overall major findings of the current study are that RV involvement in TTC tends to occur in patients with a more severe degree of LV involvement, and hence the hemodynamic impact of the presence of RV involvement is at most additive. As shown by the comparative data in Tables 8 and 9, presence of RV involvement in TTC is associated with greater myocardial injury (as evidenced by troponin T and NT pro BNP release), more marked catecholamine stimulus (cf metanephrine concentration) and a trend towards lower LVEF. Thus it is not surprising that RV involvement was a univariate correlate of hypotension. Similarly, LOS was markedly prolonged in patients with RV involvement (Table 10).

The current findings differ to some extent from previous reports in the literature all of which related to substantially smaller series of patients with TTC (196). Notably, Fitzgibbons et al (196) also demonstrated that RV involvement was associated with greater length of hospital stay, and also with more marked elevation of BNP levels. However, these investigators did not note any evidence of more extensive LV dysfunction, or of biomarker release and consequently did not pursue multivariate analyses of correlates of RV involvement.

Although abnormalities of RV wall motion were present in approximately one third of patients, severe extensive RV dysfunction (RV Score ≥7) occurred in only 7% of patients. Increased RV score predicted decreases in LVSV (Fig 2B), consistent with the categorical impact of RV involvement.
Backwards stepwise multiple logistic regression was performed to evaluate further the putative role of RV dysfunction in the development of hypotension, associated changes in LVSV and LVEF, and the potential impact of these hemodynamic variables on LOS. The strongest independent (inverse) correlate of systolic BP was LVEF. However, presence of RV involvement was an independent and inverse correlate of both LVSV and LVEF itself. Hence the implications of RV involvement on hypotension are largely indirect, being mediated primarily via associated reduction in LVEF.

The additional finding that extent of elevation of plasma metanephrine concentrations predicts reduced LVEF and low systolic BP is hardly surprising, but it is uncertain whether the association reflects primarily the extent of the catecholamine stimulus initiating TTC (73, 74) or presence of catecholamine release because of hypotension. However, on multivariate analysis, neither catecholamine not NT-pro BNP levels were significant determinants of calculated systemic vascular resistance.

Finally, the study revealed that extent of elevation of NT-pro BNP levels was the only independent correlate of LOS. Both we (53) and the others (187) have noted the remarkable and prolonged (53) release of BNP and NT pro BNP in TTC patients. It is likely that the release is engendered primarily as a result of myocardial inflammation rather than distension (53). While the precise mechanism(s) by which such inflammation may have prolonged LOS were not delineated, it is clear that initial hemodynamic impairment per se is not usually a determinant of prolonged hospitalization.

In our recently published analysis of hypotensive response in TTC, no single factor appeared to play a large part (192). The current data suggest that lower LVEF and lesser degree of metanephrine release are independent determinants of hypotension. An additional factor may be inadequate chronotropic response to cardiac output: as found by ANCOVA, hypotensive TTC patients generally fail to develop a tachycardia relative to normotensive patients. Whether this reflects inappropriate mechanoreceptor stimulation, as previously described in
chronic heart failure (197), and whether this is also associated with inappropriate vasodilation cannot be determined with in the current study methodology.

4.5 Study limitations

The study also has a number of potential limitations. The primary purpose of echocardiographic evaluation of the patient cohort was directed towards the potential diagnosis of TTC rather than the evaluation of RV function, and it was judged that approximately 20% of TTE were of inadequate quality to characterize RV involvement. Furthermore, the time course of RV dysfunction in TTC was not evaluated, nor has this been studied by any previous investigators: it is certainly possible that performance of echocardiography 1 to 2 days post maximal hypotension in some of the patients may have distorted the relationship between RV function and hypotension. The impact of RV hypokinesis on RV stroke volume was not routinely measured, and no estimates were possible of the impact of RV distension on LV filling (via diastolic ventricular interaction) (198). Furthermore, a number of patients were initially treated with long and short acting nitrates, which may have contributed to falls in systemic blood pressure.

4.6 Conclusions

In conclusion, approximately one third of patients with TTC have regional RV hypokinesis; such patients have more extensive LV injury, and the latter (in combination with RV dysfunction) induces fall in LVSV and the propensity to hypotension. Importantly, speed of recovery from acute attacks of TTC to the extent of hospital discharge is not markedly dependent on initial hypotension, or on presence of RV involvement, but is likely to reflect extent of myocardial inflammation and resultant release of BNP.
Chapter: Meta-Analysis and systematic review of clinical correlates of acute mortality in Takotsubo cardiomyopathy
5.1 Introduction

Takotsubo cardiomyopathy (TTC) is increasingly recognized as a potential cause of acute chest pain and/or dyspnea in the aging population, especially women (7). While the “true” incidence of TTC remains a subject of dispute (19) and is potentially affected by issues such as the precise definition used (199), it is clear that it is not a rare condition. However, the diagnosis of TTC is markedly subject to potential selection bias, because it is substantially expedited by coronary angiography/cardiac catheterization, which serves to exclude relevant myocardial ischemia and to demonstrate presence of regional left ventricular hypokinesis.

Together with the increased recognition of the disorder, it has become apparent that TTC often occurs without ST elevation on index ECGs (and thus in patients who may not undergo emergency coronary angiography). It may occur in a more atypical form in the very elderly and it frequently is precipitated, not only by severe emotional stress, but also by concurrent/antecedent acute illness (200): the common pathophysiology potentially being a pulse of increased catecholamine release and resultant myocardial inflammation (16).

Both short and long term outcomes in patients with TTC are generally good, but not uniformly so. In the long-term, there is frequently slow recovery (164) and a significant risk of recurrence (201). Acutely, there is an essentially unknown rate of early life-threatening tachyarrhythmias (8), but the main risk is development of shock (142), which is known to carry a significant mortality rate. Indeed, as TTC is engendered by increased catecholamine release, usual pharmacotherapy for shock may be inappropriate. However, in most individual analyses of TTC cohorts with hypotension/shock, it has proved impossible to identify strong predictors of mortality.

Previous studies have revealed that the release of catecholamines which precipitates onset of TTC may be engendered by the occurrence of life-threatening extra-cardiac disease states
Furthermore, it has been suggested that males with TTC may represent a particularly high-risk subset (203).

Only one recent major study has evaluated the in-hospital mortality related to TTC in the American population by diagnosing TTC from National Inpatient Sample (NIS) discharge database using International Classification of Diseases (204). However, this method is prone to coding errors and wrong diagnosis. In addition to that, information on mortality comes from small case reports (8). We therefore performed a meta-analysis and systematic review to determine the global incidence of in-hospital mortality and to consolidate the evidence.

In the current study, we sought to determine utilizing a systematic review/meta-analysis approach:

1. The “overall” risk of in-hospital mortality in TTC patients,
2. Clinical correlates of variability in this risk,

And we prospectively elected to evaluate in particular the putative impact of:

a. TTC occurring secondary to other life-threatening disorders
b. TTC in males
c. Advanced age
d. TTC precipitated by medication-induced increases in catecholamine levels.
5.2 Methods

This systematic review and meta-analysis is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines (205).

5.2.1 Study eligibility

We included comparative studies of any design (randomized control trials, cohort, case control and cross sectional). Eligible studies had to provide documentation on in-hospital mortality in patients diagnosed with TTC as well as mode of death for all the patients who died in hospital after diagnosis of TTC. Furthermore, only studies with \( \geq 10 \) patients were considered eligible. Inclusion was restricted to publications in the English language or when translation of the foreign language publications was provided. When data was reported from overlapping study samples (e.g., multiple publications from the same group), the most recent study or one with the highest number of patients was included in the analysis. Single case reports and previous systematic reviews on TTC were not included.

