Takotsubo Syndrome: Precipitants, Clinical Course and Emerging Treatments

A thesis submitted by

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Abstract

Introduction

Clinically, patients with Takotsubo Syndrome(TTS) presents similarly to that of acute coronary syndromes(ACS). Recent evidence has demonstrated that not only is TTS a common condition, like ACS, it is also associated with significant morbidity and mortality in the short- and long-term. It is now also recognised that there are various precipitants and risk factors of TTS, and like ACS, this list continues to grow. However, the exact pathophysiology, natural history, as well as treatment options for TTS remain incompletely understood. The studies described in this thesis were carried out to delineate a number of aspects of the precipitation, complications and potential treatment of TTS.

Methods

We investigated (a)novel risk/precipitating factors for ACS and TTS, (b)prognostic impact of variability in severity of attacks of acute TTS and the rate of myocardial recovery, and (c)pharmacological strategies for limiting severity of acute attacks, as well as accelerating the recovery in TTS. We sought correlations of high ambient temperatures, pollution, and proximity to bushfires(as individual and cumulative stressors) with the incidence of ACS and TTS. We also investigated the impacts of exogenous catecholamines and catecholamine-potentiating drugs(CPD) on TTS. In regards to the impacts of variable severity of acute attacks of TTS, we sought correlations between severity of TTS attacks and the incidence of hypotension acutely, as well as the recovery in quality of life at 3 months' follow up. Finally, we designed a double-blinded randomised controlled trial to help determine pharmacological strategies for the treatment of TTS.

Results

Novel risk/precipitating factors: Incidence of ACS increased with not only increased ambient temperatures in warmer months of the year(r_s=0.26, p=0.005), but also in the presence of high ambient temperatures, pollution and bushfires in combination(r_s=0.25,p=0.005). We found no significant analogous correlations with TTS presentations however, with the caveat of small numbers. We also found that precipitation of TTS in association with drug-induced incremental catecholamine exposure was common(18% of total case-load), and associated with a non-significant trend(log rank X^2=2.3, p=0.13) towards increased long-term mortality.

Impacts of variable severity of acute attacks of TTS: Hypotension/shock occurred commonly(35%) in TTS patients acutely, and correlated with markers of attack severity including lower LVEF(p=0.009), higher plasma troponin-T(p=0.008) and NT-proBNP concentrations(p=0.046). Hypotension/shock was also a strong predictor of in-hospital mortality(p<0.001). However, the magnitude of acute TTS attacks did not significantly correlate with quality of life after 3 months.

Design of randomised controlled trial: We utilised N-acetylcysteine(NAC) acutely, a potent anti-oxidant and a source of hydrogen sulphide to limit nitrosative stress, and then an ACE inhibitor(ramipril), due to its anti-inflammatory properties. Recruitment for NACRAM is now advanced, but treatment allocation remains blinded.

Conclusions

Our understanding of the differential pathophysiology of ACS and TTS continue to evolve and grow. We have confirmed that TTS is not only associated with both substantial mortality in the short-(especially in the presence of hypotension) and long-term, and also that TTS leads to significant morbidity in the long-term. Whilst the NACRAM clinical trial represents the first prospective therapeutic investigation in TTS, additional future studies are also needed to develop interventions to prevent the occurrence and recurrence of TTS.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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 the future, be used in a submission in my name, 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.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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List of Abbreviations

^{18}F-FDG	^{18}F-fluorodeoxyglucose
3-NT	3-nitrotyrosine
5-HT	Serotonin
ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
ADMA	Asymmetric dimethyl arginine
Akt	Protein kinase B
ANOVA	Analysis of variance
ARB	Angiotensin receptor blocker
ATP	Adenosine triphosphate
BNP	B-type natriuretic peptide
cAMP	Cyclic adenosine monophosphate
CAS	Coronary artery spasm
CMR	Cardiac magnetic resonance imaging
CPD	Catecholamine potentiating drugs
DNA	Deoxyribonuclein acid
DM	Diabetes mellitus
ECG	Electrocardiograph
EDRF	Endothelium-derived relaxing factor
eGFR	Estimated glomerular filtration rate
ERK	Extracellular signal-regulated kinase
fMRI	Functional magnetic resonance imaging
Gi	G-inhibitory
GLS	Global longitudinal strain
GPCR	G-protein-coupled receptor
Gs	G-stimulatory
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulphide
HOCl	Hypochlorous acid

Hs-CRP	High sensitivity C-reactive protein
ICD-10	International Classification of Diseases (10^{th} revision)
IQR	Inter-quartile range
L-NAME	L-NG-Nitro-arginine methyl ester
LGE	Late gadolinium enhancement
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVOT	Left ventricular outflow tract
MACE	Major adverse cardiac events
MCS-SF36	Mental component summary of SF-36 questionnaire
MI	Myocardial infarction
MINOCA	Myocardial infarction with no obstructive coronary arteries
MMP	Matrix metalloproteinase
MRS	Magnetic resonance spectroscopy
NAC	N-acetylcysteine
NAD^+	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
NOS	Nitric oxide synthase
NSTEMI	Non-ST-elevation myocardial infarction
NT-proBNP	N-terminal pro B-type natriuretic peptide
O_2^-	Superoxide
$ONOO^-$	Peroxynitrite
PARP	poly(ADP-ribose) polymerase
PCS-SF36	Physical component summary of SF-36 questionnaire
PCWP	Pulmonary capillary wedge pressure
PEA	Pulseless electrical activity
PET/CT	Positron emission tomography/computed tomography
PKA	Protein kinase A
PI3K	Phosphoinositide 3-kinase
$PM_{2.5}$	Particulate matter less than 2.5µm

QoL	Quality of life
RWMA	Regional wall motion abnormalities
SAM	Systolic anterior motion
SCAD	Spontaneous coronary artery dissection
SD-1	Syndecan-1
SF-36	Short Form 36 questionnaire
sGC	Soluble guanylate cyclase
SI	Signal intensity
STEMI	ST-elevation myocardial infarction
TTS	Takotsubo syndrome
TXA_2	Thromboxane A2
TXNIP	Thioredoxin-interacting protein
UAP	Unstable angina pectoris

List of publications arising

Published studies reflecting results of the original studies in this thesis:

1. **Ong GJ**, Nguyen TH, Stansborough J, Surikow SY, Horowitz JD. Incremental "Therapeutic" Myocardial Exposure to Catecholamines: Incidence and Impact in Takotsubo Syndrome. Cardiovascular drugs and therapy. 2020;34(1):95-100.

2. **Ong GJ**, Girolamo O, Stansborough J, Nguyen TH, Horowitz JD. Incidence and clinical/laboratory correlates of early hypotension in takotsubo syndrome. ESC Heart Failure. 2021;8(3).

3. **Ong GJ**, Nguyen TH, Stansborough J, Surikow S, Mahadavan G, Worthley M, Horowitz JD. The N-AcetylCysteine and RAMipril in Takotsubo Syndrome Trial (NACRAM): Rationale and design of a randomised controlled trial of sequential N-Acetylcysteine and ramipril for the management of Takotsubo Syndrome. Contemporary clinical trials. 2020;90:105894.

Additionally, the following relevant review of the subject matter has been published:

Ong GJ, Nguyen TH, Kucia A, Liu S-F, Surikow S, Girolamo O, Chong C-R, Chirkov Y, Schenck-Gustafsson K, Frenneaux M, Horowitz JD. Takotsubo Syndrome: Finally Emerging From the Shadows? Heart, Lung and Circulation. 2021;30(1):36-44.

Chapter 1: Literature Review

1.1 The concepts of "acute coronary syndrome"

1.1.1 History and evolution

Symptomatic acute myocardial ischaemia, or pain due to acute inadequacy of myocardial blood flow, was probably first described by the ancient Egyptians(1), but of course there was no particular clinical or pathophysiological insight into this condition for many years.

In the modern context, the term "acute coronary syndrome (ACS)" refers to a spectrum of clinical conditions with rapid and unanticipated emergence of symptoms consistent with myocardial ischaemia. Eventual diagnoses, depending on presence/absence of resultant myocardial injury, range from unstable angina pectoris (UAP) to acute myocardial infarction (MI).

In the early to mid-1900s, electrocardiography(ECG)(2) and "cardiac biomarkers"(3) were introduced and revolutionised the diagnosis of MI by improving the detection of myocardial injury or necrosis. Since then, different definitions of MI have been used, leading to controversies and confusion. From the early 21st century, there have been numerous attempts at improving the classification and definition of MI. The latest iteration - The 4th universal definition of MI(4), attempted to define and delineate MI into 5 main types, shown in Table 1.1. However, this definition has also been challenged(5), largely in relation to distinguishing Type 2 MI from acute myocardial injury occurring independent of impairment of coronary blood flow and/or of increases in myocardial oxygen "demand". The essential problem with the idea that "MI" should reflect myocardial necrosis specifically due to an ischaemic process is that in practice, it is often impossible either to ascertain or to totally exclude the existence of such a process.

Table 2.1: Types of MI as defined by the 4th Universal Definition of Myocardial Infarction

Type	**Definition**
1	Spontaneous MI related to ischaemia due to a primary coronary event such as plaque erosion and/or rupture, fissuring or dissection.
2	MI secondary to ischaemia due to either increased oxygen demand or decreased supply.
3	Sudden unexpected cardiac death often with symptoms suggestive of myocardial ischaemia.
4a	MI associated with percutaneous coronary intervention (PCI)
4b	MI associated with coronary stent thrombosis
4c	MI associated with coronary in-stent-restenosis (ISR)
5	MI associated with cardiac surgery

As regards pathophysiology, little insight was available on UAP/ acute MI until the early 20th century when Obrastov and Strazhesko as well as Herrick described MI-associated presence of thrombus within a coronary artery, resulting in, in some cases, complete occlusion of the affected coronary arteries(6, 7).

1.1.2 Pathophysiological correlations

Why should there be a propensity towards coronary artery thrombosis in patients with coronary atheroma? The first approaches towards the solution of this problem came with the seminal experiments of Furchgott and co-workers(8) in delineating the presence of at least one humoral

vasodilator factor within the vascular endothelium, and the subsequent demonstration by Moncada that regions of arteries with atheroma lacked evidence of "endothelium-derived relaxing factor (EDRF)" activity(9). Subsequently, it was demonstrated that nitric oxide (NO) and/or a closely related S-nitrosothiol accounted for EDRF activity, and that NO exerted not only vasodilator, but also anti-aggregatory (and anti-inflammatory) activity(10). Thus it could theoretically be postulated that atheroma-associated lack of NO effect might render the affected artery prone to not only thrombosis but also local vasoconstriction. Indeed, over the past 50 years, theory about the pathogenesis of various types of ACS has reflected variable emphasis on the anti-thrombotic, anti-inflammatory and vasodilator effects of NO, usually, and somewhat paradoxically, in isolation rather than combination.

1.1.3 The "vulnerable" plaque

In the early 1980s, Davies et al. reported that almost three quarter of patients with sudden myocardial ischaemic deaths had evidence of intraluminal thrombosis, and of the patients without coronary thrombosis, a majority had evidence of "plaque fissuring" (implying the formation of an opening from the vascular lumen to the tunica intima)(11). Furthermore, thrombus where present, was found at sites of varying stenoses. More importantly, all thrombosis occurred at sites of either "fissuring" or rupture of lipid-rich atheromatous plaques. Interestingly, in addition to plaque rupture, endothelial denudation or erosion was also found to be responsible for the formation of coronary thrombosis(12).

The role of the endothelium in the pathophysiology of ACS therefore had become increasingly important, not just in regards to NO synthesis and release, but it also became apparent the vasodilatory effect of NO was dependent on an intact endothelial glycocalyx(13). Furthermore, the endothelial glycocalyx was also found to play a role in attenuating leucocyte and platelet adhesion to the vascular endothelium. Not only does damage to the endothelial glycocalyx increase platelet-

endothelial adhesion(14), inhibition of glycocalyx shedding with a matrix metalloproteinase inhibitor (doxycycline) also reduced leukocyte-endothelial adhesion(15). Indeed, in the context of MI and ACS, there is certainly no paucity of evidence of an important role for underlying shedding of the endothelial glycocalyx(16, 17).

1.1.4 The "reversible occlusion" in acute ST-elevation myocardial infarction (STEMI)

Not all MI can be attributed to coronary thromboses. Utilising invasive selective coronary angiography in the 1980s, De Wood et al. found that total coronary occlusion (often attributed to coronary thrombosis) was frequently present during the early hours of acute MI. However, the frequency of this finding decreased from 87% to 65% when coronary angiography was performed over the following 12-24 hours(18). This not only implied coronary artery recanalization after thrombus formation in MI, it also suggested that early coronary artery vasospasm may play a major role as a contributor towards transient occlusion of infarct-related coronary arteries. Furthermore, platelets activated following thrombus formation release thromboxane A2 (TXA_2) and serotonin (5-HT), which also promote coronary arterial smooth muscle constriction(19).

1.1.5 Coronary artery spasm (CAS)

Even though the notion of angina secondary to coronary arterial vasospasm had been put forward over a hundred years ago by Osler(20) and Gallavardin(21), it wasn't until the 1950s when Prinzmetal and his colleagues describe in detail, a case series of patients with a "variant" form of angina pectoris attributed to transient occlusion of coronary arteries due to an increase in vessel wall tone(22). In this variant type of angina, pain described was not brought on by effort, and was often associated with marked ST segment changes on ECG during the acute pain "attacks". Post

mortem examination of the patients revealed evidence of old myocardial infarctions, but with non-obstructive atherosclerotic disease seen within the corresponding coronary arteries.

Subsequent to the advent of invasive coronary angiography, further work investigating angina related to coronary vasospasm was undertaken by Maseri et al. in the 1970s. They demonstrated instances of severe coronary artery vasospasm inducing acute myocardial ischaemia, successfully reversed following administration of sublingual nitroglycerin(23). Endo et al. also demonstrated that patients with variant angina could be successfully managed with the calcium channel antagonist nifedipine, but not with coronary artery bypass grafts(24). This finding provided further evidence that coronary artery atherosclerosis is not always the cause of myocardial ischaemia or infarction.

Again, NO appears to play a significant role in the pathophysiology of CAS. Moriyama and colleagues were able to demonstrate that endothelial NO-mediated vasodilation was decreased in patients with CAS(25). Much more recently, Imam et al. demonstrated that platelet NO signalling was impaired in patients with CAS, and that this NO resistance was further accentuated during acute symptomatic crises(26).

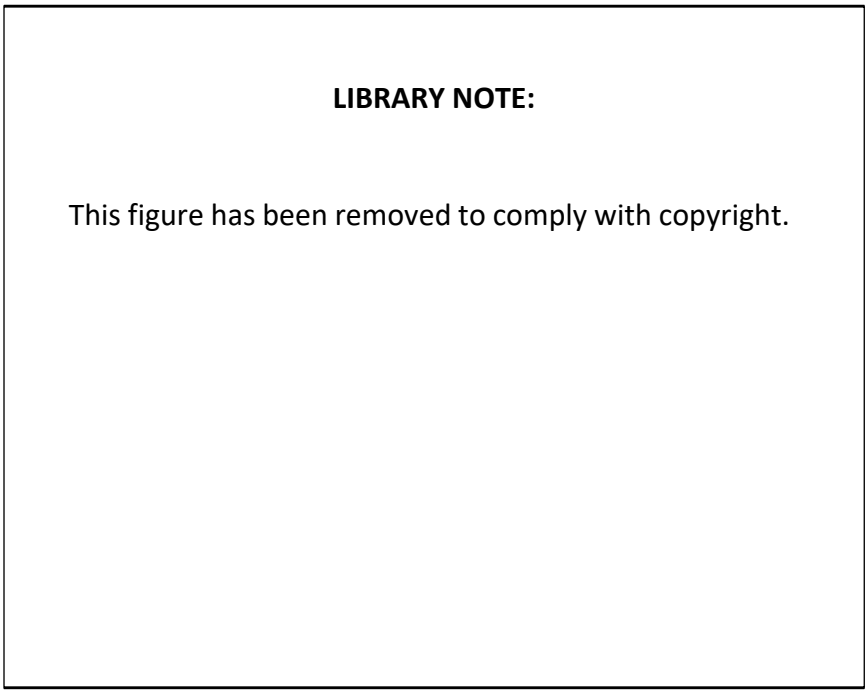
Given the apparent impairment in NO signalling, it is therefore not surprising that there are other factors contributing to endothelial dysfunction. Indeed, Imam and colleagues also demonstrated a substantial shedding of the endothelial glycocalyx during acute symptomatic “attacks” of CAS as measured by increased plasma syndecan-1 (SD-1) concentrations. Damaged glycocalyx provides an environment favouring platelet activation and adhesion, and can lead to coronary vasoconstriction via TXA_2 and 5-HT release.

1.1.6 Spontaneous coronary artery dissection (SCAD)

Distinct from atherosclerotic disease and coronary vasospasm, SCAD causes acute myocardial ischaemia and infarction, apparently independent of NO activity. SCAD was first described in the

early 1930s by Pretty at autopsy of a 42 year old female(27), and is now a well-known cause of myocardial ischaemia and infarction. It is characterised by the spontaneous formation of intra-mural haematoma within a coronary artery, either caused by an intimal tear leading to the generation of a false lumen allowing the entry of blood, or due to spontaneous haemorrhage arising from the vasa vasorum within the vessel wall. This results in obstruction of the vascular lumen, and subsequent reduction in blood flow (Figure 1.1).

Figure 1.1. Cross-sectional views of the coronary artery. A, Normal coronary artery. B, Coronary artery with intramural hematoma. C, Coronary artery with intimal tear. Spontaneous coronary artery dissection is characterized by the spontaneous formation of an intramural hematoma, which can lead to compression of the true lumen and myocardial infarction. An intimal tear may be present. (Image reproduced from Hayes et al, Circulation 2018)



The pathophysiology of SCAD is not completely understood, but SCAD tends to occur in patients (especially women) without risk factors for atherosclerosis, or evidence of atherosclerosis in the coronary arteries. Rather, it is associated with fibromuscular dysplasia(28), pregnancy(29), as well as other arteriopathy/connective tissue disorders(30). Furthermore, SCAD has also been reported to occur particularly in situations of intense emotional and physical stress(30). In the context of this thesis, SCAD represents a condition which must be distinguished clinically from not only atherosclerotic coronary artery disease, but also Takotsubo Syndrome (TTS).

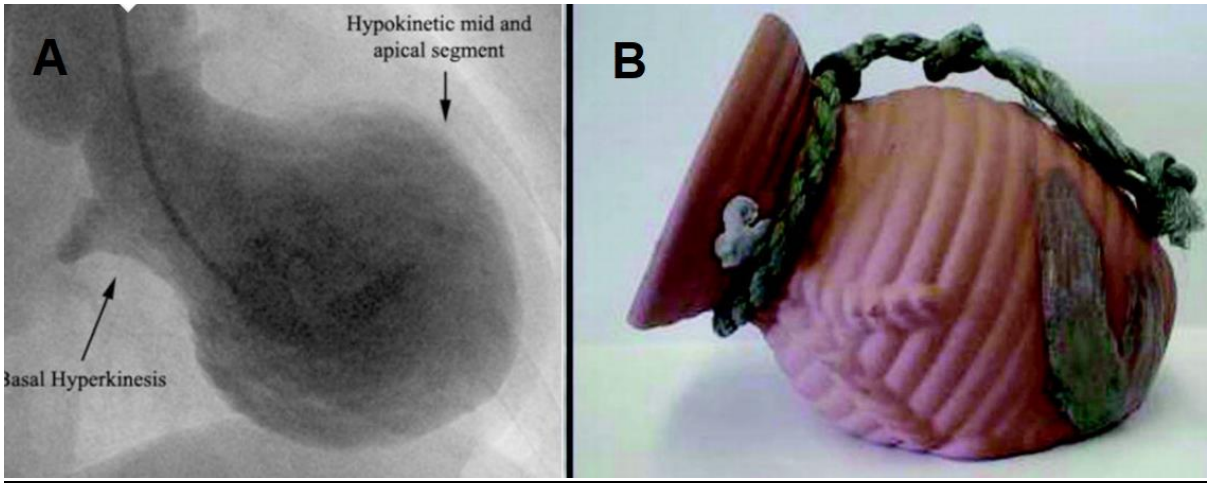
1.2 Takotsubo Syndrome (TTS)

TTS, also known as stress cardiomyopathy, “broken heart” syndrome, or apical ballooning syndrome, is an acute inflammatory cardiac condition that can often be clinically indistinguishable from ACS. Its history, epidemiology, diagnosis, clinical course and pathophysiology are all central to the research studies discussed in this thesis.

1.2.1 History

The term “tako-tsubo” cardiomyopathy was first adopted in 1990 by Japanese investigators who named the condition on the basis of the similarity in the shape of the left ventricle (LV) to a Japanese octopus pot (Figure 1.2). However, the earliest reports of TTS probably date back to the 1950s, when Rona and co-workers described a myocardial infarction-like condition induced with isoprenaline in an animal model (31). A few decades later, some researchers also noted an increased incidence of presumptive acute MI at times of natural disasters such as earthquakes(32), or within the general population at times of large sporting events(33). Although the diagnosis of acute MI was not questioned at the time, it is likely in retrospect that many of these cases reflected TTS, as was recognised more recently at the time of the Christchurch earthquakes in 2010 and 2011(34).

Figure 1.2. Left ventriculogram (systolic frame) (A) of a patient with TTS, showing marked ballooning of the left ventricle apex during systole, resembling the shape of a Japanese octopus pot, shown in (B). (Image reproduced from https://uvachemistry.com/2015/12/06/takotsubo-broken-heart-syndrome/]



1.2.2 Epidemiology

TTS is no longer thought to be a rare condition. In fact, it accounts for approximately 2% of all patients presenting to hospital with suspected ACS(35), and as high as 10% of all females with suspected ACS presentations(36). TTS also represents 10% of females ≥50 years of age who presented with suspected ST-segment elevation "MI"(37). Whilst it has emerged that TTS attacks are often precipitated by emotional or physical stress, and occur most commonly in post-menopausal women, this is by no means a constant association. About a third of patients develop TTS without an obvious trigger(38). Furthermore, children as young as 15 months of age have been diagnosed with TTS(39), and in contrast to the adult population, TTS tends to equally affect both sexes in the paediatric population(40).

