Tako-Tsubo (Stress) Cardiomyopathy: Pathophysiology and Natural History.

By

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Abstract

Introduction. Tako-Tsubo cardiomyopathy (TTC), also known as apical ballooning syndrome, is a recently described form of acute cardiac dysfunction of uncertain pathogenesis, which occurs with greatest frequency among post-menopausal women. Presentation generally mimics that of an acute myocardial infarction (AMI) but is independent of the presence of fixed coronary artery disease and is classically preceded by severe stress. While patients with TTC with ST elevation are typically diagnosed at emergent cardiac catheterization, the majority does not exhibit initial ST elevation. It is not known whether TTC can be reliably distinguished for AMI non-invasively on the basis of clinical and laboratory tests. Although there is considerable uncertainty about the pathogenesis of TTC, pronounced catecholamine release and an acute inflammatory process are implicated. Systolic dysfunction most commonly affects the apex of the left ventricle and has generally been considered self-limiting and fully reversible. Although obvious hypokinesis resolves and left ventricular ejection fraction tends to return to normal, data that challenge this view include abnormal elevation of natriuretic peptide concentrations, 3 months from the index event, together with the late persistence of some inflammatory cells on LV biopsy.

Methods. In three experimental chapters, this thesis examines aspects of (a) diagnosis (b) pathogenesis and (c) recovery, in a cohort of 125 TTC patients (mean age 67 years; 95% female). As regards diagnosis, it was hypothesized that an arbitrarily derived 'TTC score', incorporating NT-proBNP levels, might facilitate early differentiation from a cohort of females with AMI (n = 56; mean age 70 years). The primary comparison was based on data available at 24 hours post-admission. In a subset of 49 TTC patients, acute multisequential

cardiac magnetic resonance imaging was performed and repeated at 3 months. Pathogenetic investigations:- Extent of oedema was quantified both regionally and globally from T_2 weighted images, with comparison to data from 10 age-matched female controls. Correlations were sought between oedema and the extent of hypokinesis, catecholamine release, N-terminal proBNP release and markers of systemic inflammatory activation. Functional recovery was assessed via 2D speckle-tracking echocardiography (n = 36) and 15 patients, ≥1 year from their index TTC admission, underwent T_1 mapping via CMR in order to address the question of whether residual fibrosis is present after TTC.

Results.

A. Diagnosis: TTC scores were significantly different (TTC group median was 4, vs. 2 in the ACS group; $P < 0.0001$). Receiver operator curve analysis demonstrated an area under the curve (AUC) of 0.74 ($P < 0.0001$), with 62% sensitivity and 75% specificity for a score ≥4; when stressor exposure was scored in both groups, AUC was 0.89 ($P<0.0001$), with 78% sensitivity and 82% specificity (TTC score ≥4). The TTC score separated groups when haemodynamic compromise was absent (AUC 0.80, $P<0.0001$), but not when hypotension or heart failure were evident (P = NS).

B. Pathogenesis: In the acute phase of TTC, T_2-weighted signal intensity was greater at the apex than at the base ($P < 0.0001$) but was nevertheless significantly elevated at the base ($P < 0.0001$), relative to control values; over three months, T_2-weighted signal decreased substantially but remained abnormally elevated ($P = 0.02$). Regional extent of edema correlated inversely with radial myocardial strain. There were also direct correlations between global T_2-weighted signal and plasma normetanephrine ($r=0.33$, $p=0.028$), peak NT-proBNP ($r=0.40$, $p=0.0045$), C-reactive protein ($r=0.34$, $p=0.023$) and troponin T release ($r=0.29$, $p=0.045$).

C. Recovery: Patients exhibited lower global longitudinal strain than controls [mean 17.9 ± 3.1 (SD)%, versus 20.3 ± 1.6; $P = 0.0057$], but did not differ significantly from controls in values of apical twist. Three month global longitudinal strain correlated with the extent of residual NT-pro-BNP elevation ($r=0.38$, $P=0.027$), but did not correlate with markers of the acute severity of the TTC attack. Finally, patients with a remote history of TTC,

demonstrated significant intramyocardial fibrosis (V_e = 0.24), versus controls (V_e = 0.21, P = 0.013), but extent of which was not correlated with global longitudinal strain.

Conclusions. (1) The TTC score, while not of itself diagnostic, may facilitate the differentiation of TTC in patients with presumed ACS, but with diminished efficacy in the presence of haemodynamic compromise. (2) TTC is associated with slowly resolving global myocardial edema, the acute extent of which is correlated with regional contractile disturbance and acute release of both catecholamines and NT-proBNP. (3) Imaging data after TTC indicate that, at 3 months, recovery is substantial, but not complete; at ≥1 year there is evidence of diffuse interstitial myocardial fibrosis. Further efforts to expedite diagnosis, delineate pathogenesis and evaluate residual disability may assist in the development of appropriate treatment regimens.

Declaration

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

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Christopher J. A. Neil, 17th December 2012

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Statement of contribution to research

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

Execution

I performed all the recruitment and organization of patients into the studies, with the assistance of Ms Jeanette Stansborough (research nurse). I collected all clinical data, also with assistance from Ms Stansborough. I performed echocardiographic studies and cardiac magnetic resonance scans at The Queen Elizabeth Hospital. Dr Yuliy Chirkov performed the platelet aggregometry studies. Metanephrine assays were performed by Dr Malcolm Whiting at SA Pathology, Adelaide. Collagen biomarker assays was performed by Dr Michael Metz at ClinPath, Adelaide.

Analysis

All data were collated and analyzed by myself. Inter-observer analyses were performed with Dr Thanh Ha Nguyen, Mr Matthew Chapman and Ms Tharshy Pasupathy.

List of published studies

This thesis is based in part on the following original studies, which exist in published form:

1. Nguyen TH, Neil CJ, Sverdlov AL, et al. N-terminal pro-brain natriuretic protein levels in takotsubo cardiomyopathy. The American Journal of Cardiology 2011, 108, 1316-1321.

2. Neil CJ, Nguyen TH, Sverdlov AL, et al. Can we make sense of takotsubo cardiomyopathy? An update on pathogenesis, diagnosis and natural history. Expert Rev Cardiovasc Ther 2012, 10, 215-221.

3. Neil CJ, Nguyen TH, Kucia A, et al. Slowly resolving global myocardial inflammation/oedema in Tako-Tsubo cardiomyopathy: evidence from T_2-weighted cardiac MRI. Heart 2012, 98, 1278-1284.

4. Neil CJ, Chong CR, Nguyen TH, Horowitz JD: Occurrence of Tako-Tsubo cardiomyopathy in association with ingestion of serotonin/noradrenaline reuptake inhibitors. Heart, Lung & Circulation 2012, 21, 203-205.

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List of abbreviations used

MI	Myocardial Infarction
STEMI	ST-Elevation Myocardial Infarction
NSTEMI	Non-ST-Elevation Myocardial Infarction
TTC	Tako-Tsubo Cardiomyopathy
ACS	Acute Coronary Syndrome
LV	Left Ventricle
CAD	Coronary Artery Disease
TIA	Transient Ischaemic Attacks
ANP	Atrial Natriuretic Peptide
BNP; NT-proBNP	B-Type Natriuretic Peptide; Amino-Terminal Prohormone Of BNP
ET-1	Endothelin 1
NPR-C	Natriuretic Peptide Receptor C
cGMP	Cyclic Guanosine Monophosphate
AR; βAR	Adrenoceptor; Beta-Adrenoceptor
SR	Sarcoplasmic Reticulum
SERCA	Sarco(Endo)Plasmic Reticulum Calcium ATPase
NET	Norepinephrine Transporter
COMT	Catecholamine O-Methyl Transferase
MAO	Monoamine Oxidase
VMA	Vanillylmandelic Acid
HPLC	High Performance Liquid Chromatography
LVEF	Left Ventricular Ejection Fraction
WMSI	Wall Motion Score Index
PCWP	Pulmonary Capillary Wedge Pressure
LGE	Late Gadolinium Enhancement
CMR; CE-CMR	Cardiovascular Magnetic Resonance; Contrast-Enhanced CMR
SPECT	Single Photon Emission Computed Tomography
SNT	Sympathetic Nerve Terminal
LVOT	Left Ventricular Outflow Tract
LAD	Left Anterior Descending
PDA	Posterior Descending Artery
NOS	Nitric Oxide Synthase
Pl3K	Phosphoinositide 3-Kinase
PKB	Protein Kinase B
PARP	Poly-ADP Ribose Polymerase
GRK5	G Protein-Coupled Receptor Kinase 5
LPS	Lipopolysaccharide
TNF-α	Tumour Necrosis Factor Alpha
IL	Interleukin
SPAIR	SPectral Attenuated Inversion Recovery
SENSE	SENSitivity Encoding
T_2-W SI	T_2-Weighed Signal Intensity
2DS	2D-speckle tracking
V_e	Extracellular volume

Chapter 1

Literature review

1.1 Acute myocardial injury: ischaemic and non-ischaemic causes

Within contemporary medicine, perhaps no single condition has as great an impact on global health, or has prompted so concentrated a research effort, than acute myocardial infarction (MI) (Murray and Lopez, 1997, Ferreira, 2010). Although descriptions of other manifestations of ischaemic heart disease, such as angina pectoris (Heberden, 1772), preceded it, the first descriptions of acute MI emerged independently from two Russian authors in 1910 (Obraztsov and Strazhesko, 1910) and from Herrick, who in 1912 observed clinical and electrocardiographic changes in six patients, attributing these to sudden occlusion of a coronary artery, based on autopsy findings in one (Herrick, 1912). Paul Dudley White astutely observed the unity of this sudden presentation, with angina as described by Heberden, in that both proceeded from underlying atherosclerotic plaque (Davies, 1996); vasospasm, however, as described by Prinzmetal, constitutes an additional mechanism, with or without associated non-occlusive atheroma (Prinzmetal et al., 1959). The explosion of knowledge that has followed these observations, resulting ultimately in a reduction of the lethality of acute MI, would likely have fascinated each of these clinicians.

In large part, this progress may be attributed to (i) the scale of the problem, in epidemiologic and economic terms, (ii) the relative simplicity of clinical features, extensively corroborated by human pathological studies and (iii) an ischaemic mechanism of injury, which can be easily recapitulated for study in experimental animals. Parallel developments in clinical enzymology (La Due and Wroblewski, 1955, Vincent and Rapaport, 1965, Freeman and Opher, 1965, Konttinen and Somer, 1973) contributed to landmark clinical diagnostic criteria (Committee, 1959, Armstrong, 2008, Alpert et al., 2000, Thygesen et al., 2007). Not to be overlooked, the time-dependence of the efficacy of MI treatment (Gersh et al., 2005), has added significant impetus to the early diagnosis of acute MI.

Therefore, the concept that myocardial ischaemia and MI represent the most important cause of new onset chest pain is both epidemiologically sound and medicolegally compelling. The use of release of markers of myocardial cell death (such as creatine kinase, it MB isoform, and troponins I and T) for the diagnosis of MI has likely reinforced

the notion that such release is *synonymous* with acute MI and, thus, necessarily the result of an ischaemic process, and undermined the consideration of other non-ischaemic causes (Jeremias and Gibson, 2005, Ammann et al., 2004). Indeed, although clinicians recognize this problem, even in the most recently published 'universal definition' of MI, such biomarker elevations are effectively characterized as infarcts (Thygesen et al., 2007).

However, it is important to recognize, on the basis in the first instance of isolated tissue data, that myocardial cell death may be apoptotic, rather than necrotic (La Vecchia et al., 2000). Over the last 10 years, a large number of conditions in which coronary artery disease may be absent, have been variably associated with episodic myocardial cell death, possibly of apoptotic origin (see Table 1.1). Some of these may also be association with chest pain, indistinguishable from that of ischaemia (Jeremias and Gibson, 2005).

Therefore at present the diagnosis of *ischaemic* myocardial injury cannot purely be based on the finding of injury *per se*, but the differentiation between infarction and non-ischaemic cellular death remain a substantial challenge. One area of particular difficulty – and also of considerable interest – has been the entity that constitutes the subject of this thesis: Tako-Tsubo cardiomyopathy.

1.2 Tako-Tsubo cardiomyopathy as a non-ischaemic acute cardiac injury

Tako-Tsubo cardiomyopathy (TTC), also known as stress cardiomyopathy or apical ballooning syndrome, is a unique disorder of myocardial function, of uncertain pathogenesis. Once regarded as a rare form of reversible left ventricular (LV) dysfunction, TTC is now known to account for a substantial proportion of patients with presumed acute coronary syndrome (ACS) (Dib et al., 2009b, Parodi et al., 2007). TTC was only recognised as a distinct disorder in a 1990 series by Japanese investigators (Sato, 1990), describing apical left ventricular dysfunction without obstructive coronary disease. Periapical contractile deficit is classical, but predominantly mid-ventricular (Cacciotti et al., 2007) and purely basal (Copetti et al., 2007) patterns are now well described. Despite a high incidence of an antecedent psychosocial stress, the syndrome also appears to be precipitated by concomitant severe physical illness (Gianni et al., 2006b, Sharkey et al.,

2010b) and can occur without an obvious stressor. The last 20 years has seen TTC progress from the subject of occasional case reports into an established diagnostic category (Maron et al., 2006) (International Classification of Diseases code 429.83) and the focus of over 2,000 manuscripts annually (Shao et al., 2012) (see Figure 1.1).

No single clinical or electrocardiographic feature is pathognomonic. Thus, diagnosis currently rests on conformity to accepted criteria, including the exclusion of obstructive coronary disease (Bybee et al., 2004a, Madhavan and Prasad, 2010a). The majority of clinical series demonstrate abnormal catecholamine levels, during the acute phase of TTC (Nef et al., 2010a, Wittstein et al., 2005), lending support to their role in this disorder; however, evidence for a causal role of this elevation is currently incomplete. Among the other mysterious aspects of TTC, are its predilection for post-menopausal females, who account for approximately ~90% of cases (Bybee KA, 2004, Gianni et al., 2006b, Nef et al., 2010a, Sharkey et al., 2010b, Wittstein et al., 2005) and the bases for the diverse patterns of regional contractile deficit (Cacciotti et al., 2007, Copetti et al., 2007), for which no satisfactory structure-function relationship has thus far been proposed.

In contrast to myocardial infarction, myocardial necrosis is usually minimal and treatment is supportive, in the expectation of rapid LV contractile recovery (Bybee et al., 2004a, Gianni et al., 2006a). This, together with an overall low incidence of acute-phase complications and mortality, has tended to reinforce a benign view of TTC natural history, typified by such appellations as “a sheep in wolf’s garb” (Dhar et al., 2007). However, early risks include *torsade de pointes* (Samuelov-Kinori et al., 2009a) and hypotensive shock (Wittstein et al., 2005), with or without dynamic obstruction to LV outflow (Thorne et al., 2007, Nunez-Gil et al., 2009). A substantial risk of late recurrence also exists (Elesber et al., 2007). Furthermore, aspects of recovery, such as residual symptomatology and the potential for subtle persistent LV dysfunction have been largely overlooked.

In this chapter, TTC will be reviewed in detail, as a basis for experimental chapters dealing with aspects of diagnosis, acute pathogenesis and the long-term impact of this condition on myocardium.

1.2.1 Historical aspects

The term “Tako-Tsubo-like cardiomyopathy” (TTC) was coined by Sato et al in 1990 (Sato, 1990) in a text-book chapter dedicated to the description of a newly recognized and unexplained phenomenon; the same group then published a series of five female patients, four of whom developed the condition after severe emotional stress, the following year in the English literature (Dote et al., 1991). As is now well known, the Japanese term ‘Tako-Tsubo’ denotes the resemblance between the characteristic shape of the LV in systole, as imaged in the acute-phase of the disorder, to a pot used for trapping octopuses.

In 1997, Pavin *et al* reported two similar cases in European women, and made a salient connection to earlier literature, by applying the term ‘stress cardiomyopathy’ (Pavin et al., 1997). In a histopathological study, Cebelin and Hirsh (Cebelin and Hirsch, 1980) had coined this expression to describe 11 cases of sudden cardiac death, preceded by severe emotional distress, in whom myofibrillar degeneration could be identified. A 1986 New England Journal of Medicine case report, which described a female patient apparently suffering acute heart failure as a result of severe emotional stress (1986), is among several probable reported examples of this phenomenon, which predate the Japanese experience. The first North-American case report, demonstrating the classical appearance of TTC on left ventriculography, carried the evocative title, ‘A Broken Heart’, resulting in a synonym, ‘Broken Heart Syndrome’ (Brandspiegel et al., 1998), whereas the first American series was particularly important in that it established the occurrence of this disorder in the context of critical illness, as distinct from emotional stress alone (Sharkey et al., 1998).

Under these various titles, numerous case reports and several influential case series were subsequently published: notably, a large Japanese multicentre series (Tsuchihashi et al., 2001), the first European series(Desmet et al., 2003), several small but highly descriptive studies by Akashi and colleagues (Akashi et al., 2003, Akashi et al., 2004b, Akashi et al., 2002, Akashi et al., 2004a), a case series/systematic review from the Mayo Clinic, offering the first clinical diagnostic criteria (Bybee et al., 2004a) and popularized the term ‘Apical Ballooning Syndrome’. As will be discussed, distinct variants of regional LV dysfunction have since been described and are named according to the site of maximal contractile deficit: the basal variant (also known as ‘inverted TTC’ or the ‘artichoke heart’) (Bonnemeier et al., 2007, Copetti et al., 2007, Nanda et al., 2007) and mid-ventricular

variant (also dubbed the 'onion-shaped heart') (Botto et al., 2008, Cacciotti et al., 2007, Brunetti et al., 2008): these are now extensively described and generally believed to be akin to TTC, partly on the basis of a shared clinical and histopathological profile (Marechaux et al., 2008).

In summary, via a long process, clinicians worldwide have been inducted into the recognition of a unique cardiac disorder. In keeping with this, the 'rising incidence' of TTC is likely to be a function of the diagnosis of cases that would previously have been misclassified as MI, threatened MI or unstable angina. Whilst arguments have been made for or against the applicability of each of these terms (de Gregorio et al., 2009, Akashi et al., 2010, Nef et al., 2010b), given the variability LV regional expression beyond apical involvement and occasional absence of any identifiable antecedent stressor, the term TTC will be preferred in this thesis, to denote the whole spectrum of the disorder.

1.2.2 Differentiation from myocardial infarction: early studies

A central issue concerning TTC has always been the 'infarct-like' presentation of the disorder. In approximately 80% of patients in most series before 2005 (Bybee et al., 2004a, Gianni et al., 2006a), the initial ECG showed ST segment elevation, prompting treatment of patients as acute MI. Historically, therefore, the diagnosis of TTC has been in large part contingent on the initial exclusion of MI. The evolution of information and its application in this regard is discussed below.

1.2.3 Evolution of definition

The 1959 World Health Organization definition of acute MI was pivotal for prospective research into this condition and in its treatment, in unified terms (Committee, 1959). Nevertheless, it was formulated at a time when decades of clinical experience and study were available. In contrast, clinical definitions and diagnostic criteria for TTC were advanced prior to concentrated prospective study, with clinical and pathological aspects of the syndrome remaining to be elucidated. The dominant set of diagnostic criteria are

those proposed by the Mayo clinic, initially in 2004 (Bybee et al., 2004a) and subsequently revised in 2008 (Prasad et al., 2008) and 2010 (Madhavan and Prasad, 2010a, Hurst et al.). Japanese (Kawai et al., 2007) and Italian (Novo et al., 2008) authors have also contributed to this discussion. In common with other attempts, the "Mayo criteria" propose four categories of diagnostic information, with each being required to satisfy a diagnosis (see Table 1.2).

Thus, in the current (2010) Mayo-proposed definition of TTC (Table 1.2), two of the four proposed components of the diagnostic process involve the exclusion of other disease states (acute MI, phaeochromocytoma and myocarditis), one involves ECG changes and biomarkers anomalies, which are indistinguishable from those of MI and only the specification of the regional distribution of left ventricular hypokinesis carries any positive aspect of diagnostic specificity. Although authors have questioned the validity of these criteria from various perspectives, (Burgdorf et al., 2008b, Caforio et al., 2009, Gaibazzi et al., 2009), the more difficult question is, *can we supply a better 'gold standard' of diagnosis*?

1.2.3.1 The problem of 'mandatory' cardiac catheterization

By requiring the exclusion of 'obstructive' CAD, the Mayo criteria make TTC, to a large extent, a diagnosis of exclusion. To some extent this reflects the historic practice of diagnosing TTC *a posteriori*, in patients catheterized for suspected unstable coronary disease, at a time when (arguably) TTC would very seldom have been considered *ahead of angiography*. However, as a function of improved clinical pattern recognition, *a priori* consideration of TTC is now clearly possible. The implicit argument that MI is a more serious condition, probably also underpins an approach based on exclusion: the chance of missing evolving MI, is minimized, and anti-atherosclerotic therapies can be rationally prescribed. On the other hand, criteria for the diagnosis of ACS/MI (see section 1.1) have always been *inclusive*, rather than exclusive, calling for identification by clinical, electrocardiographic and biomarker evidence, whilst catheterization is discretionary.

It is highly likely that the evolution of acute angiographic practices in cardiology have brought about the approach to diagnosis based on 'negative' features (i.e. lack of explanatory obstructive CAD). A comparison with the entity of cardiac injury associated with subarachnoid haemorrhage (which likely often conforms to a diagnosis of TTC, as discussed in Section 1.3.2.4) is noteworthy, in that this syndrome can be acceptably diagnosed without coronary angiography, on a probabilistic basis, incorporating 'positive' features recognized in the syndrome. This condition was described earlier than TTC and prior to widespread mandatory catheterization practices in ACS (Mehta etc). Thus, although not the only factor, the acceptability of the differing routes of diagnosis (for what may well be different expressions of a unified pathology), in each condition, may depend to a large extent on investigative trends in cardiology.