5.2.2 “Primary” vs. “secondary” TTC

For the purpose of this meta-analysis we defined “secondary TTC” as development of TTC in a patient already suffering from a potentially life-threatening medical/surgical condition, and “primary TTC” as that which developed in the absence of such acute critical illness.

5.2.3 Data Sources and search strategy

A comprehensive search strategy was designed with the assistance of an expert librarian from the University of Adelaide. A thorough computer based search was performed using OVID MEDLINE, EMBASE, Google Scholar and PubMed databases. Search terms included (Takotsubo Cardiomyopath* or Broken Heart Syndrome or Stress Cardiomyopathy or Apical Ballooning Syndrome or Takotsubo Cardiomyopathy*) and (Mortality or Mortalit* or Death* or Fatalit* or Incidence* or Prevalence* or Statistic*). No limit to the start date was
applied and the search was conducted up to 10 July 2013. We hand searched the references cited in the previous reviews and important articles on TTC. We contacted the corresponding authors of the studies where relevant information was missing.

5.2.4 Study selection and data extraction

One reviewer (Kuljit Singh) screened all titles and abstracts independently. This was followed by the full text review of the selected articles by the same reviewer. Same reviewer (KS) extracted data independently from selected studies using a standardized, pilot tested extraction template. The following data was extracted: study characteristics (author, country, study design, study population, number of participants and objective of the study), participant characteristics (age and gender), clinical characteristics (ACS type for TTC, predisposing physical/emotion stressors, left ventricle ejection fraction, acute complications), in-hospital mortality (number of in-hospital deaths and mode of demise) and predictors of mortality and acute complications.

5.2.5 Quality assessment

The quality of included studies was assessed using a sub-set of the Tooth et.al, manuscript titled 'Quality of Reporting of Observational Longitudinal Research' (206), including only the 23 quality domains relevant to a meta-analysis of observational studies. We assessed biases using classifications of 'low risk of bias' when data for the criterion were reported, 'high risk of bias' when data was not reported and 'unclear risk of bias' when the criterion was not relevant to the study design. Review Manager software version 5.2 was used to generate the risk of bias graph.

5.2.6 Statistical analysis

Continuous variables were reported as means ± standard deviation (SD), while skewed data were described as medians ± interquartile range. Categorical variables were reported using
odds ratios (OR) with 95% confidence intervals (CI). Risk estimates were presented using OR, risk ratio (RR) and risk difference (RD) with 95% CI. Heterogeneity between studies was assessed by a combination of the $I^2$ statistic, Cochran’s Q test and observation of the data for each outcome. If formal meta-analysis was not possible due to a skewed distribution of the number of patients between each study, we disregarded the individual studies and used data as if obtained from a single study. Studies with missing data were excluded from meta-analyses and details extrapolated in the quality and risk of bias assessments. We then used logistic regression to assess the relationship. Significant interaction between variables was considered when $P < 0.05$. All calculations were performed using Review Manager software version 5.2
Figure 25: Schematic of database search and identification of studies.
5.3 Results

5.3.1 Literature identification

The literature search on TTC and mortality yielded 486 citations (Figure 25) of which, 382 citations were in English and were related to humans. Abstracts were reviewed for the 382 short listed citations and 78 articles were chosen for full text review. Of the 78 full-text manuscripts reviewed for eligibility, 35 met all of the inclusion and exclusion criteria (Table 13). An additional five manuscripts were identified through hand searching leading to a total of 37 studies included in this analysis. Five authors were contacted for missing data, with one responding and providing the requested information.

All the studies designs were observational, including 22 retrospective studies, 13 prospective and two partially retrospective and partially prospective studies. Sample size ranged from 10 to 256 participants (total 2120). Fifteen of these studies were from Europe, 13 were from Asia, and eight were from the United States of America and one from Australia.

5.3.2 Basic characteristics

Basic characteristics of the patients are detailed in Table 14. Among the whole cohort of 2120 patients with TTC, 87% (n = 1859) were women. The mean age of patients diagnosed with TTC was 68 years (95% CI 67 to 69 years). There was a significant association between the presence of physical/emotional stress and development of TTC (OR 6.23, 95% CI 4.04 to 9.63, p < 0.00001, 27 studies, \( \hat{I}^2 = 85\% \); Figure 26). Approximately 52% of patients presented as NSTEMI rather than STEMI. Chest pain (63.4%) was the most common presenting symptom followed by dyspnea (23.2%). In 13.3% of patients the mode of presentation was incidental finding of abnormal ECG, syncope, palpitations, hypotension, shock and vomiting.
According to the wall motion abnormalities, although TTC occurs relatively frequently as apical and mid-ventricular forms, 20 studies specified the regional pattern of TTC of these, apical TTC was found in 75% (n = 939) and other variants were found in 25% of cases (n = 313). The average left ventricular ejection fraction during acute TTC episodes was 40.2% (95% CI 38.3% to 42.1%) rising to 60.5% (95% CI 59.2% to 61.8%) among the studies that followed up TTC patients to recovery.

### 5.3.3 Mortality

#### 5.3.3.1 Overall

There were 96 deaths in the whole cohort. On meta-analysis, the in-hospital mortality was 4.5% (95% CI 3.1% to 6.2%, $I^2 = 60.8\%$). We performed comparisons between prospective
and retrospective studies and between single-center and multi-center studies to determine whether there was any heterogeneity due to these factors (Table 15). The in-hospital mortality of multi-center studies (3.8%, 95% CI 1.8% to 7.1%, 7 studies, $I^2 = 74.8\%$) and prospective studies (4.8%, 95% CI 2.3% to 8.2%, 12 studies, $I^2 = 71.5\%$) tended to be less consistent than single center studies (4.9%, 95% CI 3.1% to 7.1%, 28 studies, $I^2 = 58.5\%$) and retrospective studies (4%, 95% CI 2.1% to 6.4%, 18 studies, $I^2 = 54.6\%$).

5.3.3.2 Clinical correlates of mortality rates

Due to low numbers, pooled analysis was performed for the cause of acute death, divided into cardiac and non-cardiac origins. Among all deaths, 38% (n = 35) were directly related to cardiac complications and 62% (n = 58) due to underlying co-morbid medical conditions. Ventricular arrhythmias, shock and refractory heart failure contributed to most of the cardiac mortality. In patients dying because of TTC related cardiac complications, 40% (n = 14) died because of ventricular arrhythmias, 34% (n = 12) due to shock and heart failure and 26% (n = 9) had other cardiac complications (ventricular wall rupture, mural thrombus related embolic complications etc.).

5.3.3.3 “Primary” vs. “secondary” TTC

Twenty-seven studies reported data on “primary” and “secondary” TTC, 60% of these 1674 patients were designated to have “primary” and 40% were found to have “secondary” TTC. Acute illnesses associated with the “secondary TTC” included sepsis, intracranial hemorrhage, exacerbation of asthma/emphysema, acute gastrointestinal bleed, bowel obstruction and surgical emergencies.

From the 667 individuals with secondary TTC, 65 (10%) died in the hospital. In comparison among primary TTC patients, in-hospital mortality was only 1% (12 out of 1007). Thus, participants with a “secondary” diagnosis of TTC were significantly more likely to die
compared to those with a “primary” diagnosis (RD -0.11, 95% CI -0.18 to -0.04, p= 0.003
27 studies, I² = 84%, Figure 20).