Physical stressors such as acute illnesses triggering TTS attacks are also common, accounting for approximately 25% of TTS cases(41). Examples of physical insults described in association with TTS include life-threatening illnesses such as intracerebral bleeds(42), respiratory failure(43), and even more recently, but controversially, COVID-19 infections(44, 45). Under these circumstances, males are more commonly involved and mortality rates are substantially higher(46), perhaps reflecting the combined impacts of the precipitating illness plus the resultant TTS. The variable prognostic implications of TTS according to absence or presence of associated severe, potentially life-

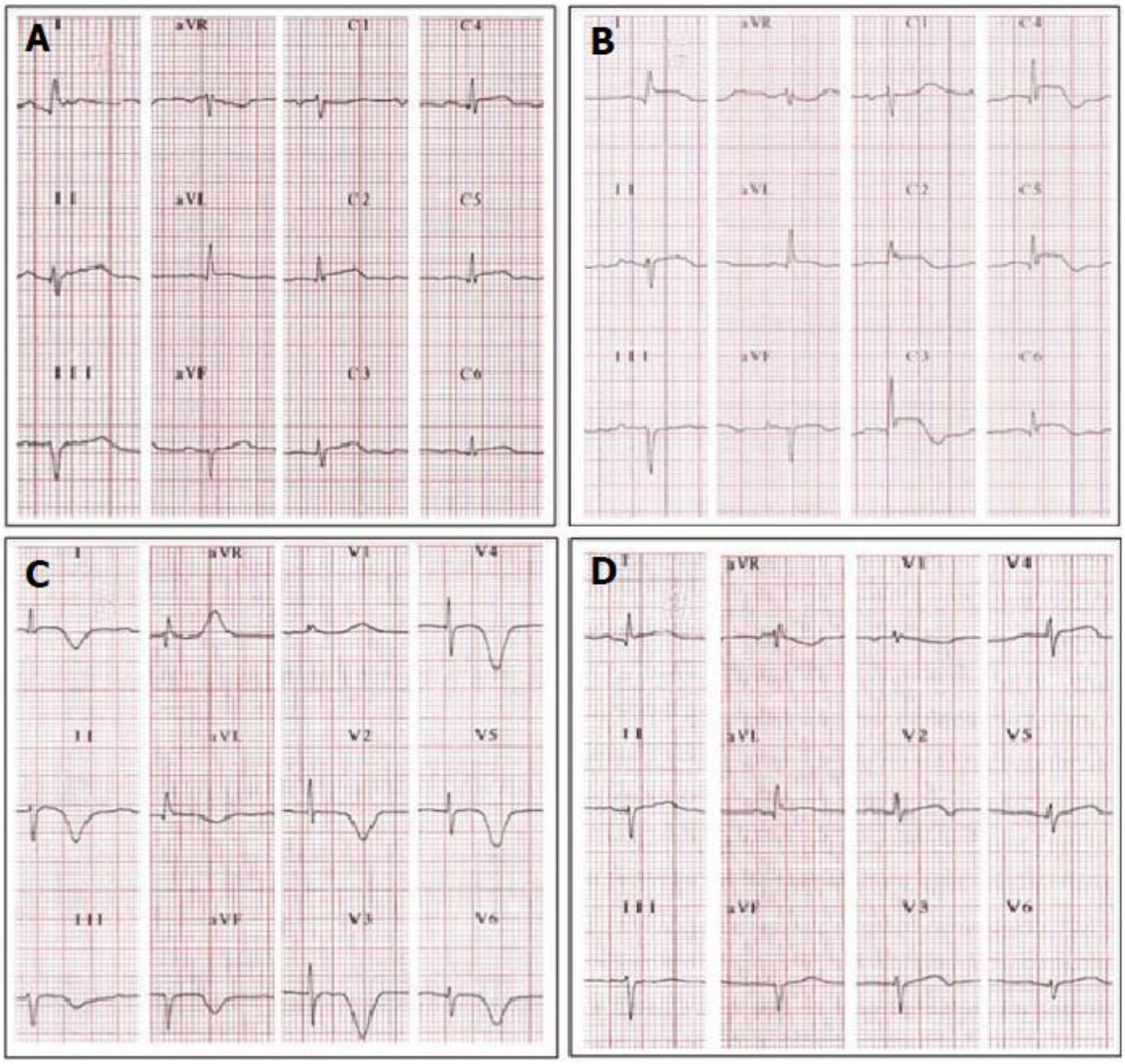
threatening, extracardiac disease has led to a subclassification into “primary” and “secondary” TTS respectively(46).

“Secondary” TTS as a result of heat-stroke has also been described(47). In fact, contrary to MI, the incidence of which increases during the winter months(48), the incidence of TTS appears to be higher not just during the summer months(49, 50), but also on warmer days(51).

1.2.3 Clinical Presentation and Diagnosis

TTS typically presents similarly to ACS, with symptoms of chest discomfort and/or dyspnoea. This is accompanied by elevation of cardiac biomarkers including plasma troponin, B-type natriuretic peptide (BNP) and N-terminal pro B-type natriuretic peptide (NT-proBNP), as well as ECG changes such as ST-segment elevation or depression which then evolves to widespread T-wave inversion with associated QT-segment prolongation over the next 24 - 48 hours (Figure 1.3). Unfortunately, despite previous attempts at doing so(52), there is currently no strong evidence that patterns of acute ECG changes in isolation, facilitate differentiation between TTS and acute MI.

Figure 1.3: Progressive changes in the ECG of a patient with TTS. A: Initial ECG after 3 hours of symptoms. There is diffuse ST-segment elevation; B: 24 hours after onset of symptoms. The ST-segment elevation seems to be more prominent. The T waves start to invert ; C: Third day. The T waves are now inverted, deep, wide and symmetrical in most leads. The corrected QT-interval is prolonged (520 milliseconds); D: ECG 3 weeks later. The T waves are almost normal and the QT-interval is no longer prolonged. (Image reproduced from https://www.wjgnet.com/1949-8462/full/v8/i7/WJC-8-413-g002.htm)



However, beyond the predominant occurrence in post-menopausal women and the history of acute stressful circumstances immediately preceding the attacks, there are a number of unusual early clinical features, which should draw attention to the possibility that the diagnosis is TTS. For example, the most common complication occurring in the acute stages of TTS is the onset of substantial hypotension with poor peripheral perfusion (in other words, shock). This is more frequently seen in TTS than in acute MI(53). This development of shock in TTS is superficially mysterious, as it implies vascular refractoriness to the inotropic and vasoconstrictor effects of released catecholamines, and despite the presence of shock, LV filling pressures are rarely severely elevated(54). In addition,

plasma BNP/NT-proBNP concentrations are usually markedly elevated(55, 56). The degree of NT-proBNP elevation is often out of proportion to plasma troponin elevation(57), and is also significantly elevated when compared to patients with acute MI(58).

Despite these differences, it is often difficult to distinguish TTS from ACS. For that reason, there have been numerous attempts at improving the precision of diagnosis accuracy of TTS. The most widely cited diagnostic criteria were proposed from the Mayo Clinic first in 2004, and subsequently modified in 2008 (Table 1.2)(59). However, these criteria have been extensively criticised, predominantly due to the likely exclusion of patients with concurrent incidental/"bystander" obstructive coronary artery disease(60), a major issue in an ageing population. More recently, a group of international experts on TTS (InterTAK) developed new international diagnostic criteria, with the hope of improving the identification and stratification of TTS (Table 1.3)(60). These updated criteria specifically mention that presence of significant coronary artery disease is not a contradiction to the diagnosis of TTS, and also recommend the use of cardiac magnetic resonance imaging (CMR) in the diagnostic work-up, predominantly as a confirmatory test. The Mayo Clinic Criteria also fail to consider the relatively rare "inverted" form of TTS, where there is predominant basal hypokinesis(61). However, to date, there is no evidence that the wide utilisation of the InterTAK diagnostic criteria for TTS has lead to any substantial increase in the frequency of its diagnosis.

Table 1.3: Proposed Mayo Clinic diagnostic criteria for TTS (2008).

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. [a]
Absence of obstructive coronary disease or angiographic evidence of acute plaque rupture. [b]

New ECG abnormalities (either ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin.
Absence of phaeochromocytoma and myocarditis.

[a] *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. In these cases, the diagnosis of TTS should be made with caution, and a clear stressful precipitating trigger must be sought.*

[b] *It is possible that a patient with obstructive coronary atherosclerosis may also develop TTS, although thought to be rare. In these cases, the diagnosis of TTS should be made with caution, and a clear stressful precipitating trigger must be sought.*

Table 1.4: International Takotsubo Diagnostic Criteria (InterTAK Diagnostic Criteria)

Patients show transient[a] LV dysfunction (hypokinesia, akinesia, or dyskinesia) presenting as apical ballooning or midventricular, basal, or focal wall motion abnormalities. RV involvement can be present. Besides these regional wall motion patterns, transitions between all types can exist. The regional wall motion abnormality usually extends beyond a single epicardial vascular distribution; however, rare cases can exist where the regional wall motion abnormality is present in the subtended myocardial territory of a single coronary artery (focal TTS).[b]
An emotional, physical, or combined trigger can precede the takotsubo syndrome event, but this is not obligatory.
Neurologic disorders (e.g. subarachnoid haemorrhage, stroke/transient ischaemic attack, or seizures) as well as pheochromocytoma may serve as triggers for takotsubo syndrome.
New ECG abnormalities are present (ST-segment elevation, ST-segment depression, T-wave inversion, and QTc prolongation); however, rare cases exist without any ECG changes.
Levels of cardiac biomarkers (troponin and creatine kinase) are moderately elevated in most cases;

significant elevation of brain natriuretic peptide is common.
Significant coronary artery disease is not a contradiction in takotsubo syndrome.
Patients have no evidence of infectious myocarditis.[b]
Postmenopausal women are predominantly affected.

[a] *Wall motion abnormalities may remain for a prolonged period of time or documentation of recovery may not be possible. For example, death before evidence of recovery is captured.*

[b] *Cardiac magnetic resonance imaging is recommended to exclude infectious myocarditis and achieve diagnostic confirmation of takotsubo syndrome*

1.2.3.1 Diagnostic imaging modalities in TTS

Invasive coronary angiography

Invasive coronary angiography was first introduced in the mid 1900s, but it wasn't until the late 1900s when it had evolved into not just a diagnostic procedure, but also a therapeutic one as well, initially because of the inception of therapeutic intracoronary infusion of thrombolytic agents for evolving acute MI(62) and then of coronary angioplasty(63). It is a procedure performed to access, and study the coronary circulation by injecting radiocontrast agent via catheters into the coronary arteries under fluoroscopy (real-time X-ray). Coronary angiography is commonly performed in patients with suspected ACS. In the context of TTS, especially in patients with ST-segment elevation on ECG, invasive coronary angiography is performed urgently to rule out evolving acute myocardial infarction as the cause of the ECG changes.

Echocardiography

Echocardiography is a diagnostic imaging modality that utilises ultrasound technology, and can provide accurate information on cardiac morphology, function, and haemodynamic status non-invasively. Since its introduction in the 1950s by Hertz and Edler, echocardiography has become one of the central diagnostic investigations in the field of cardiology. Two-dimensional (2D) echocardiography allows a real-time assessment of heart function. In TTS, echocardiography is utilised to not only assess the degree of contractile dysfunction, but also to localise and quantitate regional wall motion abnormalities (RWMA). Furthermore, echocardiography can also be used to detect potential complications of TTS including pericardial effusion(64), LV outflow tract (LVOT) obstruction(65), systolic anterior motion (SAM) of the mitral valve leading to mitral regurgitation(66), as well as the presence of LV mural thrombus(67).

Where 2D echocardiographic images are suboptimal, and visualisation of the endomyocardial border challenging, contrast echocardiography, involving injection of echo contrast (usually a suspension of lipid microspheres) via a peripheral vein, can be useful. It also improves the detection of LV mural thrombi(68). In addition, myocardial contrast echocardiography (MCE) can also be a helpful tool in differentiating TTS from acute MI(69).

More recently, measurement of subtle variation in LV function using strain imaging by speckle tracking echocardiography has increasingly become part of routine practice, as it appears to be a more sensitive, reproducible and load-independent measure of LV function when compared to left ventricular ejection fraction (LVEF)(70). Indeed, despite normalisation of LVEF in TTS patients after 3 months, there is often persistent impairment in global longitudinal strain (GLS), which also correlated with persistent elevation of plasma NT-proBNP concentrations and impairment in quality of life(71), thus demonstrating that LVEF is a relatively crude measure of LV function.

Cardiac Magnetic resonance Imaging (CMR)

CMR represents the current diagnostic "gold standard" for qualitative and quantitative assessment of RWMA and accurate quantification of RV and LV volumes and function. In addition, a major strength of CMR is the ability to assess different causes of myocardial injury from myocardial tissue characterization. In the context of TTS, CMR can be used to demonstrate extensive myocardial oedema and acute increases in LV wall thickness without associated late gadolinium enhancement (LGE), a typical characteristic of acute MI and myocarditis(72). Furthermore, complications of TTS such as pericardial effusions or LV thrombus can also be easily appreciated. The key advantages of performing early CMR imaging in patients with suspected TTS are summarized from both qualitative and quantitative points of view in Table 1.4. Most importantly, the absence of subendocardial or transmural LGE corresponding to regions of hypokinesis/akinesis is useful to exclude recent MI(72), while an extraordinary feature of TTS is the (prolonged) presence of global oedema, throughout the left ventricle: the overall (summative) intensity of this oedema has been utilized as a measure of severity of TTS attacks at the myocardial level(73). Figure 1.4 demonstrates the utility of CMR imaging to distinguish TTS from acute MI, as well as documenting the severity of inflammatory/oedematous change within the myocardium in TTS patients, via demonstration of extensive LV oedema in the absence of evidence of localized acute MI.

Table 1.5: Summary of qualitative and quantitative assessments on CMR in TTS.

Qualitative	Presence LV systolic dysfunction and associated RWMA
	Presence of myocardial oedema
	Absence of MI pattern LGE
	Presence of pericardial/pleural effusions/LV thrombus
Quantitative	Reduced LV ejection fraction
	Increased T2 weighted signalling as an assessment of degree of myocardial oedema

	Increased LV wall thickness(74)

Figure 1.4: CMR images comparing TTS to acute MI.

Images A and B are short axis T2 weighted STIR images, with the thin arrows pointing to areas of high T2 signal indicative of myocardial oedema, consistent with presence of inflammation. Image A was obtained from a TTS patient, demonstrating global myocardial oedema, and thus representing changes consistent with a global myocarditis, whilst Image B shows changes in a patient with a recent LAD territory acute MI, showing a localised, peri-infarct area of high T2 signalling. Images C and D are end-diastolic 4 chamber views post gadolinium administration. Image C belongs to a TTS patient, showing no evidence of late gadolinium enhancement(LGE), whilst Image D demonstrates extensive subendocardial LGE in the LAD territory, consistent with MI (thick arrow). (Image reproduced from Ong et al. Heart, Lung and Circulation 2020)

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1.2.4 Natural History

Acute phase

Contrary to early reports, it is now increasingly accepted that TTS is not a benign disease, either acutely, subacutely or in the long term. As regards the acute phase in isolation, development of hypotension with or without shock is common(54), and represents the main basis for in-hospital mortality, which remains around 3-4% in most large patient series(46, 75). This is intriguing, given

the association with increased release of catecholamines in TTS, as will be described in the later chapters. A number of studies have demonstrated that hypotension has little or nothing to do with impaired LV systolic function, and that both pulmonary capillary wedge and left ventricular end-diastolic pressures are rarely elevated to the extent which would normally result in development of pulmonary oedema via disturbance of the Starling Equation(54).

TTS patients also occasionally develop respiratory failure as a result of pulmonary oedema(76) which develops despite the absence of conventional haemodynamic aberrations, as evidenced by pulmonary congestion despite a relatively low pulmonary capillary wedge pressure (PCWP). This likely reflects the haemodynamic impacts of vasodilator autacoids, and of transient permeabilization of blood vessel walls with resultant fluid extravasation. Further evidence supporting this is the common finding of pericardial(77) and/or pleural effusions(78) acutely in TTS.

The other serious, and potentially fatal early complication of note in TTS is the transient occurrence of both tachy-and brady-arrhythmias over the first 48 hours(79). These rhythm disturbances which include ventricular fibrillation, ventricular tachycardia (including torsade de pointes), asystolic and pulseless electrical activity (PEA), have been previously documented in TTS patients suffering from cardiac arrest(80). In a large international registry of TTS patients, the frequency of development of early cardiac arrest in TTS was approximately 5%, and is associated with a substantially higher short- and long-term mortality when compared to those without cardiac arrest(80). However, cardiac arrest tends to occur at or soon after presentation, leading to the vexed question of causation and also to the actual incidence of pre-hospital occurrence of ventricular fibrillation(81).

Last but not least, cases of LV mural thrombosis with subsequent systemic embolization leading to cerebral infarctions have also been reported(67).

Chronic phase

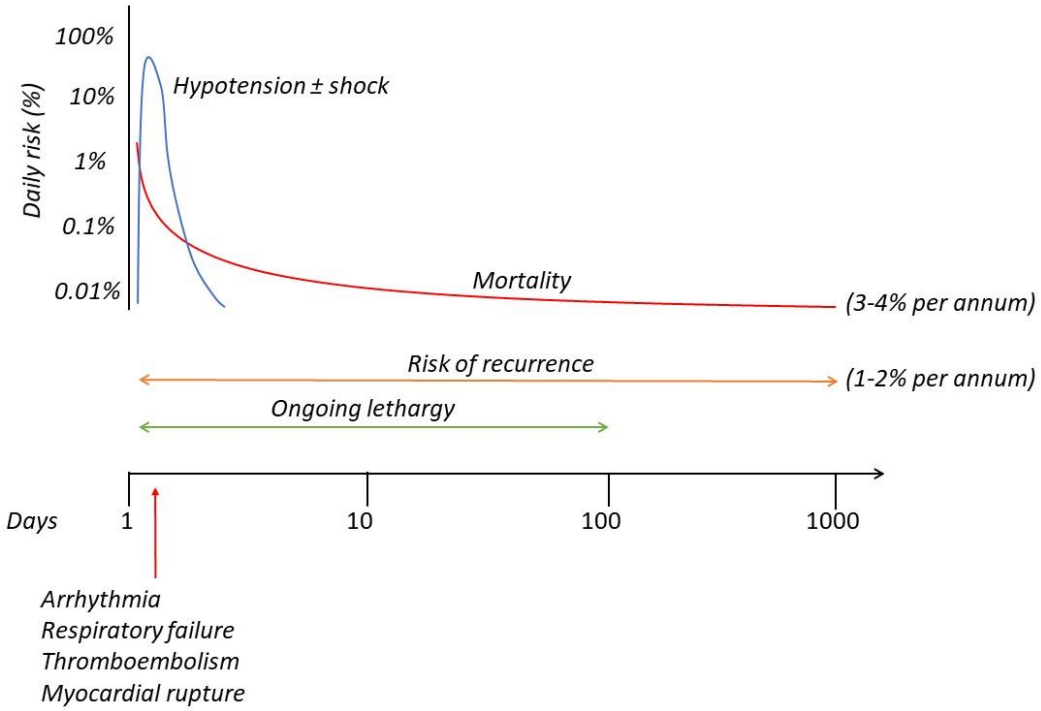
Clinical progress after the first 48 hours generally includes substantial recovery of myocardial and vascular function, as measured by resolution of regional LV hypokinesis on echocardiography(82), and also of hypotension.

However, most patients continue to feel lethargic for at least 3 months following the initial TTS attack, and many complain of ongoing exertional dyspnoea(71). This indirect evidence of ongoing disease activity is not surprising, given the persistent elevation of plasma NT-proBNP concentrations, impairment in LV systolic function as measured by GLS, and evidence of myocardial inflammation/oedema 3 months after TTS attacks(71, 73). Furthermore, myocardial energetics, as measured by phosphorus magnetic resonance spectroscopy (MRS), remain impaired not only beyond 4 months after TTS attacks(83), but also in the long-term as well(84).

There is also a risk of recurrence of TTS, which appears to be approximately 1-2% per annum(85). The extent of myocardial dysfunction during the index admission is directly associated with an increased risk of recurrence, whilst the use of angiotensin converting enzyme (ACE) inhibitors on discharge is associated with reduced incidence of recurrence.

More importantly, it has progressively emerged that the long-term mortality risk after attacks of TTS is similar to that after acute MI(86), and that a substantial proportion of these deaths is of cardiovascular origin. Figure 1.5 summarises key aspects of the early and late clinical features and complications of TTS.

Figure 1.5: Timeline of complications of TTS.



1.2.4.1 TTS as a paraneoplastic syndrome

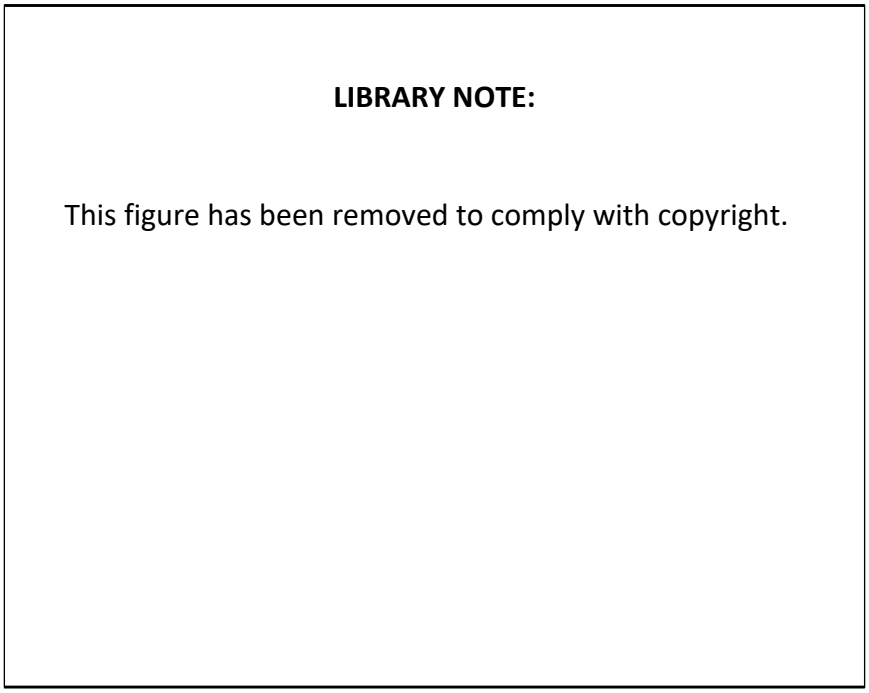
There is increasing evidence that a history of malignancy, whether prior or active, is common among patients with TTS. The prevalence of malignancies has been reported to be around 10-20% of patients with TTS(87, 88), and in one study, malignancies were subsequently diagnosed in 10% of TTS patients during a mean follow up period of 4 years(88). Furthermore, cancers also appear to be more common in TTS when compared to patients with ACS(89).

More importantly, the outcomes of TTS patients with cancers are worse, with higher rates of major adverse cardiac events (MACE) in-hospital(90, 91), as well as on follow-up(87, 88). In fact, not only is cancer associated with a substantial risk of all-cause mortality, cardiovascular mortality also appears to be substantially higher(92).

The exact mode of interaction between malignancies and TTS has not been established, although there are several hypotheses. Potentially interacting signal transduction pathways contributing to

propensity for both TTS and carcinogenesis are depicted in Figure 1.6. Indeed, some malignancies (and their treatments) may be associated with increased catecholamine production, and may therefore contribute to the development of TTS. The pathophysiology of TTS will be further discussed in the following chapter.