1.2.3.2 Is exclusion of fixed coronary disease enough?

Standard coronary angiography does not offer complete exclusion of *all* possible coronary pathologies, such as coronary vasospasm. Although this is not believed to be a dominant tendency in the TTC population (Tsuchihashi et al., 2001, Gianni et al., 2006b), it does occur and may be a factor in any one individual. Provocative testing (requiring administration of acetylcholine at angiography) is generally not performed in acute unstable situations (during which interpretation is difficult), but can be performed as a secondary procedure, where the index of suspicion for vasospasm is high. Some have also suggested the use of intracoronary vascular ultrasound, to assess non-obstructive coronary atheroma (Cuculi et al., 2010). However, it is not immediately apparent how this serves to elucidate a mechanism or a diagnosis, beyond existing practices and the satisfaction of criteria.

1.2.3.3 Can TTC coexist with CAD, phaeochromocytoma or myocarditis?

The diagnostic criterion of the absence of fixed obstructive CAD, has been questioned by recent authors (Winchester et al., 2008, N'Guetta et al., 2010, Gurlek et al., 2007) and has, in fact, been contravened in several prominent case series, including Wittstein's New

England Journal article (Wittstein et al., 2005) and a series originating from the Mayo clinic itself (Del Pace et al., 2011)! From the point of view of research – and especially where pathogenesis is under consideration – a 'diagnosis of exclusion' is arguably the best practice for ensuring the purity of the sample. However, there is no known reason whereby TTC and CAD, given that they are both relatively common in post-menopausal females, must be mutually exclusive.

The consequences of utilizing an exclusive approach to the diagnosis of TTC may be far-reaching. Specifically, such an approach:-

i. would bias against making the diagnosis of TTC in very elderly, infirm or critically unwell patients, in whom coronary angiography may be relatively difficult and/or dangerous

ii. might distort the apparent epidemiological characteristics of the patient population with TTC, favouring its occurrence in individuals with few risk factors for CAD. Whether this is actually the case is potentially important with regard to the pathogenesis of TTC (Gaddam et al., 2011, Cuculi et al., 2010).

The issue (Mayo criterion 4) of exclusion of phaeochromocytoma and/or myocarditis is essentially a semantic one, given the extensive evidence that (a) TTC is likely provoked by exposure to high catecholamine concentrations and (b) TTC is characterized by the presence of inflammatory changes within involved myocardium. In regards to differentiation of viral myocarditis from TTC, Caforio and colleagues described a case, which fulfilled both World Health Organization criteria for myocarditis on endomyocardial biopsy, as well as the Mayo Criteria for TTC (in all respects other than myocarditis) (Caforio et al., 2009). Although virus-negative, these authors interpreted the case as myocarditis "mimicking" TTC; another report concluded similarly with regard to a case with the basal variant (Karamitsos et al., 2011). Thus, although the idea of myocarditis manifesting as regional contractile dysfunction in a typical TTC-like pattern (or variant) is novel, it is clear that overlapping features will make differentiation challenging.

1.2.3.4 Excluding an ischaemic basis for regional contractile dysfunction in TTC

The demonstration of compatible regional contractile dysfunction is the essential requirement, or *sine qua non*, for a diagnosis of TTC – but it is nevertheless not sufficiently specific to be *pathognomic*. Without trivializing arguments for the exclusion of CAD, it is clear that single vessel coronary disease rarely offers an explanation for the configuration of ECG changes and/or contractile deficit observed in TTC. In fact, too low a threshold for attributing a presentation to CAD, even mild or moderate fixed disease, is an obvious cause of failure to diagnose TTC, especially if ventriculography is therefore not undertaken (Madhavan and Prasad, 2010a). It has been suggested that transient obstruction to a large 'wrap around' left anterior descending artery (supplying the apex and the distal (~25%) portion of the inferior wall), might produce a ventriculogram resembling that of apical TTC: however, this finding is present in approximately 27% of TTC patients (Hoyt et al., 2010). Various patterns of double and triple vessel coronary disease might also produce this pattern. However, it is more difficult to argue similarly for TTC in which the apex is 'spared', i.e., the mid-ventricular and basal variants, which together account for at least a quarter of cases (Singh et al., 2010). Since these variations are not sufficiently explained by CAD, provided that non-invasive imaging could provide sufficient certainty of the wall motion abnormality, a case could even be made for foregoing invasive investigation altogether (Cuculi et al., 2010).

The ideal solution to providing an alternative 'gold standard' would be the identification of a highly specific – or pathognomonic – feature of TTC. An approach, which may suffice for the meantime, would be to lay emphasis on the transient wall motion abnormality being *both* more extensive than any one coronary artery territory *and* out of keeping with the extent and severity of the coronary disease observed. This is appropriately addressed in the latest revision of the Mayo Clinic Criteria (Madhavan and Prasad, 2010a). In cases in whom invasive coronary angiography is contraindicated (and, perhaps also, where CT-coronary angiography is not available or not suitable) an additional requirement might be for the demonstration of the *resolution* of wall motion deficit. Furthermore, the exclusion of myocardial infarction can be achieved by different approaches: several authors have recommended cardiac magnetic resonance imaging with late post-Gadolinium enhancement, for this utility (Eitel et al., 2011b).

Akashi et al have recently called for a revision of diagnostic criteria, based on expert consensus (Akashi et al., 2010). If a near-pathognomonic investigational pattern emerges from prospective research, the question may arise as to whether certain patients, in whom the diagnosis can be made non-invasively with very high certainty, might actually avoid urgent angiography or, potentially, the unnecessary administration of fibrinolytic therapy. The potential – and potential pitfalls – of various investigational modalities in providing such information, will be discussed in sections following (see Section 1.4), but again, such a strategy is unlikely to emerge unless a greater pathogenetic understanding is also achieved.

1.3 Tako-Tsubo cardiomyopathy: clinical aspects

1.3.1 Epidemiology

As mentioned, superficially, although the diagnosis of TTC is being made with increasing frequency, growing awareness of this diagnosis, coupled with early invasive and non-invasive imaging practices in healthcare systems, rather than an actual increase in disease incidence, is sufficient explanation. Among male and female patients with symptoms suggestive of ACS, most estimates of the proportion of TTC cases have clustered around 2%, with concordance between North American (Bybee et al., 2004b, Hurst et al.) and Japanese data (Ito et al., 2003, Matsuoka et al., 2003, Akashi et al., 2004b, Akashi et al., 2010). However, several more recent studies stand in contrast. Data from the Minneapolis Heart Institute Foundation, for instance, indicate that 5% of females (largely post-menopausal) with apparent STEMI, actually have TTC (Sharkey, 2008, Sharkey et al., 2008). When apparent NSTEMI is included, recent estimates suggest a prevalence ranging from 5 to 12% of the total caseload (Dib et al., 2009a, Parodi et al., 2007). These divergent estimates may simply illustrate the effect of more relevant selection of a target group upon the Bayesian probability of a diagnosis, i.e. postmenopausal females presenting with chest pain/dyspnoea/positive troponin.

It is clear that TTC is far more common than previously thought and that the full spectrum of TTC must be appreciated if the true incidence is to be elucidated. In this regard, it is

worth noting that the majority of series predominantly describe patients who present via Emergency Departments with typical symptoms and are processed according to a working diagnosis of ACS, before investigations clarify the actual diagnosis. Furthermore, TTC can occur without chest pain (analogous to 'silent ischaemia') and can occur in general medical, critical care and perioperative contexts (Sharkey et al., 1998, Bainbridge and Cheng, 2009), i.e. settings in which cardiologists may be only peripherally involved and for which cardiac catheterization may be pursued less aggressively. An observational study of 100 consecutive critical care patients raises the possibility that a sizeable number of patients in these clinical contexts — up to a third in this study — have a TTC-like condition complicating their course (Park et al., 2005). Thus, whilst it is necessary for such a surprising finding to be reproduced, it may be that only the 'tip of the iceberg' is currently appreciated.

1.3.2 Acute presentation

Patients with TTC tend to present with chest pain (~68% of recognized cases) or dyspnoea (~18%) (Sharkey et al., 2010a, Gianni et al., 2006b). A truly normal ECG would appear to be rare, and abnormal cardiac troponin I or T appears to be virtually universal, although one review reported negative troponin assays in some patients diagnosed with TTC (Gianni et al., 2006b). Finally, as noted, a minority have either atypical chest pain or present without pain and are diagnosed as a result of abnormal biomarkers or ECG changes: neither of these aspects are specifically addressed in the literature.

1.3.2.1 Gender disparity

The basis of the disparity has yet to be explained adequately, and its elucidation is likely to be critical for understanding the cardiac injury associated with TTC. Early systematic reviews, estimated that among patients diagnosed with TTC, approximately 90% were female (Bybee et al., 2004a, Gianni et al., 2006b). Few explanations have been offered for this, apart from the teleological argument that men are biologically better protected against stress-induced cardiotoxicity throughout the centuries (Stollberger and Finsterer,

2011). Somewhat more substantively, the same authors speculated that higher rates of sudden unexpected cardiac death in males, as evident in autopsy series and in the context of epilepsy (Monte et al., 2007), may lead to under-diagnosis of men with TTC, based on disparate pre-hospital mortality. An alternative epidemiological explanation, based on extrapolation from animal-model data, regarding the potentially protective effect of estrogen supplementation, proposes that declining rates of hormone replacement might be exposing post-menopausal females to a greater risk of TTC (Mendoza and Novaro, 2010). Indeed, although limited by the lack of medication reporting in case reports and series, Kuo et al reviewed TTC literature from 1990 to 2008 and failed to find a single female receiving oestrogen replacement therapy (Kuo et al., 2010).

At present, however, the incidence of TTC in *middle-aged* females has not been specifically correlated to the menopausal state. However, the possibility of sex-hormone deficiency (analogous to menopause) in younger females with TTC is at least raised by several cases reported with concomitant anorexia nervosa (Ohwada et al., 2005, Bonnemeier et al., 2006a), which is associated with secondary amenorrhoea. Further questions as to the pathogenetic relevance of the gender disparity are dealt with in Section 1.5.8.1.

1.3.2.2 Age and cardiometabolic risk profile

Patients at both extremes of age can experience TTC, with mean ages of onset ranging from 62 to 75 years (Bybee et al., 2004a). Premenopausal women are not uncommonly diagnosed and neonatal cases have been reported (Greco et al., 2011, Hernandez et al., 2010). In pooled data, the average prevalences of cardiovascular risk factors are as follows: a history of hypertension in 43% of patients, diabetes in 11%, dyslipidaemia in 25%, and current or past smoking in 23% (Gianni et al., 2006b). In the several case-comparator studies that exist, whereby risk factors can be compared to females presenting with obstructive ACS, TTC patients appear to have a similar incidence of diabetes mellitus and hypertension (Pirzer et al., 2011, Wittstein et al., 2005, Madhavan et al., 2009). One small study indicates a favourable/less atherogenic serum lipid profile among patients with TTC, as compared to ACS patients (Gaddam et al., 2011): the intriguing possibility that TTC patients are metabolically distinct and somewhat protected from atherosclerosis has been

raised (Cuculi et al., 2010) but not explored thus far. As mentioned previously, it is also possible that selection bias imposed by the diagnostic exclusion of fixed coronary artery disease might contribute to the findings.

1.3.2.3 Antecedent psychological and physiological stress in TTC

Although the typical scenario of severe psychological trauma has given rise to the terms of stress cardiomyopathy and broken heart syndrome, the element of emotional trauma is by no means uniform, in TTC presentations. It is clear that a wide variety of psychological stressors, both individual and communal, are possible (see Table 1.3). It is also possible that this aspect of TTC presentation could be underestimated, due to non-disclosure or underreporting, or overestimated, due to the inherent subjectivity involved.

This can be illustrated with reference to two papers, one reporting 29-40% of patients in whom no preceding emotional or physical stressful event was identified (Gianni et al., 2006b), whereas another retrospective study, employing active follow-up, detailed 100 patients in which at least one stressful factor were identified in all but two (Wittstein, 2008)! The nexus between stressors and precipitation of TTC may have ben exaggerated by biased post-hoc methodology in some series, such as this. The potential for increased early suspicion/recognition of TTC, based on clinician's prospective knowledge of an antecedent stress, has not been evaluated. One could argue that a lower threshold for relevant investigation should apply in circumstances of known antecedent stress, provided that the relationship between psychological antecedents with ACS, is equally acknowledged (Moller et al., 1999, Bunker et al., 2003, Matthews et al., 2004).

1.3.2.4 TTC complicating medical/surgical illness

Acute neurological disorders and phaeochromocytoma

At least three well-recognized conditions of reversible cardiac injury, associated with epileptic seizure, subarachnoid haemorrhage and phaeochromocytoma, have been re-evaluated in the light of the emergence TTC as a diagnostic entity. Acute neurological

disorders, such as generalized seizure, intracerebral bleeding and to a lesser extent stroke, have been associated with the evolution of widespread ST-T wave abnormalities, together with cardiac biomarker positivity and non-obstructed coronary arteries (Davies et al., 1991). Some authors have previously assumed these to be "false positive" ECG signs, possibly in association with transient coronary spasm (Pollack, 2006).

Kono et al were the first to study LV wall motion in 19 post-ictal patients, noting an apical wall motion abnormality in twelve (Kono et al., 1994), all of which resolved on follow-up echocardiography. In order to characterize seizure-associated TTC, Stollberger and colleagues compared data from 36 case reports, to those of a composite TTC population, drawn from 12 published series (n = 974) (Stollberger et al., 2011). They reported a relatively increased rate of hypotensive shock (25%, versus 8% in this study) among those with preceding seizures, although a diagnostic selection bias could easily apply to more haemodynamically unstable subjects. In addition, recurrent TTC was higher than has been reported (14%, versus 3% in this study), possibly due to the recurrent nature of the stressor.

Phaeochromocytoma was first linked to the established entity of catecholamine cardiomyopathy, by Schaffer et al (Schaffer et al., 1981) and Matsuura et al (Matsuura et al., 1983). In an elegant case study in 1989, Iga and colleagues correlated elevated catecholamine levels with LV wall motion abnormalities, resolving on serial echocardiography, in a female patient with phaeochromocytoma (Iga et al., 1989). Nanda et al reported a single case of phaeochromocytoma in an 18-year-old woman (Nanda et al., 1995), in which rapid myocardial functional recovery was attributed to aggressive medical therapy. Interestingly, although publication bias may be sufficient explanation, the concurrence of phaeochromocytoma with *basal* TTC has been reported with disproportionate frequency, since the description of the latter variant (Kim et al., 2010).

The special case of stroke

The consideration of stroke/TIA is an important one, because it has been described both as a cause and a complication of TTC (Grabowski et al., 2007, Lee et al., 2009b). Secondary

stroke is obviously plausible, with treatment/preventative implications; however, the distinction between primary and secondary can be difficult in any one patient. Leaving aside this question, the exact incidence of TTC among patients with acute stroke/TIA, is not known. Given the prevalence of intensive stroke units, in which *routine* transthoracic echocardiography is very frequently the policy, one might think that that would be easy to establish. However, as will be covered in the discussion of investigational modalities, there are reasons why this test might be insensitive, especially if a prior suspicion of TTC is not present.

The potential relevance of psychological comorbidity

Reports of concomitant depression and anxiety (Nguyen et al., 2009, Behrens et al., 2008) are interesting in the light of their potential modulation of the sympathetic nervous system, although studies have differed as to which predominates among TTC patients. Mudd et al reported a greater incidence (40%) of major depressive disorder among TTC patients (Mudd et al., 2007), whereas Summers et al reported greater rates of premorbid anxiety among TTC patients versus those suffering STEMI (56 vs 12%) (Summers et al., 2010); on the other hand, Del Pace et al reported equal rates of anxiety traits between TTC and STEMI patients, when a validated questionnaire was used (Del Pace et al., 2011).

With regard to panic disorder, given that acute attacks are associated with marked cardiac noradrenaline spillover (Baumert et al., 2009), it is perhaps surprising that very few cases of TTC among this group are apparent from the literature, or evident in the above series. Furthermore, the prevalence of depression and panic were equivalent in post-menopausal women in the Women's Health Initiative study (Smoller et al., 2003). On the other hand, panic disorder is frequently associated with chest pain and can be associated with coronary spasm/myocardial infarction (Mansour et al., 1998). Similarly, the arousal associated with post-traumatic stress disorder has not been reported as a trigger for TTC. The lack of prospective data in cohort studies of patients with anxiety/depression/panic disorders is a serious limitation to our understanding of this area.

1.4 Associated abnormalities

1.4.1 ECG changes and arrhythmogenicity

1.4.1.1 ST-T wave abnormalities: specificity for TTC

Although earlier series suggested an overall incidence of ST segment elevation in initial ECG's of approximately 70-80% (Gianni et al., 2006b, Pilgrim and Wyss, 2008), the true incidence may be closer to 40% of cases (Dib et al., 2009b, Nguyen et al., 2011a). Although subtle differences may exist, the ST-segment morphology in TTC is difficult to distinguish from STEMI (Bybee et al., 2007, Barker et al., 2009b). T-wave abnormalities are seen in approximately 64% (Gianni et al., 2006b), but are again variable. Some of this variation may be a consequence of evolution of ECG changes, in relation to the time of onset, in which T-wave inversion appears to eventually replace ST-elevation (Mitsuma et al., 2007); Q waves, if present (~20%), tend to resolve (Robles et al., 2007). The distribution of ECG changes is, usually, precordial (approximately 80%) (Gianni et al., 2006b); inferior, inferolateral and patterns 'widespread' ST-T wave abnormalities are not uncommon and, as yet, no component of the ECG pattern of TTC is known to confer diagnostic specificity.

In terms of the dichotomy between ST and non-ST elevation cases of TTC, it is more than likely that at least some studies are affected by a bias in health care systems, in that patients with ST elevation would be expected to undergo emergent angiography (±PCI), in contrast to patients categorized as non-ST-elevation myocardial infarction (NSTEMI). It may well be that the actual majority of TTC patients, in fact, present with non-ST elevation. Indeed, the work of Dib *et al* suggests that two thirds of TTC do not have ST-elevation at presentation (Dib et al., 2009a). This unresolved issue has significant implications for the timely diagnosis and appropriate treatment of TTC as a group, in addition to prospective research into this condition.

1.4.1.2 QT prolongation and torsade de pointes

Importantly, there is strong evidence of TTC-induced prolongation of the QT interval and its association with *torsade de pointes* (Denney et al., 2005, Wittstein et al., 2005,

Elkhateeb and Beydoun, 2008, Mahfoud et al., 2009). The QT interval appears to be most prolonged at day three (Kurisu et al., 2004). The mechanism is not understood, but may well relate to heterogeneous activation and altered repolarization-reserve of the left ventricle (Behr and Mahida, 2009). The incidence of early *torsade de pointes* and ventricular fibrillation in TTC is unknown, given the uncertainty of diagnosis in patients who die in the pre-hospital period.

In view of the potential lethality of this complication, some attempts have been made to examine the determinants of *torsade de pointes* in the context of TTC. Whilst one review of 14 cases suggested that male patients with TTC were at greater risk, this finding may equally reflect publication bias (Samuelov-Kinori et al., 2009b). Fascinatingly, a retrospective review of ECG characteristics in 105 patients with TTC (Dib et al., 2008), in addition to confirming QT prolongation, suggested a higher incidence of arrhythmia in those exhibiting greater R-R variability, implying enhanced vagal modulation of heart rate. This possibility should be entertained seriously, given that the QT interval is known to increase with bradycardia. That the avoidance of bradycardia (as is recommended in the management of QT-prolonged patients generally), should be carefully considered in TTC also, is highlighted by the cases of two TTC patients, both of whom suffered *torsade* with AV block and required pacing support (Kurisu et al., 2008).

Although QT prolongation appears to reach its peak at day three, arrhythmic presentations seem frequently to be either the initial presenting event or to occur in the first 24 hours (Kucia et al., 2010). In this regard, inverse rate adaptation of ventricular depolarization – the expected relationship between heart rate and QT interval – was found to be minimal on third-day Holter studies (Bonnemeier et al., 2006b), which is difficult to explain. Finally, some case reports have described the co-concurrent diagnosis of stress cardiomyopathy and congenital long QT syndrome (Sasaki et al., 2006), which is known to be affected by sex steroids and emotional stress (Kurokawa et al., 2009). The potential that both TTC and many types of congenital QT prolongation-based arrhythmias may be stimulated by enhanced catecholamine release may be relevant (Seethala et al., 2011).

1.4.2 Laboratory findings

1.4.2.1 Markers of myocardial necrosis

As previously stated (Section 1.3.2), the vast majority of currently recognized cases of TTC have mild elevation of biomarkers of cardiac myonecrosis.

1.4.2.2 Natriuretic peptide elevation

Background and physiological considerations

The release of natriuretic peptides in TTC raises both physiological and pathophysiological issues which merit detailed review. Although the endocrine function of the heart was first conceived of 30 years before, it was not until 1981 that de Bold and colleagues observed a ~30-fold increase in sodium excretion (with corresponding urine volume increase), when myocardial homogenates were intravenously injected into rats (de Bold et al., 1981, de Bold, 2011). Since then, the natriuretic peptide family has been under intense study for their diagnostic, prognostic and therapeutic potential to restore disordered homeostasis, primarily in heart failure (Maisel et al., 2002). Briefly, de Bold's group described the structure of atrial natriuretic peptide (ANP) and demonstrated its utility as a marker of cardiac failure (Seidah et al., 1984). Brain, or B-type, natriuretic peptide was described in 1988, and found to be primarily synthesized in the LV (Niwa et al., 1988, LaPointe, 2005). C-type natriuretic peptide was identified in 1990 (Sudoh et al., 1990), this being predominantly released by vascular endothelium.