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Primary</th>
<th>Secondary</th>
<th>Risk Difference M-H, Random, 95% CI</th>
<th>Risk Difference M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avasthi 2004</td>
<td>0 5</td>
<td>0 5</td>
<td>2.9% 0.08 [0.31, 0.31]</td>
<td></td>
</tr>
<tr>
<td>Apanasadiis 2006</td>
<td>0 20</td>
<td>0 3</td>
<td>2.6% 0.00 [0.03, 0.23]</td>
<td></td>
</tr>
<tr>
<td>Azzarelli 2008</td>
<td>0 14</td>
<td>0 5</td>
<td>3.6% 0.00 [0.24, 0.24]</td>
<td></td>
</tr>
<tr>
<td>Bonello 2007</td>
<td>3 11</td>
<td>0 3</td>
<td>2.0% 0.27 [0.14, 0.69]</td>
<td></td>
</tr>
<tr>
<td>Bulia 2012</td>
<td>1 42</td>
<td>1 12</td>
<td>4.5% -0.06 [0.22, 0.10]</td>
<td></td>
</tr>
<tr>
<td>Burgdorf 2008</td>
<td>0 42</td>
<td>3 8</td>
<td>2.7% 0.38 [0.70, 0.08]</td>
<td></td>
</tr>
<tr>
<td>Eleftheriadis 2007</td>
<td>2 70</td>
<td>0 30</td>
<td>5.7% 0.03 [0.03, 0.09]</td>
<td></td>
</tr>
<tr>
<td>Ellien 2006</td>
<td>0 10</td>
<td>1 4</td>
<td>1.9% 0.25 [0.67, 0.17]</td>
<td></td>
</tr>
<tr>
<td>Eshkenazi 2009</td>
<td>0 35</td>
<td>0 6</td>
<td>4.1% 0.00 [0.19, 0.19]</td>
<td></td>
</tr>
<tr>
<td>Fang 2008</td>
<td>0 4</td>
<td>2 6</td>
<td>1.3% -0.33 [0.78, 0.11]</td>
<td></td>
</tr>
<tr>
<td>Hsu 2010</td>
<td>0 9</td>
<td>0 5</td>
<td>3.3% 0.08 [0.26, 0.26]</td>
<td></td>
</tr>
<tr>
<td>Jansou 2010</td>
<td>0 16</td>
<td>0 11</td>
<td>4.9% 0.00 [0.14, 0.14]</td>
<td></td>
</tr>
<tr>
<td>Jie 2012</td>
<td>0 31</td>
<td>6 6</td>
<td>4.1% -1.00 [0.20, 0.80]</td>
<td></td>
</tr>
<tr>
<td>Komar 2007</td>
<td>0 20</td>
<td>3 15</td>
<td>3.9% -0.28 [0.64, 0.01]</td>
<td></td>
</tr>
<tr>
<td>Kwon 2012</td>
<td>0 52</td>
<td>18 156</td>
<td>5.7% -0.12 [0.17, 0.00]</td>
<td></td>
</tr>
<tr>
<td>Lee 2010</td>
<td>0 6</td>
<td>3 4</td>
<td>1.8% -0.75 [0.19, 0.31]</td>
<td></td>
</tr>
<tr>
<td>Lee 2010</td>
<td>1 27</td>
<td>7 29</td>
<td>4.4% -0.20 [0.09, 0.03]</td>
<td></td>
</tr>
<tr>
<td>Mitchell 2007</td>
<td>0 19</td>
<td>0 3</td>
<td>2.8% 0.08 [0.33, 0.33]</td>
<td></td>
</tr>
<tr>
<td>Mitsuura 2010</td>
<td>0 12</td>
<td>0 9</td>
<td>4.4% 0.00 [0.17, 0.17]</td>
<td></td>
</tr>
<tr>
<td>Parodi 2011</td>
<td>1 45</td>
<td>1 34</td>
<td>6.0% -0.01 [0.08, 0.08]</td>
<td></td>
</tr>
<tr>
<td>Previtali 2011</td>
<td>0 123</td>
<td>1 5</td>
<td>2.5% -0.20 [0.55, 0.15]</td>
<td></td>
</tr>
<tr>
<td>Primelshofen 2010</td>
<td>0 16</td>
<td>0 15</td>
<td>5.1% 0.00 [0.12, 0.12]</td>
<td></td>
</tr>
<tr>
<td>Sharkey 2010</td>
<td>2 64</td>
<td>1 67</td>
<td>6.7% 0.01 [0.04, 0.07]</td>
<td></td>
</tr>
<tr>
<td>Speddicato 2008</td>
<td>0 26</td>
<td>0 3</td>
<td>2.6% 0.08 [0.33, 0.33]</td>
<td></td>
</tr>
<tr>
<td>Tsuchihashi 2001</td>
<td>0 18</td>
<td>1 29</td>
<td>5.3% 0.03 [0.14, 0.07]</td>
<td></td>
</tr>
<tr>
<td>Vidi 2008</td>
<td>0 12</td>
<td>2 16</td>
<td>4.1% 0.13 [0.32, 0.07]</td>
<td></td>
</tr>
<tr>
<td>Vidu 2012</td>
<td>1 29</td>
<td>1 5</td>
<td>2.3% 0.13 [0.57, 0.21]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>769</td>
<td>484</td>
<td>100.0% -0.11 [0.18, 0.04]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Q(df=4)= 0.02, I²= 147.94, d= 26.00 (P < 0.0001), I²= 82%.
Test for overall effect: Z = 3.02 (P = 0.003)

Figure 20: Pooled analysis with RD and 95% CI comparing acute mortality in primary and secondary TTC groups.

TTC=Takotsubo cardiomyopathy
CI= Confidence interval
RD+ Risk difference

5.3.3.4 Gender differences

Of the 265 men diagnosed with TTC, 22 (8%) died in the hospital (Table 16). Male gender was associated with higher acute mortality relative to female gender (OR 2.6, 95% CI 1.49 to 4.60, p = 0.0008, 14 studies, I² = 0%, Figure 21), with males found to have a 6% absolute
increase risk of in-hospital mortality compared to females (RD 0.06, 95% CI 0.01 to 0.11, 
p= 0.02, 14 studies, $\Gamma^2 = 30\%$, Figure 22). However, insufficient reporting of data did not 
permit us to determine whether the distribution of “primary” vs. “secondary” TTC was gender 
specific.
Figure 21: Pooled analysis with OR and 95% CI comparing acute mortality in males and females diagnosed with TTC.

TTC=Takotsubo Cardiomyopathy
CI= Confidence Interval
OR=Odds Ratio

Figure 22: Pooled analysis with RD and 95% CI comparing acute mortality in males and females diagnosed with TTC.

TTC=Takotsubo Cardiomyopathy
CI= Confidence Interval
RD= Risk Difference

5.3.3.5 Advanced age
Only 10 of the 37 studies reported age of patients dying in the hospital. Two of these did not report the mean age of the whole group. The mean age of patients dying in hospital tended to be higher (72 ± 7 years) than that of the whole cohort (65 ± 7 years). Individual comparison of the 8 studies revealed that the non-survivor group was older, however, this difference was statistically significant in only one study (Table 17).

5.3.3.6  Catecholamine use

Only 9 studies reported the use of catecholamine. However, data of catecholamine use in dying patients was not available to assess the impact on in-hospital mortality.

5.3.4  Complications

Atrial fibrillation, bradycardia, shock, left ventricular outflow tract (LVOT) obstruction, moderate to severe mitral regurgitation, mural thrombus and ventricular arrhythmias were the main complications. Patients who suffered from shock were treated with intra-aortic balloon pump (IABP), inotropic agents or a combination of both.