Figure 1.6: A schematic view of potential reciprocity between TTS and cancer both at the level of initiation and progression. (Image reproduced from Nguyen et al. Cardio-Oncology 2019)



Abbreviations: GPCR – G-protein-coupled receptor; cAMP – cyclic adenosine monophosphate; Gs – G-stimulatory; Gi – G-inhibitory; PKA – Protein kinase A; ERK – Extracellular signal-regulated kinase; PI3K – Phosphoinositide 3-kinase; Akt – Protein kinase B.

1.2.5 Pathophysiology

The exact pathophysiology of TTS is not yet completely understood. However, given the circumstances surrounding the onset of most episodes of TTS, including the development of TTS following infusion of catecholamines(93), it was not totally surprising when Wittstein and colleagues suggested (on the basis of investigations in a small cohort of TTS patients) that TTS was associated with very marked elevation of plasma catecholamine concentrations, and that TTS therefore represented a form of "catecholamine cardiotoxicity"(94). Over the last few years, the mean extent of elevation of plasma catecholamine concentrations associated with TTS has been challenged(95), but the ultimate issue is the nature, and the extent of catecholamine effect on the vasculature, myocardium, as well as the central nervous system in TTS: this issue cannot be easily addressed in human subjects, except perhaps via therapeutic trials involving blockade of catecholamine effect. Furthermore, there is some evidence that blockade of catecholamine signalling pathway may result in some "paradoxical" effects(96).

1.2.5.1 Vasculature

TTS was initially attributed to multivessel coronary artery spasm, and certainly coronary vasoconstriction is sometimes observed during the acute stages of TTS (97, 98). The theory of a causative role for coronary spasm, although still sometimes promulgated, has a number of obvious weaknesses. For example, ischaemia due to abnormal coronary reactivity would tend to induce regions of LV hypokinesis congruent with the distribution of particular major coronary arteries, and a number of investigators have failed to detect evidence of myocardial ischaemia during the acute phase of TTS(69, 99, 100). Furthermore, crises of coronary artery spasm only occasionally trigger

myocardial necrosis like TTS. However, these facts have not completely abolished enthusiasm for the idea that TTS is fundamentally a transient ACS(101).

Another, more definitive, recent piece of evidence of vascular involvement in TTS is the demonstration of transient glycocalyx "shedding"(102) and associated transient endothelial dysfunction. Shedding of the endothelial glycocalyx during acute attacks of TTS facilitates monocyte/macrophage adhesion to the vasculature and subsequent transmigration into the myocardium, leading to myocardial inflammation and oedema(103). The resultant increase in vascular permeability can also lead to the development of pleural and pericardial effusions commonly seen in TTS. Furthermore, glycocalyx shedding would lead to disturbances of vascular rheology, abolishing laminar blood flow and stimulating increased expression of the pro-inflammatory protein and inflammasome activation thioredoxin-interacting protein (TXNIP).

Finally, β2-adrenoceptor activation is coupled, via postsynaptic Gi-mediated interactions, to activation of nitric oxide synthase(NOS)(104), and when hyper-stimulated by catecholamines, as in TTS, is associated with the potential for supranormal endogenous NOS activation(105). This may potentially lead to inappropriate peripheral vasodilatation, and can theoretically contribute partly to the development of hypotension in TTS.

1.2.5.2 Myocardium

Supra-normal tissue levels of catecholamines also appear to have a direct toxic effect on the myocardium leading to impaired contractility. In a rat model of TTS, Paur and colleagues demonstrated that administration of high-dose adrenaline induced impairment of myocardial contractility in a typical TTS-like regional akinesia, via "biased" post-receptor signalling in activated cardiac β2-adrenoceptors, with selective, Gi-protein activation(96). Shao and colleagues later confirmed this discovery(106), and also showed that administration of high dose isoprenaline led to

increased intra-myocardial lipid accumulation(107), particularly in areas of akinesia, suggesting diminished local lipid catabolism.

Furthermore, the resultant increased release of NO from β2-adrenoceptor stimulation, in the presence of the superoxide (O_2^-), (also incrementally produced by the actions of catecholamines on the myocardium(108)), may induce the formation of peroxynitrite ($ONOO^-$). Peroxynitrite induces associated redox stress and protein nitration(109). Indeed, Surikow and colleagues have demonstrated that, in a rat model of TTS, both generation and effects of $ONOO^-$ are markedly increased(110). This has also been observed in TTS hearts post-mortem(111). Importantly, $ONOO^-$ induces deoxyribonucleic acid (DNA) damage, and thus indirectly contributes to the activation of the DNA repairing enzyme poly(ADP-ribose) polymerase(PARP)(112). Activation of PARP is energy-consuming and may therefore be responsible for the impairment of myocardial energetics which is seen in patients with TTS on MRS as described earlier(83).

Surikow and colleagues also demonstrated a significantly greater concentration of TXNIP in the myocardium of isoprenaline-treated rats(110). The combination of redox stress and the disruption in vascular laminar flow from glycocalyx "shedding" in TTS likely explains the increase in TXNIP expression(113), and ultimately contributing to myocardial inflammation. In addition, there is evidence of increased macrophage infiltrates, predominantly in the form of the pro-inflammatory M1-subtype(114, 115). It seems likely that this macrophage infiltration plays an important role in maintaining myocardial inflammation for several months following acute attacks of TTS.

A schematic view of current understanding of the pathogenesis of TTS, as affecting both vasculature and myocardium, is shown in Figure 1.7.

Figure 1.7: Schematic view of pathogenesis of TTS in relation to common clinical outcomes. (Image reproduced from Ong et al. Heart, Lung and Circulation 2020)

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Abbreviations: O_2^- – Superoxide; MMPs – Matrix Metalloproteinases; $ONOO^-$ – Peroxynitrite; β2 – β2 adrenoceptors; NOS – Nitric oxide synthase; NO – Nitric oxide; PARP-1 – Poly [ADP-ribose] polymerase 1; NAD^+ – Nicotinamide adenine dinucleotide; ATP – Adenosine triphosphate

1.2.5.3 "Brain-heart connection"

Given the involvement of catecholamines in the development of TTS, as well as its association with neuropsychiatric disorders(38), a potential role of the central and autonomic nervous systems has been raised. Indeed, there have been studies reporting abnormalities in the function- activity relationship in areas of the brain related to both emotions and sympathetic nervous system in patients with TTS. In 2014, Suzuki and colleagues first reported significantly increased cerebral blood flow in the basal ganglia, hippocampus, and brain stem in TTS patients acutely(116). Subsequently in 2018, using brain functional magnetic resonance imaging (fMRI), Hiestand and colleagues demonstrated substantial anatomical differences between TTS patients and healthy control subjects in areas of the brain involved in emotional processing, cognition, and the autonomic nervous system(117). Also using brain fMRI, Templin and colleagues in a later study showed that brain

regions associated with the sympathetic and parasympathetic networks are less functionally inter-connected in patients with TTS, compared with controls, and that this aberration persists months to years after TTS episodes(118). More recently, utilising ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT), Radfar and colleagues demonstrated that not only was amygdala activity associated with subsequent development of TTS, individuals with higher amygdala activity developed TTS earlier compared to those with lower amygdala activity(119).

Clinically, patients with TTS displayed a reduction in heart rate variability both acutely and 3 months after attacks, suggesting altered sympathetic activity and sympathovagal balance(120). Additionally, in a series of experiments assessing sympathetic and parasympathetic activity in response to physical and emotional stimuli, Kaufmann and colleagues demonstrated that not only do TTS patients have excessive sympathetic responsiveness, they also had evidence of reduced parasympathetic modulation of the heart rate(121). Again, these findings were present even years after the initial TTS attack.

Overall, although still not yet definitive, these studies therefore have provided some evidence that may explain why some patients are more susceptible to develop TTS. The overall suggestion is that patients with TTS have chronic autonomic signalling dysfunction, favouring sympathetic over parasympathetic discharge from the central nervous system. However, these findings contrast to the hypotheses of Paur et al(96), which focus on aberrant *post-receptor* β_2-adrenoceptor signalling, and do not fully explain triggering of TTS episodes in the presence of phaeochromocytoma, or by acute exposure to exogenous catecholamines.

1.2.6 Key residual issues concerning Takotsubo Syndrome

There remains several residual questions in TTS, including

(1) Issues regarding pathogenesis

a. The relative importance of catecholamines vs aberrant post β2-adrenoceptor signalling
b. Is there a genetic/miRNA component in TTS? And what are the bases for aging and female preponderance?

(2) Issues regarding public health
a. What is the risk of TTS during periods of crises including natural disasters, or even at times of a pandemic?
b. What is the role of the health system in light of the aging population

(3) Development of therapeutics
a. How do we improve patient outcomes acutely, and how do we improve recovery post TTS?
b. How do we prevent the occurrence, or recurrence of TTS?

(4) Education
a. How do we improve the knowledge of both health practitioners and patients in TTS, particularly in regards to the importance of early recognition of TTS, as well as symptomatology post-attacks?

1.3 Scope of the studies in this thesis

The overall objectives of this thesis were to:

(a) Investigate novel (non-traditional) risk factors or precipitants for both ACS and TTS (Chapters 2.1 and 2.2),

(b) Investigate the impacts of variability in attack severity and rate of myocardial recovery among TTS patients (Chapters 3.1 and 3.2), and

(c) Investigate pharmacological strategies for limiting severity of acute attacks, as well as accelerating the recovery in TTS. (Chapter 4)

Chapter 2: Risk factors or precipitating factors of acute coronary syndromes and Takotsubo Syndrome

The death of former President of the United States of America, Franklin Roosevelt at age 63 from hypertensive heart disease and stroke in the mid 1900s led to the initiation of one of the most recognised epidemiological studies on the development of coronary artery diseases – the Framingham Heart Study. The first results, published in 1957, introduced the concept of "coronary risk factors", and showed that advanced age, hypertension, hypercholesterolaemia, smoking, diabetes, obesity, and a lack of physical activity were associated with increased risk of developing cardiovascular diseases(122).

Over the next few years, the list of coronary risk factors continued to expand, and now includes pro-thrombotic states such as hyperhomocysteinaemia(123), and various pro-inflammatory states including rheumatological conditions(124), chronic kidney disease(125) and even cancer(126). It is also clear that our current understanding of coronary risk factors remains incomplete(127).

TTS, on the other hand, irrespective of its putative risk factors, is often precipitated by emotional and physical stressors (or sometimes, a combination of the two). Whilst some patients with TTS report no identifiable trigger, a hallmark of TTS is its association with a preceding stressful event. Examples of various precipitants of TTS published thus far are shown in Figure 2.1.

Figure 2.1: Examples of emotional and physical triggers that have been reported in patients with TTS. (Image reproduced from Ghadri et al. European Heart Journal 2018)

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One of the most commonly reported, and widely known, emotional triggers of TTS is the recent occurrence of a death in a family. Hence, TTS is also colloquially known as “broken heart” syndrome. It is therefore a reasonable assumption that the historical reports of increased rates of “MI” after

the death of a significant family member may in fact largely represent TTS(128). Furthermore, TTS attacks also likely account for the “widowhood effect”, which described the increased risk of mortality of widows and widowers soon after the death of a spouse(129).

Besides the grieving reaction, it is also generally accepted that TTS is associated with a variety of other neuropsychiatric disorders. Its association with depression, for instance, is particularly interesting, since depression can be considered a state of “catecholamine deficiency”(130), and furthermore, many antidepressants exert inhibitory effects on catecholamine re-uptake into nerve endings, thus increasing tissue catecholamine concentrations within the synaptic cleft. It is possible therefore that the medications used for the treatment of these disorders are responsible for precipitating TTS attacks. Indeed, the use of some antidepressants, be it at therapeutic or supratherapeutic doses, have been associated with not only TTS attacks(131, 132), but also sudden cardiac death(133, 134).

In addition, the incidence of “MI” has been reported as being markedly greater at times of natural disasters such as earthquakes and hurricane storms(135). In Australia, Dennekamp and colleagues also found an increased rate of out-of-hospital cardiac arrests after exposure to air pollution associated with bushfires(136). As mentioned in the previous chapter, it is possible that many of these cases actually reflect TTS attacks. More recently, another (admittedly small) Australian study found that not only did rates of ACS presentations increase during the 2019/2020 bushfire season, presentations from bushfire-affected areas were more commonly female, and more likely to be diagnosed with TTS and myocardial infarction with non-obstructive coronary arteries (MINOCA)(137).

The following sections in this chapter focus specifically on the impacts of ambient temperature, pollution and the proximity to bushfires on the incidence of ACS and TTS, as well as catecholamine-potentiating drugs (CPD) and exogenous catecholamines on TTS.

2.1 "Bushfire Season" in Australia: correlations between impacts of variability in ambient temperature, air pollution and proximity to bushfires on incidence of acute coronary syndromes and Takotsubo Syndrome

2.1.1 Introduction

A number of "coronary risk factors" have been shown both to predict the development of coronary atheroma and also the clinical emergence of consequences of atherogenesis such as stable myocardial ischaemia or acute coronary syndromes (ACS) including unstable angina pectoris (UAP) and acute myocardial infarction (MI). The list of these factors continues to expand, notably with recent demonstration that long-term increases in air pollution represent a substantial coronary risk factor(138-140). Regarding the impact of specific pollutants, there are extensive data to the effect that chronic exposure to fine particle pollution (due to increased atmospheric concentrations of particulate matter of less than 2.5µm ($PM_{2.5}$) represents a coronary risk factor, independent of other "conventional" factors such as smoking, hypertension, hypercholesterolaemia and diabetes(140, 141).

There is also some evidence that *acute* increases in air pollution may also impose synchronous, or slightly delayed, increases in incidence of acute ischaemic events. For example, Ishii et al(142) evaluated the effects of transient increases in $PM_{2.5}$ concentrations throughout Japan and found that an increase of 10µg/m^3 was associated with approximately an 11.5% increase in incidence of Type 1 acute MI. These investigators also evaluated the possible extent of hysteresis between exposure to increased particulate matter and presentation with acute MI, and showed that peak risk, which occurred in spring, was associated with a "lag" of approximately 2 days between exposure to increased air pollution and peak incidence of diagnosis of acute MI.

Acute and chronic changes in ambient temperature have also been evaluated as a potential risk factor for precipitation of acute MI. In general, results of studies have suggested that in isolation, there is an increased risk of acute MI during both acute and chronic falls in temperature(143, 144). However, Wang et al(145) have recently reported that development of "heat stroke" is associated with evidence of acute MI in approximately one third of patients. Furthermore, a longitudinal study from Augsburg, Germany from 1987 to 2014 suggested a gradual transition from cold-related to heat-related association with acute MI risk during that period(146).

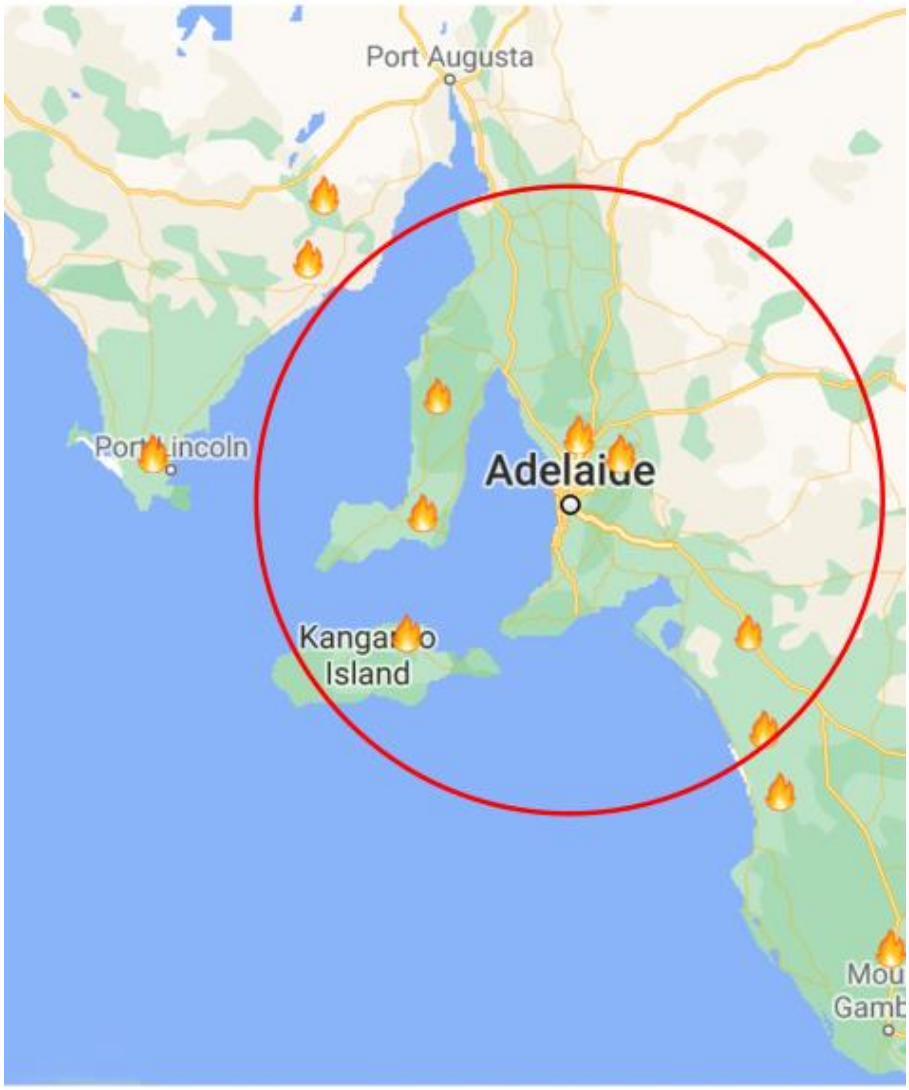
In addition to ongoing evaluations of the roles of particulate matter and of variations in ambient temperature as potential precipitants of acute MI and other forms of ACS, the occurrence of fires, whether as a result of industrial accidents and of forest fires (also commonly known as wildfires or bushfires), is an increasing problem in many parts of the world, especially during the summer months. This represents an additional potential precipitant not only for acute respiratory but also potentially for cardiovascular emergencies, such as out-of-hospital cardiac arrest and hospital presentation with ACS/acute MI, potentially partially via association with increased ambient temperature and release of a wide range of particulate matter and polluting chemicals(147). However, associations between the occurrence of fires and the precipitation of cardiovascular, as distinct from respiratory events, remain less than conclusively established thus far(147). Furthermore, it is statistically challenging to determine to what extent the health impacts of the occurrence of fires relate to the fires *per se,* or also to the common association with elevated temperature, the release of pollutants, and/or to heavy physical activity and sympathetic activation associated with immediate proximity to the fires.

The condition of Takotsubo Syndrome (TTS), which represents an acute inflammatory response of the cardiovascular system (first vasculature, then myocardium) to pulse exposure to catecholamines, and which occurs especially in ageing women following emotional or physical stress (148), is theoretically more likely to occur in the environment created by the combination of fires, extreme

heat and air pollution. However, to date, there have been no detailed studies of that putative association.

Australia has had a long history of association between very hot summer temperatures and risk of fires (locally termed “bushfires”), mainly in the countryside. These risks are set against a relatively low background level of air pollution, which in theory would be expected to increase in association with bushfires. The summer period of 2019-2020 was unusually hot through much of the country, and there was a series of disastrous bushfires in the eastern and southern regions, especially in January 2020. Adelaide, the capital of, and only large city in, South Australia, was relatively close to several large bushfires within the adjacent 200km, as shown in Figure 2.1.1.

Figure 2.1.1: Geographical map of South Australia and the locations of bushfires during the summer period of 2019-2020. Bushfires within a 200km radius of Adelaide are shown by the red circle.



In the currently reported study, data on patient admissions with suspected acute myocardial ischaemia/infarction or TTS from the Emergency Departments of all Adelaide public hospitals during a 120-day period from 1st November 2019 (late spring) to 28th February 2020 (end of summer) have been analysed to evaluate putative isolated and combined impacts of ambient temperatures, atmospheric $PM_{2.5}$ density and proximity to bushfires and the incidence of ACS/acute MI and TTS.

2.1.2 Methods

Discharge summaries (including discharge diagnoses) for patients presenting to the Emergency Departments of the four tertiary public hospitals for adults in Adelaide between the dates of 1st November 2019 and 28th February 2020 with symptoms potentially representing acute myocardial ischaemia were evaluated and categorised according to discharge ICD-10 codes. The bases for the designation of the final diagnoses, including fluctuation in cardiac troponin T concentrations, together with electrocardiographic, echocardiographic, cardiac catheterisation and cardiac myocardial resonance imaging (CMR) data during admission, were also scrutinized to establish that these diagnoses were consistent with relevant in-hospital investigations.

Patients in whom there was evidence of acute myocardial injury on the basis of troponin elevation were categorised according to whether there was evidence of Type 1 acute MI based on the 4th Universal Definition of MI(4), or definite evidence of TTS (on the basis of absence of concordant coronary artery disease, together with regional left ventricular wall motion anomalies and characteristic appearances on CMR examination(148)).

Patient demographics and comorbidities were recorded, as well as estimated glomerular filtration rates.

As regards the putative risk factors being examined, environmental data, including maximal daily temperatures and $PM_{2.5}$ concentrations, were supplied by the South Australian Environmental Protection Authority. Data regarding the occurrence and proximity to Adelaide of bushfires were provided by the South Australia Country Fire Service and patients' places of residence were evaluated to determine whether or not they resided within 200km of an active bushfire.

The study protocol was approved by the relevant Human Research Ethics Committee.

Data Analyses

Data were first evaluated for possible univariate correlations between the three potentially fluctuating environmental factors (temperature, $PM_{2.5}$ density and proximity to fires) and daily frequency of presentations with each diagnostic entity. To evaluate the possibility of a short period of hysteresis between exposure to an environmental precipitant and the emergence of a clinical syndrome, comparisons with performed both synchronously and with a delay period of 24 hours. Proportional comparisons were made using Chi-squared test. Univariate correlations with maximal temperature and $PM_{2.5}$ density were performed using linear or non-linear regression with Fisher's or Spearman's correlation coefficient as appropriate, while impact of proximity to bushfires was evaluated by non-paired Student's t-test or Mann Whitney test as appropriate.

The next step in data analysis was to determine whether, as expected, the occurrence of bushfires was correlated both with greater ambient temperature and with greater $PM_{2.5}$ concentrations. These analyses were performed using non-paired Student's t-tests. The potential for a differential impact of potential risk factors in combination was performed by evaluation of the incidence of acute coronary syndromes on days of zero, one, two and three risk factors, compared via Spearman's correlation.

To evaluate the possibility that the impacts of the three factors investigated (temperature, $PM_{2.5}$ concentrations, bushfires) might be gender-specific, analyses were repeated according to patients' genders, using 2-way ANOVA.

Analyses were performed separately for the total of various forms of ACS (including UAP, and acute S-T elevation and non-ST elevation myocardial infarctions) and for TTS.