There is a substantial spectrum in the stimuli for natriuretic peptide release. Although ANP and BNP are classically regarded as sensors and regulators of excessive pressure and volume loads, it is now clear that ischaemia, inflammation, as well as redox and paracrine factors (including catechols, AII and ET-1), modulate their release (Zhang et al., 2004, Mehra et al., 2006, de Bold, 2009). Unlike ANP, which is stored in granules, BNP is synthesized by successive cleavage of larger precursor peptides (pre-proBNP1-134 and proBNP1-108) and secreted in bursts (Yasue et al., 1994). Final cleavage occurs during exocytosis and is mediated in uncertain proportions by two serine proteases: the

transmembrane protein, corin (Semenov et al., 2010, Wu et al., 2002), and a cytosolic enzyme, furin (Sawada et al., 1997). This results in simultaneous release of both BNP1-32 (i.e., the active hormone) and the larger amino-terminal of the prohormone of BNP (NT-proBNP), the latter having no known physiological function.

The clearance of BNP is rapid (plasma elimination half life ~20 min) in comparison to that of NT-proBNP (half life ~20 hours), which has implications for their use as diagnostic markers. BNP is degraded by ectodermal neutral endopeptidase or undergoes receptor-mediated clearance at the kidneys, at the NPR-C receptor; NTY-proBNP is renally cleared. Plasma BNP and NT-proBNP measurements may aid the diagnosis of heart failure in emergency departments (Januzzi et al., 2006b), but not with the specificity that was first hoped (Maisel and Daniels, 2012). This is likely because of the above-mentioned variety of chemical and paracrine stimuli for release of BNP/NT-proBNP, in addition to that of LV pressure and volume overload. Increasing age and female gender are themselves predictors of elevated BNP/NT-proBNP in the context of ACS and heart failure (Maisel and Daniels, 2012).

BNP promotes natriuresis, vasodilation, renin-inhibition, and anti-hypertrophic and anti-fibrotic functions, via its effect on particulate guanylate cyclase, to produce the second messenger, cGMP. Additional cardioprotective and lusitropic effects of natriuretic peptides have been reported (LaPointe, 2005). However, in heart failure, the hoped-for benefit of exogenous recombinant BNP1-32, was not evident (O'Connor et al., 2011), possibly because of resistance at target organs, prevalent in that condition (Chen, 2007). Furthermore, it has recently been demonstrated that BNP may exert cGMP-dependent adverse effects by stimulating catecholamine release (Chan et al., 2012): this unsuspected property may partially antagonize some of the functions of BNP, such as those listed above, *in vivo*.

BNP and NT-proBNP in TTC

A number of investigators have reported that at the time of diagnosis, plasma levels of brain natriuretic peptide (BNP) (Akashi et al., 2004a, Grabowski et al., 2008, Madhavan et

al., 2009, Morel et al., 2009, Wittstein et al., 2005) or its precursor N-terminal proBNP (NT-proBNP) (Nef et al., 2008a, Nguyen et al., 2011a) are markedly elevated in the majority of cases of TTC. The reported increases in NT-proBNP/BNP levels in TTC are surprising, given that left ventricular filling pressure is not markedly elevated (Akashi et al., 2003, Yoshida et al., 2007, Nguyen et al., 2011a). Autoradiography data from a rat model of TTC, demonstrate rapid LV myocardial expression of BNP mRNA (Ueyama, 2004). An important issue is whether BNP release is global or whether it predominantly arises from hypokinetic zones of myocardium, which may be exposed to greater systolic wall stress. Although no perfect methodology exists for the estimation of wall stress, two human imaging studies suggest that the stimulus for BNP release is not principally mechanical, which raises the possibility of cellular injury (Madhavan and Prasad, 2010a, Nguyen et al., 2011a).

1.4.2.3 Elevation of catecholamine levels

The synthesis and release of catecholamines

Catecholamines are secreted as stress hormones or neurotransmitters in three forms: dopamine, noradrenaline or adrenaline. These substances have varying affinities for their adrenoceptor (AR) targets and thus, exert differing effects. The oxygen-dependent synthesis of catecholamines occurs in the adrenal medulla, or within neurons. The initial and rate-limiting step is the synthesis of DOPA from the amino acid tyrosine, by the iron-dependent enzyme tyrosine hydroxylase. Dopamine is then synthesized by the decarboxylation of DOPA and transported to secretory vesicles where it is hydroxylated to form noradrenaline. The addition of a methyl group is required to produce adrenaline: this step predominates in the adrenal medulla and is foregone in sympathetic neurons. Release from storage vesicles in the sympathetic varicosity, occurs in response to membrane depolarization, via calcium influx. As stated in the previous section, it has recently been shown that cyclic GMP, a product of both BNP activation of particular guanylate cyclase and nitric oxide activation of soluble guanylate cyclase, can stimulate catecholamine release (Chan et al., 2012).

Physiologic effects of catecholamines

The major physiologic effects of catecholamines are (i) tachycardia (via an effect at the sinoatrial node), (ii) positive inotropy/lusitropy (via effects on atrial and ventricular cardiomyocytes), (iii) vasoconstriction/pressor effects (via effects on vascular smooth muscle cells) and (iv) reduction of the atrioventricular conduction time (via an effect at the atrioventricular node). Noradrenaline also plays a role in coordinating the baroceptor reflex.

In healthy cardiomyocytes, adrenergic stimulation occurs mainly via β_1AR, which account for 70-80% of βARs expressed on cardiomyocytes. β_2ARs account for ~20% but this proportion can be increased in advanced heart failure, by selective down regulation of the β_1 subpopulation (Bristow et al., 1986). β_3ARs usually account for 2% of receptor density, but this proportion may be increased in acute inflammatory states (Moniotte et al., 2007), with diabetes (Amour et al., 2007) and with advanced age (Chen et al., 2010b).

βAR-mediated effects in modulating excitation-contraction coupling and inotropy are well studied. Briefly, β_1 and β_2 receptors are coupled to G_s proteins, which stimulate adenylate cyclase. Thus, a second messenger cAMP then activates protein kinase A (PKA), which phosphorylates various targets involved in calcium cycling. Of these, the phosphorylated voltage-gated L-type calcium channel, crucially, allows greater influx of calcium across the sarcolemma (the 'calcium spark'). The main reservoir of calcium within the cardiomyocyte is the sarcoplasmic reticulum (SR), which releases a transient burst of calcium (the 'calcium transient'), which, in concert with ATP, activates the contractile apparatus. In systole, SR calcium release is triggered by the calcium spark and is proportionate to both the calcium spark and its baseline calcium load, i.e., the amount of calcium actively sequestered in diastole via the ATP-dependent pump, sarco(endo)plasmic reticular calcium ATPase (SERCA). The function of this pump is crucially related to β_1 and β_2-stimulation, via PKA and phospholamban.

Inhibitory coupling and desensitisation of β-ARs

Although the excitatory coupling of β_1- and β_2-ARs hold true in the main, this is not always the case. In a phenomenon termed ligand- or stimulus- trafficking, intense stimulation brings about coupling of β_2-ARs to inhibitory G proteins (G_s), via phosphorylation (Iaccarino et al., 1999). This process reduces the amount of cAMP synthesized and is up-regulated in heart failure (Feldman et al., 1988). Activation of G_i has the potential to couple these receptors to other important cell survival signaling pathways, such as the MAP kinases, leading some to posit that ligand-trafficking is a cardioprotective mechanism and has formed the basis of a major hypothesis regarding the pathogenesis of TTC (Paur et al., 2012, Lyon et al., 2008) (see Section 1.5.7.2)

In addition, although small in number, myocardial β_3-receptors can also couple interchangeably to G_s or G_i proteins, as well as to NO synthase (Moens et al., 2010).

Neuronal and extraneuronal handling of catecholamines

As stated elsewhere, ~90% of noradrenaline is taken back into the bouton via norepinephrine transporters (NET) (uptake 1 and 2) (Cingolani et al., 2011), although this capacity appears to be somewhat reduced in women (Mitoff et al., 2011). The fate of remaining noradrenaline is one of the following: (i) extraneuronal enzymatic metabolism (5%) and (ii) escape into plasma (8%). In mammals, metabolism of noradrenaline occurs via of the following enzymatic pathways:

i. Catechol-O-methyltransferase (COMT)
    - Resulting in the non-acidic derivative normetanephrine (NMN)

ii. Monoamine oxidase (MAO)
    - Resulting in 3,4-Dihydroxymandelic acid, 3-Methoxy-4-hydroxymandelic acid (also known as vanillylmandelic acid, VMA) or 3-methoxy-4-hydroxyphenylethylene glycol.

In peripheral non-neuronal tissue, VMA is the major metabolite of catecholamines, and is excreted unconjugated in the urine; levels of MHPG are minor (although this is not so in the central nervous system).

The term 'metanephrines' refers to the methoxy analogues of epinephrine and norepinephrine: metanephrine and normetanephrine (Dopamine, methylated by COMT, is not referred to as a metanephrine). O-Methylation outside the neuroeffector junction and creates a non-acidic methylated compound which is stable in tissue and in the circulation, before being excreted in urine. The metanephrines are not totally inactive, physiologically: metanephrine exhibits 15-25% of the pressor activity of adrenaline; normetanephrine is 1/600 as active as norepinephrine on feline blood pressure (Champagne et al., 1960, Schildkraut et al., 1965); normetanephrine can also inhibit the NET, uptake-2 (Rahman et al., 2008). Oxidative deamination by MAO, followed by reduction to VMA, yields an acidic derivative, which has no known biologic activity and which is excreted renally (Kopin, 1985).

In the case of noradrenaline escaping enzymatic processing, conjugation (by glucuronidation or sulfatation) takes place in the blood (Kopin, 1985). As the conjugation process is assumed to deactivate and stabilize catecholamines, prior to urinary excretion, the idea that reduced conjugation may predispose to worse outcomes in heart failure and perioperative myocardial ischaemia is attractive. However, no evidence exists for a detrimental effect of free catecholamines, over and above their total concentrations (Dobnig et al., 1995). Ratge et al reported that 70–80% of total circulating noradrenaline and adrenaline and approximately 98% of circulating dopamine are conjugated (Ratge et al., 1986). Standard measurements of plasma catecholamines usually do not take into account the conjugated fraction, versus that which is free. However, determination of free and conjugated concentrations (e.g. by HPLC) has allowed study in heart failure and in the perioperative period (Sametz et al., 1999).

Precipitation of inflammation/oxidative stress by catecholamines

In the context of this thesis, not only the inotropic effects, but also the pro-oxidant/pro-inflammatory effects of catecholamines on the myocardium, are centrally important. While prolonged exposure to high concentrations of catecholamines has been associated with induction of necrosis and precipitation of heart failure (Packer, 1992) (see Section 1.5.5.2), there is considerably less information about the effects of short term pulses of catecholamine secretion.

Catecholamines in acute TTC

The well-known study of Wittstein *et al* (Wittstein et al., 2005) compared plasma concentrations of epinephrine, norepinephrine and metanephrine in 13 TTC patients against a group of 7 STEMI patients in Killip III class at admission. The major finding was that of a 2-3-fold elevation catecholamine levels. Akashi et al had produced the first such series (Akashi et al., 2003) and similar catecholamine dynamics were also seen in critically ill patients with sepsis showing LV apical ballooning (Park et al., 2005). However, several other studies have failed to demonstrate a difference (Madhavan et al., 2009, Akashi et al., 2008). The challenges of catecholamine measurement aside, it is conceivable that Wittstein's TTC group, one quarter of whom required intra-aortic balloon pump therapy, were in a more critically ill condition than most TTC patients and, hence, as a group, had higher catecholamine concentrations than are usual, even in an acute context.

1.4.3 Echocardiography

Doppler, M-mode and 2D (B-mode) echocardiography are now standard tools for the assessment of myocardial and valvular conditions. For a concise description of technical aspects and measurement conventions, the reader is referred to guidelines and reviews (Lang et al., 2005, Baumgartner et al., 2009). Most series of TTC have drawn on echocardiography to some extent, usually to document abnormal LV function acutely, with or without follow-up studies to describe recovery. Despite the segmental nature of LV systolic dysfunction in TTC, the main expression of results in most published series has been LV ejection fraction. Leaving aside concerns regarding measurement variability (Hare

et al., 2008), LVEF may be particularly unsuited as a measure of the extent and severity of TTC. This is because LVEF is a global measure, which is influenced by compensation hyperfunction, as is frequently seen in the basal segments of the apical variant: thus, the deficit of affected segments is likely to be masked or offset by the value of LVEF, accept in very severe cases. A simple alternative is the wall motion score index, whereby each segment of the LV (conventionally divided into 17 segments) is scored according to the degree of deficit: given that supranormal wall thickening is not differentiated from normal wall thickening, WMSI is likely to reflect the overall burden wall motion deficit (Cerqueira et al., 2002, Madhavan et al., 2011).

LV diastolic volumes, which tend to be *mildy* enlarged in the acute phase (Park et al., 2009, Rigo et al., 2009), before resuming a normal size and shape in recovery. In view of the expected direct relationship between end-diastolic volume and contractile function, up until a plateau (Starling, 1918), this might seem to be a functional paradox. The pathophysiological interpretation of this observation has not been considered, but it noteworthy that the same is seen in other non-ischaemic disorders, acute myocarditis and sepsis cardiomyopathy; in the latter (discussed in Section 1.5.9), it has been suggested that dilatation of the cavity might be compensatory, by recruiting more stroke volume.

1.4.3.1 Doppler echocardiography

The velocity of bulk flow within the cardiovascular system can be directly appreciated via Doppler ultrasound/echocardiography, which utilizes the Doppler principle. In brief, in common with all ultrasound, this consist of (i) emission (from a probe), (ii) reflection (at a tissue interface) and (iii) reception of the ultrasonic energy (at the same probe). The reflective elements, in the case of Doppler ultrasound, are those of the cellular constituents of blood, which are moving. The frequency of ultrasound energy undergoes a 'shift', in proportion to the change in velocity of the blood, which enables the derivation of the average velocity of the blood (relative to the probe). Pressure differences between chambers, can be inferred via the modified Bernoulli equation, which relates pressure to volume: $\Delta P = 4v^2$.

The relevance of Doppler echocardiography to TTC is its potential to measure intraventricular gradients in the case of acute obstructive TTC. As discussed in Section 1.5.1.1, when a relative narrowing is produced as a result of hypertrophy or hypercontraction, a chamber (in this case the LV) can be functionally 'compartmentalized', resulting in higher pressure in the distal compartment and rapid acceleration of blood past the narrowing. Thus, intraventricular Doppler is a non-invasive adjunct to LV catheter pressure measurements and should be routinely performed in TTC patients undergoing transthoracic echocardiography. Having said this, there are no systematic reports of Doppler findings in TTC, probably because true obstructive TTC is uncommon.

1.4.3.2 Diastolic function

On average, in contrast to systolic function, echocardiographic indices of diastolic function does not appear to be markedly impaired in the acute phase of TTC (Burri et al., 2008), although not in all studies (Meimoun et al., 2011). Although the available data do not permit a definitive conclusion, the relative preservation of *overall* LV relaxation in TTC would partially explain the observation that PCWP (a reflection diastolic LV pressure) is seldom severely elevated (Akashi et al., 2004a, Nguyen et al., 2011a). The latter is itself surprising, given that the systolic wall motion abnormalities of TTC generally exceed those of MI; the typical degree of wall motion impairment seen TTC would be equivalent to a large infarct, the latter of which would often be associated a 'restrictive' LV filling pattern on transmitral Doppler (Cerisano et al., 2001) (indicative of severe global LV stiffness/diastolic dysfunction) and, not infrequently, pulmonary oedema. The reported incidence of true pulmonary oedema, as discussed in Sections 1.5.5.3 and 1.6.1.1, may be a relatively uncommon event.

Diastolic function is also important in the context of assessing the completeness of recovery, given that indices such as E/E' should be sensitive to more subtle longer-term myocardial dysfunction. Interestingly, in two studies, global diastolic indices did not change significantly between the acute phase and 1-3 months (Loiske et al., 2011, Meimoun et al., 2011).

1.4.3.3 Advanced echocardiography/myocardial mechanics

Normal myocardial function involves a complicated series of electrically coordinated events, which can be collectively called 'myocardial mechanics' (Mor-Avi et al., 2011). Ventricular 'pumping' is, in fact, the sum of (i) longitudinal shortening (of the LV, predominantly by long-axis oriented subendocardial fibres), (ii) circumferential squeezing (largely via the action of mid-wall fibres) and (iii) radial thickening (produced by the expanding short axis dimensions of myocytes, contracting *en masse*), together with (iv) rotational components (Helle-Valle et al., 2005). Perhaps with the exception of rotational indices, all of these components have been able to be studied for some time, by the application of relatively simple echocardiographic techniques, e.g. M-mode echocardiography (Henein et al., 1993, Henein and Gibson, 1995). However, currently, two main echocardiographic methods are recommended for the assessment of myocardial mechanics, tissue Doppler and 2-dimensions (2D) speckle tracking. Both of these have been validated against sonomicrographic measurements, but speckle tracking appears to have the key advantage of superior repeatability characteristics and the reader is directed to reviews for detailed methodological description (Marwick, 2006, Mor-Avi et al., 2011). The main measures of interest are those of deformation, or strain, which is defined as the percentage change in length of a segment, relative to its original length; 'strain rate' is the speed at which this occurs. These parameters are further described as global or regional, specifying the direction of the deformational movement studied, relative to the cardiac axis, e.g., 'mid-ventricular circumferential strain-rate' or 'global longitudinal strain'. Thus, a comprehensive and quantitative understanding of the myocardial mechanics of TTC can be obtained through these tools. Findings in TTC, utilizing speckle tracking (Burri et al., 2008, Baccouche et al., 2009, Mansencal et al., 2009) or tissue Doppler encoding (Pasanisi et al., 2007) will be discussed using this framework.

Longitudinal dysfunction in TTC

Since the work of Gibson and others (Alam et al., 1992, Gibson et al., 1979, Henein and Gibson, 1995, Henein et al., 1993) , the functional significance of the longitudinal shortening of the LV in systole (about 10-12mm in normal individuals) has been recognized. Physiologically, this aspect of cardiac motion is believed to be a function of the longitudinally orientated myofibers of the subendocardial layer of LV myocardium [i.e., fibers which are parallel to the central long axis of the LV (Henein et al., 1993, Henein and Gibson, 1995)]. Perhaps because of the vulnerability of the subendocardial layer to injury, longitudinal function appears to be both delayed and reduced in numerous conditions. In particular, although measured in different ways, interest has been focused on the prognostic significance of abnormal long axis function (e.g. in heart failure (Willenheimer et al., 1997) or after MI (Brand et al., 2002)). Longitudinal systolic function is depressed in systolic LV dysfunction (in proportion to LVEF), but can also be reduced in those with a preserved ejection fraction, where diastolic dysfunction is apparent (Willenheimer et al., 1999, Petrie et al., 2002). The quantification of long-axis function may delineate subtle but significant LV dysfunction, for example in diabetes mellitus (Fang et al., 2004).

Given the above, long axis function assessment is likely to be useful for the study of TTC. The available investigations concur that indices of longitudinal LV function are impaired in the acute phase (Burri et al., 2008, Heggemann et al., 2009b, Mansencal and Dubourg, 2009, Meimoun et al., 2011, Loiske et al., 2011). Burri et al studied 5 patients in the acute phase of TTC utilizing velocity vector imaging (based on a speckle tracking methodology) and reported both LV systolic and diastolic longitudinal dysfunction (Burri et al., 2008). Surprisingly, acute longitudinal (but not radial) impairment was also evident at the cardiac base, which has generally been considered “spared” in the typical apical ballooning variant.

Mansencal and colleagues utilized speckle tracking to compare segment-by-segment long-axis tissue velocity, in TTC versus acute anterior MI (Mansencal and Dubourg, 2009). Not surprisingly, average longitudinal shortening was abnormal in both conditions, more in the apical, versus mid-LV or basal segments. However, in TTC, tissue velocities did not vary *between radial segments,* at each respective level. They interpreted this ‘non-systematized’ distribution of impairment (extending beyond a single epicardial coronary distribution) as being potentially useful for differentiating TTC from MI. The findings of

Heggemann et al are similar (Heggemann et al., 2011) – in essence, a quantitative demonstration that the contractile dysfunction of TTC exceeds the bounds of a coronary territory.

Radial and circumferential deformation indices

Whilst affected myocardium typically appears akinetic or severely hypokinetic, several investigations, utilizing 2D strain measurements by echocardiography, have noted post-systolic shortening, a non-specific finding, consistent with impaired but viable tissue (Heggemann et al., 2009a, Marechaux et al., 2009). Moreover, this subtle abnormality frequently persisted at the time of follow-up (~1 month) (Heggemann et al., 2009a), which challenges the notion of rapid and complete recovery of myocardium. Overall, however, radial and circumferential strains are initially impaired, but appear to return to normal control values at one-month follow-up (Mansencal et al., 2009).

Rotational functional indices in TTC

LV twist results from the dynamic interaction of myofibers, in two main orientations, described as oppositely wound helices (Helle-Valle et al., 2005). It is suggested that the helical subepicardial fibres, which have a larger arm of movement than subendocardial fibres, determine twist direction, at any one level in the LV (Sengupta et al., 2008, Nucifora et al., 2010). Thus, rotation at the apex is counter-clockwise (as viewed from an inferior position), whereas rotation is clockwise at the base: average angular displacement of myocardium, relative to the central axis, is conventionally expressed in positive or negative values, for counter-clockwise and clockwise rotation, respectively. LV twist is therefore the sum of apical and basal rotation, but is largely driven by apical twist, which is of a greater magnitude (Helle-Valle et al., 2005). LV torsion is defined as LV twist, normalized to LV diastolic length (Mor-Avi et al., 2011). All of these parameters are influenced by LV shape and volume (van Dalen et al., 2010).