Shock was the most common complication in patients diagnosed with TTC and was found in 9.3% of the patients. The preference for treatment of shock with IABP or inotropic medication or both varied among different studies. The frequency of IABP insertion and the use of inotropic medication was 4.4% (n = 53/1194) and 16.8% (n = 130/778) respectively among the patients diagnosed with TTC. The second most common complication was moderate to severe mitral regurgitation (8.2%, n =56/680) followed by LVOT obstruction, found in 6.8% (n = 90/1326) and atrial fibrillation, which occurred in 6.7% of TTC patients. The risk of sustained ventricular arrhythmias (4.2%, n = 53/1259) and mural thrombus (2.3%, n = 28/1198) was low.
5.3.5 Quality assessment

Overall, study quality was quite good with 14 out of the 26 criterion's having at least 75% of all studies reporting a low risk of bias for bias (Figure 23, Figure 24). Six criterion were not well reported across 75% of studies or more, including justification of the number of included participants, reasons for non-inclusion of subjects, reporting of confounders, accounting for confounders in the analyses, accounting for missing data in the analyses, impact of bias assessed qualitatively and impact of bias assessed quantitatively.
Figure 23: Risk of bias assessment presenting review author’s judgments about each quality criterion presented as a percentage across all included studies. Criterions were based on an adaption of the categories reported in the Tooth et.al. 2005 paper ‘Quality of reporting of observational longitudinal research’.
Figure 24 Risk of bias summary with review author’s judgments about each quality criterion for each included study. Criteria were based on an adaption of the categories reported in the Tooth *et al.* 2005 paper ‘Quality of reporting of observational longitudinal research’
5.4 Discussion

The main purpose of this systematic review was to attempt to identify correlate variables in in-hospital mortality among patients hospitalized with TTC. Understanding of TTC as a differential diagnosis of acute coronary syndrome has advanced considerably worldwide over the last 10 years, with the diagnosis entertained far more frequently than previously the case (207). The process of diagnosing TTC has been facilitated by the widespread practice of emergency cardiac catheterization for patients with suspected acute myocardial infarction and, by the availability of cardiac MRI scanning, which permits both exclusion of acute myocardial infarction and identification of the widespread cardiac edema, which characterizes TTC (16).

The previous literature had made it clear that the major modalities of in-hospital death in patients with TTC were via development of lethal tachyarrhythmias and/or shock (8, 142), and indeed the current systematic review supported this concept. The versed issue was: who develops the arrhythmias/shock? In this regards there was somewhat fragmentary information, but it had been suggested widely that male gender, while relatively rare among Caucasian TTC patients, was associated with increased mortality (208). It was also known that TTC occurs frequently as a result of catecholamine release secondary to severe extracardiac conditions (107), and we chose to focus on this entity, which we designated “secondary” TTC, as a potential source of differential mortality rates.

Our study suggests that overall in-hospital mortality for TTC is approximately 4.5%. However, mortality is four-fold greater for males than females, in accordance with previous findings (209). More importantly, “secondary” TTC as associated with 10-fold increase in mortality relative to “primary” TTC. Unfortunately, the available data do not permit delineation of two important related issues: whether TTC in males is frequently “secondary”, and whether patient’s dying with “secondary” TTC do so predominantly because of their
severe antecedent non-cardiac illnesses. Prospective evaluation of the TTC/antecedent illness interplay in cohorts of patients with “secondary” TTC is therefore desirable to elucidate the latter issue in particular.

A third objective of the current study was to evaluate the impact of advanced age on TTC related mortality. Although it appears increasingly that propensity towards TTC is related to the physiology of the aging heart, rather necessarily than to menopausal physiology, little information is available regarding either incidence or outcomes of TTC in the very elderly. One previous study showed a significant increase in mortality with increasing age of TTC patients (140). In the current evaluation, there was a non-significant trend towards increased mortality with advanced age: limited characterization of patients in individual studies represented an impediment to full data analysis.

It is now appreciated that increased release of catecholamine is a triggering factor for TTC in most or all of the cases. This represents a practical dilemma for the management of patients who develop shock in association with TTC: theoretically incremental catecholamine administration may be harmful. Indeed, two studies (140, 210) with higher use of catecholamine administration had higher mortality. Furthermore, one study suggested that organic nitrate administration also was a correlate of increased mortality (211), while ACE inhibitor use was protective (212). The current review was unable to capture data on the entire study cohort regarding these aspects of therapeutics.

The main limitations of the current study arise from lack of clinical detail in many of the component publications, and from the fact that mortality in immediate hospitalization/pre-hospitalization periods (that is, before cardiac catheterization) would preclude making the diagnosis of TTC. The missing information in the papers and ruling out non-English literature from the analysis were the other main limitations.
The main finding of the study, that “secondary” TTC carries a high mortality, raises a number of interesting issues regarding the diagnosis of this condition. For example, it remains uncertain how often “myocardial infarcts” occurring in the context of extra cardiac surgery of cerebrovascular events are actually due to TTC. As such “infarcts” carry appreciable mortality it is appropriate for greater efforts to be directed to the diagnosis, treatment and ultimately prevention of “secondary” TTC.
### Selection criteria

<table>
<thead>
<tr>
<th>Selection criteria</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
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<tr>
<td>Population</td>
<td>Definition of TTC fulfilled</td>
<td>Definition of TTC not fulfilled</td>
</tr>
<tr>
<td></td>
<td>Data of in-hospital mortality available</td>
<td>No data on mortality available</td>
</tr>
<tr>
<td></td>
<td>Cause of in-hospital death stated in all the patients</td>
<td>Cause of death not stated</td>
</tr>
<tr>
<td>Study design</td>
<td>Observational or case control studies with $\geq 10$ patients</td>
<td>Single case reports, review articles, conference abstracts, statements and editorials.</td>
</tr>
<tr>
<td>Study quality</td>
<td>Acute outcome data of TTC patients available</td>
<td>No data on acute outcome</td>
</tr>
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</table>