2.1.3 Results

Patient demographics

Over the 120-day study period, a total of 504 patients were admitted with ACS and 35 with TTS. Patient data are summarised in Table 2.1.1. Approximately two thirds of patients admitted with ACS were men. Patients with TTS tended to be older than those with ACS, were more likely to be women, and had longer durations of hospitalization. A total of 10 patients were adjudicated as having myocardial infarction without obstructive coronary artery disease (MINOCA) rather than TTS. In-hospital mortality rates were low for both ACS and TTS patients.

Table 2.1.1: Patient demographics.

	Total (n=539)	ACS				TTS (n=35)	P value
		STEMI (n=151)	NSTEMI (n=251)	UAP (n=92)	MINOCA (n=10)		
Age [Years; Median (IQR)]	69 (59-78)	66 (56-73)	71 (60-81)	67 (58-76)	69 (59-83)	72 (67-78)	0.12
Sex (Males, %)	338 (62.7%)	109 (72%)	166 (66%)	56 (60%)	3 (30%)	4 (11%)	<0.0001
eGFR [Median (IQR)]	77 (57-90)	76 (55-90)	77 (57-90)	81 (54-90)	90 (75-90)	79 (56-90)	0.92
Troponin-T [ng/l; Median (IQR)]	240 (61-1041)	1583 (456-4927)	202 (96-584)	30 (30-33)	199 (94-437)	373 (193-672)	0.23
DM (n, %)	165 (30.6%)	30 (19.9%)	86 (34.3%)	39 (42%)	2 (20%)	8 (24%)	0.29
Duration of	2.3 (1.6-	2.8 (1.9-4)	2.2 (1.5-4)	1.7 (1-3)	2.7 (1-4.1)	3.6 (2.8-	<0.0001

hospitalization [Days; Median (IQR))	4)					7)	
In-hospital mortality (n, %)	24 (4.5%)	16 (10.6%)	6 (2.4%)	2 (2%)	0 (0%)	1 (3%)	>0.99

Abbreviations: eGFR - estimated glomerular filtration rate; DM - diabetes mellitus. P values represent difference between ACS and TTS patients. Note the lowest limit of quantitation of Troponin-T was 30ng/L: levels of Troponin-T below 30 are reported as 30. P values refer to comparison of data for ACS and TTS patients.

Ambient conditions

The Australian Bureau of Meteorology reported that 2019 was Australia's warmest year on record, and with the emergence of bushfires (mainly in January/February 2020), high temperatures continued throughout much of the study period. Median maximal daily temperature was 23.9°C, but temperatures varied widely, exceeding 30°C on 25% of study days. Maximal daily temperature was 43°C. Daily maximal $PM_{2.5}$ concentrations were usually quite low in comparison with those seen in most cities around the world. For example, over the study period, maximum $PM_{2.5}$ densities stayed below the World Health Organisation recommended maximal safety level(149) of 25μg/m^3 on all but 14 days, while $PM_{2.5}$ concentrations exceeded 100μg/m^3 on only 2 days. Bushfires occurred within 200km of Adelaide on 47 of the 120 days evaluated.

Relationships between environmental factors and frequency of ACS admission:

(a) Impact of maximal daily temperature, evaluated in an univariate manner, is shown in Figure 2.1.2A. There was a direct relationship (r_s=0.26; p=0.005) between ambient temperature and frequency of ACS admissions.

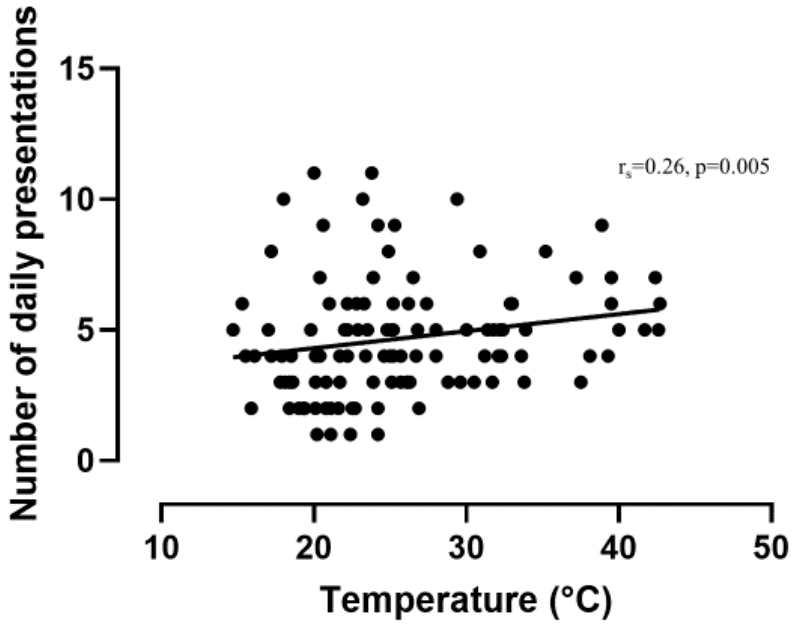
(b) Over the entire monitoring period, increases in $PM_{2.5}$ density (Figure 2.1.2B) were associated with a non-significant and non-linear trend towards increased daily frequency of ACS admission (r_s=0.13**;** p=0.18). Probability of admission was significantly increased (Median 4 vs 5 admissions per day: p=0.028) on "unsafe $PM_{2.5}$ concentration[>25 $\mu g/m^3$]"(149) versus lower $PM_{2.5}$ concentration days.

(c) Presence of bushfires (Figure 2.1.2C) was also associated with a trend (p=0.091) towards an increase in daily frequency of ACS admissions.

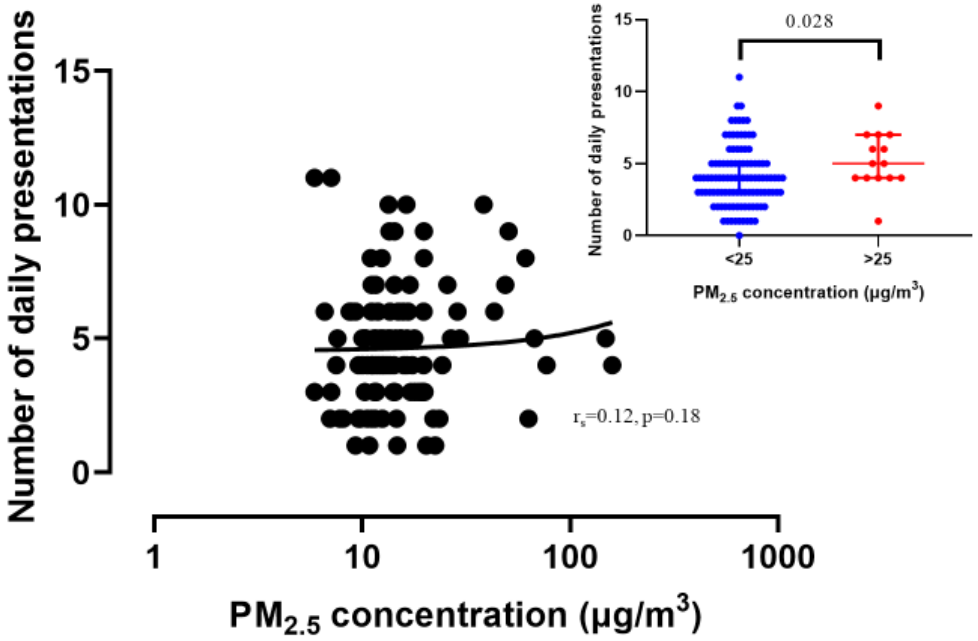
(d) The impact of the three factors evaluated in isolation, in pairs and combined is summarised in Figure 2.1.2D, showing an overall additive impact of presence of all increasing numbers of risk factors in combination (r_s =0.25, p=0.005)

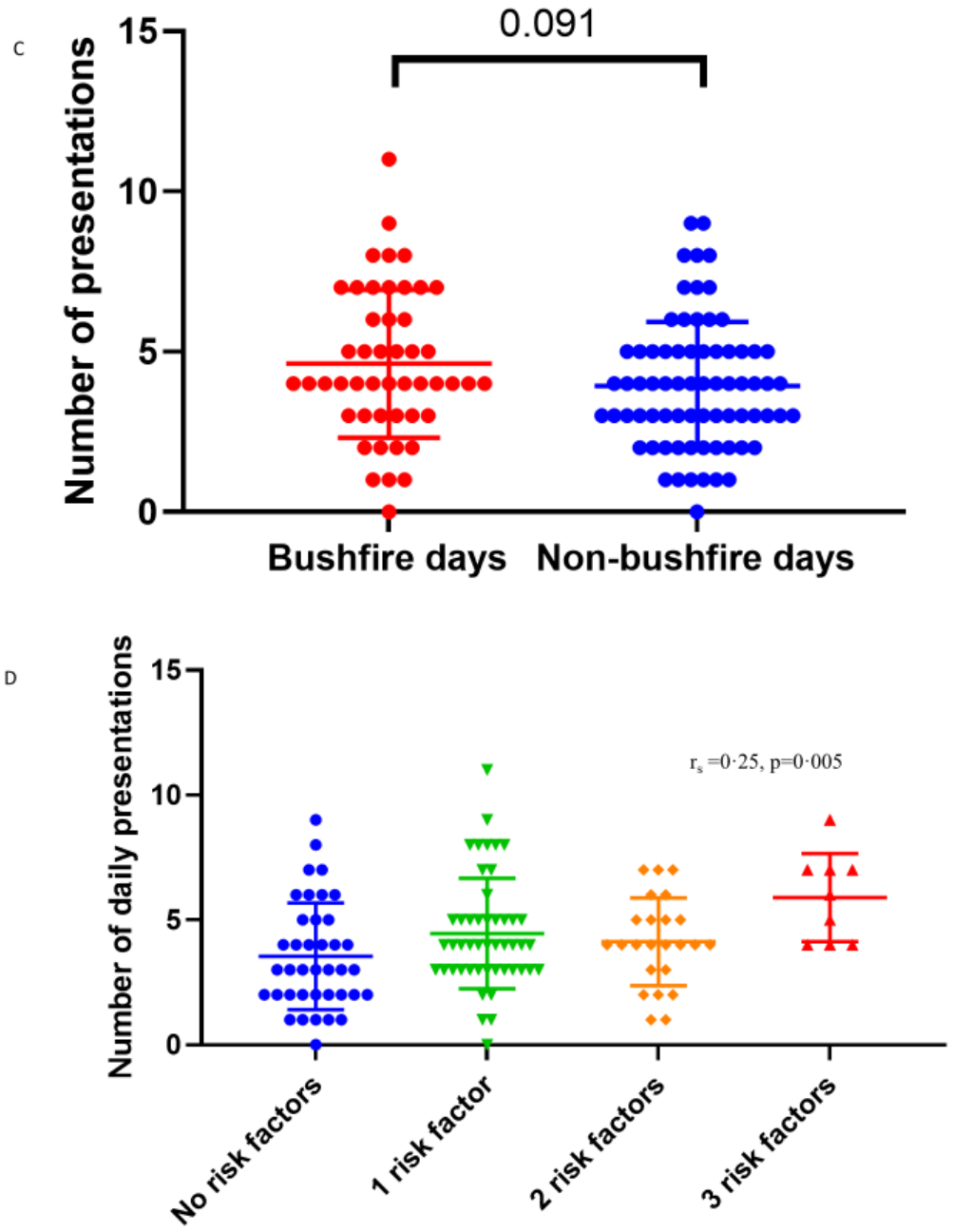
Figure 2.1.2: Relationships between environmental conditions and daily ACS presentations

A


Number of daily presentations
15
10
5
0
r_s=0.26, p=0.005
10
20
30
40
50
Temperature (°C)

B


Number of daily presentations
15
10
5
0
1
10
100
1000
r_s=0.12, p=0.18
$PM_{2.5}$ concentration (µg/m^3)
0.028
Number of daily presentations
15
10
5
0
<25
>25
$PM_{2.5}$ concentration (µg/m^3)



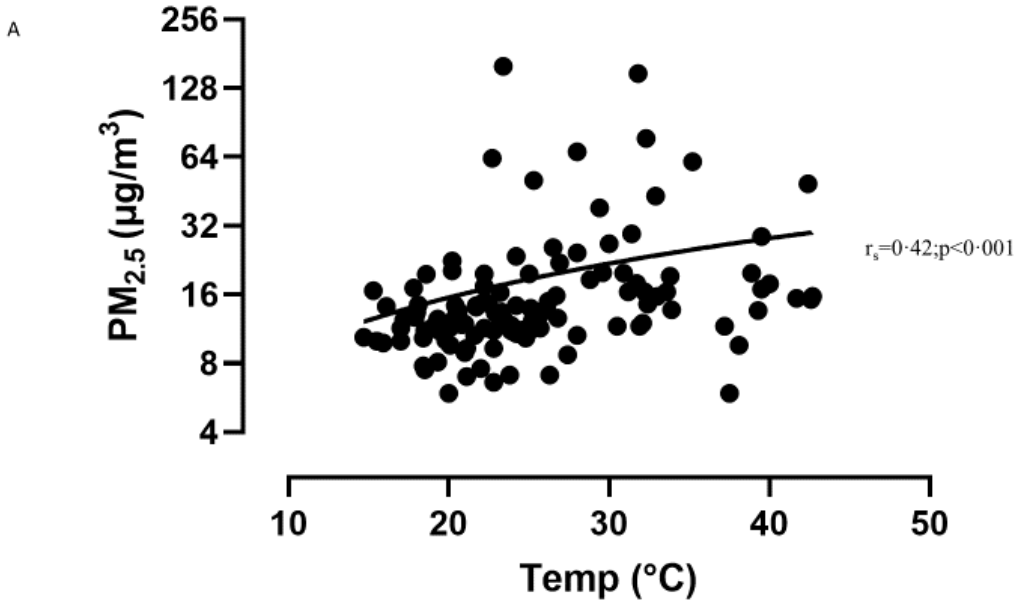
(A) Correlation between maximum daily temperature and number of daily ACS presentations, (B) Correlation between maximum daily $PM_{2.5}$ concentrations (log_{10} scale) and number of daily ACS presentations: Inset: variability between frequency of ACS presentations and "safe" versus "unsafe" $PM_{2.5}$ concentrations (C) Daily ACS presentations on bushfire vs non-bushfire days (results shown in median and IQR). (D) The impact of all 3 factors individually, and in combination on number of daily presentations (high temperature days is defined as days with maximum temperature above the

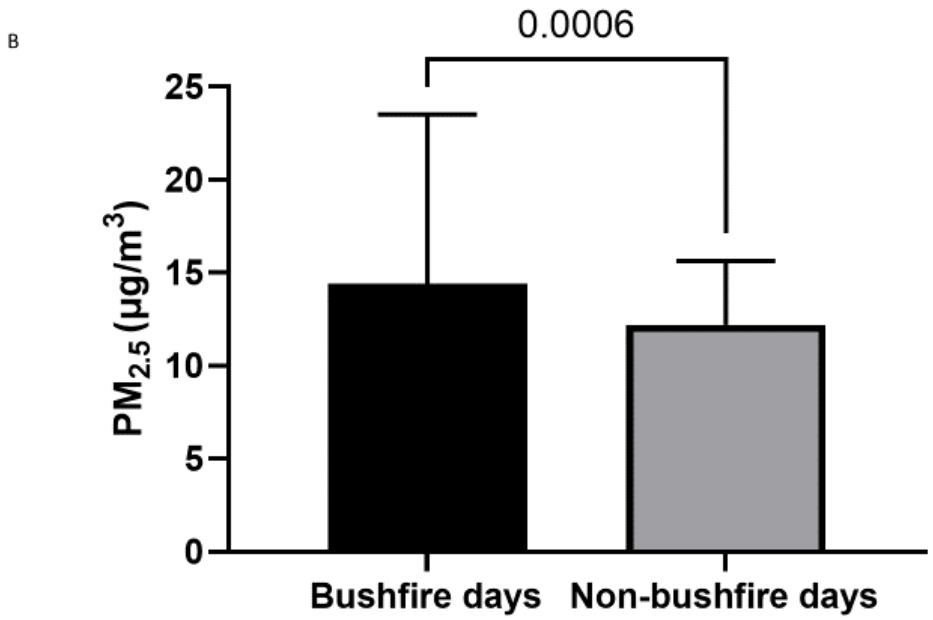
mean maximum temperature during the study period, high $PM_{2.5}$ is defined as days with the maximum $PM_{2.5}$ concentration exceeding 25μg/m^3). All statistical data are shown on the Figure.

Interactions between temperature, pollution and bushfires as regards ACS risk

As expected, all three factors under investigation were directly correlated with each other: $PM_{2.5}$ concentrations were greater (Figure 2.1.3A: r_s=0.42;p<0.0001) on days of high temperature, while in turn $PM_{2.5}$ concentrations were greater on bushfire than non-bushfire days (Figure 2.1.3B).

Figure 2.1.3: Interaction between temperature, pollution and bushfires.





(A) Maximum daily $PM_{2.5}$ concentrations (log_2 scale) vs maximum daily temperatures, and (B) Maximum daily $PM_{2.5}$ concentrations on bushfire days vs non bushfire days. All statistical data are shown on the Figure.

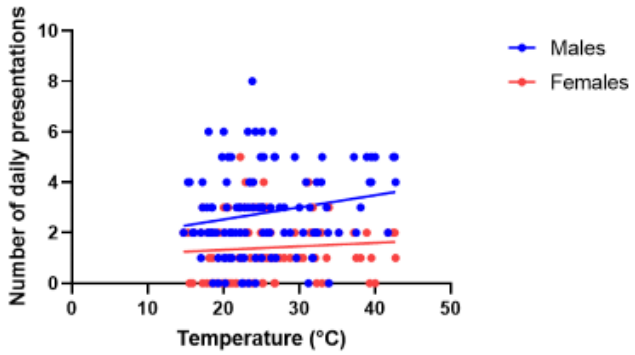
Impact of gender on changes associated with ACS

Gender-specific analyses, summarised in Figure 2.1.4, showed that the increases in incidence of ACS with increasing temperature occurred disproportionately in men (ANOVA: F=1.62, p=0.026). This differential impact was not statistically significant when total numbers of risk factors were considered (Figure 2.1.4B).

Figure 2.1.4: Gender-based differences in impact of risk factors on ACS risk. (A) Impact of temperature variability on incidence of ACS in males and females. (B) Impact of increasing numbers of risk factors on incidence of ACS in males and females. Data were analysed by 2-way ANOVA, and

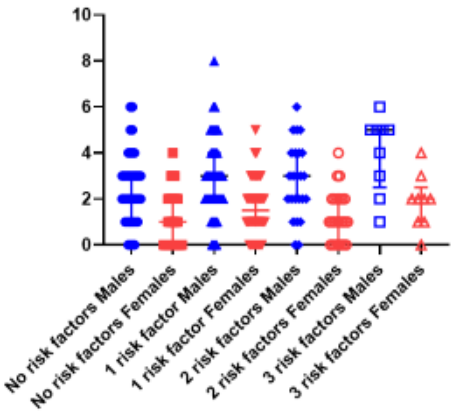
the resultant statistical analyses for interactions between maximum daily temperature and gender(A), and number of risk factors and gender(B) are shown in the respective figures.

A



ANOVA	F	P-value
Temperature	1.68	0.018
Gender	82.29	<0.01
Interaction	2.28	0.026

B



ANOVA	F	P-value
Risk factors	3.76	0.012
Gender	47.58	<0.01
Interaction	0.59	0.62

Is there 24-hour "hysteresis" between triggering factor and onset of ACS symptoms?

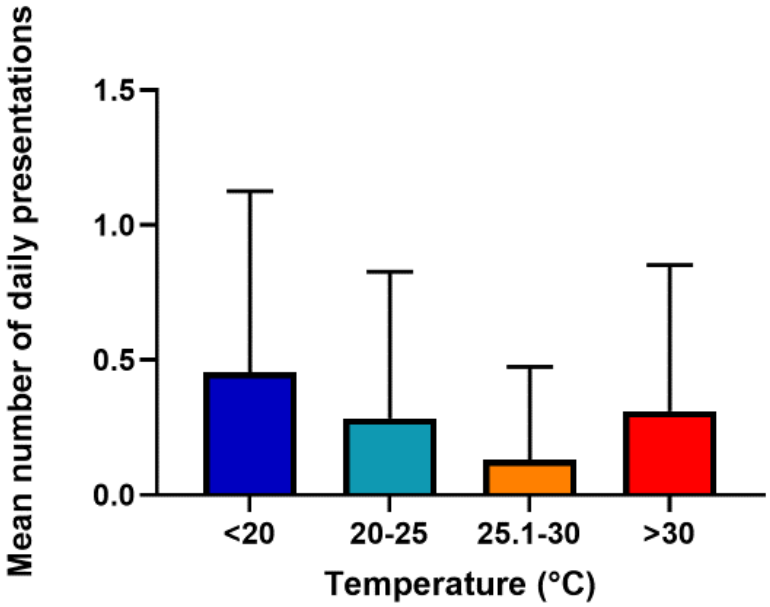
There was remarkably little day-to-day fluctuation in any of the three factors under study, and also very little difference between ACS incidence on consecutive days. Therefore, it was not possible to adequately test the hypothesis that impact of acute exposure to environmental risk might be subject to a short hysteresis period, as previously reported(144, 150)**.**

Daily fluctuation in incidence of TTS

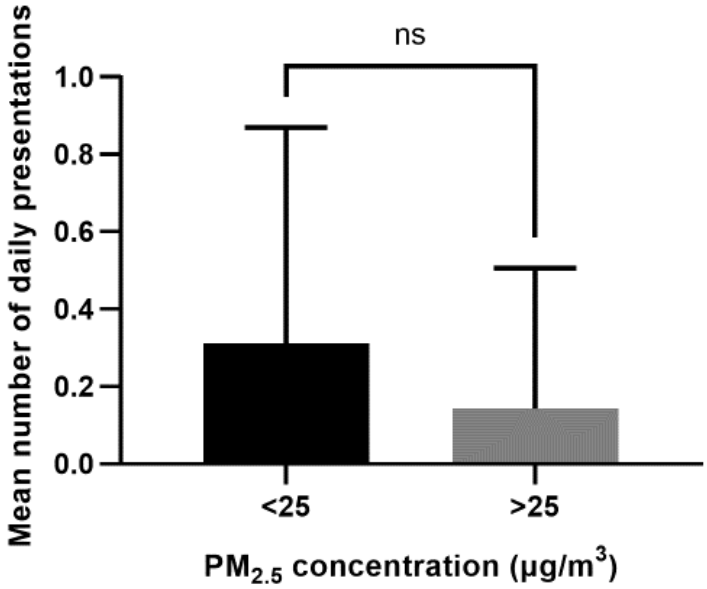
Impact of variability in temperature and $PM_{2.5}$ concentrations, as well as that of presence/absence of bushfires, is shown in Figure 2.1.5A-B. None of the factors under current investigation were associated with substantial fluctuation in presentation rates with TTS.