One study assessed LV twist and its components in detail, employing serial studies in TTC, with reference to a group of normal control and a group of post-MI patients (Meimoun et al., 2011). Apical twist was generally reduced at the onset of TTC, but recovered at one month. Of interest, a novel pattern of reversed apical rotation (i.e. clockwise) was identified in the acute phase, but in only 3 patients.

1.4.4 Cardiovascular magnetic resonance imaging

Magnetic resonance, as a clinical imaging technique, combines high spatial resolution with moderate temporal resolution; the use of cardiovascular magnetic resonance (CMR) has seen a significant growth over the past decade, due to both increasing availability and the ability to provide incremental information in an increasing number of conditions (Pennell et al., 2004). In addition to wall motion analysis and accurate volumetric quantitation, 'tissue characterization', via T_1-weighted contrast-enhanced CMR, T_2-weighted oedema-sensitive imaging and iron-sensitive T_2* techniques, is relevant to a range of cardiomyopathic processes and infarct imaging (Karamitsos et al., 2009, Friedrich, 2008, Friedrich et al., 2008), justifying to some degree, the lighthearted label of a "one-stop shop" for cardiac imaging (Poon et al., 2002).

1.4.4.1 Evaluation of myocardial necrosis or scar

The use of gadolinium-based contrast agents, which increase signal in T_1 weighted MR by shortening the longitudinal relaxation time of surrounding protons, enhances signal in proportion to its concentration, within a given tissue. Within myocardium, the relative volume of distribution of chelated Gd^{3+} can be increased in two main ways: (a) the disruption of cell membranes (to allow Gd^{3+} into the intracellular compartment), i.e., necrosis, and (b) the expansion of the extracellular matrix, i.e., oedema, scar formation or diffuse reactive fibrosis. The most common form of imaging in cardiac clinical use, which employs Gd^{3+} for the above purposes, is designated late gadolinium enhancement (LGE), given that images are acquired after ~5-30 minutes after a bolus, to allow 'washout' of

Gd^{3+} from unaffected myocardium and retention in segments with necrosis/fibrosis (Moon et al., 2004, Klein et al., 2007).

Regarding the frequency of LGE in TTC, there is considerable variation in reports. Whilst some authors suggest that TTC patients *very rarely* exhibit myocardial LGE (Syed et al., 2008), one Japanese series suggests that LGE positivity occurs *very frequently* in TTC (i.e., almost 50%) (Naruse et al., 2011); the differences are almost certainly methodological and it may be that the latter observation reflects tissue oedema, rather than necrosis/scar. Occupying the middle ground, some authors, have suggested that subendocardial foci of LGE are *occasionally* visible in TTC patients (Haghi et al., 2006, Rolf et al., 2009, Eitel et al., 2011b). Furthermore, these foci denote an expansion of extracellular matrix content on LV biopsy (Rolf et al., 2009). Quantitative methods for deriving and true T_1 relaxation time, however, are now available (Iles et al., 2008) and may prove useful for elucidating the question of intramyocardial fibrosis, both in the short and long-term, following the insult of TTC. Overall, the early suggestion that the principal usefulness of LGE-CMR in TTC is the exclusion of new or old myocardial infarction, has been borne out (Eitel et al., 2009, Eitel et al., 2011b, Joshi et al., 2010, Koeth et al., 2008).

1.4.4.2 Oedema imaging

Oedema is known to be present during the acute phase of TTC:- this can be demonstrated on the basis of histology (Nef et al., 2007b) and also T_2-weighted imaging by CMR (Otsuka et al., 2008, Rolf et al., 2009, Abdel-Aty et al., 2009). T_2-weighted imaging is based on the measurement of the multiple echo amplitudes; T_2 relaxation and, consequently, T_2-based image-contrast, derives from the relative abundance of stationary protons, greatest in water and in aliphatic chains of lipids. Of note, “fat-suppression” can be achieved, effectively negating the signal attributable to fat, such abnormal tissue signal intensity reliably reflects oedema. Hence, In myocardium, the association of T_2-weighted signal and histological oedema has been validated in a canine model of MI, utilizing T_2 weighted black-blood sequences (Tilak et al., 2008).

The diagnostic and pathogenetic implications of oedema in acute TTC have been partially explored in imaging studies. Abdel Aty et al originally observed the presence of oedema on T_2-weighed slices of heart in eight patients (Abdel-Aty et al., 2009). This, together with the early experience with LGE in TTC, led to an extensive collaborative study (incorporating data from 6 centres in >250 patients) of the diagnostic potential of multisequential CMR in Mayo criteria-defined TTC (Eitel et al., 2011b). Essentially, oedema was visualized in 81% of patients, was not confined to coronary territories and tended to follow the zones of wall motion abnormality. LGE was rare and pericardial effusion was surprisingly common (43%).

1.4.5 Nuclear imaging findings

1.4.5.1 Nuclear perfusion imaging

Differing regional patterns of myocardial perfusion, utilizing 99mTc-MIBI or 201Tl single photon emission computer tomography (SPECT), have been reported. Several investigators describe relatively reduced tracer uptake in affected LV apical myocardium, despite normal coronary arteries, which recover within days or weeks (Bybee et al., 2006, Ito et al., 2005, Kurisu et al., 2003). However, other groups have reported the opposite (Cimarelli et al., 2008, Burgdorf et al., 2008c). These differences, which may or may not be explained by the timing of investigations, raise the possibility that TTC may be misdiagnosed as apical ischaemia/infarction in some cases, on the basis of SPECT, if angiographic confirmation is not pursued. If we accept that there are indeed perfusion defects in TTC, this does not necessarily imply subnormal blood supply to the affected regions. Resting perfusion imaging with SPECT allows qualitative comparison of regional perfusion, where differences can be appreciated in one region, *relative to another,* rather than with reference to normative levels of perfusion. The affected apical report may in fact have normal rates of perfusion, whereas the basal region may have supranormal perfusion, thus creating the regional difference.

1.4.5.2 Cardiac sympathetic neuroimaging

MIBG is an analogue of noradrenaline, containing a radiolabelled Iodine moiety, which is taken up by NET, uptake 1. Its main clinical use is in the imaging of neuroendocrine tumours, including phaeochromocytoma, which sequester the tracer. However, the regional density of cardiac sympathetic neurons can also be studied and the rate of washout/uptake can be quantified as a heart-to-mediastinum ratio. Utilizing the later method, investors have consistently documented increased cardiac sympathetic activity in the acute phase of TTC (Iga et al., 1995, Akashi et al., 2004b, Ito et al., 2005, Cimarelli et al., 2008, Burgdorf et al., 2008c). However, in regional terms, a defect is seen in the apex, interpreted as regional cardiac denervation (Moriya et al., 2002, Pessoa et al., 2006, Scholte et al., 2006), which for a given patient, exceeds the size of the zones of reduced perfusion (99mTc SPECT) and reduced fatty acid uptake (as assessed by uptake of the 123I-β-methyl-p-iodophenylpentadecanoic acid tracer, 123-I-BMIPP (Biswas et al., 2009)). These defects can persist for ~3 months, but are resolved by 6 months (Moriya et al., 2002). The mid-ventricular variant of TTC is associated with analogous findings (Moriya et al., 2009). Whilst superficially, this may be at odds with the finding of an increased global rate of wash-in/uptake, this finding should be qualified as a regional difference in MIBG uptake. Denervation is a possible explanation, but MIBG uptake may also be reduced competitively in the presence of locally increased concentrations of noradrenaline (as seen when imaging patients are taking tricyclic antidepressants (Sisson et al., 1987)). An analogous observation has been made in a single female TTC patient, utilizing the PET tracer, 11c hydroxyephedrine, which also localizes to sympathetic nerve terminals and which demonstrated an apical defect early after TTC (Prasad et al., 2009). Overall, although these observations are interesting from a pathogenetic point of view, it is unlikely that routine imaging would yield clinically useful information.

1.4.5.3 "Metabolic" imaging findings

Although a 'metabolic omnivore' (Ashrafian and Neubauer, 2009), the predominant energy substrate of the mammalian heart is fatty acids; proportionally, consumption of glucose and pyruvate is minor. 13-N ammonium positron emission tomography (PET), coupled

with 18-F flurodeoxyglucose imaging, demonstrates reduced coronary flow in the acute phase of TTC, together with local apical reduction of resting cardiac glucose uptake (proportional to its consumption as an energy substrate) (Feola et al., 2006, Feola et al., 2008). Analogous findings to those of glucose uptake are available, demonstrating reduced free fatty acid uptake/lipolytic metabolism, to a greater regional extent than myocardial hypoperfusion (Ito et al., 2005, Kurisu et al., 2003), utilizing the combination of 123I-BMIPP and 99mTc SPECT. This may signal an "inverse flow-metabolism mismatch", a finding that resembles the metabolic state of "stunned" or hibernating myocardium (in the context of ACS and severe CAD, respectively).

1.5 Pathogenesis

The nature of TTC implies exposure to, and/or susceptibility to, a 'noxious stimulus'. The regional nature of the disorder suggests either (i) a regional exposure to such a stimulus *or* (ii) local injury in response to a global exposure. Although no unifying hypothesis presently explains TTC, three theories regarding its pathogenesis are most prominent in the literature and have been briefly summarized in Table 1.4. In this regard, TTC is most widely seen as a form of acute catecholamine cardiotoxicity; in fact, each of these theories incorporate catecholamine excess, directly or indirectly, in what they propose. In the following sections, pathogenetic issues beyond the three theories in Table 1.4 will also be considered. In addition, the notion that the entity of TTC may, in part, be a protective response, in order to avoid more severe consequences, has rarely been considered and will therefore be evaluated.

1.5.1 Anatomical and histological descriptors

1.5.1.1 Anatomical problems

All attempts to provide a unifying theory of the pathogenesis of TTC must address the correspondence between regional cardiac involvements with the mechanism(s) proposed, in anatomical terms. This problem is further complicated by the fact of variable

configurations of LV dysfunction, which can even occur in the same patient, on different occasions (From et al., 2011, Blessing et al., 2007). If, however, we simply consider the apical form of TTC, the issues can be summarized as below.

Anatomical implications of the 'catecholamine hypothesis

In development, sympathetic neurons invade the myocardium, following the route of the coronary arteries (Chow et al., 1995, Hildreth et al., 2008). Hence it follows that the density of sympathetic nerve terminals (SNT's, also called varicosities or boutons) is greater at the cardiac base and diminished towards the apex (Matsuo et al., 2009b) and that SNT's are more abundant in the subepicardium than the subendocardium. Correspondingly, a gradient of both efferent sympathetic neuron density and of catecholamine concentration exists, from base to apex (Mori et al., 1993). In addition, boutons are more concentrated in the anterior, compared with the inferoposterior, LV wall (Janes et al., 1986a, Matsuo et al., 2009b). Thus, the (usually) periapical distribution of myocardial injury in TTC is discordant with that of local *neurogenic* catecholamine release. However, it is possible that regional responsiveness of myocardium to blood-borne catecholamines may vary inversely with patterns of local concentration (Lyon et al., 2008). Indeed, *circulating* catecholamines are implicated in TTC associated with phaeochromocytoma or inotropic therapy.

Specifically, spatial distribution of boutons appears to vary inversely with the density of adrenergic receptors in myocardium, so that lower usual concentrations of the catecholamine ligand tend to correspond with greater cardiomyocyte sensitivity. β-ARs are *upregulated* in the context of denervation (Regitz et al., 1991) or β-blockade (Bristow et al., 1982, Bristow et al., 1986, Golf and Hansson, 1986), but *downregulated* in the context of chronically high cardiosympathetic activity (Colucci et al., 1981, Bristow et al., 1982, Fowler et al., 1986). This spatial distribution of sensitivity to a given stimulus appears to be a means of achieving uniform levels of stimulation in an end organ, where a stimulus may be delivered in a non-uniform fashion. Evidence for this was provided by Mori et al, who demonstrated increased apical contractile responsiveness (relative to the base) to direct sympathetic ganglia stimulation, noradrenaline infusion and a forskolin derivative (as an

independent stimulator of cAMP production), in open-chested dogs (Mori et al., 1993). This fact may actually render the apical myocardium even *more susceptible* to catecholamine stimulation, if exposure occurs via the vascular system and/or blood pool. However, it must be added that none of the above-mentioned studies extend to the cardiotoxic effect of catecholamines.

Anatomical considerations in the vascular/ischaemic stunning theory

Any "vascular theory" faces the problem that TTC does not involve myocardial segments that share a coronary arterial supply. Thus, such a theory would require distal vascular spasm occurring in all 2-3 major coronary arteries in concert, rather than in a single coronary (Sato, 1990); alternatively diffuse constriction of small vessels may be implicated (Angelini, 2008). However, in both of these cases, one would arguably expect diffuse subendocardial ischaemia, rather than selective apical dysfunction (Buckberg et al., 1972). The remainder of the issues connected with this set of theories is discussed in Section 1.5.2.

LVOT obstruction as a causative mechanism

A description of two cases, by Armstrong and Marcovitz, detailed a novel complication of apparent MI, whereby the concurrence of shock with a transient systolic murmur was explained by dynamic LVOT obstruction (rather than an acquired ventricular septal defect or ischaemic mitral regurgitation) (Armstrong and Marcovitz, 1996). Anteroapical wall motion deficits were attributed to MI; hypertrophic cardiomyopathy was excluded, whereas hyperactive basal segments in the LV were explained as compensatory. From 1996 to 2002, 26 similar patients were reported among Caucasians, many of whom were managed with thrombolysis (Hrovatin et al., 2002). However, Villareal et al later described three similar white females *without* ST-elevation and subsequent demonstration of normal coronaries (Villareal et al., 2001). Although the link to TTC was not appreciated, the disorder was explained in its own right, by invoking a sequence of severe sympathetic discharge and LVOT obstruction, *followed by* regional stunning.

LVOT obstruction, with a mid-cavity obstruction, is present in 11-25% of TTC patients at the time of their initial evaluation (Bybee et al., 2004a, Tsuchihashi et al., 2001, Gianni et al., 2006b, El Mahmoud et al., 2008b). Since the above observations, made in the context of presumed MI, several authors have applied a similar sequence of events to the pathogenesis of TTC, proposing that the triad of (i) abnormal myocardial functional architecture (i.e. a proximal septal bulge) (Merli et al., 2006, Villareal et al., 2001, Ionescu, 2008), (ii) reduced LV volumes (possibly with relative hypovolaemia) and (iii) a slack MV apparatus (Merli et al., 2006), might predispose to TTC or actually be causative. Merli *et al* proposed that the combined effect of this underlying anatomy, with basal hyperactivity and transient obstruction would be injurious via extreme periapical wall stress and further suggested that this phenomenon may be unrecognized in the majority because of the resolution of pressure gradients before detection on echocardiography or catheterization (Merli et al., 2006). However, while this theory may be partially relevant to the presence of hypotension in some patients with TTC, it does not represent a plausible primary basis for the variable patterns of hypokinesis seen in TTC. It is far more likely that LVOT obstruction is a variable consequence of TTC, with a partial basis in LV morphology: the haemodynamic importance of this complication will be discussed in Section 1.6.1.2.

The 'Brutsaert hypothesis': an alternative suggestion

The unique finding of axisymmetric a-/hypo-kinesis in a non-vascular segmental distribution, as seen in TTC, raises the possibility of a regional noxious stimulus. To date, the major suggestion, which has not centred on either the vasculature or the sympathetic nervous system, has come from Brutsaert (Brutsaert, 2007). Given that the endocardial surface is an important signalling interface (Brutsaert, 2003), and given that the ratio of endocardial surface to transmural thickness increases markedly toward the apex (in part because of the gradient of trabeculation from base to apex), Brutsaert postulated modulation of the process by factors released from the endocardial endothelium. To date no further experimental work has been reported to further evaluate this hypothesis, although several endothelial/endocardial autacoids may have pro-inflammatory and negative inotropic effects (Mohan et al., 1995a).

1.5.1.2 Evidence from histology and ultrastructural analysis

Autopsy and biopsy studies reveal fairly consistent changes in conventional electron microscopy, as summarized in Table 1.6. Experimental findings have revealed that the histological changes (see Table 2) in stress cardiomyopathy are quite similar to those associated with catecholamine cardiotoxicity in both human studies (Yamanaka et al., 1994) and low-dose animal models (Movahed et al., 1994). Indeed, these findings concur with those of the original post-mortem description of 'stress cardiomyopathy' by Cebelin and Hirsh (Cebelin and Hirsch, 1980) and with an earlier series, in which Nef and colleagues documented myocardial infiltrates (Rolf et al., 2009). Histological findings have been reviewed extensively from a forensic pathology point of view elsewhere (Fineschi et al., 2010).

More recently, extracellular accumulation of collagen I, angiotensin II expression, immunohistochemical evidence of superoxide generation (Nef et al., 2008b) was reported. Further biochemical findings derived from biopsy studies will be discussed in Section 1.5.4, in combination with data from animal models.

1.5.2 Is there a problem with myocardial perfusion?

Abnormal myocardial perfusion has been documented in the acute-phase of TTC in multiple ways. In addition to this, abnormalities of coronary reserve appear to persist to at least 6 months (Galiuto et al., 2010). Angiographic frame count analyses are reduced acutely, as described by multiple authors, thus indirectly suggesting microvascular dysfunction (Owa et al., 2001, Elesber et al., 2006, Bybee et al., 2004b). As discussed in Section 1.4.5.1, while abnormalities of myocardial perfusion in SPECT studies are inconsistent, ammonium PET suggests decreased coronary reserve. **The question is not, therefore, whether the microvasculature of the heart is abnormal, but whether the problem is primary (i.e. vasospastic) or secondary: the latter could easily be the case, given the presence of inflammation/oedema (see Section 1.4.4.2).**

1.5.2.1 Diffuse or multivessel epicardial coronary spasm

The tendency of brief ischaemia, followed by reperfusion, to induce reversible myocardial dysfunction is known as "myocardial stunning" and was first described by Braunwald and Kloner (Braunwald and Kloner, 1982). The initial observers of TTC invoked the idea of multivessel coronary spasm, coupled with myocardial stunning, as a potential mechanism (Sato, 1990). Initial support for a vasospastic mechanism of injury in TTC suffered when the first systematic provocation study was reported, showing significant inducible coronary spasm in only 10 out of 48 patients (Tsuchihashi et al., 2001). Angelini reported acetylcholine-induced diffuse coronary spasm in five patients with TTC (Angelini, 2008). However, these were somewhat atypical, having all experienced recurrent episodes of chest pain. The relationship of these findings to the majority of TTC cases is therefore uncertain.

1.5.2.2 Coronary reserve and microvascular dysfunction

Coronary reserve is defined as the capacity of the coronary vascular bed to reduce coronary resistance and permit increased blood flow (hyperaemia) in response to a demand or stimulus (e.g. exercise, positive inotropy or coronary vasodilation). Whatever the measurement technique, coronary reserve, expressed as the ratio of the hyperaemic state to the basal/resting state, begins to fall in the presence of a fixed coronary stenosis >45-50% (Gould et al., 1990). Abnormal coronary reserve in the *absence* of a coronary stenosis, therefore, strongly implies vasodilatory dysfunction at the microvascular level. In acute TTC, whilst exceptions should be noted (Sganzerla et al., 2008), the majority of studies have demonstrated microvascular dysfunction and/or reduced coronary flow reserve, as below:

i. Reduction in TIMI frame count (Owa et al., 2001, Bybee et al., 2004b)

ii. Reduction in TIMI perfusion grade (Elesber et al., 2006)

iii. Reduced coronary reserve by intracoronary Doppler techniques (Kume et al., 2005)

iv. Increased intracoronary resistance (Daniels and Fearon, 2011)

v. Reduced coronary reserve by myocardial contrast echocardiography (Galiuto et al., 2010)

vi. Reduced coronary reserve by nuclear imaging (Yoshida et al., 2007)

The results of two echocardiographic studies are noteworthy. Studying apical TTC patients (n = 15, approximately 3 days after presentation), Galiuto and colleagues were able to demonstrate a relative perfusion deficit in the affected (periapical) region (Galiuto et al., 2010). Apical perfusion was augmented within ~90 seconds of adenosine infusion (as shown in Figure 1.2). However, in parallel, WMSI was transiently augmented by adenosine, which does not possess major direct inotropic properties. Some corroboration comes from a case-control study by Rigo and colleagues, employing transthoracic pulsed Doppler of the LAD and PDA in basal and hyperaemic conditions (achieved via dipyridamole 0.84 mg/kg over 6 h), both within 24 hours and at discharge (Rigo et al., 2009). Although statistical significance was not reported for this observation, mean WMSI improved with hyperaemia at both time points. Dipyridamole inhibits reuptake of adenosine into platelets, red blood cells and endothelial cells leading to increased extracellular concentrations of adenosine; however, both have multiple effects.

Similar analogous effects of adenosine and dipyridamole are not known to occur, for instance, in myocardial stunning. In proposing a mechanistic explanation, it must be taken into account that both adenosine and dipyridamole have multiple actions, which are beyond the scope of this discussion (Hori and Kitakaze, 1991). One potential explanation is the “Gregg phenomenon”, whereby myocardial contractile force is coupled to coronary flow (Gregg, 1963). Given the concurrent presence of oedema and inflammation, the potential for microvascular resistance via extrinsic compression must not be overlooked: this situation likely co-exists with vasoconstriction early after TTC.

Finally, Rigo et al also reported the intriguing finding that six months after TTC, coronary flow reserve remained reduced (Rigo et al., 2009), as displayed in Figure 1.2.