**Table 13: Study selection criteria.**
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<thead>
<tr>
<th>No.</th>
<th>Source</th>
<th>Country</th>
<th>TTC Patients</th>
<th>Age</th>
<th>Gender</th>
<th>ACS Type</th>
<th>Variant of TTC</th>
<th>Left Ventricular ejection fraction</th>
<th>Emotional/Physical stress</th>
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<tr>
<td>1.</td>
<td>Spedicato et al. 2008(213)</td>
<td>Italy</td>
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<td>4</td>
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<td>18</td>
<td>11</td>
<td>29 0</td>
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<td>Israel</td>
<td>13</td>
<td>68</td>
<td>1</td>
<td>12</td>
<td>12</td>
<td>1</td>
<td>13 0</td>
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<td>3.</td>
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<td>France</td>
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<td>68 ±15</td>
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<td>13</td>
<td>6</td>
<td>8</td>
<td>40.6 ± 7.2%</td>
</tr>
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<td>7</td>
<td>47</td>
<td>41</td>
<td>13</td>
<td>40 14</td>
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<td>Taiwan</td>
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<td>9</td>
<td>3</td>
<td>9 3</td>
</tr>
<tr>
<td>6.</td>
<td>Sato et al. 2006(218)</td>
<td>Japan</td>
<td>16</td>
<td>71.5 ± 9.4</td>
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<td>7</td>
<td>40 ± 8%</td>
</tr>
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<td>Italy</td>
<td>19</td>
<td>65 ±13</td>
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<td>17</td>
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<td>10</td>
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</tr>
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<td>8.</td>
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<td>13</td>
<td>22 0</td>
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<td>65.8 ± 14.0</td>
<td>57</td>
<td>15</td>
<td>54</td>
<td>154</td>
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<td>25</td>
<td>33</td>
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<td>64 +/-12</td>
<td>6</td>
<td>32</td>
<td>19</td>
<td>19</td>
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<td>64</td>
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<td>59</td>
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<td>61 ± 7</td>
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<td>Elesber et al.</td>
<td>USA</td>
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160
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<th>Interquartile Range</th>
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<th>90th Percentile</th>
<th>95th Percentile</th>
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<th>Percental Increase</th>
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<td>61.26±8.77%</td>
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<td>Vriz et al 2013</td>
<td>Italy</td>
<td>25</td>
<td>21</td>
<td>20</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>32 ± 11%</td>
<td>&gt; 50%</td>
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<td>Sharkey et al 2010</td>
<td>USA</td>
<td>136</td>
<td>13</td>
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<td>69</td>
<td>36 ± 9</td>
<td>77%</td>
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<td>64.4 ±7.3%</td>
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<td>18</td>
<td>28±10</td>
<td>51±14</td>
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<td>27</td>
<td>Vidi et al 2009</td>
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<td>14</td>
<td>20</td>
<td>41 ± 11</td>
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<td>58 ± 8</td>
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<td>Singh et al 2010</td>
<td>USA</td>
<td>114</td>
<td>8</td>
<td>22</td>
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<td>58</td>
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<td>32%</td>
<td>65%</td>
<td>26</td>
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<td>Country</td>
<td>Average Age ± SD (Years)</td>
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<td>34</td>
<td>Lee et al. 2009 (232)</td>
<td>Singapore</td>
<td>55</td>
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<td>6</td>
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<td>Mitchell et al. 2007 (234)</td>
<td>USA</td>
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<td>Athanasiadis et al. 2006 (235)</td>
<td>Germany</td>
<td>65 ± 10</td>
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<td>16</td>
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</table>

Table 14: Summary of basic characteristics of 37 studies included in this systematic review.
<table>
<thead>
<tr>
<th>Grouping</th>
<th>Number studies</th>
<th>In-hospital death (%)</th>
<th>I squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>37</td>
<td>4.5 (95% CI, 3.1-6.2)</td>
<td>60.8%</td>
</tr>
<tr>
<td>Retrospective studies</td>
<td>18</td>
<td>4 (95% CI, 2.1-6.4)</td>
<td>54.6%</td>
</tr>
<tr>
<td>Prospective studies</td>
<td>12</td>
<td>4.8 (95% CI, 2.3-8.2)</td>
<td>71.5%</td>
</tr>
<tr>
<td>Single Centre studies</td>
<td>28</td>
<td>4.9 (95% CI, 3.1-7.1)</td>
<td>58.5%</td>
</tr>
<tr>
<td>Multi Centre studies</td>
<td>7</td>
<td>3.8 (95% CI, 1.4-7.1)</td>
<td>74.8%</td>
</tr>
</tbody>
</table>

Table 15: In-hospital mortality in the whole cohort and in subgroups.
<table>
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<tr>
<th>No.</th>
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<th>Gender of dead patients</th>
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<td>Total</td>
<td>Cardiac</td>
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<td>Elian et al. 2006(214)</td>
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<td>Bonello et al. 2007(215)</td>
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<td>Hsu et al 2010(217)</td>
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Table 16: Total number, cause and gender variability of in-hospital deaths in Takotsubo patients.
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<th>P value</th>
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<td>66.8 ±11</td>
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<td>65 ± 5.65</td>
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<td>68 ±13</td>
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<td>73 ±10</td>
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<td>Joe et al.</td>
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<td>74.5 ±10.6</td>
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Table 17: Comparison of mean age among the whole group and non-survivors in the 8 individual studies.

SD = Standard of deviation
6 Chapter: A meta-analysis of recurrence of Takotsubo Cardiomyopathy
7.1 Introduction

The clinical course of TTC after the first 24 hours is usually one of rapid symptomatic improvement. Chest pain usually diminishes, and a number of studies have shown rapid improvement in the LV regional wall motion over a period of 7 days. On the other hand, it has recently emerged that complete recovery is usually delayed for at least 3 months, with evidence of persistent LV systolic dysfunction associated with ongoing symptoms of lassitude, exertional dyspnea and sometimes chest pain; approximately 50% of patients remain symptomatic 2 years post acute attacks.

Recurrent acute attacks of TTC are thought to occur in approximately 1 to 3% of patients per annum. However, in the absence of large prospective trials the information on the incidence and clinical correlates of recurrence remains unclear. Most of the data on the recurrence rate of TTC has been evaluated from observational studies, which is prone to selection bias. The results of 2 relatively large studies with nearly 4-year follow-up have shown different slightly different recurrence rate. While the Mayo Clinic data has shown the recurrence rate to be nearly 3-4% per year, the results from Tuscany registry document the rate of recurrence to be lower than 1% (11, 236, 237). Furthermore, given the small data sets and short observation time has made it difficult to evaluate the correlates of recurrence.

The initial treatment following the diagnosis of TTC is essentially the same as adopted for heart failure. However, there is no clear data on the efficacy of the currently used therapy on the recurrence of TTC. Increased circulating levels of catecholamines in some of the cases of TTC have formed the basis of use of β-blockers following the diagnosis of TTC. However, β-blocker medication has neither been found to reduce the initial nor recurrent episodes of TTC in small case reports or observational studies (7, 201). On the other hand, use of angiotensin converting enzyme inhibitors (ACEi) might help in the recovery of TTC and reduce episodes of recurrence via renin angiotensin system (RAS) and by their direct anti-inflammatory
properties on the myocardium. However, because of paucity of the data and lack of studies evaluating the role of ACEi therapy following TTC diagnosis, no clear statement can be made about its use in TTC at the moment.

We performed a meta-analysis and systemic review to consolidate the current evidence and to evaluate the determinants of recurrence of TTC and cause of its variability. We also prospectively evaluated the impact of discharge medications, left ventricular ejection fraction (LVEF) and type of stressor during initial episode on recurrence.

### 7.2 Methods

This systematic review and meta-analysis is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines.

#### 7.2.1 Inclusion criteria

We included comparative studies of any design (randomized control trials, cohort, case control and cross sectional). **Eligible studies needed to have \( \geq 5 \) patients and a minimum follow-up of 3 months with confirmed documentation of recurrence of TTC.** Inclusion of studies was not limited to English and foreign studies were included if translation to English was available. When data was reported from interrelating study samples (e.g., publications from the same group), the study with the highest number of patients and the longest follow up was included in the analysis. Single case reports, case series with less than 5 patients or where the follow up was not conducted were not included. Systematic reviews of TTC were not included in the analysis.

#### 7.2.2 Search strategy

A thorough computer based search was performed using OVID MEDLINE, EMBASE, Google Scholar and PubMed databases was made with the help of a senior librarian of University of Adelaide. Search terms included (Takotsubo Cardiomyopathy*[TW] OR Broken Heart Syndrome [TW] OR Stress Cardiomyopathy [TW] OR Apical Ballooning
Syndrome[TW] OR Tako-Tsubo Cardiomyopath* [TW]) and (Recurrence [MH] OR Recurrence*[TW] OR Relapse*[TW] OR Reappearance*[TW] OR Incidence*[TW] OR Prevalence*[TW] OR Recurrent*[TW]). No limit to the start date was applied and the search was conducted up to 31 January 2014. We hand searched the references cited in the previous reviews and important articles on recurrence on TTC. We contacted the corresponding authors of the studies where relevant information was missing.