Figure 2.1.5: Relationships between environmental conditions and daily TTS presentations.

A


Mean number of daily presentations
1.5
1.0
0.5
0.0
<20
20-25
25.1-30
>30
Temperature (°C)

B


ns
Mean number of daily presentations
1.0
0.8
0.6
0.4
0.2
0.0
<25
>25
PM2.5 concentration (µg/m3)

c

(A) Association between quartiles of maximum daily temperature and number of daily TTS presentations, (B) Mean number of TTS presentations on days with "safe" versus "unsafe" $PM_{2.5}$ concentrations, and (C) Mean daily TTS presentations on bushfire vs non-bushfire days. All results shown as mean ± SD.

2.1.4 Discussion

In the chronic context, it is now well-established that extent of a number of subtypes of air pollution, for example as measured by atmospheric concentrations of $PM_{2.5}$ particles, represent risk-factors for the eventual development of both ischaemic heart disease and lung disease. However, data concerning the combined acute impact of the various inter-linked components of climate change, including increasing temperatures, increased $PM_{2.5}$ concentrations, and alarming increases in the risk of bushfires, often adjacent large urban populations, remain lacking. Furthermore, studies conducted in the acute setting have thus far tended to concentrate on single factors, and therefore to ignore the possibility that increased temperature, increased $PM_{2.5}$ concentrations, and proximity of bushfires, may have cumulative or even multiplicative impacts. The current study was undertaken to evaluate the extent of such a potential interaction between risk factors.

The setting for the study was Adelaide, a city in South Australia where there has been a well-documented trend towards increases in daily temperatures over the past 30 years, and where the occurrence of bushfires in summer has been a longstanding concern, but where levels of air pollution, as measured by mean $PM_{2.5}$ concentrations, remain within the "safe" range at most times. This setting is likely to be of particular importance given that it is most conducive for detection of impact of fluctuation in $PM_{2.5}$ concentrations, assuming that this impact has been reported to fluctuate most sharply below 25$\mu g/m^3$ (151). Furthermore, this setting (of acute changes in environment superimposed on a low basal concentration of $PM_{2.5}$) is potentially relevant to the circumstance of many cities of moderate size in otherwise rural settings, where the occurrence of bushfires in the summer months is an emerging public health issue(152).

The study, although focusing throughout on maximal temperatures, $PM_{2.5}$ concentrations, and proximity or otherwise of bushfires, sought dual correlates: both with the development of ACS and that of TTS. Both of these disorders present in a similar manner in the majority of patients(60) but have markedly different pathogeneses: ACS results predominantly from thrombosis on "vulnerable"

atheromatous plaques(11), while TTS represents from transient, probably catecholamine-induced, inflammation of the coronary vasculature followed by myocardial inflammation which is usually of more prolonged duration(148). However, it is theoretically conceivable that risks of both ACS and of TTS may be modulated by some or all of the ambient factors under consideration in this study.

What can we learn from previously published studies? As regards ACS, there are extensive, and superficially confusing, data implicating both unusual falls and increases in ambient temperature as positive correlates of daily risk of ACS(153). Most interestingly, a recent study from Augsburg, Germany(146), documented a gradual transition over the past 20 years towards increased impact of increases in temperature. On balance, it is probable that both marked falls and marked increases in ambient temperature may increase incidence of ACS. For example recent studies from both Japan(154) and Brazil(155) have documented increased ACS with falls in temperature, while a substantial meta-analysis(156) has shown evidence of bidirectional trends at temperature extremes. Heat stroke, a common admission diagnosis in patients hospitalised on high temperature days, has also been shown to be associated with underlying myocardial injury, and presumably acute MI, in a minority of patients(145, 156). As regards the gender distribution of ACS patients, many previous reports have documented an approximately 2:1 male preponderance. The finding that the impact of temperature on risk of ACS was disproportionately increased in men as temperature rose may reflect greater exposure of men to outdoor temperatures: the current study design does not permit further exploration of this finding.

The short-term impact of changes in $PM_{2.5}$ concentrations in isolation has been reported as being relatively small in previous publications. For example, a large Chinese study(151) reported that the mean $PM_{2.5}$ concentration was 63.4μg/m^3 on days of fatal acute MI, as against 62.1μg/m^3 on days without fatality, and that the risk of fatal AMI fell by only approximately 5% on days when $PM_{2.5}$ concentration was as low as 20μg/m^3. Data on the impact of bushfires on cardiovascular risk are scarce, and complicated by variability in study design (for example as regards the mean proximity of

subjects to the fires), plus the complexities imposed by the association between fires and increases in air pollution levels(157).

As regards TTS**,** available data concerning impact of the parameters studied by us are quite limited. On the one hand, it appears that the incidence of TTS is affected by increases in ambient temperature, perhaps to a greater extent than that of ACS. For example, Novo et al(50) reported that incidence of TTS in Italy increases in summer, and indeed that the ratio of TTS to ACS cases also increases during that period of time. Similarly, in Japan(158) the diagnosis of TTS is made most commonly in summer and in early autumn. Furthermore, there are 2 case reports in the literature of TTS as a complication of heatstroke(159, 160). However, TTS is often induced by various types of acute stress, and hypothermia has also been reported as a trigger(161).There are no definitive data related to the impact of bushfires on TTS, but there are reports to the effect that "acute MI without obstructive coronary disease" became more common during recent Australian bushfires(137), and that previous fires may have been associated with increased incidence of such "infarcts"(142). There are no previous publications related to the impact of air pollution on incidence of TTS.

The actual findings from the current study were of considerable interest, especially as regards interactions with daily incidence of ACS. In the context of an unusually hot period of time, there was a strong and direct relationship between daily temperature in isolation and incidence of ACS. Overall, each increase in temperature of 1°C was associated with approximately a 1.2% increase in risk of ACS. The temperature: incidence relationship appeared to be linear over the temperature range occurring during the study period.

The univariate data as regards the interaction between $PM_{2.5}$ concentrations and incidence of ACS were consistent with a weak, curvilinear relationship, which was most apparent (and statistically significant) above the "safe" concentration of 25µg/m^3, as might be expected from previously published Chinese data for variability in acute MI mortality risk(151). Similarly, in isolation, there were numerically, but not significantly, more ACS cases on "bushfire" than on "non-bushfire" days.

However, the parameters under investigation were inter-linked: temperatures were higher, and $PM_{2.5}$ concentrations greater, on "bushfire" days. Hence it was impossible to extrapolate from any univariate correlation to apportion effect size without considering interactions, the main issue to be investigated in the study. When incidence of ACS was considered against all of the various combinations and permutations of putative risk factors, as depicted in Figure 2, it was apparent that there is a summative risk between increased temperature, $PM_{2.5}$ concentration, and presence of bushfires. In the presence of all 3 risk factors, the incidence of ACS was approximately 1.4 times greater than the mean for the period under investigation.

As regards TTS, 35 patients were diagnosed over the 120 days under evaluation. While this number reinforced recent recognition that TTS is far from a rare disorder, with this number of cases, we were unable to detect any significant fluctuation in incidence according to daily temperature, $PM_{2.5}$ concentration or presence of bushfires. It is recognised that this "negative" result may reflect Type 2 error.

The actual mechanism(s) by which risk of ACS increases with increasing ambient temperatures remains unknown, but one possible explanation for this may be increased blood viscosity at higher ambient temperatures(162). Indeed, several physiological changes found at higher ambient temperatures such as increased platelet and red cell counts, as well as cholesterol concentrations, may contribute to thrombogenesis(162). As regards TTS, plasma concentrations of catecholamines have not only been found to increase with passive heating(163), but also to be substantially higher in cases of fatal hyperthermia(164), thus providing a possible mechanism for TTS attacks on warmer days.

Overall, a number of limitations apply to the current data, which should impose caveats on interpretation of the findings, not least of the impact of the three parameters evaluated. First, this was a study involving only the warmer months of the year in the Southern Hemisphere: no conclusions can be drawn regarding year-round variability in temperature or pollution. Second, the

setting was Adelaide, where atmospheric $PM_{2.5}$ concentrations are usually low: fluctuations tended to occur within the "steep region" of the previously documented concentration: effect curve(151), and may be less obvious for cities with higher average $PM_{2.5}$ concentrations. Third, the presence of bushfires was evaluated only categorically, rather than in terms of exact proximity, extent of individual responsibility for fire-fighting, associated local increases in temperature and air pollution, and extent of individual stress experienced in each case. However, ACS admission frequency did not vary significantly when date for fires <50km from Adelaide were considered (data not shown).

One of the prospectively defined objectives of the study was to consider the potential impact of 24-hour hysteresis between exposure to risk factors and onset of cardiovascular impact. This proved impossible to assess properly because there was only minimal day-to-day variability in each parameter. It has also been claimed by some investigators that acute MI without obstructive coronary stenoses may be more common in the aftermath of bushfires(137, 142): only limited conclusions can be drawn in this regard, because not all patients with myocardial injury underwent coronary arteriography, while it is not possible to ensure that such "MINOCA" cases were not actually TTS, since not all of them underwent CMR imaging. Similarly, it is important to recognise that the pathophysiology of myocardial injury in this series of cases could not be defined precisely: for example, acute exposure to air pollution may theoretically predispose to onset of vascular endothelial dysfunction and to platelet activation/aggregation in the absence of atheromatous plaque rupture(165).

In conclusion, the current study serves to demonstrate that increased temperatures during an Australian summer represent a risk factor for simultaneous onset of ACS, and also to suggest strongly that the associated presence of bushfires and of increased atmospheric $PM_{2.5}$ adds substantially to that risk. These findings are likely to be relevant to many other parts of the world, and should inform health care planning for the increasingly common presence of such risk factors in the context of progressive global warming.

2.2 Incremental "Therapeutic" Myocardial Exposure to Catecholamines: Incidence and Impact in Takotsubo Syndrome

The contents in this chapter are similar to published work listed in page 15.

2.2.1 Introduction

As mentioned previously, myocardial inflammation and oedema during acute TTS in all probability represent the indirect result of aberrant β_2- adrenoceptor/G_i protein signalling(96), and may be precipitated by both endogenous (associated with emotional and physical stressors), or exogenous catecholamine surges, compounded by vascular permeabilization as a result of acute glycocalyx "shedding". Indeed, plasma catecholamine concentrations are significantly elevated in patients with TTS, perhaps even when compared to patients with heart failure secondary to MI(94).

A number of previous case reports have emphasised the precipitation of some cases of TTS by exposure of susceptible patients to exogenous catecholamines(93), or to drugs with catecholamine-potentiating effects(43, 131, 166). However, such individual reports, or categorisations of outcomes based upon agglomeration of case reports(132, 167), tend to emphasise more spectacular cases, and provide no insight as to incidence or overall impact on outcomes.

Recently, the InterTAK investigators evaluated the relative impacts of specific precipitating factors, patient demographics and baseline haemodynamic status on short- and long-term prognosis in TTS(168). While this study has contributed to better clinically-based prediction of long-term risk in TTS patients, it did not consider factors such as background of long-term pharmacotherapy, nor did it address causation on a molecular basis.

In the current study, we sought to determine the incidence, causes, and specific impact of antecedent treatments with the potential to increase catecholamine effects on the myocardium on short and long-term outcomes in a registry-based cohort of consecutive TTS patients.

2.2.2 Methods

Patient identification, inclusion and exclusion criteria

The study protocol was approved by the local Human Research Ethics Committee (HREC 2009094). Consecutive patients who presented between 1st of August 2009 to 1st of April 2019 to three tertiary referral hospitals in Adelaide, South Australia, with a clinical diagnosis of TTS were identified. Diagnostic criteria used were: (i) History suggestive of TTS, (ii) elevated plasma troponin concentrations with markedly elevated plasma NT-proBNP concentrations, (iii) Electrocardiographic and echocardiographic findings suggestive of TTS, (iv) the absence of coronary artery stenoses on coronary angiography that may be responsible for the regional wall motion abnormality, and (v) cardiac magnetic resonance imaging (CMR) confirming the diagnosis of TTS(169) and excluding other causes. Informed consent was obtained prior to recruitment. Baseline clinical data and patient characteristics were obtained including age, sex, comorbidities and medications taken prior to presentation. Patients were then followed up yearly via phone. They were categorised to 2 subgroups, according to the presence (CA/CPD+) or absence (CA/CPD-) of catecholamine or catecholamine-potentiating-drug administration. Patients in whom TTS occurred secondary to clearly life-threatening extra-cardiac disorders ("secondary" TTS) were excluded from this analysis, irrespective of whether or not catecholamines were administered as components of treatment of the disorders concerned.

The following categories of pharmacotherapy were considered as potential catecholamine-potentiating drugs:

(a) Sympathomimetics (e.g. methylphenidate, phentermine(170))

(b) β_2-adrenoceptor agonists (e.g. salbutamol(43))

(c) Anti-depressants, specifically agents that increase plasma concentrations of catecholamines (e.g. tricyclic antidepressants(171), serotonin-noradrenaline reuptake inhibitors(172))

Relevant investigations

Analyses were based on:-

(i) Admission plasma concentrations of the catecholamine metabolite normetanephrine as an index of extent of catecholamine exposure

(ii) Indices of haemodynamic impact and extent of inflammation

a. Acute echocardiographic left ventricular ejection fraction (LVEF)

b. Incidence of early hypotension as defined by a systolic blood pressure of <90mmHg within the first 24 hours post presentation, and

c. Peak plasma NT-pro BNP concentrations

(iii) Extent of early recovery, based on echocardiographic global longitudinal strain (GLS) over 3 months post attack

(iv) TTS recurrence and mortality rates during follow up

Statistical analyses

To compare the 2 groups, we used unpaired t-tests in normally distributed samples and Wilcoxon-Mann-Whitney tests in those with a skewed distribution. We used the Chi-squared (χ^2) test or Fisher's exact test to analyse proportional data. A log rank test was performed to compare survival data between the 2 groups. The limit of statistical significance was set at $p<0.05$. Data are expressed as mean ± SEM unless otherwise stated.

2.2.3 Results

After excluding other cases of definite "secondary" TTS (n= 20), a total of 301 patients with TTS were identified within the study period. Fifty-five of these patients (18.3%) had prior catecholamine administration or theoretical catecholamine potentiation, and were therefore included in the CA/CPD+ subgroup. The remaining patients were included in the CA/CPD- subgroup. Baseline characteristics of patients in both subgroups are summarised in Table 2.2.1. There were no significant differences between the two subgroups: in both subgroups approximately two thirds of TTS cases had predominantly apical hypokinesis.

Table 2.2.1. Patient characteristics

	CA/CPD+(n=55)	**CA/CPD- (n=247)**	**P-value**
Age (years; mean ± SD)	66 ± 14	69 ± 13	0.12
Gender (M : F)	4 : 51	18 : 229	1.00
Diabetes Mellitus (n; %)	11 (20%)	37 (15%)	0.36
Hypertension (n; %)	28 (51%)	125 (51%)	0.99
eGFR [mL/min; median (IQR)]	63 (60-817)	62 (59-85)	0.99
Type of TS (% apical)	64%	65%	0.83

Patients were followed up for a median of 3.0 years, and the duration of follow up was also similar in both groups.

Agents implicated are summarised in Table 2.2.2. All patients who received tricyclic antidepressants were on relatively low doses, typically <100mg/day of amitriptyline.

Table 2.2.2. Catecholamine potentiating drugs. Note that 3 patients had exposure to multiple agents

Drug therapy	**Number of patients affected**
Tricyclic antidepressants	24
Nebulised β_2 adrenoceptor agonists	15
Serotonin-noradrenaline reuptake inhibitors	10
Exogenous CA	2
Mirtazapine (Noradrenergic and specific serotonin antidepressant)	6
Indirect sympathomimetic (methyphenidate)	1

Plasma normetanephrine concentrations were significantly higher in the CA/CPD+ group:- median 1149pmol/L (IQR 799pmol/L – 1713pmol/L) vs 938pmiol/L (IQR 610pmol/L – 1345pmol/L) in the CA/CPD- group, p=0.03 (Figure 2.2.1). Plasma metanephrine concentrations did not vary significantly between the CA/CPD+ and CA/CPD- groups (medians 200pmol/L and 210pmol/L respectively).

Figure 2.2.1. Comparisons of plasma normetanephrine concentrations during index admission.

**p=0.03*

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Acute haemodynamic and inflammatory impact

The proportion of patients who developed hypotension during their admission with TTS was not statistically different between groups, with an incidence of 28.6% in the CA/CPD+ group compared to 23.2% in the CA/CPD- group (95% confidence intervals for difference +19%, -8.3%).

Comparisons of peak plasma NT-proBNP concentrations and acute LVEF are depicted in Figure 2.2.2 (A) and (B) respectively. Consistent with previous reports, peak plasma NT-proBNP concentrations were markedly elevated beyond the normal range. Median concentrations were similar in both groups (3974ng/L vs 4730ng/L, p=0.47). Acute LVEF was also not significantly different between the 2 groups, with a mean of 47% in the CA/CPD+ group and 46% in the CA/CPD- group.

Figure 2.2.2. Comparisons of acute haemodynamic [acute LVEF, (A)] and inflammatory impact [peak plasma NT-pro BNP, (B)]. There were no significant differences between groups for either parameter.

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Recovery and long term outcomes

Extent of recovery of left ventricular systolic function over 3 months, based on changes in the sensitive echocardiographic parameter GLS was -5.4% for the CA/CPD+ group, and -4.5% for the

CA/CPD- group (p=NS), suggesting that excessive catecholamine exposure does not modulate rate of recovery.

Only 8 patients experienced TTS recurrence, distributed 1:7 according to CA/CPD+ and CA/CPD- groups (p=NS).

There was a total of 42 deaths during the study period, 11 (20%) within the CA/CPD+ group, and 31 (12.6%) within the CA/CPD- group. Figure 2.2.3 shows the Kaplan-Meier survival curve for each group:- there was a non-significant trend (Log rank X^2=2.3, p=0.13) towards greater mortality in the CA/CPD+ group.

Figure 2.2.3: Kaplan Meier curve comparing the survival distributions between the 2 groups, p=0.13 for difference.

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3.1.4 Discussion

To our knowledge, this is the first study exploring the impact of catecholamine administration or theoretical catecholamine potentiation on short- and long-term outcomes in TTS.

Pathogenesis of TTS is still incompletely understood, although there is clinical and experimental evidence that “pulse exposure” to a variety of endogenous or exogenous catecholamines is frequently the initiating factor(94, 96). However, the current finding that plasma normetanephrine concentrations are particularly elevated in the CA/CPD+ subgroup may be interpreted in several ways.

On the one hand, it is possible that this reflects greater catecholamine stimulus than in CA/CPD- patients. However, it is also possible that greater elevation of normetanephrine concentrations reflects in part persistence of increased sympathetic drive beyond the time of the onset of the attack. These data therefore should remind us of the less-than-clear-cut relationship between systemic circulation of remarkably increased concentrations of catecholamines immediately post TTS attacks, as initially reported by Wittstein et al(94), but not fully confirmed by any investigator since then(95). Given that it is now known that the cellular inflammation and myocardial energetic impairments associated with TTS usually subside slowly(83, 114), it is entirely possible that elevation of plasma normetanephrine concentrations >12 hours post onset of symptoms represents, to a large extent, presence of ongoing stimuli (such as the precipitating agent), and/or a continued sympathetic response (as in the response to hypotension).

It would be assumed that a larger stimulus (whether “initiating” or “perpetuating” in nature) would lead to more severe attacks, as judged by haemodynamic impact (eg hypotension, fall in LVEF, NT-proBNP release). However, there was little evidence for this (apart from a numerical excess proportion of hypotensive patients). In general, substantial stimuli account for the occurrence of “secondary” TTS, mainly in males and with larger long-term mortality rates(46). In the current series, separate consideration of CA/CPD+ patients left only a small residual “secondary” TTS group, which

was excluded from analysis. Indeed, the marginal increase in long-term mortality rates seen in CA+/CPD+ patients raises the issue of whether it is appropriate that they be regarded as a form of "secondary" TTS, with its implication of considerably increased mortality rates(46).

These data are particularly important in the context of the current study, because the factors which have been identified thus far as early components of the pathogenesis of TTS (catecholamine release(96) and nitrosative stress(111)) may be present predominantly in the short-term. Other factors such as macrophage/monocyte infiltration, increased expression of the pro-inflammatory mediator, thioredoxin interacting protein (TXNIP)(110, 111) and myocardial oedema per se, may contribute to slow haemodynamic recovery. In this context, the current results suggest that recovery following acute attacks was not different in the presence of CA/CPD.

It has recently been demonstrated that long-term mortality post TTS is approximately equal to that post-MI(38, 86, 173). In the current study, mortality was approximately 4.7% per year, and tended to be greater among CA/CPD+ patients. Determinants of long-term mortality in entire patient cohorts include initial normetanephrine concentrations, but also size of index attacks(174). However, a larger patient cohort would be needed before definitive conclusions could be drawn regarding mortality differences.

Our study confirms that there is substantial long term mortality risk in patients with TTS, and suggests that perhaps patients exposed to CA/CPD may be at an even greater risk compared to the remainder of TTS patients. The reason for this finding is not clear. One possibility is that chronic catecholamine exposure in the vicinity of cardiomyocytes predisposes to biased β2-adrenoceptor phosphorylation(175). Furthermore we cannot be precisely sure in all cases whether patients' prior pharmacotherapy represented the mechanism of induction of TTS attacks, or simply a marker of risk:- similarly, mortality risk may represent the sum of that imposed by the pre-existing disease states plus sequelae from TTS(176, 177).

There are several caveats to our study. Firstly, normetanephrine has a short plasma half-life of about 90 minutes(178). As TTS is sometimes diagnosed late (i.e. after ruling out ACS), the degree of elevation of plasma catecholamine concentration may have been under-appreciated. However, this does not explain the difference seen as the diagnostic difficulty applies for patients in both groups, and furthermore, the perpetrating CPD would not have been discontinued yet at the time of blood collection. Secondly, no dose-response type data were available. We identified several different therapies at different doses that may have potentiated the acute TTS attacks, and each of them would have contributed to TTS at varying degrees. Additionally, we do not have complete data regarding any ongoing long term exposure to CA/CPD agents. Lastly, as recurrence and mortality rates were low, the lack of statistical significance between groups may be related to Type 2 error.

Overall, we have found that precipitation of TTS in association with drug-induced incremental catecholamine exposure is common, and represents a potentially preventable cause of TTS. As with the TTS population as a whole, CA/CPD+ patients carry substantial risk of ongoing mortality, and may actually be at greater risk than the remainder of TTS patients.

Chapter 3: Prognostic impact of variability in attack severity and rate of myocardial recovery in Takotsubo Syndrome

3.1 Incidence and clinical/laboratory correlates of early hypotension in Takotsubo Syndrome

The contents in this chapter are similar to published work listed in page 15.