1.5.3 Biochemical bases of hypokinesis: calcium, energetics and *stunning*

The concept of "myocardial stunning" has been invoked in TTC, but without clear evidence of *antecedent* myocardial ischaemia. This term originated in the laboratory, to explain transient contractile deficit after brief ischaemia in salvaged myocardium (Braunwald and Kloner, 1982, Bolli, 1990). In these classical experiments, the duration of a post-ischaemic "stunned" period was proportional to the period of anoxia, i.e., whereas active shortening began to resume ~20 seconds after 1 minute of experimental ischaemia, 15 minutes of ischaemia resulted in ≥ 1 hour of stunning. Ultrastructural damage (particularly mitochondrial) occurred in response to even brief ischaemia-reperfusion (~5 minutes) in animal models, but little in the way of myocardial oedema is seen with ischaemia relieved by 15 minutes. Clinically, the concept of myocardial stunning was subsequently applied to different clinical situations, within the framework of coronary heart disease, as summarized by Kloner and Przyklenk (Kloner and Przyklenk, 1991): for example, after coronary angioplasty, during unstable angina with intermittent ischaemia and reperfusion, during effort or stress angina, in the case salvaged myocardium after reperfusion for acute MI, or after cardiopulmonary bypass grafting.

In addition, the term has been used beyond the circumstance of ischaemia, in connection to the often prolonged left atrial dysfunction following restoration to sinus rhythm, from atrial fibrillation, by cardioversion (Kloner and Przyklenk, 1991). Some authors oppose the application of this term outside of the post-ischaemic context (Bolli, 1990).

The analogy to myocardial stunning has been applied to TTC by multiple authors, including in the manuscript describing the first case series (Dote et al., 1991, Angelini, 2008); in keeping with the original idea of myocardial stunning, a similar mechanism has been pre-supposed, i.e. ischaemia-reperfusion, without definite evidence that such a mechanism is applicable in TTC.

However, are there similarities between stunning in reperfused myocardium and reversible systolic dysfunction in TTC? Both experimental and clinical ischaemia-reperfusion injury give similar histological pictures, with contraction band necrosis (Rossi

and Matturri, 1985, Karch and Billingham, 1986, Verma et al., 2002), in contrast to the coagulation necrosis typically seen in completed infarction. Similar contracture can be reproduced in the experimental settings of ischaemia-reperfusion injury (Verma et al., 2002), in the "calcium paradox" (i.e. exposure of myocytes to a progressively hypercalcaemic medium, leading eventually to dysfunction and death) (Daly et al., 1987) and, most importantly, in high-dose catecholamine exposure (appearing within 5-10 minutes of high dose isoprenaline, in mongrel dogs) (Todd et al., 1985b, Todd et al., 1985a). Contraction band necrosis in the absence of coronary disease has also been associated with resuscitation attempts (Karch, 1987), drowning (Lunt and Rose, 1987), intracranial haemorrhage (Baroldi et al., 1997), sudden cardiac death (Fineschi et al., 1999) and sympathomimetic drugs (Fineschi et al., 1997). In pathological practice, contraction bands lesions in accidental death, are interpreted as an effect of agonal adrenergic stimulation (Baroldi et al., 2001).

Whether via ischaemia-reperfusion injury or excessive βAR stimulation by catecholamines, as a trigger for calcium influx, it has been suggested that calcium overload is relevant to TTC. Disordered calcium homeostasis has certainly been implicated in myocardial stunning and reperfusion injury (Kloner and Przyklenk, 1991) and some attempt has been made to evaluate biochemical determinants of calcium handling in human TTC and animal models thereof. Both sarcolipin overexpression and phospholamban dephosphorylation, which have been observed in early post-TTC biopsies (Nef et al., 2009b), would be predicted to negatively impact calcium sequestration in the sarcoplasmic reticulum (via negative regulation of the sarco-(endo)plasmic reticular calcium ATPase, SERCA), leading to a propensity for cytoplasmic calcium overload during changes from steady state heart rate or βAR stimulation.

Irrespective of the presence/absence of antecedent ischaemia, there is potential for TTC to be associated with myocardial energetic impairment. In the presence of intense catecholamine stimulation, high-energy phosphate depletion begins to occur in myocardium after ~5 minutes (Todd et al., 1985b). In addition, redox stress is associated with mitochondrial dysfunction and high-energy phosphate depletion (Yokota et al., 2009). Energetic impairment also occurs frequently in the presence of other forms of heart failure and appears to play a central role (Neubauer, 2007).

Early after TTC, it appears that affected segments display reduced substrate uptake (see Section 1.4.5.3), but actual metabolic data are limited. Fragmentary data are currently available, as regards the occurrence/severity/time-course of energetic impairment in human TTC (Horowitz and Frenneaux, 2011).

1.5.4 Does catecholamine exposure "cause" TTC?

Associative evidence linking TTC with supranormal catecholamine levels is compelling:- there are only occasional reports of normal catecholamine levels in series, perhaps implying that elevation is generally transient (Madhavan et al., 2009, Morel et al., 2009). Furthermore, some of the catecholamines are released from myocardium: Kume and colleagues reported elevation of noradrenaline concentrations in coronary sinus blood samples (relative to aortic concentrations; thus, a transmyocardial gradient) (Kume et al., 2008).

The main evidence for a "causative" role for catecholamines is that (a) *de* novo human TTC can directly follow catecholamine administration and (b) TTC-like changes can be induced in animals by exposure to catecholamines (see Section 1.5.5.4). In a both a rat and a primate model of TTC, β_1-AR ($\pm\alpha_1$-blockade) has been reported to be protective (Ueyama et al., 2002, Izumi et al., 2009b). In the human situation, this seems partially at odds with the fact that TTC occurs frequently in patients already receiving β-AR antagonists (Sharkey et al., 2010a).

It is clear that exogenous catecholamine administration, including intravenous infusion of high doses of noradrenaline or dobutamine in intensive care (Abraham et al., 2009) or administration of adrenaline as a result of EpiPen® use (Zubrinich et al., 2008), can precipitate TTC. Reports of TTC occurring in recipients of proadrenergic drugs, such as sympathomimetics (Alsidawi et al., 2011, Sharkey et al., 2010a), certain antidepressants (Neil et al., 2012b), or even the venom of the Irukandji jellyfish (which acts via increased sympathetic activation) (Tiong, 2009). Although, in isolation, these situations cannot be regarded as definitive evidence for a catecholaminergic cause (due to possible confounding by an *intercurrent* illness or experience), the accumulation of such reports Is

highly suggestive. However, the most compelling evidence that exogenous catecholamines *in isolation* can precipitate TTC comes from case reports in which catecholamines were administered *diagnostically*, in the context of dobutamine stress echocardiography (see Table 1.7).

The above list, however, raises yet another question: which endogenous catecholamine, adrenaline or noradrenaline, is mainly responsible? This is a pathologically relevant consideration, mainly in view of the differential potency of adrenaline and noradrenaline at the β_1AR, versus the β_2AR, respectively. The frequent finding of higher noradrenaline than adrenaline levels has lead to the use of the term "neurogenic", in relation to the catecholamine release observed in TTC (Wittstein et al., 2005), but states in which adrenaline predominates can also be associated with TTC, notably EpiPen® use (Zubrinich et al., 2008) and phaeochromocytoma. This issue is also important with regard to the pathophysiological theory of Lyons and colleagues (Lyon et al., 2008, Paur et al., 2012), who present a model of cardiac dysfunction, which presupposes/requires that adrenaline is the active "toxin", which will be discussed in relation to animal models of TTC (Section 1.5.5.4).

What is primarily lacking, with regard to a "catecholamine hypothesis", is an explanation for (i) the fact that only a minority of individuals react to catecholamines in this way, and (ii) the regional heterogeneity of the disorder within the LV, which contrasts with the global distribution of previous descriptions of cardiomyopathy due to phaeochromocytoma (Gatzoulis et al., 1998).

1.5.4.1 "Non-classical" actions of catecholamines: oxidative stress

The majority of postulated mechanisms for catecholamine cardiotoxicity in TTC have been based on "classical" functions of catecholamines, such as positive inotropy or calcium loading, or altered coupling of transmembrane βARs, yielding negative inotropy. **A question of critical importance is whether catecholamine exposure may induce oxidative stress and potentially LV systolic dysfunction via conventional βAR stimulation (independently of auto-oxidation).** There is evidence that myocardial NADPH oxidase can

be activated by β_1AR activation in a cAMP-dependent fashion (White et al., 2010). On the other hand, *chronic* noradrenaline exposure increases cardiac anti-oxidant defenses (glutathione peroxidase, glutathione reductase and superoxide dismutase) (Neri et al., 2007). The fact that, in human TTC, abnormal myocardial superoxide production has been demonstrated histologically (Nef et al., 2008b), raises the question of whether, and to what degree, oxidative stress participates in the initiating injury and early phase of TTC.

Cyclisation and auto-oxidation of catecholamines: potential role in the induction of oxidative stress

Under physiological conditions, numerous copper-containing proteins (c-cytochrome oxidase, caeruloplasmin, xanthine oxidoreductase) and reactive oxygen species (e.g. superoxide anion or hydroxyl radical) participate in the oxidation and intra-molecular cyclisation of adrenaline or noradrenaline, to form adenochrome (Bindoli et al., 1989, Genova et al., 2006). When rat hearts were perfused with radiolabelled ^{14}C-adrenochrome, a significant proportion of the radioactivity was localized in myocardial mitochondria (Taam et al., 1986, Fliegel et al., 1985). Furthermore, adenochrome can participate in redox cycling (Genova et al., 2006), resulting in enhanced free-radical production and potentially further oxidation of catecholamines (Bindoli et al., 1999); in the case of the heart, this may relate to the alteration in sarcolemmal membrane permeability associated with catecholamine toxicity (Rona, 1985). The oxidation of adrenaline to adrenochrome can occur with exposure to stimulated neutrophils (Matthews et al., 1985) and is prevented by ascorbic acid, which is concentrated in adrenal medulla and central nervous system (Bindoli et al., 1989).

The aetiology of catecholamine cardiomyopathy may be multifactorial, with contributions of microvascular dysfunction and calcium overload (Rona, 1985). However, the role of adrenochrome has been studied extensively. For example, Langendorff-perfused rat hearts exposed to adenochrome (10-25mg/kg) showed rapid decline in developed tension, followed by an increase in resting tension (Yates et al., 1981). The same was not true for similar doses of epinephrine, metanephrine, dihydroxymandelic acid or vanillylmandelic acid. Ultrastructural damage was present within 10 minutes of infusion, with alterations in

mitochondrial appearance. Isoproterenol injection may also induce ultrastructural changes, which can be prevented by pretreatment with vitamin E or zinc (Singal et al., 1982). Whilst these mechanisms have been validated in experimental settings, the "adrenochrome hypothesis" for catecholamine cardiotoxicity has been challenged, in that relatively high concentrations of adrenochrome (~10^{-4} M) are required to produce cardiac damage and it is questionable whether anything approaching these concentrations would occur *in vivo* (Rona, 1985).

In summary, despite the abundance of evidence that acute administration of catecholamines, such as isoprenaline, can produce cardiac injury in animal models, precise mechanisms, including the potential generation of auto-oxidation products, remain unclear. Although superoxide generation is evident in the myocardium of acute TTC patients, both the pathogenetic significance of this observation and the mechanisms upstream of it, have yet to be clarified.

1.5.5 Other potential biochemical effectors

1.5.5.1 Neuropeptide Y

Wittstein et al demonstrated that levels of the co-transmitter, neuropeptide Y, were increased in TTC patients, in parallel with catecholamine levels (Wittstein et al., 2005). The fact that neuropeptide Y has a primarily sympathetic origin (Zukowska-Grojec et al., 1988) and induces prolonged vasoconstriction may explain some of the findings related to abnormal microvascular function or coronary reserve, post-TTC (Zukowska-Grojec et al., 1991).

1.5.5.2 Nitric oxide

In describing the functional relationship between nitric oxide (NO) and catecholamines, attention has already been drawn to the recent finding that NO, via cGMP generation, is able to potentiate sympathetic noradrenaline release (Chan et al., 2012). However, conversely, NO release may also be partly under adrenergic control (Kanai et al., 1997,

Oddis et al., 1995). Although little information is available regarding NO biology in TTC, the potential for a contribution of NO to its pathogenesis will be considered below.

NO is known to modulate cardiac contraction/relaxation by directly acting on cardiomyocytes or via endothelial cells (Mohan et al., 1995b, Mohan et al., 1995a). NO is produced from L-arginine by three isoforms of the enzyme NO synthase (NOS). In the vascular system, constitutive or endothelial NOS (eNOS), serves to produce NO, whereas this is achieved by neuronal NOS (nNOS) in the nervous system, including the parasympathetic nervous system (Melikian et al., 2009). Vasodilation, the primary function of vascular NO, is achieved via its diffusion into smooth muscle cells, where it interacts with soluble guanylate cyclase to produce its second messenger cyclic GMP. In parallel with this paracrine action, NO transport by haemoglobin effectively gives NO an 'endocrine' action (Angelo et al., 2008).

Inducible NOS (iNOS) is elaborated by a variety of cells in response to inflammatory stimulation and plays a vital bactericidal role in the immune system. NO production by iNOS, in contrast to eNOS, is not dependent on calcium regulation and produces much greater concentrations of NO, with differing pathophysiological effects. The prototypical condition in which iNOS has been studied, septic shock, in which NO is perceived in a negative light, has led to the concept of iNOS-derived NO as the 'evil twin' of the otherwise harmless and beneficial vascular NO (Kan and Finkel, 2000, Finkel, 1996). Relevant to TTC, iNOS/NO are implicated in reversible myocardial depression in septic and post-traumatic shock (Merx and Weber, 2007, Finkel et al., 1992), as well as in viral myocarditis (Finkel, 2000), as will be discussed in a later section (Section 1.5.5.5). Furthermore, both β_2AR and β_3AR are coupled to constitutively expressed myocardial eNOS, such that NO release may follow catecholamine exposure (Moens et al., 2010).

1.5.5.3 Potential proadrenergic effect of BNP

The origin and actions of BNP have been covered in a previous section (Section 1.4.2.2). BNP, *inter alia*, exerts vasodilator effects, promotes the restoration of homeostasis and the limitation of cardiac injury. This may well be relevant in TTC and may, for instance, explain

the surprisingly low incidence of pulmonary oedema seen in TTC. However, since it has recently emerged that BNP can induce the release of catecholamines (Chan et al., 2012), a negative impact of BNP is also possible in the context of TTC.

1.5.6 Factors engendering rapid recovery: biochemical determinants

The presence of cardioprotective mechanisms in TTC may be fundamental to the rapid resolution of cardiac dysfunction in TTC, despite initial intense inflammation within the myocardium. Cardioprotective mechanisms *in states of cardiodepression* have been studied in myocardial stunning and myocardial hibernation (Depre and Vatner, 2007). Myocardial hibernation was at one time proposed to represent a condition in which chronic/intermittent myocardial ischemia resulted in a new metabolic equilibrium, in which myocardial necrosis was prevented by down-regulation of function (i.e., the ‘‘smart heart’’ hypothesis) (Rahimtoola, 1985). However, the genomic basis of cardioprotection extends beyond metabolic control. A survey of TTC-related literature reveals that, in this condition (or attempts to model it), some pro-survival mechanisms are indeed activated.

Cardioprotective processes, as exemplified by pre-conditioning, involve recruitment of a system of prosurvival kinases [phosphatidylinositol-3-OH kinase (PI3K)-Akt (also known as protein kinase B, PKB) and the p42/p44 extracellular signal-regulated kinases (Erk1/2)], collectively termed the reperfusion injury salvage kinase (RISK) pathway (Yellon and Hausenloy, 2007). Pharmacologic activation of the RISK pathway diminishes experimental infarct size, via a reduction in apoptotic and necrotic cell death (Yellon and Baxter, 1999, Hausenloy and Yellon, 2004). In this regard, Nef and colleagues documented activation of RISK pathway components in post-TTC LV myocardium, potentially limiting both cellular damage and post-inflammatory fibrosis (Nef et al., 2008b). This study showed up-regulation of Akt/PKB signaling, and its downstream targets, nuclear factor kappa-B and the transmembrane mitochondrial protein, B-cell lymphoma-extra large BcL-XL, with intact expression of energy metabolism genes. The authors postulated on the one hand, a significant pathogenetic contribution of catecholamine-triggered oxidative stress, balanced by RISK pathway activation and resultant cardioprotection.

Analogous observations, derived from human and animal model studies, include the following:

i. Haem oxygenase 1, which is induced in conditions of oxidative stress and is protective against ischaemia and reperfusion injury in transgenic mice (Juhasz et al., 2011), is expressed in immobilized rats (Ueyama et al., 2009).

ii. Natriuretic peptides and heat shock proteins, which are also considered cardioprotective via diverse mechanisms (Gerczuk and Kloner, 2012, Muller and Dhalla, 2010) and are also induced after immobilization (Ueyama et al., 2003b).

Whilst it is clear that TTC involves some acute injury to myocardium, it is likely that cardioprotective mechanisms are simultaneously activated: these has been given limited consideration thus far, but are likely to be critically important in modulating the rapid recovery from severe LV systolic dysfunction that occurs in most cases of TTC.

1.5.7 Relevance of postulated animal models

1.5.7.1 Observations from rodent models of catecholamine cardiotoxicity

Various animal models are relevant to TTC. These models, which utilize rodents, dogs and primates, are variously based on high levels of exposure to *exogenous* catecholamines or exposure to severe stress, with corresponding *endogenous* catecholamine production. Some of these models have been specifically adapted to gain insight into TTC. Insights from other studies, in which TTC was not of primary interest, but from which relevant insights can be drawn, will also be discussed.

The isoprenaline-treated rat has been used in various research applications, since its original description by Rona in 1959, for the study of heart failure remodelling. All of these models utilize supraphysiological doses of catechol (usually milligram per kilogram doses of isoprenaline, given as a subcutaneous or intraperitoneal injection) (Rona et al., 1959). Rats, thus exposed, develop variable amounts of coagulation necrosis/neutrophilic infiltration, similar to infarction, with the resultant scar being subendocardial and predominantly apical/mid-ventricular, similar to that reported in human catecholamine

cardiomyopathy. Associated inflammatory activation is marked (Grosjean et al., 1999): the heart/body weight ratio increases within hours, suggesting myocardial oedema, in the presence of inflammatory cell infiltrate (Panagia et al., 1985). Rapid structural and functional changes are also evident in subcellular organelles. These have been best described by Tanaka and colleagues, who observed cells with a mixture of these patterns, with respect to myofibrillar structure: normal cells, cells with a normal myofibrillar arrangement but normal myofibrils and finally, cells exhibiting myofibrillar destruction (Tanaka et al., 1980). Irrespective of the degree of myofibrillar damage, distinct organellar alterations were observed: mitochondrial changes (swelling, loss of cristae and myelin figure), dilation of the sarcoplasmic reticulum and T-tubules and nuclear changes; an increase of glycogen granules was also noted.

These high-dose catecholamine models have various applications, namely, the creation of a model of heart failure, the assessment of cardiotoxicity mechanisms specific to catecholamines and the study of regenerative capacity of the heart. However, the extensive degree of myocardial necrosis fails to recapitulate, precisely, the clinical scenario of TTC: specific efforts to achieve this will now be reviewed.

1.5.7.2 Application to TTC: rodent models

Tanaka and colleagues were the first to note cardiac lesions, analogous to those seen with exogenous catecholamine exposure, in rats exposed to repeated immobilization stress and water immersion (Tanaka et al., 1980). They further noted similar ultrastructural changes, when comparing these models. Specifically with regard to the study of TTC, the major body of work has come from Ueyama and co-workers (Ueyama et al., 2011, Ueyama et al., 2003b, Ueyama et al., 2009, Ueyama et al., 2002, Ueyama et al., 2003a, Ueyama, 2004, Ueyama, 1999) who have utilized immobilized rats to recapitulate stress-induced reversible cardiac dysfunction, at least in terms of ECG changes and left ventriculographic appearance.

Their findings can be briefly summarized as follows:

i. Immobilization stress–induced ECG and ventriculographic changes can both be obviated by pretreatment with combined blockade of α_1 and β_1ARs, but not by either alone, or by a calcium channel blocker (verapamil) or glyceryl trinitrite

ii. In ovarectomized rats, supplemental oestrogen partially attenuated these immobilization-induced changes

iii. Immobilization has been shown to induce rapid myocardial expression of

- p44/p42 mitogen-activated protein kinase
- immediate early genes (e.g., c-fos and c-jun)
- heat shock protein 70
- natriuretic peptide genes (ANP and BNP)
- haem oxygenase 1

iv. Utilizing a combined DNA microarray and real-time reverse transcription-polymerase chain reaction methodology, gene expression in rat heart and aorta, was examined in the first three hours.

- 30 of 37 (81%) of genes upregulated in the heart were prevented by beta-1-AR blockade
- 47 out of 48 (98%) of genes upregulated in the aorta were prevented by α_1AR blockade.

In regards to the last finding, at least as far as the disease process is reflected by myocardial gene expression, the signal transduction pathway triggered by β_1AR stimulation was extensively implicated; these authors did not consider the potential role of β_2AR.

Less well known, but complimentary, is the work of the group at the West Virginia School of Medicine, who produced a novel rat model of stress cardiomyopathy (Chen et al., 2009, Kan et al., 2005), utilizing repeat acute stressor exposure, separated by one week, involving two hours immobilisation alternating with exposure to an open space (without

shelter). Reversible cardiomyopathy, described in terms of invasive haemodynamic data and reduced fractional shortening by M-mode echocardiography was prevented by a p38-MAPK inhibitor, SB-203580.

Additionally, Ellison and colleagues utilized a lower dose bolus isoproterenol (5mg/kg, compared to >50mg/kg in most rat models of catecholamine cardiotoxicity) in the rat to examine catecholamine-induced effects on myocardium, and cardiac stem cells in particular (Ellison et al., 2007). Similarities with the TTC phenotype included initial ST-elevation, and recovery of global parameters of LV function, by day 6, in the ~80% of rats which survived. However, the extent of subendocardial necrosis seen in this model – with 17% loss of myocyte mass – clearly exceeds damage usually sustained in TTC. Nevertheless, this study documented cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP), both hallmarks of apoptotic cell death, one day after catecholamine exposure, the later of which is a potential pharmacological target.