All titles and abstracts were searched individually. This was followed by the full manuscript review of the selected articles by the reviewers. One reviewer (KS) extracted data independently from selected studies using a standardized, pilot tested extraction template. The following data was extracted: study characteristics (author, country where investigation was conducted, study design, number of participants and objective of the study), participant characteristics (age and gender), clinical characteristics (ACS type for TTC, predisposing physical and emotion stressors, LVEF, acute complications, acute mortality, length of stay, discharge medications), recurrences (number and time of recurrence after initial diagnosis) and complications during long term follow up (death, chest pain, dyspnea, fatigue and heart failure).

Same reviewer (KS) assessed quality of included studies using a sub-set of the Tooth et.al, manuscript titled 'Quality of Reporting of Observational Longitudinal Research'(206), including only the 23 quality domains relevant to a meta-analysis of observational studies. We evaluated potential biases using classifications of 'low risk of bias' when data for the criterion were described, 'high risk of bias' when data were not stated and 'unclear risk of bias' when the criterion was not relevant to the study design. Review Manager software version 5.2 was used to make the risk of bias graph. Any classification, which was not straightforward, was solved by discussion with a third reviewer KC.

7.2.3 Statistical analyses

Continuous variables were reported as means ± standard deviation (SD), while skewed data
were described as medians ± interquartile range. Categorical variables were reported using odds ratios (OR) with 95% confidence intervals (CI). If formal meta-analysis was not possible due to a skewed distribution of the number of patients between each study, we overlooked the individual studies and used data as if obtained from a single study. Studies with missing data were excluded from meta-analyses and details extrapolated in the quality and risk of bias assessments. We then used logistic regression to assess the relationship. Significant interaction between variables was considered when $P < 0.05$. All calculations were performed using Review Manager software version 5.2, SPSS software and Microsoft Excel. We divided the studies above and below the median level of percentage of ACE/ARB, BB and recurrence rate and performed Chi square test. The annual incidence of recurrence for each individual study was calculated. Formula for a CI for a population proportion was used to obtain CI for annual recurrence for each individual study.

### 7.3 Results

The literature search on TTC and recurrence yielded 298 citations. The abstracts of all the 298 citations were reviewed and 58 articles were chosen for full text review. Of the 58 articles chosen for the full text review, 34 were selected as per the inclusion and exclusion criteria. Six out of 34 studies were noted to be duplicated (from the same group with overlapping patient data sets) and were excluded. Addition 3 articles were found on the hand search leading to a total of 31 articles in the systematic review (Figure 25). Authors were contacted to obtain details of the patients with recurrence where it was not reported in the study.
All the studies designs were observational, including 20 retrospective, 10 prospective and one partially retrospective and partially prospective. Sample size ranged from 12 to 224 participants (total 1664). Twenty-one of these studies were from Europe, 5 were from Asia, and 4 were from the United States of America and 2 from Australia.
Basic characteristics of the studies are detailed in Table 18. Among the whole cohort of 1664 patients with TTC, 91.5% (n = 1523) were women. The mean age of patients was 66 years (95% CI 63 to 69 years). In nearly 74% of patients TTC was preceded by physical/emotional stress and in remaining 26% no obvious stressor was identified. Approximately 52% of patients presented as STEMI and the rest as NSTEMI. Presenting symptoms included chest pain in nearly 73% of cases and dyspnea in 18%, while in nearly 8% of patients TTC was diagnosed incidentally without either chest pain or dyspnea. Moderate LV systolic dysfunction was present in most of the patients with mean LVEF of 40% (95% CI 39% to 41%).

Acute complications were common. Death during index admission occurred in 3.5% of TTC cases. Acute heart failure was noted in 28% of cases, acute pulmonary edema in 21% and shock in 8% of the patients. Intubation, insertion of intra-aortic balloon pump and inotropic support during the shock/heart failure was common and the frequency of use varied from 7% to 18% in the studies mentioning this complication. Average length of hospital stay was 6.4 days (95% CI, 4.8 to 8 days). Nearly 80% of patients were discharged on anti-platelet medications (aspirin or clopidogrel). Angiotensin converting enzyme inhibitor/Angiotensin receptor blockers (ACEi/ARB) and Beta adrenergic antagonists (BB) were prescribed to nearly 67% and diuretic to 27% of TTC patients at discharge.

All the studies followed up TTC patients post-discharge for a minimum of 3 months (inclusion criteria) and mean follow up among the whole cohort was 24.5 months (95% CI, 19 months to 33 months). 74 cases of recurrence of TTC were detected during follow-up in the dataset of 1639 patients. The adjusted annual incidence of recurrence per 100 cases of
TTC was calculated for all the individual studies (Figure 26). There was significant heterogeneity ($I^2 = 77\%$) among the studies and annual incidence of recurrence was roughly 1.5%. Extensive data of cases of recurrence were reported in only nine studies. These nine studies comprised a pool of 504 patients and 23 associated events of recurrence of TTC at time intervals varying from 3 weeks to 72 months post index admission. The mean age of patients with recurrence was 65.5 years (95% CI, 62.1 to 68.9 years). Nearly all cases of recurrences occurred in women. This information was used to evaluate the incidence of recurrence over 6 month intervals from the time of diagnosis to a maximum follow-up of 6 years (Figure 27). Cumulative incidence of recurrence increased from 1.2% at first 6 months to nearly 5% at 6 years. The incidence of recurrent chest pain in the absence of imaging and

Figure 26: Adjusted rate of recurrence per 100 patients per year among the studies included in the systematic review (Shown in percentage).
biochemical evidence of TTC was much higher and occurred in 14% of the patients requiring contact with cardiologist and/or hospital admission.

Figure 27: Accumulative incidence of recurrence of Takotsubo cardiomyopathy at 6-month intervals.

We then evaluated the potential impact of BB and ACEi/ARB the incidence of recurrence. The proportion of patients discharged on BB and ACE/ARB on diagnosis of TTC and incidence of recurrence in 19 studies is described in Table 19. There was no significant association between the rate of recurrence and use of BB medication (P=0.28) (Figure 28). However, there was a negative correlation between use of ACE/ARB use and rate of recurrence (P = 0.016, r = -0.45) (Figure 29). This correlation between reduced rates of
recurrence and ACE/ARB treatment at discharge was also confirmed by Chi Squared test; there was negative correlation of ACE/ARB with recurrence rate (P=0.02) and no correlation of recurrence rate with BB use after discharge.

![Graph showing correlation of percentage of patients discharged on β-adrenergic blockers with incidence of recurrence. Each dot represents a separate study. (R = -0.207, P =0.28)](image)

**Figure 28:** The graph showing correlation of percentage of patients discharged on β-adrenergic blockers with incidence of recurrence. Each dot represents a separate study. (R = -0.207, P =0.28)

The average LVEF during the first episode of TTC was much lower in the patients who had recurrence 33% (95% CI, 29% to 37%) than the whole pool 40% (95% CI, 39% to 41%). Despite a higher recurrence rate of TTC in cases where initial TTC was precipitated by emotional stress than physical illness, there was no statistically significant difference between the two groups. It was also noted that during recurrence the stressor could be different from the initial episode.

Nearly 8% (128/1593) of patients who survived index admission died during the follow up. Of the 128 deaths during follow-up, 27 were because of cardiac causes, 2 were related to TTC (one patients died during recurrence of TTC), 82 were because of non-cardiac issues and cause for 17 deaths was not specified. Only 4 studies reported on symptoms of dyspnea during the follow-up in these 11 percent of patients complained of dyspnea during follow up
LVEF did not normalize in a small proportion of patients (2.5%) who continued to have symptoms of heart failure. The information on symptoms of fatigue was only reported by one study.