3.1.1 Introduction

As discussed in earlier chapters, complications of TTS can occur both early and late. In the early stages, there is a risk of hypotension and shock(179), tachyarrhythmias (80), heart block(180), and the development of mural LV thrombus(67). There are also reports of left ventricular rupture(181). In the longer term, patients often experience persistent impairment of quality of life(71). There is also a substantial risk of late recurrence, occurring in 1-2% of patients per annum(85). The long-term mortality rate following TTS episodes is similar to that after ACS(86).

By far the most common, and most life-threatening in-hospital complication is the development of hypotension and shock. Surprisingly, the exact cause of early hypotension in TTS is not completely understood, with previous studies finding no significant correlation between systolic blood pressure and either extent of LV systolic dysfunction or diminution in cardiac output(54). In the absence of mechanistic understanding, the management of shock in TTS is also challenging, as catecholamine administration is both harmful and likely to be ineffective on theoretical grounds(182). Early hypotension not only represents a common cause of in-hospital mortality, but has also been implicated as a predictor of late mortality risk(183).

In the current study, we utilized a large and detailed database of TTS patients to identify both the incidence of early hypotension/shock and clinical/laboratory parameters which correlate with the risk of this complication in individual patients.

3.1.2 Methods

This study is approved by the local Human Research Ethics Committee. Data from consecutive patients admitted with TTS to three tertiary hospitals in Adelaide, Australia, were analysed. Patients had provided informed consent to participate in the South Australian TTS registry. Diagnosis of TTS was made on the basis of Mayo Clinic criteria(59), supplemented wherever practicable by performance of CMR imaging within 5 days of admission(78). Baseline patient data, including age, sex, comorbidities and prior therapy, were documented, as were in-hospital clinical data, including lowest recorded systolic blood pressures. Hypotension was defined as a systolic blood pressure of ≤90mmHg. Patients who developed TTS in relation to a potentially life-threatening physical illness were classified as "secondary" TTS(46), whilst the remainder of the patients were classified as "primary" TTS.

Patients' venous blood samples were collected in the acute setting. Plasma concentrations of normetanephrine were measured at the time of diagnosis. NT-pro-BNP, CRP, and troponin-T were measured serially, and peak concentrations during the hospital stay were recorded.

Transthoracic echocardiography was also performed during the acute presentation. Standard apical two-, three-, and four-chamber views were obtained with special attention to LV endocardial definition. LVEF was then calculated using the Simpson's biplane method.

For univariate comparison of patients who developed hypotension with those who did not, we used unpaired t-tests/Wilcoxon tests or Chi-squared tests/Fisher's exact test as appropriate. This was followed by a backwards stepwise multivariate linear regression analysis, forcing into this analysis the parameters indicated in *Table 3.2.1*, irrespective of the results of univariate comparisons. Potential status of mutually correlated parameters as confounders on multivariate analyses was evaluated utilizing Pearson's or Spearman's correlation coefficient as appropriate. This methodology

was designed to facilitate evaluation of potential correlates of hypotension according to differences in patient demographics, type of TTS, associated disease states, admission treatments, and markers of TTS attack severity. The limit of statistical significance was set at $p<0.05$. Data are presented as mean ± SD or median (inter-quartile range) as appropriate.

Table 3.1.1: Parameters included in multivariate analysis as potential correlates of development of hypotension.

Parameters included in the multivariate analysis	
Patient demographics	Age Sex
Clinical characteristics	Primary/Secondary TTS Apical/Non-apical hypokinesis
Comorbidities	Hypertension Diabetes mellitus
Admission medications	ACE inhibitors/ARBs
Laboratory parameters	Estimated GFR Plasma NT-proBNP, troponin-T, CRP, and normetanephrine concentrations
Echocardiographic parameters	Acute LVEF

3.1.3 Results

Data from 319 TTS patients were analysed. Patients' demographics and baseline characteristics are summarised in *Table 3.1.2*. A total of 113 (35%) patients were hypotensive early during hospitalisation. Of those patients, 20 (17%) required admission to Intensive Care Units, where all

received infusions of positive inotropic agents. Hypotension occurred more frequently among female than male patients, and tended to be less frequent among patients with histories of hypertension. On the other hand, prior treatment with ACE inhibitors or ARBs was not associated with any increase in frequency of hypotension.

Table 3.1.2: Patient demographics and baseline characteristics: comparison of patients who developed versus those who did not develop hypotension. Significance values relate to comparisons of normotensive and hypotensive patients.

	Total (n = 319)	Hypotensive (n = 113)	Not hypotensive (n = 206)	p-value
Age [years; median (IQR)]	68 (60-77)	67 (60-77)	69 (60-78)	0.303
Male (n; %)	24 (8%)	3 (3%)	21 (10%)	0.014
Secondary TTS (n; %)	81* (25%)	33* (29%)	48 (23%)	0.237
Apical TTS (n; %)	209 (65%)	74 (65%)	135 (65%)	0.983
Diabetes Mellitus (n; %)	51 (17%)	19 (18%)	32 (16%)	0.577
Hypertension (n; %)	158 (51%)	46 (44%)	112 (55%)	0.069
Prior use of ACE inhibitors/ARB (n; %)	125 (42%)	37 (36%)	88 (45%)	0.128

* A total of 14 of patients with secondary TTS had underlying sepsis. 7 of these developed hypotension

Table 3.1.3 shows the clinical, biochemical, and echocardiographic differences between patients who developed hypotension and those who did not. A total of 8 (2.5%) patients died in hospital: all of these had developed hypotension during acute presentation (p<0.001, Fisher's exact test). Left ventricular outflow tract (LVOT) obstruction (peak ≥30mmHg LVOT gradient) was present in only 6 (2%) patients, but was significantly associated with hypotension (p=0.019).

Table 3.1.3: Clinical and laboratory differences between hypotensive vs not hypotensive patients.

	Hypotensive (n = 113)	Not hypotensive (n = 206)	p-value
Lowest systolic blood pressure [mmHg; median (IQR)]	80 (78-86)	105 (100-110)	<0.001
In-hospital mortality (n; %)	8 (7%)	0 (0%)	<0.001
Estimated GFR [mL/min; median (IQR)]	60 (57-80)	62 (60-86)	0.035
LVEF [%; mean ± SD]	44 ± 11	48 ± 11	0.009
LVOT obstruction* (n; %)	5 (4%)	1 (0.5%)	0.019
NT-proBNP [ng/L; median (IQR)]	4714 (2781-11109)	4120 (2410-7186)	0.046
Trop T [ng/L; median (IQR)]	480 (261-775)	368 (200-599)	0.008
CRP [mg/L; median (IQR)]	13 (4-54)	10 (5-31)	0.296
Plasma metanephrine [pmol/L; median (IQR)]	1060 (680-1570)	970 (650-1380)	0.491

* LVOT obstruction (peak LVOT gradient ≥30mmHg on echocardiography)

Extent of anomalies of all the parameters tested, whether indicative of haemodynamic disturbance or of inflammatory activation, tended to be greater in patients who developed hypotension. Of these, the significantly different parameters were lower estimated glomerular filtration rates (GFR) and LVEF, and higher plasma NT-proBNP and troponin-T concentrations in the hypotensive group. *Figure 3.1.1* illustrates the distribution of data for LVEF, peak NT-proBNP and troponin-T concentrations.

On multivariate linear regression analysis, female sex and lower LVEF were found to be independent correlates of the development of hypotension (β=-0.22, p=0.009 and β=-0.21, p=0.010 respectively).

Figure 3.1.1: Univariate analyses of the association between the development of hypotension and severity of acute TTS attacks (A: Acute LVEF, B: peak NT-proBNP concentrations, C: peak troponin-T concentrations). All comparisons were made by non-paired t-tests, and p-values are indicated on the figure.

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3.1.4 Discussion

The occurrence of hypotension and shock in the acute stages of TTS represents one of the most common and feared early complications of the disorder. Previous registry publications, including one emanating from the InterTAK group (and thus utilizing overlapping data from the current study),

recently showed that the development of shock is associated with a substantial risk of both in-hospital and long-term mortality(183). The current data confirm that hypotension per se, with or without clear-cut shock, represents a substantial risk factor for short-term mortality. Intriguingly, hypotension was also associated with data suggesting more severe attacks of TTS.

Consistent with the previous publication from the InterTAK group(183), we also found that acute LVEF was statistically lower in patients with hypotension both on univariate and multivariate analyses. Other parameters of severity of TTS attacks, including plasma NT-proBNP and troponin-T concentrations, however, were not statistically significant correlates of hypotension on multivariate analysis. We also found that acute LVEF correlates closely and inversely with plasma NT-proBNP and troponin-T concentrations (*Figure 3.1.2*). Therefore, the presence of low LVEF is predictive of greater elevation of NT-proBNP and troponin-T concentrations, and acts partially as a "confounder" on multivariate analysis. Conversely, peak plasma NT-proBNP concentrations of >10,000 ng/L were associated with increased probability of early hypotension (51% vs 32%; p=0.006). The other major findings from the current study are that hypotension is more likely to occur (both on a univariate and multivariate basis) in females, with no obvious association with "secondary" TTS (a disorder more commonly affecting males).

Figure 3.1.2: Evaluation of potential for "confounder" status between parameters of severity of TTS attack (A: Correlation between acute LVEF and peak NT-proBNP concentrations, and B: Correlation between acute LVEF and peak troponin-T concentrations). Data were analysed using Spearman's correlation coefficient, and significance levels are shown. Note that the upper limit of quantitation for the assay utilized to measure plasma concentrations of NT-proBNP was 35,000ng/L.

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As regards the potential mechanistic implications of the current findings, there are, unfortunately, many residual unanswered questions. The first of these is the precise *cause* of the hypotension. In this regard, results of two recent investigations are relevant. Previous publications have shown that:-

(a) hypotension is neither associated with substantial increases in right heart pressures nor decreases in (implied) cardiac output values(54), raising the issue that the problem may relate to *inappropriate peripheral vasodilatation*.

(b) hypotension is not engendered by right ventricular involvement in TTS(184), and is therefore not analogous with hypotension complicating right ventricular infarction.

Interestingly, we have also demonstrated that TTS is associated with paradoxically low plasma concentrations of the endogenous NOS inhibitor asymmetric dimethyl arginine (ADMA) and with hyper-reactivity of platelets to the anti-aggregatory effects of NO donors(105): in combination, these findings suggest that TTS may be associated with *increases in both NO generation and effect*. Furthermore, during the early stages of TTS, there is variable release into the circulation of the endothelial glycocalyx component syndecan-1, indicating inflammatory damage to the endothelium, with resultant increase in vascular permeability(102). Thus it is possible that both excessive NO-mediated dilatation and depletion of intravascular volume may contribute to the development of hypotension. These novel pathogenetic concepts related to mechanisms underlying the development of hypotension are synthesized in Figure 3.1.3.

Figure 3.1.3: Early hypotension/shock in TTS: postulated inotropic vs non-inotropic components of pathogenesis.

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Abbreviations: β2 – β2 adrenoceptors; Gi – G-inhibitory; Gs – G-stimulatory; NOS – Nitric oxide synthase; NO – Nitric oxide; O_2^- – Superoxide; $ONOO^-$ – Peroxynitrite; TXNIP – thioredoxin interacting

protein; MMPs – Matrix Metalloproteinases; PARP-1 – Poly [ADP-ribose] polymerase 1; NAD^+ – Nicotinamide adenine dinucleotide; ATP – Adenosine triphosphate; L-NAME – L-NG-Nitro-arginine methyl ester (NOS inhibitor)

These findings argue for the use of fluid replacement as the primary therapeutic option in TTS, as opposed to the more commonly used treatment of infusion of catecholamines (a theoretically undesirable idea) and other pressor agents.

It remains far from certain why hypotension, as shown from the current analysis of a large data set, reflects not only low LVEF but also other markers of a "large attack". The first possibility is that hypotension simply reflects substantial impairment of LV contractility, and that the resultant fall in cardiac output is the main precipitant of hypotension. This seems unlikely, especially as a sole precipitant, given the apparent preservation of cardiac output(54). The second possibility is that both poor left ventricular systolic function and essentially vasodilator hypotension are products of the same stimuli, and tend to be proportionate without significant direct interaction, potentially exacerbated by impaired reflex tachycardic responses. In theory, end-organ refractoriness to the positive inotropic and vasoconstrictor effects of catecholamines(185) might contribute to such a "balanced" concordance. The third, and theoretically perhaps the most attractive possibility, is that the extent of glycocalyx shedding might represent the common pathogenetic factor, causing hypotension via fluid "leakage" and myocardial inflammation via permeabilization to monocyte and neutrophil ingress and subsequent intracardiac inflammation(103, 114). Furthermore, pleural and pericardial effusions are commonly found in TTS(64, 186), and may appear independent of the impairment in cardiac output. This idea could in theory be progressed by studying the effects of inhibitors of glycocalyx shedding, such as the non-specific matrix metalloproteinase inhibitor doxycycline(187).

Overall, there is also a strong implication that a major driver of hypotension may be inappropriate vasodilatation. Specifically, as stroke volume falls (a little), the resultant fall in blood pressure should trigger baroreceptor-mediated increases in sympathetic tone and thus heart rate and peripheral vascular resistance. The fact that this does not occur may reflect, at least in part, excessive NO-mediated vasodilatation(105), but it remains possible that, despite the high catecholamine environment in acute TTS, there is a diminution in responsiveness to both α- and β-adrenoceptor responsiveness. To date, this hypothesis has not been tested.

In the absence of identification of clinical correlates of TTS-associated hypotension which clearly identify its causes, there can be no single "message" regarding either prevention or treatment of the problem. However, if we are to assume that early hypotension results both from impaired contractility (reduced stroke volume) and systemic circulatory (inappropriate vasodilatation and increases in vascular permeability) anomalies, a therapeutic approach addressing the full spectrum of these pathogenetic factors (as shown in Figure 3) seems appropriate.

While the implications of early hypotension on long-term survival were not explored in this study, it must be noted that hypotension has both short and long-term adverse prognostic implications(183). We also did not explore the implications of variations in early modes of treatment in TTS, but none of the hypotensive patients were treated with plasma volume expansion. A further limitation is that we did not fully explore the implications of the presence/absence of LVOT obstruction(188), other than confirming its univariate association with hypotension and its relative rarity. Nevertheless, these results leave unanswered the issue of whether LVOT obstruction contributes directly to the development of hypotension, or indirectly as a component of larger TTS attacks. Finally, we elected to adopt a qualitative, rather than quantitative, approach to hypotension, because the majority of patients who developed severe hypotension were eventually treated with pressor agents.

The results of the current study show that early hypotension/shock occurs in approximately one third of TTS cases, and represents a strong predictor of in-hospital death. Hypotension appears to

reflect evolution of more severe attacks of TTS, and not to result purely from reductions in LV contractility.

It needs to be recognised clinically that the acute phase of TTS is associated not only with regional impaired LV contractility (and occasionally with LVOT obstruction), but also with important but transient extracardiac anomalies. These potentially reduce peripheral vascular resistance(105), and also cause fluid extravasation into the extravascular space(102) (accounting for development of pleural and pericardial effusions). However, to date, none of the clinical strategies utilised for management of hypotension/shock have taken this into account. Development via controlled clinical trials of effective methods for simultaneously limiting hypotension and size of TTS attacks remains a major medical priority.

3.2 Determinants of variable symptomatic recovery after acute attacks of Takotsubo Syndrome

3.2.1 Introduction

Initial reports suggested that TTS was a relatively rare condition, and in practice largely represented a "diagnosis of exclusion", made after acute MI had been excluded by coronary angiography. However, a number of factors, including better characterisation of clinical features of TTS (such as typical ECG fluctuations and the presence of marked elevation of plasma concentrations of BNP and NT-pro-BNP)(56) and the potential for making the diagnosis definitively by cardiac magnetic resonance imaging (CMR)(72), have facilitated recognition of the disorder in approximately 10% of women presenting with "MI"(37).

The early development of shock, occurring in up to 15% of cases, represents the main basis for in-hospital mortality in TTS: occasional patients also die of tachyarrhythmias or of embolism from ventricular mural thrombosis(46). However it has been considered historically that in patients surviving the first 48 hours, the prognosis for full recovery is excellent. Indeed, early reports uniformly suggested, as sole criteria of "full recovery", that left ventricular wall motion abnormalities (typically apical hypokinesis) become undetectable, with normalisation of left ventricular ejection fraction within approximately 7 days, and that the only residual concern thereafter was a 1-2% annual risk of recurrence(189, 190).

Until recently, a perplexing clinical finding was that many patients report ongoing symptoms. For example, in one series, almost half of a group of TTS patients remained symptomatic after 1 year(191). While it might have been assumed that the symptoms reflected ongoing emotional stress, evidence has gradually accumulated to the effect that such symptoms are indeed commonplace(38) and likely reflect ongoing myocardial dysfunction(71).

For example, the main pathological finding of TTS evident on CMR is extensive oedema of the myocardium. Neil et al. demonstrated that this oedema resolves very slowly, and is still detectable 3 months after the onset of attacks(73). BNP and NT-pro-BNP levels also remain persistently elevated for at least 3 months(56). Furthermore, Dawson et al utilised ^{45}P-magnetic resonance spectroscopy (MRS) to demonstrate defective myocardial energetics (represented by reduced phosphocreatine to ATP ratio) persisting for at least 4 months after attacks(83). And finally, not only does impairment of LV function, as measured by the sensitive parameter of global longitudinal strain(GLS), persist at 3 months, the degree of GLS impairment also correlates with BNP elevation and the residual impairment of quality of life(71). Indeed, the recent recognition that development of patchy myocardial fibrosis long-term(84), represents a potential basis for non-resolution of symptoms at any stage post attacks in some patients.

With increased understanding of the pathogenesis of TTS, it seems potentially achievable to develop strategies to reduce severity of myocardial inflammation and thus of attacks. The question therefore arises: can we identify early in the course of the disease patients who are likely to have marked and prolonged symptomatic impairment? In the current study, we have therefore tested the hypothesis *that severity of acute physiological disturbance predicts symptomatic status after 3 months*.

3.2.2 Methods

Patient identification, inclusion and exclusion criteria

The study protocol was approved by the local Human Research Ethics Committee. Patients who presented between 1st of March 2009 to 1st of July 2015 to three tertiary referral hospitals in Adelaide, South Australia with a clinical diagnosis consistent with TTS as per the Mayo Clinic Criteria(59), were identified. Informed consent was obtained prior to the recruitment. Baseline clinical data and patient characteristics were obtained including age, sex and comorbidities.

Comorbidities were scored via the Charlson Comorbidity Index. The absence or presence of shock were also recorded. We included all patients aged 18 years and above with definite TTS who were able to complete SF-36 questionnaires.

Investigations performed during the acute attack

Patients' venous blood samples were collected in the acute setting. Plasma concentrations of normetanephrine, NT-pro-BNP, hs-CRP, Troponin T and creatine kinase were measured. Both NT-pro-BNP and creatine kinase levels were measured daily serially to determine peak levels.

Transthoracic echocardiography was performed in all patients at acute presentation and at 3 months follow-up, using Vivid 7 ultrasound system with M3S probes (1.5-4MHz sector array transducer) (GE Ultrasound, Horten, Norway). Standard apical two-chamber, three-chamber, and four-chamber views were obtained with special attention to LV endocardial definition. Images were obtained at frame rates of 50 to 80 frames/sec. Three cardiac cycles were obtained for optimal cycle selection in the off-line analysis period, and one cardiac cycle from each image was used for the analysis. GLS values were analysed using the speckle-tracking software for Vivid (2D-strain EchoPac PC v.7.0.1, GE Healthcare, Horten, Norway). The endocardial border was manually traced, and region of interest was drawn to include the entire myocardium in all cases. Images were accepted for analysis when segments approved for speckle analysis were tracked reliably. Global strain values were estimated from the average of peak regional left ventricular strain values.

CMR was performed on 1.5 T Philips Intera and Achieva systems (Philips Medical Systems, Best, Netherlands), with a five-channel phased- array coil and electrocardiographic gating. CMR was performed in patients without any contraindications to this procedure. Black-Blood-T2-weighted images were analysed by 2 independently working medical practitioners, to determine the extent of myocardial inflammation. This was done utilising certified CMR evaluation software [OsiriX Lite

(http://www.osirix-viewer.com/)]. In each patient short axis views of the left ventricle were obtained at three levels - apex, mid and base. Endocardial and epicardial contours were traced manually in all 3 levels. Myocardial oedema was then analysed to provide the mean signal intensity (SI) score for each slice, results of which were expressed relative to the SI score of their spleen, giving a SI ratio(73). This SI score ratio was termed the T2 score. Both inter- and intra-observer coefficients of variability for this methodology were less than 2%.

Follow up

Patients were followed up at 3 months after the initial episode. During this visit, SF-36 questionnaires were completed by the patients. Components of the SF-36 were identified and recorded. As in the acute episode, venous blood samples were obtained from each patient, and their plasma NT-pro-BNP levels repeated. Follow up echocardiography was also performed at this setting and left ventricular ejection fraction and global longitudinal strain were documented.

Statistical analysis

To test the central hypothesis that the severity of the acute physiological disturbance predicts symptomatic status 3 months post TTS, correlations were sought between the physical component of the SF-36 (PCS-SF36) at 3 months with parameters of the acute attack, patients' demographics and comorbidities. Correlation analyses were performed using Pearson and Spearman correlation coefficients as appropriate. We also performed t-tests to determine whether their recovery at 3 months depended on the use of renin angiotensin system blockers or β-adrenoceptor antagonists on discharge. A multivariate backward linear regression analysis was also performed, including into the analysis parameters of the acute attack, irrespective of the results on univariate analysis.

3.2.3 Results

Patient characteristics

Of a total of 212 patients diagnosed with TTS over the study period, 47 (22%) completed a SF-36 quality of life assessment at 3 months and are therefore included in this report. Patients' clinical characteristics are summarized in Table 3.2.1.

Table 3.2.1: Patient characteristics (n=47)

Age (years; mean ± SD)		66 ± 14
Gender (M : F)		3 : 44
Previous episode of TTC (n; %)		5 (11%)
Diabetes Mellitus (n; %)		7 (15%)
Hypertension (n; %)		21 (45%)
Age-adjusted Charlson Comorbidity Index (mean ± SD)		2.7 ± 2.2
ST elevation on presentation (n; %)		18 (38%)
Initial hypotension/shock (n; %)		7 (15%)
Discharge therapy	ACE inhibitor (n; %)	26 (55%)
	Angiotensin receptor blocker (n; %)	10 (21%)
	β-adrenoceptor antagonist (n; %)	13 (28%)

Severity of acute episode

This was evaluated on the basis of (a) impairment of left ventricular ejection fraction (LVEF), (b) extent of release of biochemical markers of inflammation and necrosis, (c) plasma concentrations of normetanephrine and (d) extent of myocardial oedema on CMR.

These are summarised in Table 3.2.2, with peak values compared with normal ranges.

Table 3.2.2: Severity of acute TTC attack. Skewed data are summarized by median values and interquartile ranges unless otherwise specified. Normal reference values for age-matched population are provided.