A new model: the potential role of "biased agonism" of β_2AR in TTC

In 2008, Lyon et al published a hypothesis to explain TTC, based on the phenomena of ligand- or stimulus-directed trafficking and biased agonism for adrenaline at the β_2AR (Lyon et al., 2008) (see also Section 1.5.1). They argued, first, from the anatomical viewpoint, that not only was there an increasing gradient of β_1AR density from base to apex, but that the ratio of β_2AR:β_1AR also increased apically (as reviewed in Section 1.5.1). The main events of this theory are, in sequence:- (i) the intense activation of transmembrane βARs by their catecholamine ligands, (ii) the cAMP and PKA-dependent phosphorylation of the β_2AR, leading to (iii) the alteration of post-receptor signaling from "canonical" G_s to inhibitory G_i, so as to (iv) negatively regulate adenylate cyclase and reduce intracellular cAMP, on further activation by adrenaline. Of note, the first step can involve both adrenaline or noradrenaline (or a synthetic catecholamine), so that intense initial noradrenaline β_1AR could theoretically bring about the coupling of β_2AR:G_i (Daaka et al., 1997). However, once in place, further activation of β_2AR relies on adrenaline alone: hence, the term "biased agonism".

Very recently, this group published their findings from a new rat model, in which comprehensive testing of this hypothesis was carried out (Paur et al., 2012). Certain problems exist, however, in reconciling this model with pre-existing animal work, as well as in translating the findings into the context of human TTC. Briefly, male rats, treated with an intravenous bolus or adrenaline 4.28 x 10^{-8} mol/100g demonstrated a TTC-like pattern of reversible apical contractile depression/basal hypercontractility on 2D echocardiography. Notably, a higher dose of noradrenaline (1.43 x 10^{-7} mol/100g) did not induce similar changes. Confirmation of greater β_2AR number and functional responses was carried out in isolated apical cardiomyocytes (compared to basal cardiomyocytes), in contrast to previous findings with β_1AR. In vivo, a subgroup of animals pretreated with pertussis toxin (a G_i protein inhibitor) did not demonstrate the adrenaline-induced changes, suggesting involvement of G_i activation in the toxic effect of adrenaline; however, this led to increased mortality. Likewise, pretreatment with the p38 MAPK antagonist, SB203580, led to rapid haemodynamic deterioration/death, although not preventing apical dysfunction; the latter finding is the opposite of that of Chen and colleagues, with the same agonist, in immobilization stress (Chen et al., 2009). In contrast, levosimendan rescued the acute cardiac dysfunction without increasing mortality.

Lyon and colleagues thus emphasize the centrality of adrenaline (as distinct from noradrenaline) and potentially β_2AR, rather than β_1AR stimulation, in the initiation of contractile dysfunction in TTC. Notably, the previous frequent referral to TTC as a "neurogenic" myocardial stunning, implies that noradrenaline is the main actor in this condition and this assumption may have arisen from observations of early case series, demonstrating higher noradrenaline concentrations than those of adrenaline (Wittstein et al., 2005, Guglin and Novotorova, 2011). It is relevant that noradrenaline reaches the myocardium via local release from sympathetic neurons *and* via blood pool exposure, as a result of whole body "spillover" (Esler, 1993, Ferrier et al., 1993); in contrast, adrenaline derives largely from the adrenal glands, affecting the heart solely via the circulation. Very high circulating catecholamine levels, such as are seen in phaeochromocytoma, inhibit sympathetic nerve terminal re-uptake via NET (Eldadah et al., 2004): this would likely also enhance cardiomyocyte exposure to noradrenaline. Despite this caveat, in this model, reversible apical dysfunction is said to be "adrenaline specific".

Proponents of this model also need to reconcile it with other attempts to model TTC in rodents (Ueyama, 1999) and, particularly, primates (see below) (Izumi et al., 2009a), which have focused on β_1AR activation and to some degree α_1AR (see above). In any case, it would be expected that adrenaline exposure would correlate with measures of acute severity, if adrenaline:β_2AR:G_i signaling were mainly responsible for human TTC. Such data are only available for normetanephrine concentrations, which directly related to consequent NT-pro-BNP release in human TTC (Nguyen et al., 2011a). In addition, it must be noted that the sustained nature of overt hypokinesis in TTC (i.e. days to weeks, at most) is not reflected by this single bolus model, in which hypokinesis normalized within an hour. On the other hand, this is not inconsistent with the findings of Barletta et al, who demonstrated provocation of transient WMA in *recovered* TTC patients (versus controls) in response to cold pressor testing (Barletta et al., 2009b).

It is also possible but unlikely, that sustained hypokinesis in human TTC is the result of sustained increases in adrenaline release, which might be associated with diminution of contractile response to catecholamines. In the acute phase of TTC, contractile performance of affected segments can be augmented, as demonstrated by simple post-extrasystolic potentiation (Kurisu et al., 2007a). The fact that this contractile performance is refractory to dobutamine (Fujiwara et al., 2007) but can be enhanced with levosimendan (Padayachee, 2007b) (see Table), is certainly consistent with the model of Paur et al, who demonstrate the ability of levosimendan to “rescue” TTC-like LV dysfunction.

Therefore, the mechanism(s) by which hypokinesis is maintained after normalization of catecholamine concentrations, is of great interest in “unraveling” TTC. Essentially, the issue is the kinetics of recovery of the cellular processes underlying hypokinesis. Therefore, the contribution of intramyocardial inflammation, as demonstrated by others, and ourselves, should not be overlooked (Morel et al., 2009, Eitel et al., 2011b, Neil et al., 2012a).

In summary, the model proposed by Paur et al is internally consistent and their findings highly informative: but they may tell us more about the *initiation* of apical dysfunction in TTC, than they do about the maintenance of LV abnormalities in the acute phase. In the light of other models, it is probable that different cardiotoxic mechanisms coexist and vary between catecholamines and between different levels of exposure. As stated elsewhere,

the presence of (i) cardioprotective/cardiodepressive mechanisms in TTC (as explored by Paur et al) and (ii) the finite duration of inflammatory responses, are implicit in the short-term recovery of TTC (see Section 1.5.6).

1.5.7.3 A non-human primate model of TTC

Izumi et al have described a model of acute reversible apical LV dysfunction, employing repeated noradrenaline infusions (10micromol/kg/min) in cynomolgus monkeys (gender not specified). Interestingly, 9 of the monkeys died within 48 hours and 6 did not develop significant cardiac impairment, leaving 10 available for study. ECG monitoring showed analogous changes to human TTC (mainly ST-elevation, with some T-wave inversion) and apical dysfunction was demonstrated via 2D echocardiography. Among the genes selectively upregulated in the apex (relative to basal tissue) were BNP, SERCA, the angiotensin II receptor and mitochondria-related genes, such as peroxisome proliferator-activated receptor 1a and cytochrome c. Osteopontin was also expressed in the extracellular matrix, as has been shown in human biopsy studies (Nef et al., 2008b). Interestingly, in the light of the work of Paur and colleagues (Paur et al., 2012), although both β_1 and β_2AR would have been activated by exogenous adrenaline, apical hypokinesis and abnormal gene expressions were attenuated by selective β_1AR antagonism (metoprolol). Within this model it is therefore likely that β_1AR activation, rather than that of β_2AR, plays the principal role.

1.5.8 Key unanswered questions

1.5.8.1 Insights into predisposition in females

Consideration of the gender disparity in TTC is critical to understanding pathogenesis. In addition, the fact that most female TTC patients are post-menopausal (a time of female life characterized by a transition from parasympathetic to sympathetic dominance in the autonomic control of the cardiovascular system) has yet to be adequately explained. To date, no sex hormonal basis for TTC has emerged, nor has any epidemiological link

emerged with post-menopausal hormonal therapy. However, the differential handling of, and possibly differential susceptibility to, catecholamines, in oestrogen-deficient female hearts, remain important considerations in the pathogenesis of TTC.

In regards to sexual dimorphism in the sympatho-adrenal axis, and cardiac sympathetic outflow in particular, a number of observations can be cited:

i. Females have greater parasympathetic tone, less sympathetic control of heart rate, but higher heart rate at rest (Cingolani et al., 2011); in premenopausal life, sympathetic outflow varies during the menstrual cycle (Hinojosa-Laborde et al., 1999).

ii. Basal plasma noradrenaline levels are higher females, whereas adrenaline levels are higher in men; sex differences are maintained under conditions of standardised exercise, mental stress and cold-pressor testing (Davidson et al., 1984).

iii. Consistent with this, cardiac spillover of tritiated noradrenaline in women, exceeds that of men, in health, as well as in the context of heart failure (Mitoff et al., 2011).

iv. A major reason for the above differences may be reduced NET/uptake-1 contribution to cardiac noradrenaline turnover in women: heart rate responses during head-up tilt were potentiated more by NET inhibition (with reboxetine) in men, than in women (Schroeder et al., 2004).

The potential differential susceptibility of female vasculature and myocardium to catecholamine effects/toxicity have been extensively reviewed elsewhere (Hinojosa-Laborde et al., 1999). However, some observations relevant to TTC are as follows:

i. Forearm vasoconstriction in response to noradrenaline is attenuated in pre-menopausal women, as a result of increased β_2AR responsiveness, including associated NO release (Kneale et al., 2000), whereas in postmenopausal women, stress-mediated vasoconstriction may be enhanced (Kaski, 2006).

ii. In the heart, ovarectomised rats exhibit increased β_1AR expression versus sham-operated rats (Chu et al., 2006); the impact of oestrogen deficiency on cardiac *expression* of calcium handling proteins has been variously reported, but robust

experimental evidence of reduced SERCA *activity* after menopause has been provided in rat models (Bupha-Intr and Wattanapermpool, 2006).

iii. Finally, females have relatively reduced pressor responses to exercise, but exhibit a greater lipolytic response, as determined by serum free fatty acid levels (Davis et al., 2000): this may implicate differential stimulation of non-cardiac sympathetic outflow between genders. This may be relevant, given the detrimental effect of high concentrations of circulation fatty acids on cardiac metabolic efficiency (Grynberg and Demaison, 1996).

Although data are incomplete, if enhanced neurogenic catecholamine exposure in female hearts (via diminished reuptake) were coupled with an aging-associated increase in cardiac β_1AR responsiveness/impaired calcium handling, these facts would explain much, if not all, of the predominant occurence of TTC in aging females.

1.5.8.2 What underpins individual susceptibility to TTC?

Is there a genetic predisposition?

An inherited propensity to develop TTC is suggested by the observation of TTC in three sets of first degree relatives, two sisters (Pison et al., 2004), and two mother–daughter pairs (Cherian et al., 2007a, Kumar et al., 2010); in none of these reports was there any known inherited medical predisposition towards TTC, such as a familial endocrine tumour syndrome. One study examined genetic polymorphisms in ARs, and concluded that such polymorphisms were not pathogenetically relevant among TTC patients, [although apparently important in the expression of cardiac injury after subarachnoid haemorrhage (Zaroff et al., 2006)]. This study was limited to the α_1 and β_1AR subsets (Sharkey et al., 2009); Handy et al provided complementary information with regard to the β_2AR by gene sequencing, but in only one patient (Handy et al., 2009). In a cohort of 22 TTC patients, Spinelli and colleagues confirmed the above observations with regard to the adrenoreceptor family (with the notable exception of data regarding the β_3AR), but additionally studied polymorphisms of GRK5 (see Section 1.4.2.3) (Spinelli et al., 2010). TTC patients exhibited a higher prevalence of leucine (versus glutamine) substitution at

amino acid 41 of the non-catalytic domain, which may imply differential βAR desensitization among TTC patients.

Individual hypersensitivity to stimuli: propensity to recurrence

The question remains as to whether individuals with TTC are vulnerable because of a *supraphysiological generation* of/exposure to a noxious stimulus (e.g., catecholamines) or because of an *unphysiological response* to a physiological stimulus?

Recently, three case-control studies have addressed these questions in a controlled manner, demonstrating reversible abnormal physiological responses to adrenergic stimulation in TTC (see Table 1.6). The first of these employed mental stress itself (Sciagra et al.), whilst the second employed brief immersion of the hand in ice water (cold pressor test), as a means to provoke sympathetic nervous system activity (Barletta et al., 2009b); both assessed myocardial function simultaneously. The third assessed vascular function in response to various mental stress challenges (Martin et al., 2010). None of these patients experienced chest pain or a recurrence of TTC, despite the induction of a minor cardiac contractile deficit (a transient fall in ejection fraction ~5%, as per Barletta et al). It is therefore noted that the studies above have described *asymptomatic/subclinical and acutely reversible changes* in parameters of cardiovascular function, which is in contrast to the clinically discernible form of TTC (associated with pain and more prolonged cardiac dysfunction).

As such, these findings raise important questions about the nature of TTC and the propensity of certain patients to develop it. The finding of abnormal endothelial responsiveness at rest/baseline (Martin et al., 2010) is of significance for understanding long-term cardiovascular risk in these patients and, therefore, appropriate long-term management; the same would apply to the issue of platelet function in TTC, which has hitherto been unreported. However, the central issue underlying these three studies is whether TTC patients are subject to 'unphysiological' responses in response to release of catecholamines.

1.5.8.3 Basis for acute hemodynamic heterogeneity

Although several haemodynamic aspects of TTC have been mentioned in passing, it is important to emphasize that no one pattern is consistent. An acute episode may be characterized by hypotension *or* hypertension and with the variable presence of LVOT obstruction, mitral regurgitation, RV dysfunction and pulmonary congestion: understanding this heterogeneity is likely to facilitate risk stratification.

A proportion of TTC patients develop early and severe compromise. One study sought to examine the severity of cardiac failure and hypotension in TTC, based on the application of the Killip classification in 118 patients (Madhavan et al., 2011, Killip and Kimball, 1967). The combined proportion of Killip classes II, III and IV, was ~45%. Approximately 1 in 5 patients developed Killip IV/cardiogenic shock, whereas Killip III/pulmonary oedema was actually *uncommon* in this sample (<5%). Mean LV ejection fraction was lower and the wall motion score index and RV systolic pressure measurements were marginally (but significantly) increased, in those with Killip ≥ II. The role of RV systolic function, which might compound a low cardiac output state, was not evaluated. The low incidence of pulmonary congestion in this series concurs with the invasive haemodynamic measurements discussed in section 1.4.3.2: combining the two relevant study samples, 9 out of 66 subjects exhibited a pulmonary capillary wedge pressure ≥ 17mmHg (Akashi et al., 2004a, Nguyen et al., 2011a). On the other hand, the mean cardiac index appears to be substantially depressed in the acute phase (for example, 1.9 ml/min/m^2, in the series of Akashi et al, 2004).

The issue of "obstructive TTC" was the focus of one retrospective series of 32 patients. In this, patients with LVOT obstruction (n = 8) universally had a proximal septal bulge, with systolic anterior motion of the mitral valve and mitral regurgitation, thus analogous to hypertrophic obstructive cardiomyopathy (El Mahmoud et al., 2008a). While the prevalence of LVOT obstruction in this series may be exaggerated, it is also possible that other estimates from pooled data (11-15%) suffer from underreporting (Bybee et al., 2004a, Gianni et al., 2006b). Finally, the use of positive inotropic agents will obviously impact on dynamic outflow tract obstruction, either inducing or exacerbating obstruction. Fefer and colleagues were able to summarize 62 cases in the literature, but the incidence of use of positive inotropic agents in this subset was not clear (Fefer et al., 2009).

A unique series, of 47 patients from New York, focused more attention of mitral regurgitation in acute TTC, which was present in one quarter of patients (Izumo et al., 2011). *Only half* of these patients demonstrated obstruction/systolic anterior motion of the mitral valve, whereas the remainder exhibited mitral regurgitation *without* obstruction. In the latter group, the authors noted a lower ejection fraction, with higher coaptation distance and tenting of the mitral valve, indicative of "mitral valve tethering". Thus, the mitral incompetence in these cases was likely the result of apical displacement of the papillary muscles in systole, akin to the mechanism for regurgitation seen in dilated cardiomyopathy. Estimated right heart pressures were elevated in those with mitral regurgitation of either mechanism, but correlates of their clinical condition were not reported in detail.

The questions which arise from this, are:

i. Is hypotension fundamentally engendered by low cardiac output and/or by vasodilation?

ii. Is the presence (in some cases) of outflow tract 'obstruction' clinically significant as a partial basis for low CO?

iii. What is the reason for the relative rarity of severe pulmonary congestion?

None of these issues have been addressed by appropriately detailed acute hemodynamic studies thus far. The need for resolution of these issues may carry implications such as (a) the choice between the use of intra-aortic balloon pump and non-catecholamine positive inotropic agents for TTC with shock (Padayachee, 2007a) and (b) the potential role of fluid supplementation in TTC.

1.5.9 Potential links to other forms of acute cardiomyopathy

Approaching the issue of non-ischaemic myocardial injury, Finkel suggested a framework of understanding several conditions from the perspective of inflammation (Finkel 1996). Reversible myocardial depression seen in patients with severe sepsis is associated with local and systemic elaboration of proinflammatory cytokines and nitric oxide (NO).

Numerous studies in experimental animals imply that this relationship is causal. Taking sepsis cardiomyopathy as a prototype condition, Finkel suggested that to some degree, common mechanisms might underlie myocardial dysfunction in diverse conditions involving myocardial inflammation: Sepsis, Trauma, Ischaemia, cardiac Transplant rejection, myoCarditis and congestive Heart failure (the "*STITCH syndrome"*).

Sepsis cardiomyopathy

Myocardial depression in sepsis (Finkel, 1996, Hunter and Doddi, 2010), can be a fatal complication in septic patients: these are usually distinguished by a low cardiac index, often refractory to inotropic support, with biventricular dilatation and systolic dysfunction (Parker and Parrillo, 1983, Parker et al., 1990, Morelli et al., 2005). Unlike TTC, this condition usually affects the heart globally, rather than regionally. In survivors, cardiac function usually recovers in 7-21 days (Merx and Weber, 2007). Although sepsis cardiomyopathy has been considered a complication of the minority of septic patients ("hypodynamic" shock), this may only be a matter of degree: even patients with so-called "hyperdynamic" shock exhibit myocardial dysfunction, relative to non-septic controls. Transient dilatation of the LV, in this context, may preserve cardiac output via the Starling mechanism: this has been suggested to be an adaptive response (Flierl et al., 2008, Rudiger and Singer, 2007).

Animal models have facilitated understanding of the myocardial depression seen in the complex human context. Parillo et al examined the effects of sera from shock patients on isolated rat myocytes (Parrillo et al., 1985) and found depression of contraction, which was reversible with a change of medium. The same group, again using isolated rate myocytes, later showed similar reversible effects with recombinant cytokines, TNF-α and IL-1β (Kumar et al., 1996). Similar work with IL-6 also documented reduced calcium transient in cultured chick cardiomyocytes (Kinugawa et al., 1994). From these studies, attention has been given to cytokines as potential mediators of sepsis-associated myocardial depression, which are released locally by several types of nucleated cells in response to surgical, traumatic, ischemic or septic insults, and can circulate (Finkel, 1996).

However, the cellular mechanisms underlying cytokine-mediated cardiomyopathy appear to centre on cytokine induction and downstream events. Inducible NO synthase (iNOS), which is expressed in response to endotoxin and a variety of cytokines, plays a prominent role in the peripheral circulation in septic shock. Evidence that iNOS is also involved in myocardial depression, includes the abolition of cytokine-induced negative inotropy with NOS inhibitors, such as NG-monomethyl-L-arginine (L-NMMA) (Hare and Colucci, 1995). In another study, rat macrophages with or without pre-exposure to endotoxin were co-incubated with rat cardiomyocytes for 24 hours. Myocyte shortening was significantly reduced in cells incubated with endotoxin-exposed macrophages, but this effect was abolished by addition of L-NMMA (Balligand et al., 1993). More prolonged (>18 hour) exposure of isolated cardiomyocytes to LPS, TNF-α, or IL-1β may also increase iNOS levels (Stein et al., 1996); the addition of dexamethasone (which reduces iNOS transcription via NFκB inhibition) was protective in this context. Although the exact target of iNOS-generated NO in sepsis cardiomyopathy is not known, simultaneous high fluxes of NO and the superoxide anion result in the generation of peroxynitrite. Peroxynitrite-induced cellular damage occurs via lipid peroxidation, protein nitration and DNA strand breaks (Beckman and Koppenol, 1996). In septic shock patients, peroxynitrite is already known to play a role in the *peripheral* circulation, contributing to hypotension and vasopressor insensitivity (Pacher et al., 2007). The demonstration that a peroxynitrite decomposition catalyst was cardioprotective in a rat model of sepsis (Lancel et al., 2004), has implicated nitrosative stress in the genesis of sepsis cardiomyopathy (Hunter and Doddi, 2010, Zanotti-Cavazzoni and Hollenberg, 2009). It also has been proposed as both a cardiodepressant and a potential therapeutic target in other myocardial disorders, such as viral myocarditis (Kooy et al., 1997, Freeman et al., 1998) or doxorubicin cardiotoxicity (Weinstein et al., 2000, Pacher et al., 2003)

In sum, it appears that cytokines play a central role in sepsis cardiomyopathy. These observations may be important for understanding TTC, from several points of view: numerous TTC patients have intercurrent septic/inflammatory conditions; there is growing documentation of myocardial inflammation/oedema (Otsuka et al., 2008, Rolf et al., 2009, Abdel-Aty et al., 2009, Neil et al., 2012a), including the presence of leucocytes (Nef et al., 2007b); increased levels of the proinflammatory cytokine, interleukin 7 (IL-7), has recently been demonstrated (Pirzer et al., 2011). However, has a role for catecholamines been

delineated in sepsis cardiomyopathy? It is notable that reduced βAR responsiveness follows exposure of cardiomyocytes to activated macrophages (Balligand et al., 1993) or cytokines (Gulick et al., 1989). Intriguingly, β_3AR are upregulated, both in human myocardium during sepsis and also in cytokines-treated murine cardiomyocytes (Moniotte et al., 2007). Specific β_3AR stimulation led to negative inotropy, which was attenuated by NOS inhibition. Thus, a contribution of adrenergic signaling, in combination with NO generation, may well be a component in human sepsis cardiomyopathy: the potential for similar mechanisms in TTC has not been considered.