Figure 29: The graph showing correlation of percentage of patients discharged on angiotensin converting enzyme inhibitor and angiotensin receptor blockers (ACE/ARB) with incidence of recurrence. Each dot represents a separate study. (R = -0.448, P = 0.016)

Overall, study quality was good with 16 out of the 26 criterion's having at least 75% of all studies reporting a low risk of bias for bias. Main criterion which were not well reported across 75% of studies or more were including justification of the number of included participants, reasons for non-inclusion of subjects, reporting of confounders, accounting for confounders in the analyses, accounting for missing data in the analyses, impact of bias assessed qualitatively and impact of bias assessed quantitatively (Figure 30, Figure 31).

7.4 Discussion

Although the entity of TTC was first recognized less than 30 years ago, it has attracted considerable interest from clinicians and basic scientists in recent years. There is general
agreement that TTC is usually precipitated by exposure to a “pulse” of released or administered catecholamines (107, 238) and that individual susceptibility varies, being greater in aging females (239).
Figure 30: Detailed risk of bias assessment showing review authors’ judgement about each risk of bias item for each included study (red = high risk of bias, green = low risk and yellow = not applicable or unclear risk).
Figure 31: Risk of bias graph containing review author’s judgment about each risk of bias item presented as a percentage across all included studies (red = high risk of bias, green = low risk and yellow = not applicable or unclear risk).

Inadequate reporting of methodological rigor were persistent for several categories which includes inadequate reporting of participant selection into the study, number of participants not justified, reasons for exclusion not stated, confounders not mentioned or not accounted for in the analyses, missing data not accounted for in the analyses, impact of biases not assessed or estimated qualitatively.
Moreover, apart from the acute complications of TTC, including shock, torsade de pointes and thromboembolism, there is also relatively slow recovery process from the underlying ventricular inflammatory process (16).

Recurrence of TTC as been noted to be a problem from the earliest series (240) and might be in theory reflect a propensity towards catecholamine release, increased downstream responsiveness of biochemical pathways initiated by exposure to catecholamines or simply propensity towards recurrent emotional stress. One important consideration with recurrent TTC is the possible association with phaeochromocytoma, (241) but in the current systematic review, no such cases were specifically mentioned.

The diagnosis of TTC and therefore of recurrent TTC has undergone recent evolution from one essentially of exclusion of myocardial infarction. Currently, not only results of echocardiography/coronary angiography, but also extent of BNP/NT-proBNP release and detection of widespread odema on cardiac MRI will assist in diagnostic protocol. In the series evaluated in the current review, the median annualized recurrent rate was around 1.5%. However, there was substantial heterogeneity between series, and it is certain that not all investigators utilized the full currently available spectrum of investigations to screen for recurrences. Hence it is certain that the figure of 1.5% per annum represents an underestimate. However, this does not exclude utility of the current findings, for two reasons.

First, it appeared that recurrence occurred significantly more commonly after attacks of TTC with associated severe LV dysfunction. There are two possible interpretations here: either severe attacks are indicative of increased patient susceptibility, or this represents the results of sensitization of patients to what was perceived as a severe index illness episode. The issue should be resolved by future series with more extensive detection methods to include all recurrences.
Secondly, and more importantly, discharge strategies that included BB therapy appeared not to influence recurrence rates, while those utilizing ACEi or ARB were associated with significant decrease in recurrence rates as shown in Figure 29. As TTC is an inflammatory process, involving in a rat model increased myocardial expression of thioredoxin interacting protein (TXNIP) and evidence of nitrosative stress, this finding is consonant with our recent basic investigations. Indeed ACEi suppress TXNIP expression (176) and may also reduce nitrosative stress.

On the other hand, pathogenesis of TTC may involve β2- rather than β1- adrenoceptor stimulation (165) and therefore the finding that (β1- selective) antagonists are ineffective is not totally surprising.

Thus the current data, despite their potential inaccuracies, shed important light on the aspect of TTC. Even if the actual recurrence rate is of the order of 3-4% per annum, it would require a multicenter randomized study to evaluate pharmacotherapy to limit the problem. In the meantime, these data provide the first clue towards treatment potential.

The main limitations of the current study arise from lack of clinical details on the episodes of recurrence in the publications. The missing information in the papers and ruling out non-English literature from the analysis were the other main limitations.
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Table 18: Summary of basic characteristics of 32 studies included in this systematic review.

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Table 19: Table showing proportion of patients discharged on β-adrenergic blockers and angiotensin converting enzyme inhibitor and angiotensin receptor blockers (ACEi/ARB) in the different studies and the incidence of recurrence.
7 Summary and future perspectives
### 8.1 Summary: major findings

This thesis has been structured around 2 major foci: (i) pathogenesis and (ii) natural history of TTC. The first few chapters of the thesis focus on the pathogenesis of the disease and in the last chapters we have concentrated on the natural history of the disease: the pathogenetic studies are oriented around data from a rat model, while the natural history ata are entirely clinical in focus.

To evaluate the pathogenesis of TTC, we developed a rat model of TTC and demonstrated presence of nitrosative stress and of TXNIP in the rat myocardium. We then demonstrated that use of selective PARP-1 inhibitor administration attenuated the isoprenaline induced LV systolic dysfunction in the rat heart. In parallel to the animal work, we also confirmed presence of nitrosative stress in myocardial biopsy samples of women dying as a result of TTC. Preliminary experiments suggested that PAR activity was significantly enhanced in the human myocardial samples of TTC patients compared to controls.

As regards our second aim, the natural history of the disease, as TTC is a biventricular problem in nearly 1/3rd of cases the significance of RV involvement was evaluated. Unlike RV myocardial infarction; RV involvement in TTC does not independently predict hypotension. On the other hand, acute hemodynamic impairment in TTC is multifactorial and could be contributed to by inappropriate vasodilation, for example because of the release of NO and NT proBNP.

We also sought to identify the correlates of (i) acute mortality and (ii) recurrence through meta-analysis. It was found that acute mortality was higher in men and in patients with “secondary TTC”. The review on recurrence of TTC revealed that use of ACE inhibitors tends to reduce recurrence rate, while commonly prescribed beta-blockers did not have any effect on recurrence.
8.2 Mechanistic issues

The critical background information regarding pathogenesis on which the current experiments were predicated is as follows:

1. Catecholamine release/administration triggers episodes of TTC in “susceptible” individuals.
2. In rodent models, this appears to occur primarily via β-2 adrenoceptor activation (157).
3. Myocardial biopsy studies and CMR evaluations reveal extensive inflammatory activation, the basis of which is uncertain (16).
4. Nef at al have provided biopsy evidence for activation of the RISK pathway; this potentially limits myocardial cell loss (259, 260). One of the known activators of this pathway is NO (261).
5. Patients with TTC exhibit low ADMA levels in plasma and are hyper responsive to NO (164), raising the possibility of potential peroxynitrite generation, but
6. Plasma 3- nitrotyrosine levels are not elevated in patients with TTC approximately 24 hours post onset of symptoms (174).

We therefore sought evidence of

a) Nitrosative stress
b) Increased expression of the inflammatory activator TXNIP

in animal models and in human myocardium

8.2.1 Key findings

Development of a rat model of TTC confirmed the transient nature of LV systolic dysfunction with the use of single dose isoprenaline. In this model, impairment of apical strain at 24 hours was associated with intra-myocardial accumulation of both 3NT, a marker of nitrosative stress
and therefore a “fingerprint” of ONOO⁻ release, and TXNIP, a pre-oxidant and principal activator of inflammatory reactions, relative to levels of expression in control rats. We also found that there was mild increase in the apoptosis following isoprenaline as compared to controls, which matches with the clinical spectrum of TTC.