	Acute TTC (n = 47)
Haemodynamics :-	
Minimal systolic BP (mmHg, mean ± SD)	98 ± 17
LVEF* (%, mean ± SD)	51 ± 11
Global Longitudinal Strain** (%, mean ± SD)	-13 ± 4 (normal -20 ± 0.28)
Biochemical :- (peak values)	
Hs- CRP (mg/L)	15 (6.6 - 46.25; normal <8)
NT-proBNP (ng/L)	3517 (2365 – 10599; normal <125)
Troponin T (ng/L)	340 (163 – 624; normal <29)
Creatine kinase (units/L)	174 (113 – 269; normal <150)
Catecholamine release :-	
Normetanephrine (pmol/L)	960 (650 – 1250; normal <900)
Myocardial oedema :-	
T2 score on MRI (signal intensity ratio; mean ± SD) †	0.67 ± 0.09 (normal 0.47 ± 0.04)

* LVEF was able to be determined accurately on admission echocardiography in n = 40 patients

** GLS was able to be calculated accurately in n = 29 patients (the remainder patients had suboptimal images)

† T2 score was calculated accurately in n = 24 patients (10 patients did not have a CMR, whilst the remainder had suboptimal images)

Quality of life assessment at 3 months

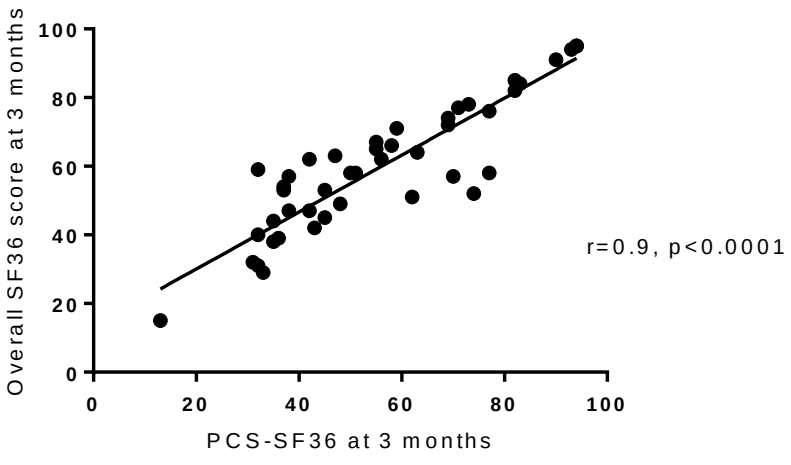
No patient died, or had an overt recurrence of TTS over the first 3 months of the study.

The primary analysis concerned the physical component scores of the SF-36 questionnaire; mean PCS-SF36 score was 56 ± 21, as against a normal mean value of 65 for the female population aged 65-74 of the region of sampling(192).

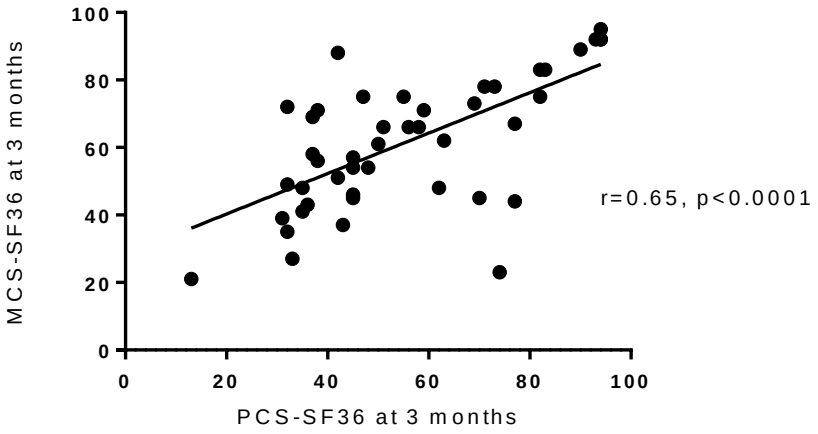
The overall SF-36 score was also impaired (mean 60 ± 19; normal mean 72)(192). There was a strong and direct correlation between PCS-SF36 and overall quality of life scores on SF-36 ($r = 0.9$, $p < 0.0001$) as shown in Figure 3.2.1. The mental component of SF-36 (MCS-SF36) also correlated with the physical component, but to a lesser extent ($r = 0.65$, $p < 0.0001$).

Figure 3.2.1: Correlation between PCS-SF36 and the overall SF-36 scores (A), Correlation between PCS-SF36 and MCS-SF36 (B) at 3 months' follow up.

A



B



Correlates of impaired physical component of quality of life at 3 months

(a) Correlations with parameters of acute attack

There were no significant correlations between the PCS-SF36 scores at 3 months, and either the minimal systolic BP on presentation (r = -0.14, p = 0.46), LVEF (r = 0.04, p = 0.79), peak plasma concentration of hs-CRP or NT-proBNP (r = -0.02, p = 0.92 and r = -0.02, p = 0.89 respectively), T2 score on acute MRI (r = -0.14, p = 0.51), plasma normetanephrine concentration (r = -0.06, p = 0.72), or markers of myocardial necrosis [Troponin T and creatine kinase (r = -0.019, p = 0.90 and r = -0.13, p = 0.37 respectively)]

(b) Correlations with patient demographics, comorbidities and discharge therapy

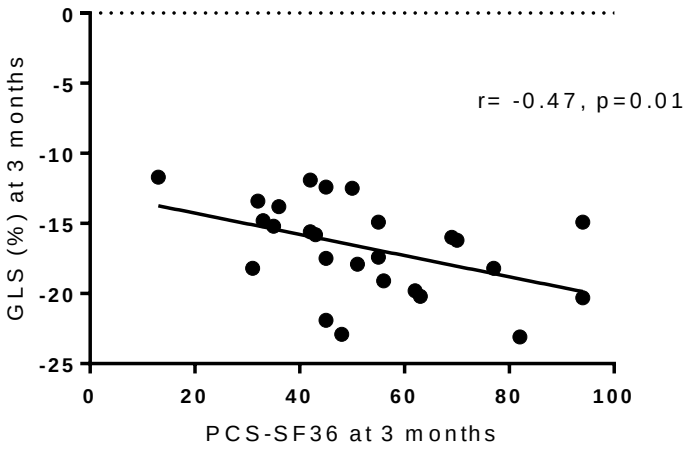
There were also no significant correlations between PCS-SF36 scores and patients' age (r = -0.20, p = 0.18), their Charlson Comorbidity Indices (r = -0.14, p = 0.34). Discharge on β-adrenoceptor

antagonists therapy, or ACE inhibitor or angiotensin-receptor blocker therapy did not significantly predict PCS-SF36 scores at 3 months. (p=0.60 and p=0.27 respectively).

(c) Correlations with LV function at 3 months

There was an inverse correlation (r = -0.47, p-value = 0.01) between GLS at 3 months and simultaneous PCS-SF36 scores (Figure 3.2.2), implying that extent of recovery of left ventricular systolic function predicted restoration of quality of life as reflected in physical activity. These results were consistent with a previous report(71).

Figure 3.2.2: Global longitudinal strain at 3 months vs PCS-SF36 at 3 months. (r=-0.47, p-value=0.01)



Multivariate analysis of predictors of impaired physical component of quality of life at 3 month

As shown in Table 3.2.3, none of the acute parameters of severity of TTS significantly predict PCS-SF36 scores at 3 months.

Table 3.2.3: Multivariate analysis. All potential covariates of PCS-SF36 score utilised in the analysis are shown.

	β	*p-value*
T2 score	*0.02*	*0.96*
Peak NT-proBNP	*0.22*	*0.48*
Peak Troponin-T	*0.25*	*0.40*
Normetanephrine	*-0.07*	*0.82*
Acute GLS	*0.17*	*0.58*
Acute LVEF	*0.04*	*0.91*
Lowest BP	*-0.46*	*0.12*

3.2.4 Discussion

Although TTS was once regarded as essentially a benign condition beyond the first 24 hours post presentation(193), that is clearly not the case: patients remain at risk for prolonged ongoing disability(73), with in-hospital mortality rates of 4.1% in a recently published large registry series(38). The bases for ongoing disability post TTS include ongoing inflammation(73), energetic impairment(83), and also subtle impairment of resting contractility(71). However it has not previously been determined whether the magnitude of the actual attack of TTS may also predict the extent of residual symptoms, as is the case after acute myocardial infarction(194).

In the current study, we confirmed that there is extreme heterogeneity among cases with regard to severity of acute attacks, whether measured on the basis of haemodynamic impairment, myocardial systemic inflammation, catecholamine release or myocardial necrosis. We also confirmed previous reports that quality of life remains somewhat impaired in many TTS patients 3 months after acute

attacks(71). We chose to evaluate putative correlations between all of the above parameters and the PCS-SF36 as determined after 3 months' recovery. In this regard, mean values for both overall SF-36 score and PCS-SF36 were marginally below age and gender-matched population means.

There was no significant correlation between PCS-SF36 values and any parameter related to magnitude of the acute attack, nor did any of these analyses approach statistical significance. This is a very important finding, effectively dissociating the extent of recovery at 3 months from the severity of the acute attack. On the other hand, PCS-SF36 was significantly and inversely correlated with concurrent GLS, consistent with previous reports(71). Thus these data suggest that the ongoing inflammation and/or energetic impairment which mediate the persistent reduction in GLS, rather than the extent of the initial episode, represent the main basis for ongoing symptomatology in TTS patients. Ultimately, this finding is consistent with the idea that the extent of myocardial necrosis during the first 24 hours post-acute MI is a major determinant of long-term extent of ventricular functional impairment, where as for TTS, there is relatively little myocardial cell death as shown from the biopsy study of Nef et al.(195), but instead ongoing and variable dysfunction of inflamed, and eventually fibrocyte-infiltrated energy-depleted myocardium.

The main caveat regarding interpretation of these data is the potential for selection bias among patients experiencing episodes of TTS. It is possible that patients with initially severe haemodynamic impairment were more likely to be excluded from the current analysis by virtue of incomplete data. Patients who passed away either during the acute episode or before the 3 month follow up period were also excluded. Furthermore, the assessment of severity of oedema on cardiac MRI occurred at a median of 5 days post onset of symptoms, which might not necessarily be the time of peak inflammation. Nevertheless, the current analysis encompasses all parameters of severity of TTS attacks currently in common clinical usage.

Ultimately these findings should impact on the development of therapeutic priorities for patients with TTS. Acutely, relevant issues include haemodynamic support, treatment of arrhythmias and

prevention of thromboembolism(46). The main relevance of the current study is that therapeutic efforts to accelerate recovery cannot be based on the severity of the acute episode of TTS. Therefore, beyond the first day of admission, it should be perceived that measures designed to accelerate reversal of myocardial inflammation and energetic impairment should theoretically take priority. In this regard, our recent finding of evidence of nitrosative stress in severe cases of TTS, with implications of activation of PARP-1(111), represents one potential therapeutic avenue. Indeed ACE inhibitors, which may ameliorate nitrosative stress(196, 197), appear to reduce recurrence risk in TTS(85). Similarly, a recent large registry report(38) documented improved outcomes among TTS patients discharged on ACE inhibitors or angiotensin receptor blockers. This therapeutic option should therefore be validated in prospective studies.

Chapter 4: Design of a randomised controlled trial to reduce attack size and to accelerate myocardial recovery in Takotsubo Syndrome

The contents in this chapter are similar to published work listed in page 15.

4.1 Introduction

As discussed in the earlier chapters, TTS represents a major cause of cardiovascular morbidity and mortality(86).

Specifically, acute attacks, typically presenting as episodes of chest pain and/or dyspnoea, can engender major problems both early and late:

Early:

TTS represents a form of catecholamine-triggered myocardial inflammation(198),which may engender lethal arrhythmias. However the main cause of in-hospital mortality is the development of severe hypotension, which causes death in 2–3% of cases(46). The mechanisms responsible for haemodynamic impairment include combinations of impaired left ventricular(LV) contractility, BNP-mediated vasodilatation(54), mitral regurgitation, and occasionally, LV outflow tract obstruction(199).

Late:

LV regional wall motion and LV ejection fraction(LVEF) usually normalise quickly(82). However, it has become increasingly clear that recovery from TTS is neither rapid nor complete, with persistent NT-proBNP elevation(56), myocardial oedema(73), persistent impairment of quality of life(71), and incomplete recovery of more sensitive markers of cardiac dysfunction such as GLS on echocardiography(71). Furthermore, myocardial energetics are impaired for at least 4 months(83), with evidence of long-term myocardial fibrosis(84). Recurrent acute attacks of TTS are also a

significant problem, occurring in 2-3% of patients per annum(85). There is also substantial long-term mortality risk, comparable to that of patients with ACS(86).

While data are currently incomplete, the initial suggestion that TTS results from multivessel coronary spasm(97) has been criticised on anatomic and coronary physiological grounds(200), but not totally excluded. Indeed, we have recently reported that TTS is associated with evidence of acute shedding of the vascular glycocalyx(102), which would likely attenuate coronary vasodilatory mechanisms. However, there is convincing evidence from both human and animal studies of both intramyocardial inflammation/oedema and cellular infiltration(110, 111), which implicates catecholamine-stimulated nitrosative stress potentially accounting for both energetic impairment and negative inotropy.

Importantly, these changes appear to resolve slowly(73), despite the transient catecholamine stimulus, raising the possibility of secondary activation of other inflammatory processes, such as the NLPR3 inflammasome, via increased expression of thioredoxin interacting protein(TXNIP)(111). Furthermore, there is increasing evidence that “large” attacks of TTS carry the most substantial long-term morbidity and mortality rates(174).

Thus, if an effective therapy for TTS is to be developed, it needs to attempt both early intervention to reduce the size of the acute attack, followed by long-term treatment to suppress ongoing inflammation and the onset of fibrosis(84).

In the NACRAM study, the design of which is described in this publication, we seek to improve the short and long-term outcomes in patients presenting with TTS by:-

Early limitation of attack size, utilising intravenous infusion of high-dose N-acetylcysteine (NAC); followed by

Acceleration of resolution of myocardial oedema and recovery of LV function, utilising the ACE inhibitor, ramipril.

We describe reasons for choice of this sequential regimen, its details, the relevant outcome measures and associated sample size calculations, together with the obstacles to be expected in a sequential double-blind placebo-controlled trial of this type.

4.2 Methods

4.2.1 Overview of study design

NACRAM is, to the best of our knowledge, the first controlled trial related to the pharmacological management of acute attacks of TTS. Specifically, it is a double-blind, placebo-controlled trial of:

Early administration of high-dose intravenous NAC to limit TTS attack severity; and/or

Subsequent oral administration of ramipril to accelerate resolution of myocardial inflammation/oedema post TTS.

NACRAM is approved by the local Human Research Ethics Committee (HREC/15/TQEH/249), and has been registered with the Australian New Zealand Clinical Trials Registry (ACTRN12616000781448).

A summary of the study design, minimal members of patients to be involved, and key investigations are shown in Figure 4.1.

Figure 4.1: Summary flow chart of the NACRAM trial design

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4.2.2 Study rationale

To date, the choice of treatments for both haemodynamic compromise early after onset of TTS and for long-term management has been idiosyncratic. For example, patients developing shock early are still often treated with catecholamine infusion, and discharged on catecholamine-potentiating antidepressants(201). However, it is now clear that a catecholamine “pulse” initiates TTS attacks(96).

Furthermore, the prolonged myocardial inflammatory activation(73) and energetic impairment(83) following TTS imply a catecholamine-independent “perpetuating” mechanism, which sometimes induces myocardial fibrosis: the latter might be seen as analogous with ongoing inflammation post-acute MI(84).

Recently, we have established three additional and potentially important aspects of the pathogenesis of TTS, partially via the use of a rodent model:-

The presence of nitrosative stress, detected via its marker 3-nitrotyrosine (3NT), within affected myocardium.

Increased infiltration of myocardium with monocytes/macrophages, together with increased myocardial content of the inflammasome activator, TXNIP. Both candidate influences prolonged inflammatory activation.

Secondary activation (via nitrosative stress) of poly(ADP-ribose) polymerase-1 (PARP-1), as a partial mediator of negative inotropic effects.

Thus, in theory, to achieve reduction in attack size and accelerated resolution of inflammation, early intervention might utilise NAC, on the grounds that it is a potent anti-oxidant which has demonstrated myocardial salvage in acute myocardial infarction (MI)(202), and sequentially an ACE inhibitor (introduced after resolution of initial hypotensive risk), on the grounds that it exerts anti-inflammatory effects and limits nitrosative stress(196).

It should also be noted that NAC acts in part as a pro-drug, releasing hydrogen sulphide (H_2S), which appears to be depleted in TTS(203).

Finally, the concept that acute glycocalyx shedding might contribute both to hypotension and to myocardial infiltration with monocytes/macrophages(204) provides an additional rationale for use of NAC as a potential means for inhibiting the activation of matrix metalloproteinases which promotes glycocalyx shedding.

4.2.3 End-point rationale

The study will have an overall objective of determining whether the sequential treatments and/or their individual components can result in improved myocardial function 3 months post TTS.

As regards each individual component of the study:

It is hypothesized that early NAC reduces the severity of TTS attacks, as measured by early CMR derived LV oedema score(73)

It is hypothesized that treatment for 3 months with oral ramipril improves recovery of the sensitive index of LV systolic function, GLS.

Secondary end-points are listed in Table 4.1. Note that correlations will be sought between early oedema score and eventual GLS, in order to evaluate the efficacy of ramipril relative to putative impact of early NAC.

Table 4.1: Study end-points for NACRAM.

	Primary	Secondary
NAC component	LV oedema score (as	Plasma normetanephrine, NT-proBNP and

	measured on CMR immediately after NAC)	hs-CRP concentrations immediately after NAC and after 3 months.
Ramipril component	Improvement in GLS over 3 months	Improvement in QoL, assessed with SF-36 at 3 months. Improvement of LV oedema score at 3 months. Improvement of plasma normetanephrine, NT-proBNP and hs-CRP concentrations at 3 months.
Overall study	Identify determinants of improvement in GLS over 3 months (multivariate analysis)	

4.2.4 Interventions

4.2.4.1 NAC

NAC can potentially be regarded as:-

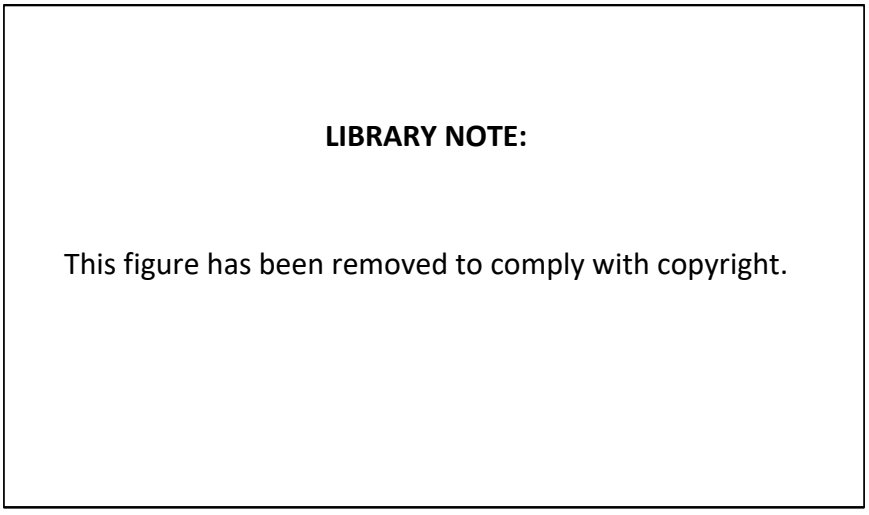
An "anti-oxidant", distinguished by substantial intracellular penetration, and selective "scavenging" of hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCI), as well as suppression of NADPH oxidase, and hence inhibition of superoxide ($O2^-$) release.

A potential source of the generation of S-nitrosothiols via combination with nitric oxide (NO), which we have shown to be abundantly released in TTS patients(105): S-nitrosothiols are potentially cardioprotective, in a partially soluble guanylate cyclase(sGC)-dependent manner.

A source of H_2S release (via breakdown to glutathione and cysteine): NAC has been shown to inhibit development of nitrosative stress which is pivotal to the negative inotropic changes in TTS.

Although it is by no means certain that restoration of H_2S availability is clinically beneficial in TTS, rodent data(203) support this possibility. Anomalies in TTS with the effects of H_2S and implications of NACRAM are summarised in Figure 4.2. Importantly, the component mediated by suppression of inflammation may be of particular importance, as increased myocardial expression of TXNIP has been documented in TTS(110).

Figure 4.2: Physiological perturbations of myocardial and vascular physiology in TTS and their potential therapeutic amelioration: implications of NACRAM.



Anomalies in TTS (shown in yellow ovals):

(i) Lack of nitric oxide synthase (NOS) suppression

(ii) $ONOO^-$ formation increased, culminating in energetic depletion

(iii) Increased NO/sGC signalling

(iv) Increased TXNIP expression, promoting inflammation

Known interactions between H_2S and NO (shown in green circles)

(1) Vasodilatation

(2) Inhibition of platelet aggregation

(3) Anti-oxidant effects

NACRAM is testing a dose of 10g of NAC given by intravenous infusion over 24 hours. This is similar to the doses previously utilised in acute myocardial infarction(205). The use of NAC in acute coronary syndromes have previously been found to reduce oxidative stress, with a trend toward more rapid reperfusion and better preservation of LV function(205). More recently early use of NAC with low dose nitroglycerine (NTG) therapy in ST elevation myocardial infarction patients undergoing percutaneous coronary intervention was associated with a reduction in infarct size and an improvement in myocardial salvage(202). In both studies, NAC use was found to be safe, with no significant differences in adverse events when compared to placebo. NAC is also widely used in other clinical situations such as paracetamol overdose, and is generally well tolerated. In contradistinction to the above studies, no NTG is infused in NACRAM, because of the substantial risk of early hypotension and because TTS represents a “high NO” environment(105).

4.2.4.2 Ramipril

Ramipril, an ACE inhibitor with proven long-term “cardioprotective” effects(206), has the potential to suppress both nitrosative stress(207) and myocardial and vascular inflammation(196, 208). A recent meta-analysis suggested that ACE inhibitor therapy reduces risk of recurrences of TTS(85). Furthermore, a large registry study has also found an association between ACE inhibitors and improved survival(38).

NACRAM is testing ramipril, given as an initial dose of 5 mg per day, and increased after 2 weeks to the maximum dose of 10 mg per day if tolerated. Ramipril at this dose has been found to reduce rates of death, myocardial infarction, and stroke in high risk patients(206).

Ramipril is widely available and commonly used for various indications. Like NAC, it is usually well tolerated. Significant adverse reactions to ACE inhibitors such as angioedema can occur, but are very rare. More commonly however, patients may develop an ACE inhibitor associated cough due to inhibition of the breakdown of bradykinin. All patients will be counselled regarding potential side effects prior to enrolment. Additionally, all patients in the ramipril component will be followed up 2 weeks following enrolment, to monitor for any potential adverse events, including hypotension or electrolyte disturbance.