Thus, despite the acknowledged differences between sepsis cardiomyopathy and TTC, there are also substantial similarities: these disorders may represent an overlapping spectrum of acute inflammatory cardiomyopathies (Finkel, 1996), with different initiating events.

1.6 Natural history of Tako-Tsubo cardiomyopathy

1.6.1 Short-term complications and outcomes

In patients admitted to hospital alive, the prognosis of stress cardiomyopathy is generally favorable, with an estimated in-hospital mortality rate of 1-2% (Gianni et al., 2006b, Nef et al., 2010a). The potential lethality of some of conditions associated with TTC (e.g. subarachnoid haemorrhage, sepsis, etc., as discussed above), also needs to be borne in mind: it is not clear to what extent critical non-cardiac illness contributes to overall mortality in the acute phase.

1.6.1.1 Acute complications of TTC

Other non-haemodynamic early complications include tachyarrhythmias, notably *torsades de pointes* (which may precede maximal QT interval prolongation) (Samuelov-Kinori et al., 2009a, Kucia et al., 2010), thrombogenic complications secondary to mural LV thrombus (Singh et al., 2007) ± stroke/systemic embolism (Cho et al., 2010). Several reports of *torsade de pointes* have been fatal (Wedekind et al., 2009, Okada et al., 2007) and several

autopsy cases have reported free wall rupture, mostly around the apex (Akashi et al., 2004c, Ohara et al., 2005), a site at which greater wall stress may be anticipated (due to increased radius of wall curvature toward apex).

1.6.1.2 Haemodynamic status in TTC

Clinically, the majority of patients are normotensive on presentation, but a substantial minority is hypotensive (Madhavan et al., 2011). Hypotension is occasionally severe and a shocked state may necessitate insertion of an intra-aortic balloon pump (Sharkey et al., 2005). Hypotension/low cardiac output appears to be a more prominent problem than pulmonary congestion (Madhavan et al., 2011) and is only partially explained by the phenomenon of mid-ventricular or LV out-flow tract obstruction in TTC.

The only systematic series employing right-heart pressures and cardiac output data, suggested a surprisingly *low* mean pulmonary capillary wedge pressure. The picture was, on average, that of peripheral hypoperfusion without pulmonary congestion (Forrester class III) (Akashi et al., 2004a); nevertheless, reports of acute pulmonary oedema do exist (Pineda Pompa et al., 2004, Park et al., 2005, Wittstein et al., 2005). Given the degree of cardiac dysfunction (reflected in the degree of wall motion impairment and low cardiac output), it is perhaps interesting that pulmonary oedema is not more common.

A dynamic intraventricular pressure gradient can occur in TTC, with an estimated frequency of 12-25% (Gianni et al., 2006b). This is due to a highly active LV base, in which stimulated radial contraction narrows the diameter of the outflow tract. Diagnosis of this complication can be either by direct measurement of intraventricular pressures at catheterization, or indirect appreciation of a within-LV pressure difference, via Doppler echocardiography: pressure differences of ≥30 mmHg have been taken to indicate severe dynamic outflow tract obstruction, based on the classification of obstructive hypertrophy cardiomyopathy. Also analogous to hypertrophic obstructive cardiomyopathy, mitral regurgitation due to systolic anterior motion of the anterior mitral valve leaflet can occur in this context, as can, post-extrasystolic potentiation which transiently worsens the gradient in obstructive TTC (Kurisu et al., 2007b).

The co-existence of obstruction with hemodynamic impairment raises the issue of this as a cause of the impairment: the contribution of obstruction to hemodynamic impairment is discussed in a later section (Section 1.5.8.3). Regardless, hypotension in TTC (with or without outflow tract obstruction) remains a significant issue. This is superficially paradoxical in the setting of high ambient concentration of catecholamines and may be mediated by factors other than reduced LV function. For example, the marked elevation of circulating levels of natriuretic peptides in TTC (Lee et al., 2009a, Madhavan et al., 2009, Morel et al., 2009), may produce peripheral vasodilatation and, in the absence of hypervolaemia, a low systemic flow state. An alternative reason for hypotension would be vagal tone, which may be expected to be elevated in the situation of cardiac distension or increased wall stress (as would be expected in TTC). Interestingly, Dib *et al* provided a hint of this in their ECG study (Dib et al., 2008), in which R-R variability (an indicator of vagal modulation) was increased.

1.6.2 Long term outcome

TTC patients have a similar four year survival to age and gender matched individuals from the general community (Elesber et al., 2007). Recurrence has been widely reported (Nef et al., 2007a, Ikeda et al., 2009, Cherian et al., 2007b). The initially reported incidence of 2 out of the 88 subjects, over a mean follow-up period of 13 months (Tsuchihashi et al., 2001), would appear to have been an underestimate, given four year follow-up data from the Mayo Clinic (Elesber et al., 2007), indicating a recurrence rate of 11.4% over that period. The latter report also drew attention to a high incidence of post-discharge chest pain, without TTC recurrence, which has implications for the education and follow-up of these patients.

Interestingly, development of fatal malignancies during follow-up has been reported to be a disproportionate event in TTC, prompting the authors to raise the potential explanation of a paraneoplastic phenomenon (Burgdorf et al., 2008a). If such an association is real, it is possible that the presence of carcinoma (or its treatment) predisposes to TTC, but equally, a common defect may predispose to *both* of the conditions (such as immunosuppression),

accounting for the overlap. However, it must be emphasized that the existence of such an association remains questionable.

1.7 Therapeutic considerations

The clinical management of TTC at the present time is guided largely by empirical principles rather than by actual controlled trials. It may be argued that the majority of TTC patients require no specific therapy, on the basis of their uncomplicated course. On the other hand, complicated or severe TTC requires very active management.

Continuous ECG monitoring for approximately the first 48 hours is advisable, because of the risk of tachyarrhythmias (Ghosh et al., 2008, Raddino et al., 2008). Similarly, although the putative benefit of anticoagulation cannot be proven in the absence of randomised trials, it is likely justified for at least 48 hours, because of the risk of LV mural thrombosis (Azzarelli et al., 2008) and embolism (Grabowski et al., 2011).

Initial supportive therapy may be needed in patients with severe hypotension. In this regard, there are sufficient grounds to discourage catecholamine inotropes: specifically, (i) contractile performance appears to be refractory to dobutamine early after TTC (Fujiwara et al., 2007), (ii) whereas catecholamines appear to be able to precipitate *de novo* TTC (evidence summarized in Table 1.9), evidence also suggests that acute administration can worsen clinical course in the acute phase and (iii) both the worsening and provocation of "obstructive TTC" (discussed in Section 1.5.8.3) are theoretical possibilities to be borne in mind. Balloon counterpulsation and possibly the non-catecholamine positively inotropic agent, levosimendan (Padayachee, 2007a) (see Table 1.8), should be considered preferable means of circulatory support.

There is no randomised data to guide preventative therapy, although recurrence (discussed in Section 1.6.2) is an obvious target. In the minority who present with out-of-hospital cardiac arrest, a dilemma as to the role of a secondary prevention defibrillator exists. As the risk of recurrent ventricular arrhythmias arising from recurrent TTC is unknown, the efficacy of such a strategy is hard to predict: it is likely that present practice

in this uncommon situation is wholly guided by physician preference. As a minimum, however, alternative causes of ventricular tachycardia should be carefully considered.

Research directed at improved early diagnosis, understanding of pathogenesis, and evaluation of factors associated with recovery and recurrence is central to the future development of acute and chronic treatment regimes for this important condition.

1.8 Scope of the current studies

The experimental chapters that follow are structured around the broad themes of diagnosis, acute pathogenesis and long-term recovery, and will therefore focus on:-

i. understanding and improving the timely discrimination of TTC from ACS (Chapter 2)

ii. examining the role of inflammation in the acute-phase of TTC (Chapter 3) and

iii. critically evaluating the time-course of recovery of LV impairment, in functional and structural terms, and whether recovery is complete or otherwise (Chapter 4).

1.9 Tables

Table 1.1 — Conditions associated with raised cardiac troponins

(analytical causes excluded)

Adapted from Ammann et al., 2004 Jeremias and Gibson, 2005.

Cardiac diseases and interventions	
Cardiac amyloidosis	Heart failure
Cardiac contusion	Hypertrophic cardiomyopathy
Cardiac surgery	Myocarditis
Cardioversion/defibrillation	Percutaneous coronary intervention
Closure of atrial septal defects	Cardiac transplantation
Coronary vasospasm	Radiofrequency ablation
Dilated cardiomyopathy	Supraventricular tachycardia
Extra-cardiac disease	
Critically illness	Renal failure
Hypotension/hypovolaemia	Subarachnoid haemorrhage
High dose chemotherapy	Sepsis and septic shock
Sympathomimetic ingestion	Stroke
Primary pulmonary hypertension	Ultra-endurance exercise (marathon running)
Pulmonary embolism	

Table 1.2 — Mayo Clinic diagnostic criteria for Tako-Tsubo cardiomyopathy

(adapted from Bybee et al., 2004 and Madhavan and Prasad, 2010)

1	Transient hypokinesis, akinesis, or dyskinesis of the left ventricular mid segments with or without apical involvement; the regional wall motion abnormalities extend beyond a single epicardial vascular distribution; a stressful trigger is often, but not always, present*
2	Absence of obstructive coronary disease or angiographic evidence of acute plaque rupture**
3	New electrocardiographic abnormalities (either ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin
4	Absence of: pheochromocytoma, myocarditis***

*Rare exceptions to these criteria are admitted, such as those patients in whom the regional wall motion abnormality is limited to a single coronary territory

**It is acknowledged that a patient with obstructive coronary atherosclerosis may also develop Tako-Tsubo cardiomyopathy. This is stated to be rare in Mayo Clinic experience, perhaps because such cases are misdiagnosed as an acute coronary syndrome.

*** The original criteria also required the exclusion of (i) recent significant head trauma, (ii) intracranial bleeding and (iii) hypertrophic cardiomyopathy

Table 1.3 — Common or salient stressors/precipitants* of TTC

	Antecedent experience
Acute psychological stress	Bereavement or loss Conflict/argument Situational anxiety/panic Disaster/earthquakes
Physical illness or exposure	Toxic/pharmacological Dobutamine, adrenaline, isoprenaline Envenomation: eg. Irukandji jellyfish (Tiong, 2009) (Northern Queensland) Endocrinopathy: phaeochromocytoma (Agarwal et al., 2011), Addison in crisis (Abi-Saleh et al., 2006), thyrotoxicosis Neurological: subarachnoid haemorrhage, epilepsy, stroke Respiratory or critical illness

*No stressor evident in ~10% (Sharkey et al., 2010a)

Table 1.4 — Major pathogenetic theories in TTC

	Proposed	Anatomical Issues/ Problems
1. Catecholamine effect	? Ca^{2+} overload ? Oxidative stress ? Biased agonism (see text)	Basis of selective apical involvement
2. Vascular spasm/myocardial stunning	? Transient ischaemia/reperfusion ? Myocardial stunning	Basis of selective apical involvement
3. Dynamic obstruction	? Anatomical/functional LVOT obstruction ? Apical injury due to systolic wall stress	Septal bulge/ LVOT obstruction not universal Lack of analogous pathology in HOCM

Abbreviations: LVOT, Left Ventricular Outflow Tract; HOCM, Hypertrophic Obstructive Cardiomyopathy

Table 1.5 — Important seven-transmembrane-spanning receptors

	β_1	β_2	β_3	α_1	α_2
Primary G protein	G_s	G_s/G_i	G_s/G_i	G_q/G_{11}	G_i
Endogenous agonist	NA > A	A > NA	NA, A	NA, A	NA, A
Tissue distribution	heart	heart, lung, vessels, kidney	adipose, heart	heart, vessels, smooth muscle	coronaries, CNS, pancreas, platelets

Abbreviations: NA, noradrenaline; A, adrenaline

(Table adapted from Rockman et al, 2002)

Table 1.6 — Histological and ultrastructural findings in patients with acute TTC

Cardiomyocyte changes
Cytolysis. Vacuoles of different sizes with cellular deterioration
Contraction band necrosis. Variable myofibrillar destruction and disarray
Focal myocardial depletion. Apoptotic and autophagy cell death excluded (Nef et al., 2007b)
Extracellular
Intramyocardial edema (Nef et al., 2007b)
Expansion of extracellular matrix. Increased Fibrinogen and Collagen I (Rolf et al., 2009)
Infiltration by mononuclear cells. Lymphocytes and macrophages (Fineschi et al., 2010)

Table 1.7 — Case-control studies examining sympathetic responsiveness in TTC

Authors/Subjects	Assessment	Findings in TTC group
Sciagra et al, 2010 n (TTC) = 22 n (NC) = 11	Nuclear imaging (SPECT) with perfusion, under mental stress	Variable inducible wall motion and perfusion anomalies (10/22 patients)
Barletta et al, 2009 n (TTC) = 17 n (NC) = 7	Echocardiography with contrast perfusion, under cold pressor test	Induction of minor wall motion and abnormalities, with lack of augmentation of coronary blood flow*
Martin et al, 2010 n (TTC) = 12 n (NC) = 12	Endothelial function by peripheral arterial tonometry	Decreased endothelial function and reactive hyperaemia in response top mental stress*

*: noradrenaline levels were shown to be higher under stress than baseline

Abbreviations: TTC, Tako-Tsubo Cardiomyopathy; NC, Normal Control

Table 1.8 — Cases of TTC/shock, with rapid improvement on Levosimendan

Author and TTC case	Preceding treatment	Haemodynamic status	
		pre-L	post-L
Padayachee, 2007			
1 *80 year-old female with shock*	DOB and NA	CI 2.69 LVEF 30%	CI 3.5-4.0
2 *80 year-old male post-operative APO*	GTN and IABP	CI 1.46 LVEDP 25	CI 3.1-4.7
De Santis et al, 2008			
3 *68 year-old female APO/shock*	DOB and IABP	CI 1.5 PCWP 18 LVEF 20%	CI 3.5 PCWP 14 LVEF 40%
Antonini et al 2010			
4 *70 year-old female with APO/shock*	DOPA	CI 1.57 PCWP 28 LVEF 20%	CI N/A LVEF 40%
Karvouniaris et al, 2012			
5 *60 year-old male with urosepsis/TTC and shock*	DOB and NA	MVO_2 sat 64% CI (max) 3.1	CI 4.3

Abbreviations:- APO, acute pulmonary oedema; L, levosimendan; DOB, dobutamine; NA, noradrenaline; DOPA, dopamine; GTN, glycerol trinitrate; IABP, intra-aortic balloon pump; LVEF, left ventricular ejection fraction; CI, cardiac index (L/min/m^2); LVEDP, left ventricular end-diastolic pressure (mmHg); PCWP, pulmonary capillary wedge pressure (mmHg); MVO_2, mixed venous oxygen saturation; N/A, not available

Table 1.9 — Precipitation of TTC with dobutamine: selected case reports

	Dobutamine infusion rate	**Case details**
Margey et al 2009	*40 mcg/min; HR 98*	61 year-old female: inferolateral STE/hyperacute anterolateral T waves; apical ballooning
Silberbauer et al 2007	*30mcg/kg/min; atropine 0.5mg*	75 year-old female: CP, marked anterior STE, transient bradycardia; periapical dyskinesis
Cherian et al 2008	*40mcg/min; HR 123*	85 year-old female: CP/tachycardia/ inferolateral STE; LVOT gradient (85mmHg); subsequent death
Abraham et al 2009	*40mcg/kg/min; HR 146*	46 year-old male (Case 1 in series): non-specific ST-T wave changes; recovery
	40mcg/kg/min; HR 125	51 year-old female (Case 2 in series): Inferolateral STD; recovery
	30mcg/kg/min; HR 126	41 year-old female (Patient 3 in series): Inferolateral STE; recovery

Abbreviations:- HR, heart rate; STE, ST-elevation; LVOT, left ventricular outflow tract; STD, ST-depression

1.10 Figures

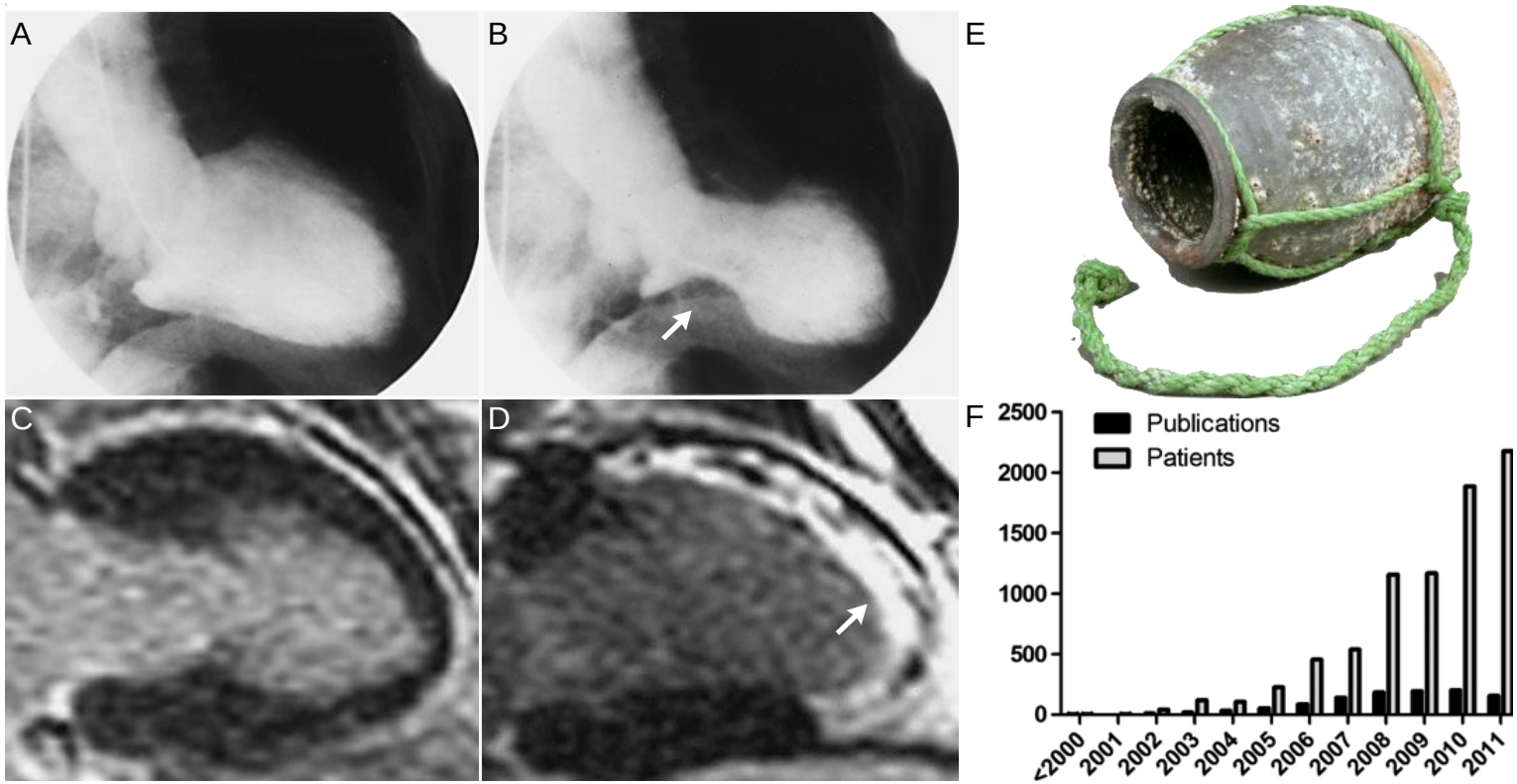

Figure 1.1 — Contrast-enhanced ventriculography during diastole (A) and systole (B), demonstrating apical and mid-ventricular akinesis, with relative sparing of the cardiac base (arrow). Panel C, contrast-enhanced cardiac magnetic resonance scanning, showing the absence of necrosis in the akinetic zone; panel D, is presented comparison: hyperenhancement (arrow) indicates necrosis, after an acute anterior myocardial infarction *(reproduced from Wittstein et al., 2005)*. Panel E shows a Japanese Octopus trap, or "Tako-Tsubo". Panel F depicts the trend of case recognition of TTC, together with the annual number of publications in the field *(reproduced from Shao et al., 2012)*.