Subsequently (once we had established the parameters of isoprenaline effect) we demonstrated that PARP-1 inhibitor (3AB) limited isoprenaline-induced impairment of contractility. 3-AB did not influence the biochemical markers of inflammation in TTC, but we did not assess energetics of PAR formation.

We found similar evidence of nitrosative stress in human myocardial samples of patients who died during the acute episode of TTC. There was increased 3-NT myocardial content in TTC patients as compared to age-matched controls. Furthermore, pilot data suggested PAR formation in human myocardium of TTC patients. However, in unselected TTC patients we could not demonstrate any difference in plasma 3 NT levels of TTC from those of controls.

### 8.2.2 Residual issues

1. These findings demonstrate the presence of nitrosative stress and suggest a mechanistic role of PARP -1 activation but do not demonstrate whether this is the major cause of inflammatory change in TTC.

2. It is possible that other means of reversing ONOO⁻ signaling (decomposition catalysts) might be more effective than PARP-1 inhibitors in this context (Figure 4).

3. It is possible that agents in current widespread clinical use, such as (N-acetyl cysteine) and ACE inhibitors might also prove useful in this context.

4. The duration of activation of PARP-1 is uncertain-hence the optimal duration of treatment remains to be established.

5. The pathogenetic role of TXNIP in the context of TTC is not yet evaluated. Perhaps the most specific way to do this is to use TXNIP knock out mice in TTC experiments.
If TXNIP plays a causal role, ACE inhibitors and verapamil may be useful in suppressing the expression (176).

6. It would be desirable to reconcile the data on plasma concentrations of 3NT in unselected TTC patients with those on myocardial content in patients with severe (fatal) TTC. Perhaps elevated levels of 3NT will be found in coronary sinus blood during acute stages of disorder.

**8.3 Clinical issues**

The critical background information regarding natural history of TTC on which the current experiments were predicated is as follows:

1. Early hemodynamic derangement-TTC is not as benign as once thought. Hypotension and shock are frequently early complications and a major cause of morbidity and mortality during the acute period of the disease (142). Although life-threatening arrhythmias and thromboembolism also represent occasional causes of early death in TTC (262), majority of the hospital mortality is due to shock. However, the mechanism of hypotension is unclear and various theories have been proposed as the cause of early hemodynamic instability without any clear answer. Furthermore, there is currently no consensus as to the optimal treatment regimen for this early phase of illness, either for the purpose of improving hemodynamics in order to prevent shock, or in order to limit myocardial injury. Since, TTC has biventricular involvement in 1/3\(^{rd}\) of cases, RV systolic dysfunction can contribute to the early hemodynamic variability.

2. Acute mortality - The clinical course of TTC after the first 24 hours is usually one of rapid symptomatic improvement. However, acute death can occur because of hemodynamic instability and ventricular arrhythmias (263). The incidence and factors
contributing to acute mortality are unknown. Furthermore, factors that can impact acute mortality, such as: underlying non-cardiac conditions, patient’s age, and the use of catecholamine to treat shock has never been evaluated.

3. The issue of recurrence: It has recently emerged that complete recovery from TTC is usually delayed for at least 3 months (16), with evidence of persistent LV systolic dysfunction associated with ongoing symptoms of lassitude, exertional dyspnea and sometimes chest pain; approximately 50% of patients remain symptomatic 2 years post acute attacks (11). Furthermore, single or multiple episodes of recurrence can occur in approximately 3% of patients per annum (152). However, the determinants of recurrence remain unclear in the absence of repeated catecholamine administration or underlying phaeochromocytoma. Furthermore, the impact of discharge therapy on the chronic complications and recurrence has not been assessed so far.

8.3.1 Key findings

From our clinical experiments in this thesis the major findings were as follows:

1. Hypotension and shock are frequently seen in the acute stage of TTC and while RV involvement contributes to the early hemodynamics impairment, it is not an independent predictor of hypotension and shock.

2. The underlying mechanism responsible for the hemodynamic impairment is probably a combination of impaired LV contractility, inappropriate vasodilation and failure to develop tachycardia by TTC patients.

3. Acute mortality of TTC is nearly 4.5% and is more common among men and in cases of “secondary” TTC.

4. Even though, recurrence of TTC is only about 2% per year, recurrent symptoms of chest pain and dyspnea are much more frequent in the absence of imagining evidence of TTC.
5. ACE inhibitors and ARB use may reduce the recurrence of TTC, but beta-blockers have not been found to have any effect on the recurrence rate.

8.3.2 Residual issues

1. Our findings suggest that early hypotension could partially reflect inappropriate vasodilation. Since, both NO and BNP are potent vasodilators, and both appear to be released substantially in TTC, their role in the mechanism of hypotension needs further evaluation.

2. Our lack of understanding of variability in the early hemodynamic status of TTC patients is paralleled by uncertainties of its treatment. Theoretically, use of catecholamines in TTC to treat hypotension can increase myocardial damage. Furthermore, use of IABP in this situation has also not been found to be very successful (146). The role of non-catecholamine myocardial inotropes such as Ca$^{2+}$ could be the next step forward.

3. “Secondary” TTC has been found to be associated with higher mortality. It is possible that preventative strategies such as avoidance of catecholamines and use of cardio protective drugs in patients who are prone to develop “secondary” TTC may reduce mortality.

4. While there is some suggestion of benefit from ACE inhibitor use to prevent chronic complications, this should be evaluated by a prospective randomized trial in future.
Addenda

8.4 Pathogenesis: Microvascular dysfunction in TTC

Given the paucity of evidence to support a pivotal role of either large vessel coronary vasospasm or of acute plaque rupture in the pathogenesis of TTC, a number of investigators have suggested that “microvascular dysfunction (MVD)” might be important. The implication is that catecholamine stress might set up dysfunction of the coronary microendothelium, which might interact with the myocardium to prolong local oxidative stress and inflammation. The notion that acute MVD may play a role in the pathogenesis of TTC rose from the findings of prolonged TIMI frame count in at least one coronary artery of patients diagnosed with TTC. The MVD theory was further supported by the findings of Kume et al, who measured coronary flow reserve volume with doppler wire in 8 patients diagnosed with TTC during the acute period and 3 weeks later. They found the coronary flow reserve volume to be decreased in the acute phase and it returned back to normal at 3 weeks. Similarly, Galiuto et al using myocardial contrast echocardiography described the presence of a severe reduction in myocardial perfusion within the dysfunctional area.

Despite all these studies supporting MVD, it remains to be known if MVD is more likely an effect rather than the cause of TTC. As discussed before, it would not be incorrect to regard TTC as a type of myocarditis where inflammation is induced by sudden rise in catecholamine levels. The involvement of the intramural coronary vasculature in inflammatory heart disease leads to reduced coronary vasodilator capacity and hence, reduced coronary reserve. Since, it is extremely difficult in practice to accurately evaluate microvascular reactivity per se in the scenario of an acute attack of TTC, the observed anomalies might be engendered in whole or in part by the presence of impaired diastolic relaxation of surrounding myocardium, with resultant extramural compressive forces. Furthermore, there is nothing in the biochemical
signaling cascade that we have proposed for TTC, which is cardiospecific (i.e., beta-2 receptors adrenoceptors are present in coronary arteries etc). The best way to delineate things fully would be to study isolated coronary microvessels from an animal model of TTC, but this has not yet been reported.
References


Rezidivierende Takotsubo-Kardiomyopathie: Variables Muster der Ventrikelbeteiligung.