4.2.5 Trial eligibility

Patients ≥18 years of age who are willing to participate, as evidenced by signing of the consent form and participation information sheet will be eligible to participate. Inclusion criteria for the study include a suspected, or confirmed diagnosis of TTS based on the International Takotsubo Diagnostic Criteria (InterTAK Diagnostic Criteria)(60). The criteria include, but are not limited to, a consistent history including symptoms of acute chest pains or dyspnoea, substantial elevation of plasma NT-proBNP concentrations, and demonstration of LV regional wall motion abnormality on cardiac imaging. Confirmation of TTS requires exclusion of relevant coronary artery disease by coronary

angiography, and demonstration of myocardial oedema and exclusion of myocardial infarction or myocarditis on CMR. Although patients can be enrolled in the study on the basis of presumptive diagnosis of TTS, they will be withdrawn if a definitive diagnosis cannot be subsequently established.

Females who are pregnant or lactating will not be eligible for the study. Exclusionary criteria for the NAC component of the study include a delay of diagnosis of >24hours after symptom onset, contraindication to NAC such as prior adverse reactions, and contraindication to magnetic resonance imaging (MRI) and gadolinium administration. Exclusionary criteria for the ramipril component include pre-existing use of ACE inhibitors or angiotensin receptor blockers (ARBs), and contraindication to ramipril including prior adverse reactions, hereditary angioedema, and severe renal impairment (defined by a calculated creatinine clearance, CrCl, of <30ml/min).

Participants will be instructed to discontinue their study medications if they develop a contraindication to the study as above, at any time points during the follow up period. However, data from all patients will be included in the intention-to-treat analyses.

4.2.6 Enrolment and randomisation

Eligible participants who are willing to participate, and have signed the informed consent form, will be randomised to either (a) NAC or placebo, (b) ramipril or placebo, or (c) both NAC or placebo and ramipril or placebo, depending on their eligibility.

Treatments will be pre-determined by a computer-generated algorithm with randomisation blocks of 10 and balanced for each of the 3 participating institutions. Randomised treatment allocations will be prepared as numbered treatment boxes containing the study drug. The study boxes will be held in the Coronary Care Unit of each of the participating hospitals, and when the decision to randomise the patient has been made, the drug pack to be used will be the next available study drug pack on the list.

4.2.7 Investigations performed

4.2.7.1 Baseline

Blood tests

Blood will be withdrawn at entry and after 24 hours for determination of (a) markers of myocardial necrosis including plasma troponin T and creatine kinase concentrations, (b) Inflammatory markers including plasma NT-proBNP and hs-CRP concentrations, and (c) plasma normetanephrine concentrations. Measurements of the above parameters will be performed by SA Pathology, the laboratory and pathology provider that services all 3 sites participating in the study.

Transthoracic Echocardiography (TTE)

TTE will be performed within the first 48 hours of hospital presentation. Standard apical two-chamber, three-chamber, and four-chamber views will be obtained with special attention to LV endocardial definition. Images will be obtained at frame rates of 50 to 80 frames/sec. Three cardiac cycles will be obtained for optimal cycle selection in the off-line analysis period, and one cardiac cycle from each image will be used for the analysis. GLS values will be analysed using the speckle-tracking software (EchoPAC v202, GE Healthcare, Horten, Norway). The endocardial border will be manually traced, and region of interest drawn to include the entire myocardium in all cases. Images will be accepted for analysis when segments approved for speckle analysis have been tracked reliably. Global strain values will be estimated from the average of peak regional left ventricular strain values. Data obtained will include LV ejection fraction, cardiac dimensions, calculated RV systolic pressure and GLS.

CMR

CMR will be performed at the respective sites of enrolment within 24 hours of completion of NAC or placebo infusion if enrolled in the NAC component, or at any time during the index presentation otherwise. CMR Black-Blood-T2-weighted images will be analysed to determine the extent of myocardial inflammation. This will be done utilising certified CMR evaluation software [CVI 42 (Version 5.10)]. In each patient short axis views of the left ventricle will be obtained at three levels: apex, mid-ventricle and base. Endocardial and epicardial contours will be traced manually in all 3 levels. Myocardial oedema will then be analysed to provide the mean signal intensity (SI) score for each slice, results of which will be compared to the SI score of their spleen, giving a SI ratio. This SI score ratio will be termed the T2 score.

SF-36 Questionnaire

All participants will complete a quality of life (QoL) questionnaire; SF-36. Different components of the SF-36 will be identified and recorded.

4.2.7.2 Follow up

All participants will be followed up at 3 months. Investigations performed at baseline including blood tests, TTE, CMR and SF-36 will be repeated at the time of follow up. Participants will also be asked about any potential adverse events.

Additionally, participants involved in the ramipril arm will also be followed up in 2 weeks. Their blood pressure and renal function including electrolytes will be checked, and adverse events documented. If deemed safe, ie not hypotensive (defined as a systolic blood pressure of <90mmHg), and without significant derangements in biochemistry such as renal function deterioration and/or

hyperkalaemia, the dose of ramipril will be doubled, and participants will receive 10mg of ramipril orally, or an equivalent placebo.

4.2.8 Analysis Plan

Treatment effects will be analysed on an intention to treat basis. Participants' baseline characteristics will be analysed to ensure that balance was achieved by randomisation.

To determine whether treatment effects differ in the active treatment arm and the placebo arm in regards to the primary and secondary end points, we will utilise unpaired t-tests in normally distributed samples and Wilcoxon-Mann-Whitney tests in those with a skewed distribution.

We will also perform univariate and multivariate analyses of early LV oedema score and GLS at follow up to assess the effect of chronic ramipril administration compared to that of early NAC administration.

The limit of statistical significance is set at $p<0.05$.

4.2.9 Statistical power

4.2.9.1 NAC component

Mean values of T2 scores previously found in TTS were approximately 0.7 ± 0.15 units. For n = 70 patients, the study will therefore have approximately 80% power to detect a reduction by 30% mean values with NAC ($p < 0.05$).

4.2.9.2 Ramipril component

Previous echocardiographic data in TTS patients revealed mean GLS values at 3 months' recovery of approximately – 18 ± 3%. With n = 80, the study has approximately 80% power to detect >2% improvement in the active treatment group, and >90% power to detect a 3% improvement at p< 0.05.

4.2.9.3 Provision for drop outs

In previous studies, NAC has been universally well tolerated except for the development of headaches when co-infused with NTG. It is expected that there will be very few if any dropouts at the NAC infusion stage.

On the other hand, development of cough as a late complication of ACE inhibitor therapy is not rare: we will anticipate up to 20% drop outs in the ramipril treatment phase, and proceed until 80 patients have completed this phase.

4.2.10 Interim analysis

The NACRAM clinical trial has now commenced recruitment, and to date, 28 participants have been enrolled in the NAC/placebo arm, and 46 participants in the ramipril/placebo arm. Study allocation remains blinded. The baseline characteristics of the participants recruited thus far are summarised in Table 4.2.

Table 4.2 Baseline demographics

	Total (n=61)	NAC/placebo (n=28)	Ramipril/placebo (n=46)

Age, years	65 ± 11	71 ± 8	63 ± 11
Gender, M:F	1:60	0:28	1:45
Prior TTS, n (%)	3 (1%)	0 (0%)	3 (7%)
Diabetes Mellitus, n (%)	6 (10%)	5 (18%)	1 (2%)
Hypertension, n (%)	18 (30%)	10 (36%)	8 (17%)
Hypercholesterolaemia, n (%)	22 (36%)	14 (50%)	13 (28%)
Charlson Comorbidity Index	2 (1.5-4)	3 (2-4)	2 (1-3)
Prior neuropsychiatric diagnosis, n (%)	20 (33%)	4 (14%)	18 (39%)
CA/CPD, n (%)	17 (28%)	7 (25%)	14 (30%)
Prior cancer, n (%)	7 (11%)	3 (11%)	6 (13%)
Primary TTS, n (%)	52 (85%)	24 (86%)	38 (83%)
Apical TTS, n (%)	38 (62%)	19 (68%)	28 (61%)
Hypotension, n (%)	20 (34%)	7 (27%)	16 (36%)
Lowest BP, mmHg	92 ± 14	93 ± 10	92 ± 15
LVEF, %	43 ± 10	43 ± 8	43 ± 9
GLS, %	-10.2 ± 6.3	-11 ± 3.5	-10.6 ± 3.4
T2 score	0.71 (0.63-0.82)	0.72 (0.63-0.81)	0.68 (0.62-0.80)
NT-proBNP [ng/L; median (IQR)]	3588 (2472-5664)	3391 (2464-5583)	3764 (2685-5762)
Troponin T [ng/L; median (IQR)]	515 (307-959)	646 (409-1002)	504 (301-970)
Plasma normetanephrine [pmol/L; median (IQR)]	816 (503-1120)	780 (505-1116)	910 (500-1210)
CRP [mg/L; median (IQR)]	5.6 (3-18.3)	3.3 (2-7.7)	8.5 (3.3-19.4)
eGFR [ml/min; median (IQR)]	83 (66-90)	76 (65-89)	89 (66-90)
SF36	57 ± 23	63 ± 25	58 ± 23

SF36-MCS	57 ± 22	65 ± 24	57 ± 21
SF36-PCS	56 ± 24	59 ± 27	57 ± 24

4.3 Discussion

To the best of our knowledge, there have been no clinical trials in TTS to date. The lack of established therapeutic options for limitation of cardiac dysfunction in TTS is a serious problem, considering TTS is neither rare or benign. With improving availability and access to multiple cardiac imaging modalities in centres worldwide, it is now possible to establish the diagnosis of TTS rapidly in most patients, and it is therefore appropriate to consider the therapeutic implications of this disorder. Furthermore, most patients with TTS experience ongoing lassitude for at least 3 months, potentially imposing a considerable burden on the community via associated health care costs and potentially inappropriate treatments(209). Hence, therapeutic intervention to accelerate recovery is clearly justified, as indeed is the use of agents to limit the extent of initial myocardial injury.

Whilst the release or administration of catecholamines or catecholamine-potentiating drugs including β_2-adrenoceptor agonists precipitates many attacks, the use of β-adrenoceptor antagonists acutely have so far been disappointing(210). Certainly, acute administration of β-adrenoceptor antagonists in TTS is contraindicated in the presence of cardiogenic shock. Cases of bradycardia-induced torsade de pointes in TTS have also been reported(79). In addition, registry-based studies involving chronic administration of β-adrenoceptor antagonists have failed to show significant benefits long term(38, 85, 211). Levosimendan, a myocyte calcium sensitiser and vasodilator, on the other hand, has shown some promising results(212-214) when administered acutely. These studies suggested an accelerated recovery of LVEF after levosimendan infusion, but many questions remain unanswered in this area.

NACRAM therefore has many potential strengths. We are testing two agents (NAC and ramipril) for the sequential treatment of TTS, in a randomised, placebo-controlled trial design. Whilst ACE inhibitors/ARBs with or without β-adrenoceptor antagonists are commonly prescribed following a diagnosis of TTS, it remains unknown whether they provide prognostically important benefits. NACRAM will help to definitively evaluate a therapeutic option, for a life-threatening condition that has so far been lacking one.

CMR has now become a major cardiac imaging modality in the diagnosis of TTS, and studies involving CMR has consistently shown persistent long-term abnormalities including myocardial oedema/inflammation(73) and energetics(83). NACRAM has therefore been designed to assess whether these parameters improve with treatment. As compared to prior studies in TTS assessing LVEF as a clinical end-point, NACRAM will utilise a more sensitive parameter for myocardial systolic function, GLS, which has been shown in TTS to be impaired in spite of the normalisation of LVEF(71). Furthermore, NACRAM will also assess improvements in quality of life, and ultimately, determinants of improvements in cardiac function in 3 months.

However, there are some limitations to the NACRAM trial design. Firstly, there are several factors that may negatively affect recruitment rates. For example, patients in circumstances where there had been a delay to diagnosis, whether or not they presented to the hospital late (>24 hours after symptom onset) would be excluded. Common pre-existing use of ACE inhibitors for various indications may also potentially slow recruitment. Secondly, a study of this size and duration is not designed, or powered adequately to assess differences in mortality and recurrence rates on NAC and/or ramipril. Nevertheless, NACRAM will provide important information regarding their extent of recovery of myocardial oedema and systolic function, both prognostically important markers(215, 216).

Chapter 5: Conclusion and future directions

The main objectives of this thesis were to investigate: (1) precipitants for both ACS and TTS, (2) the impacts of variability in attack severity and rate of myocardial recovery in TTS, and (3), pharmacological strategies for limiting severity of acute attacks, as well as accelerating the recovery in TTS.

In relation to the first objective, we evaluated the relationships between ambient temperature, fine particle ($PM_{2.5}$) pollution and proximity to bushfires on incidence of both ACS and TTS during the warmer months of the year in Adelaide, Australia (Chapter 2.1). We found that elevated temperature, both in isolation, and especially in combination with increased atmospheric $PM_{2.5}$ concentration and the presence of nearby bushfires, represent increased risks for simultaneous onset of ACS. Surprisingly, with the caveat of smaller numbers, TTS risk appears to be independent of these factors. These data are especially important in light of the emergence of climate change and global warming as cardiovascular risk factors.

We also evaluated the association between administration of exogenous catecholamines, and of catecholamine-potentiating drugs and occurrence of TTS, utilising data from a large TTS registry (Chapter 2.2). We demonstrated that TTS may have an at least partially iatrogenic basis in about 15-20% of cases. Such cases are, not surprisingly, associated with particularly elevated acute catecholamine metabolite concentrations, but behave clinically in a similar way to the remainder of a large TTS cohort, including substantial long-term mortality. The major thrust therefore, is that the induction of TTS by exogenous catecholamine administration for example, is not just a transient minor complication, but rather, a potentially life threatening problem, in both the short- and long-term.

The second objective was to evaluate the impacts of variability in attack severity and rate of myocardial recovery after acute TTS. Again, utilising a large TTS registry, we first evaluated the predictors and impacts of hypotension in TTS (Chapter 3.1) followed by the acute determinants of quality of life status at 3 months post TTS (Chapter 3.2). We showed that approximately one third of TTS patients develop early hypotension/shock, and that this occurrence is a strong predictor of in-hospital mortality. The occurrence of hypotension was associated with other markers of extensive evolving TTS attacks. We also provided further evidence that TTS is a slowly resolving disorder, with residual impairment of both myocardial function and quality of life 3 months post onset. Uniquely, quality of life status at 3 months was independent of severity of acute episodes. The implication is that it is likely other factors such as extent of ongoing myocardial inflammation and/or development of myocardial fibrosis, are principal modulators of speed of recovery. In this regard, our findings provide an additional basis for prospective randomised evaluation of anti-inflammatory/antifibrotic agents such as ACE inhibitors, angiotensin receptor antagonists or aldosterone antagonists, as means for accelerating myocardial recovery. It must be recognised, however, that while the findings of this study excluded a possible pivotal role for extent of acute haemodynamic disturbance, catecholamine concentrations in blood, or formation or NT-proBNP as predictors of late symptomatology, we were unable to examine the potential relevance of the extent of acute glycocalyx damage and resultant monocyte transmigration into the myocardium.

Therefore, the overall thrust of the investigations described in this thesis has been to focus on a number of “catecholaminogenic” factors on incidence, severity and recovery in the context of TTS. Overall, these data suggest that extent of pulse catecholamine exposure is only one of many stimuli for disease precipitation, and reinforce the concept that acute hypotension is a basis for alarm early in the clinical course of TTS, but on the other hand that TTS is “a disease of two halves”, with events beyond the acute phase, such as slow myocardial and symptomatic recovery, poorly linked with the acute pathophysiology of the disorder.

All of these findings have potential therapeutic implications, including the possibility that the optimal treatment of TTS might include separate approaches to the first 48 hours post onset as against long-term care. In Chapter 4, the design of the NACRAM trial, which addresses both acute and chronic therapeutics for TTS is described. At the time of thesis submission, 28 of the planned 70 patients in the N-Acetylcysteine(NAC)/placebo arm, and 46 of the planned 80 patients in the ramipril/placebo arm have completed the trial, and treatment allocation remains blinded. However, the theoretical significance of this study is undiminished by the other studies conducted in the context of the thesis.

5.1 Future directions

With reference to this thesis, the following issues remain:

Chapter 2: Risk factors for Acute Coronary Syndromes and Takotsubo Syndrome

An intriguing precipitant of ACS and TTS not studied in this thesis is their occurrence in the peri-operative setting. Whilst the term peri-operative "MI" have been used not uncommonly, it is likely that a substantial proportion of these patients had TTS. Indeed, post-mortem studies of patients with fatal post-operative "myocardial infarction" actually demonstrated that a large proportion of patients have no evidence of coronary arterial plaque rupture or coronary thrombosis, and this dissociation has also been observed in catheterisation data(217). Perioperative development of TTS has been described only spasmodically thus far(218), but it is appropriate that detailed prospective studies now be undertaken, with a view to better understand the incidence, mechanisms and risk factors, and developing effective preventative options for such patients. It is ironic to recall the very chequered history of intervention trials in the context of "perioperative myocardial infarction", most of these utilizing β-adrenoceptor antagonists (which are probably ineffective for TTS).

With regard to risk factors for TTS specifically, a major issue relates to the preponderance of the female gender, particularly those aged 50 and above, in TTS. Less than 10% of TTS cases are men, and these cases include a substantial proportion of "secondary" TTS(46). Whilst there have been speculative and conceptually incomplete theories on the causes of this finding, such as a protective role for oestrogen(219), there are currently no good quality animal studies exploring gender-specific aspects of this disorder, TTS has been reported in women despite post-menopausal oestrogen therapy and no abrupt postmenopausal spike in incidence of TTS has been documented. However, future research studying gender differential thresholds for β2 aberrant signalling may progress understanding in this matter.

The concept of TTS as a paraneoplastic phenomenon also merits further investigation. Recently, it has been suggested that TTS is often associated with presence of malignancy at the time of presentation(220). In fact, when compared to acute MI, TTS patients are at least 3 times more likely to have known antecedent malignancies, with breast cancers a particularly common association(220). Furthermore, it appears that a prior history of malignancy is an independent predictor of long-term mortality(89, 221). Recently published findings from our registry have demonstrated that TTS patients with antecedent malignancy (but not necessarily cancer chemotherapy) have more complicated in-hospital stays and a marked increase in late mortality rate, not only reflecting late risk associated with malignancy, but partially due to increased late cardiovascular mortality(92). These findings suggest some commonality of pathogenesis between TTS and malignancies, and should be further interrogated in future studies. There is also the question of whether this association may translate to practicalities regarding therapeutic strategies: perhaps all patients with breast cancer should be considered to be at increased risk for TTS.

Chapter 3: Natural history and pathophysiology of Takotsubo Syndrome

We have now demonstrated that the development of hypotension in TTS represents a common, and life-threatening complication of TTS. However, the exact pathophysiology of this complication remains incompletely understood. The exact role of the endothelial glycocalyx in TTS has not been established so far, apart from prior studies demonstrating increased "shedding" of the endothelial glycocalyx acutely, which may contribute to increased vascular permeability, and subsequent development of pulmonary oedema, as well as pericardial and pleural effusions and intravascular volume depletion. Future research is required to further assess the role of the endothelial glycocalyx in TTS, especially as it pertains to hypotension. For example, this could be performed by measuring the extent of "intact" endothelial glycocalyx systemically using methodology previously described(222) in TTS patients with and without hypotension. In addition, this hypothesis could also be tested by inhibiting the shedding of the endothelial glycocalyx in TTS, such as with the use of a broad spectrum matrix metalloproteinase inhibitor like doxycycline(187).

Chapter 4: Therapeutics for Takotsubo Syndrome

In regards to therapeutic options for TTS specifically, there has certainly been a lack of consensus on treatment options since its recognition 30 years ago, despite being a condition that is not only common, but also debilitating and potentially life threatening. To address this, we proposed and initiated a blinded, placebo-controlled randomised clinical trial, evaluating the effects of early infusion of NAC and subsequent treatment with the ACE inhibitor ramipril on the extent of myocardial inflammation, and recovery of LV systolic function in patients with TTS.

In addition to the need to identify patients at risk of, to prevent and ultimately to treat patients with acute TTS attacks, attention also needs to be given to prevent its recurrence, which occurs in about 1-2% per year. Whilst Singh et al.(85) had shown in a meta-analysis that use of ACE inhibitors is associated with a lower recurrence rate, the results were based wholly on observational or retrospective data. Future long-term, prospective studies would help provide more definitive, long

term treatment options for TTS patients, for example, with the administration of ACE inhibitors, or with omission of catecholamine-potentiating drugs.

Finally, the management of TTS should also focus on non-pharmacological therapy. Not only do symptoms following TTS resolve slowly, there is increasing evidence that patients feel dissatisfied, and unsupported by medical professionals post TTS attacks(223, 224). Given the increasing importance of allied health practitioners in the management of many other cardiac conditions, there is no better time to conduct a prospective clinical trial, assessing the efficacy of clinical nurse practitioner involvement, and cardiac rehabilitation post TTS.

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Addenda/Corrigenda

Following comments from the Examiners of this thesis, the following additions and corrections have been made:-

1. Limitations to analyses of clinical significance of early hypotension: Examiner 1 correctly points out that the analyses in this thesis were based upon the fact that of the 8 in-hospital deaths, all occurred in patients who developed early hypotension. While univariate analyses suggest that early hypotension represents a risk factor for early mortality, the limited numbers of early deaths in this series precluded the performance of multivariate analysis for this data set. Therefore it remains possible that factors partially associated with hypotension, such as administration of catecholamines, extent of glycocalyx shedding, and/or impairment of small vessel rheology, might have actually represented a closer association with risk of early mortality. Irrespective of extent of correlation of mortality risk with these various putative associations, the limitations of valid conclusions remain essentially predictive rather than definitive evidence of causation.
2. In discussion of the association of TTS with antecedent cancer, I regret failing to quote the following important publication: Tornvall P et al (2019): Prevalence and cumulative incidence of cancer, and mortality of patients with Takotsubo Syndrome, with focus on the index event. QJMed 112:861-7. This has now been added, see reference 221.
3. In discussing the background for the design of the NACRAM trial, which involves the administration of ramipril or placebo to patients post TTS, I did not adequately describe evidence for the anti-inflammatory effects of ACE inhibitors. In fact, there is an extensive literature revolving around the pro-inflammatory role of stimulation of the renin-angiotensin-aldosterone system. I would like to add the following publication: Kortekaas KA et al: ACE Inhibitors potently reduce vascular inflammation, results of an open proof-of-

concept study in the abdominal aortic aneurysm:PLOS One 2014:9 e11192. See reference 208.

4. Other ongoing therapeutic intervention studies in TTS: In addition to the ongoing NACRAM study, Clinical Trials.Gov currently lists 42 interventions in TTS, many of which appear to be ongoing. Importantly, NACRAM is unique among these interventions, in that it represents a double-blind randomised trial, in this case with a sequential 2X2 design.