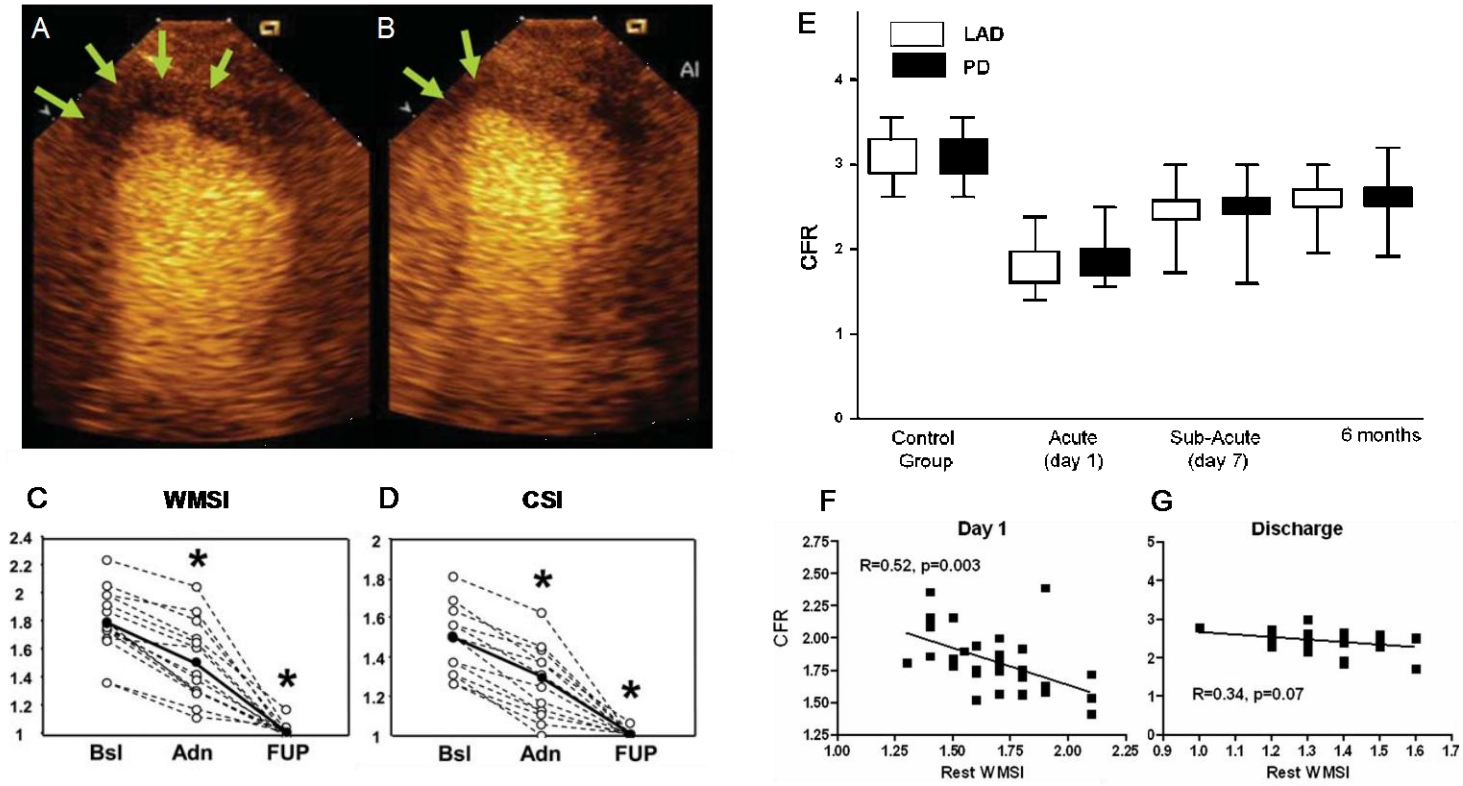

Figure 1.2 — Panels A and B depict myocardial contrast echocardiograms of the LV, showing an apical perfusion defect, ~3 days after onset of TTC (A: arrows), diminishing after 90 seconds of intravenous adenosine (Adn, 0.14 mg/kg/min) (B); remarkably, Adn transiently improved wall motion score index (WMSI) (C), in parallel with contrast score index (CSI) (D) *(reproduced from Galiuto et al., 2010).* Panel E demonstrates coronary flow reserve (CFR) (mean ± SD) in the left anterior descending (LAD) (white boxes) and the posterior descending (PD) (black boxes) arteries in control subjects, versus that in serial TTC, studied via a pulsed Doppler technique (see text); resting WMSI was correlated with CFR acutely (F), but not at discharge (G) *(reproduced from Rigo et al., 2009)*.

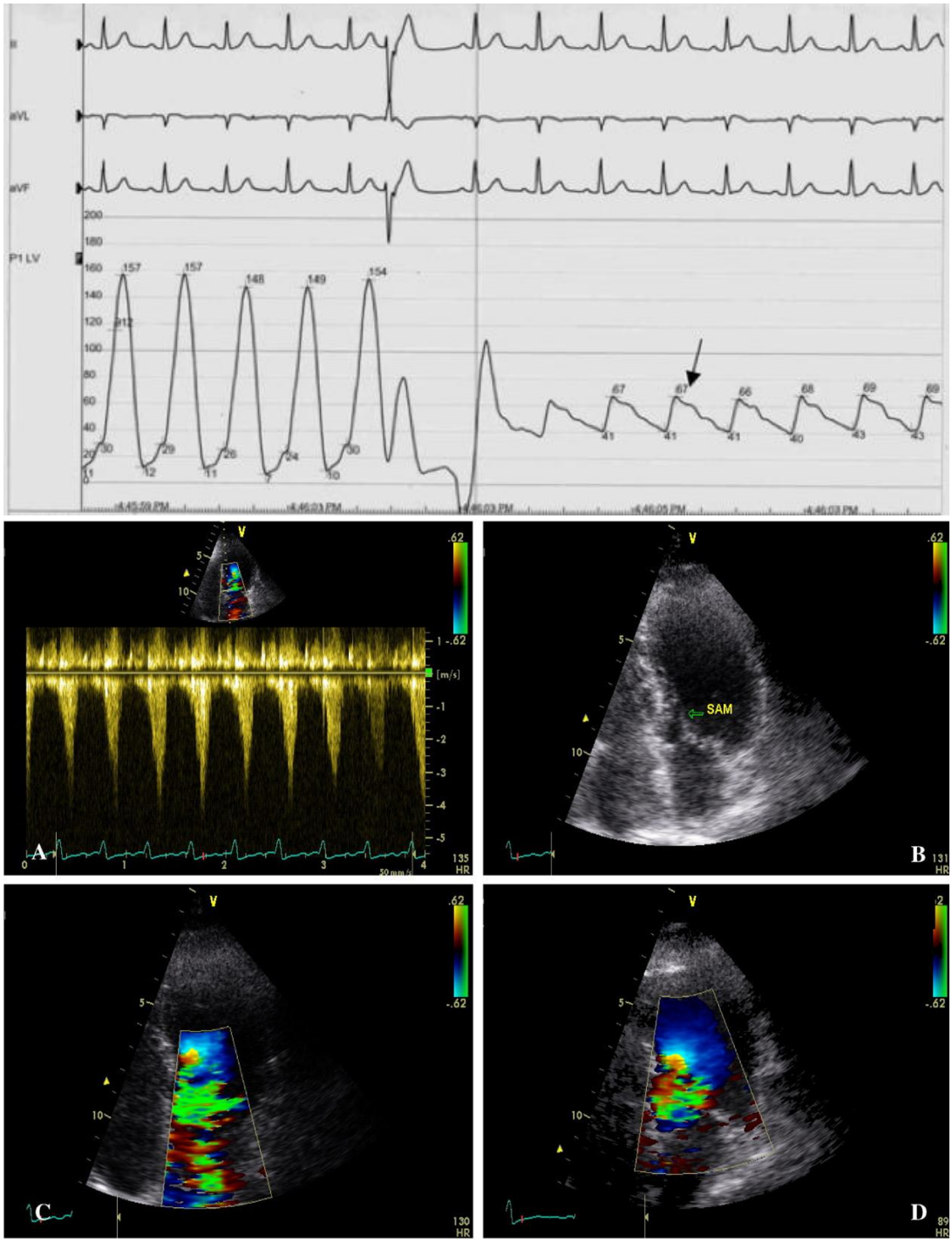

Figure 1.3 — Upper panel depicts hemodynamic tracing in a patient with obstructive TTC: on pullback of pigtail catheter from LV to aorta, a gradient of ~70mmHg is apparent; arrow shows bifid aortic waveform ("spike and dome") characteristic of dynamic left ventricular outflow tract (LVOT) obstruction (*Reproduced from Fefer et al, 2009)*. Middle and lower panels demonstrate typical late peaking pattern of LVOT obstruction on continuous wave Doppler (A), together with systolic anterior motion (SAM) of the mitral valve in apical 4-chamber view (B) and associated mitral regurgitation (C), in the same TTC patient. Dynamic LVOT obstruction and mitral regurgitation disappeared with intravenous propranolol (D) (*Reproduced from Panigada et al, 2009).*

Chapter 2

Clinical Studies in TTC: Towards Expedited Diagnosis

2.1 Background

As discussed in Chapter 1, Tako-Tsubo cardiomyopathy (TTC), also known as stress cardiomyopathy or apical ballooning syndrome, is a unique disorder of myocardial function, but which often mimics acute MI (Bybee et al., 2004a, Farouque et al., 2004). A "classic" presentation would include the following elements in sequence: (i) a severe emotional trigger, followed by the onset of (ii) severe chest pain and/or dyspnoea, prompting (iii) emergency department presentation, (iv) the detection of ST-elevation on initial ECG and (v) the acute management of STEMI, as appropriate (see Chapter 1, Section 1.2.3). In the context of contemporary STEMI management, employing cardiac catheterization, the diagnosis of TTC would be arrived at briskly and "automatically", but by a process of elimination, on the basis of non-obstructive coronary arteriography and left ventriculography. Furthermore, the expected patient would be female and in her sixth or seventh decade (Gianni et al., 2006b).

It is implicit in the rapid growth of this diagnostic category that many cases have, historically, been missed; the question is, to what extent is this diagnosis overlooked in the contemporary setting? Whilst the above patient/presentation may be considered "classic", based on the most formative early publications, the example above does not adequately reflect the substantial heterogeneity of the acute phase of TTC, or possibly even the majority of TTC cases (Sharkey et al., 2010a). Importantly, the diagnosis of TTC amongst those *without* initial ST-elevation is far from "automatic" and nor is emergency cardiac catheterization always carried out in very elderly, frail, patients with apparent STEMI. Refinement of the process of differentiation/diagnosis of TTC, is therefore of great interest.

The following chapter is derived from six years of clinical experience of TTC, in a dual center, unblended historical prospective study. This served an NHMRC-funded research program with three broad aims: to elucidate the diagnosis, pathogenesis and natural history of TTC. As such, most of the research focused on specific hypotheses. However, this chapter will comprise (i) a detailed clinical and investigational description of the patient cohort and (ii) the development and evaluation of a method of early detection of TTC.

2.1.1 Towards expedited diagnosis: rationale

It is apparent that a strategy for early diagnosis of TTC is a particularly relevant consideration in the large group without initial ST-elevation (i.e. NSTEMI), which may account for two-thirds of TTC cases (Dib et al., 2009b, Singh et al., 2010). Such a strategy must therefore take into account current practices/guidelines in the management of ACS. Early management priorities for patients suspected of NSTEMI are (i) risk-stratification and (ii) simultaneous administration of proven medical therapies: anticoagulation, antiplatelet/antithrombotic therapies, together with early administration of antianginal agents and potent HMG-CoA reductase inhibition (Anderson et al., 2007). Although a variety of approaches to risk stratification exist, those deemed at high risk of acute morbidity and mortality are recommended to undergo inpatient cardiac catheterization, generally within a 24-48 hour window from admission (Mehta et al., 2009). Angioplasty and stent implantation can then be performed on the basis of angiographic and clinical considerations.

This 24-48 hours window, which generally applies before definitive diagnosis (±treatment) in the handling of high-risk NSTEMI, offers a "potential space" for the differentiation of patients with non-ST-elevation TTC (NSTE-TTC). Whilst the advantages inherent in early diagnosis of TTC are currently uncertain, early suspicion and recognition could enable the tailoring of appropriate treatments/procedures (notably the avoidance of positive inotropic agents), as well as acute enrolment in prospective research studies. Such a tool would be an ideal means of triaging patients with a high likelihood of TTC towards a reliable non-invasive imaging diagnostic test, were one ultimately to become available. It is also likely that *only* an approach which combines (i) the *prospective consideration* of TTC, with (ii) a carefully directed series of investigations, will establish the true incidence of the disorder: it is quite possible that TTC cases are still easily attributed to incidental coronary atherosclerotic disease, or classified as either "myocardial infarction with normal coronary arteries" (Agewall et al., 2011, Chen et al., 2010a) or "non-specific troponin elevation" (in the case of 'silent/incidental' presentations) (Jeremias and Gibson, 2005).

2.1.2 Potential components of a diagnostic algorithm: candidates

(a) Electrocardiographic features

As already stated above, predictive information is only really relevant to the NSTE-TTC group. Despite this, attempts to determine specific ECG features of TTC have utilized STEMI as a comparator group, thus asking the question, can presenting ECG features differentiate TTC from STEMI? At least three studies have concluded that ECG alone does not facilitate this (Sharkey et al., 2008, Barker et al., 2009a, Carrillo et al., 2010), whilst others have suggested that it has a moderate ability to do so, either on the basis of the extent of ST elevation (Tamura et al., 2011) or reference to ST-depression/elevation in specific leads (Kosuge et al., 2010). Although numerous ECG features of TTC have been described, the use of any one feature to differentiate MI from TTC must take into account the evolution of changes over time, during the days after presentation, rather than initial features only. Whilst few data are available regarding this, it is clear that ECG changes are time-dependent: ST-elevation evolves to T-wave inversion (Mitsuma et al., 2007), with corresponding prolongation of the QT-interval over 2 days (Kucia et al., 2010, Wittstein et al., 2005); Q-waves (present in up to 32%), tend to disappear over a similar time-frame (Gianni et al., 2006b, Robles et al., 2007). Although a *diffuse* or 'widespread' pattern of ST-T wave changes is frequently described, it appears less common in the mid-ventricular variant (Kurisu et al., 2011).

(b) Plasma troponin levels

The elevation of plasma troponin levels appears to be near universal in TTC. The exceptions to this rule occur early in the history of the TTC literature and may result from the timing of blood collection (Gianni et al., 2006b), although the potential for *early false negative assays* cannot be assessed, in absence of information regarding the specific kinetics of cardiac troponin release in TTC. Thus, as a component of diagnosis, a troponin assay is unlikely to confer any specificity for TTC as opposed to ACS.

(c) BNP/NT-proBNP

As discussed in Chapter 1 (Section 1.4.2.2), some authors have reported high plasma concentrations of BNP (Akashi et al., 2004a, Grabowski et al., 2008, Madhavan et al., 2009, Morel et al., 2009, Wittstein et al., 2005) and its related peptide, NT-proBNP (Nef et al., 2008a, Nguyen et al., 2011a), in TTC and have suggested the incorporation of this data the diagnostic workup of suspected TTC, as a means of differentiation from ACS. One case-control study has assessed the potential value of BNP for the detection of TTC (Madhavan et al., 2009). In this, the use of a cohort of STEMI patients as a comparator group is a conceptual weakness, in view of the fact that the presence of ST-elevation, all else being equal, is a commitment to emergent angiography: as alluded to earlier, the insertion of a biochemical assay into this workflow is clinically meaningless. Nevertheless, the marked release of BNP in TTC, as demonstrated by these authors, appears to be of some incremental diagnostic value in this context, when appropriate cutoffs are used. This was especially so when BNP was combined with troponin release to produce a ratio (BNP/troponin), reflecting in addition the small extent of myonecrosis expected in TTC, as compared with ACS. However, it must be emphasized that this was a post-hoc analysis only, not subjected to prospective testing.

(d) Echocardiography

In the present state of the evolution of diagnostic criteria, the demonstration of compatible wall motion abnormalities represents the *sine qua non* feature of TTC. On the other hand, the shape of the LV and the distribution of wall motion abnormalities alone are not pathognomic for TTC and do not exclude other possibilities: selective apical dysfunction can occur in acute, threatened or chronic anterior MI. However, the basal and mid-ventricular variants may be more specifically characteristic of TTC as against ACS, simply because they *cannot* be explained by CAD (Cuculi et al., 2010). Similarly, the phenomenon of combined LV and RV apical dysfunction is not described in ACS, but occurs in a surprising proportion of TTC patients (~30%) (Eitel et al., 2011a, Eitel et al., 2011b).

Two studies applied more sophisticated wall motion speckle tracking analysis to the question of early differentiation from MI (Mansencal and Dubourg, 2009, Heggemann et al., 2011). This is a difficult offline process, which is not routinely available for clinical use: it is difficult to imagine its application to all cases of suspected NSTE-TTC. In contrast, the availability of basic bedside echocardiography, combined with simple contractile pattern recognition, makes it very likely to play a role in expedited diagnosis, as some authors have already pointed out (Fazio et al., 2009). In terms of the onset of LV changes relative to the onset of symptoms, little is known. The fact that TTC (with immediate imaging of characteristic LV dysfunction) has been documented during or soon after exercise (From et al., 2009) or dobutamine (Abraham et al., 2009) stress echocardiography, supports the thinking that LV dysfunction occurs very early in the 'cascade' of events.

(e) Contrast-enhanced cardiac magnetic resonance imaging

Apart from facilitating wall motion analysis (much like echocardiography), CE-CMR provides two additional advantages: the ability to visualize necrosis/scar, by the LGE technique, and the ability to visualize myocardial oedema, via T_2-weighted imaging. Although it is conceivable that it may allow the demonstration of features which are characteristic for TTC (Eitel et al., 2011b), the capacity of CMR to "rule out" or "rule in" TTC, has not been prospectively explored.

Focal necrosis, as is expected in *established* MI, is rare in TTC (Rolf et al., 2009, Eitel et al., 2011b): where it is present (<5%), it is not necessarily intense, nor subendocardially-based, as is the case of MI. Thus, within the limits of detection, CE-CMR offers the potential to exclude significant myocardial necrosis in a suspected case of NSTE-TTC. However, this does not negate the possibility of *threatened* MI, which might not trigger necrosis. Animal studies suggest that oedema occurs in the "area at risk" of infarction — confined to the compromised coronary vascular territory, where hypoperfusion is relieved before infarction occurs — and can be demonstrated by T_2-based techniques (Tilak et al., 2008, Aletras et al., 2006), without focal LGE. As most human imaging work has focused on diagnosed and reperfused STEMI, whether threatened MI can be thus reliably

demonstrated in humans is not known. However, one small study suggests that oedema (without LGE) does occur in Wellen's syndrome (Migliore et al., 2011).

2.1.3 A summative "TTC score" for early diagnosis of TTC?

Thus, several features of TTC, assessable at presentation or early in the evolution of the condition, stand out as potentially important for formulating a diagnostic suspicion. The frequent presence of antecedent stress is an interesting consideration from both a biological and clinical/diagnostic perspective. Although it must be readily acknowledged that stress can precede obstructive ACS (Bunker et al., 2003), the diagnostic utility of prospective consideration of the presence or absence of a plausible situational trigger, has not been evaluated in TTC. As mentioned above, marked BNP/NT-proBNP elevation may aid differentiation, particularly if the elevation is more than 20-fold beyond that of population norms. Finally, the prevalence of "multiregional" ECG changes (ΔECG_m) may also serve as a simple early non-invasive indicator.

2.1.4 Development of a 'TTC score'

We proposed a putative 'TTC score', giving weight to NT-proBNP elevation and specific ECG changes. In this, 1 point is assigned for troponin T elevation, 1 and 2 points for NT-proBNP ≥3,000 and ≥6,000pg/mL, respectively, and 1 and 2 points for ΔECG and ΔECG_m, respectively. Given the known association of TTC with antecedent physical/emotional stress, an additional option was to adjust the TTC score by allocating a further 1-point for the presence of such a stressor. However, given the issues of (i) probable incomplete history taking (particularly in ACS patients) and (ii) the lack of a precise definition of 'significant' antecedent stressors, the modeling of TTC scores was performed with and without differentially imputed rates of antecedent stress.

In keeping with the anticipated use of this score, only data available at 24 hours was employed in the evaluation of its potential role. TTC score distributions were first analyzed for each group (i.e., TTC versus ACS), without the factor of a stressor, elicited on history

(i.e., allowing a maximum score of 5). This was followed by analysis in which a point was allotted for the presence of a stressor in the TTC group (i.e., allowing a maximum score of 6), whereas the effect of the presence of a history of physical/emotional stress was 'modeled', by artificially allocating this at a rate of 25% (i.e., every fourth patient) and 50% (i.e., every second patient). Finally, prespecified subgroup analyses were performed without inclusion of stressor data (ST-elevation versus non-ST-elevation and Killip I versus Killip ≥ II).

2.1.5 Aims and Hypotheses

The investigations performed in this chapter were conducted as a comparison of a cohort of patients with TTC against a group of age matched female patients with obstructive acute MI (both STEMI and NSTEMI).

The central hypothesis to be tested was that an arbitrarily derived "TTC score" would differentiate TTC from AMI patients. The secondary hypothesis was that this differentiation would apply to comparisons of patients both with and without ST segment elevation on initial ECG.

Given that the diagnosis of antecedent emotional stress may be agreed to be somewhat arbitrary and also potentially subject to omission during assessment of cardiac emergencies, analyses were performed both inclusive and exclusive of this factor.

2.2 Methodology

2.2.1 Patient selection: TTC group

Patients were prospectively identified on the basis of a total clinical evaluation, in the light of currently accepted criteria, taking into account the following: (a) ST-T wave changes, with or without acute onset chest pain or dyspnoea, (b) elevation of troponin T levels, (c) detection of a characteristic wall motion abnormality and (d) the absence of an alternative diagnosis, i.e., one thought to be more likely on the basis of coronary arteriography (e.g.,

occlusive coronary disease or ruptured atherosclerotic plaque) or echocardiographic/CE-CMR findings (e.g., based on a focal pattern of myocardial oedema or subendocardially-based LGE). The type of wall motion abnormality detected had to be any of the three variants currently accepted (apical, mid-ventricular and basal) and was the *sine qua non* diagnostic feature. All coronary angiography and/or CMR imaging were reviewed with the aim of eliminating cases of acute coronary syndrome and viral myocarditis, in particular, in accordance with the Mayo clinic criteria (Madhavan and Prasad, 2010a). Where both a TTC-like wall motion abnormality *and* CAD were detected, consideration was given to the correlation between the relevant coronary artery segment distribution(s) and the wall motion abnormality: if the correlation was deemed sufficient, the case was excluded.

2.2.2 Patient selection: ACS/acute MI

In order to facilitate the evaluation of the performance of NT-proBNP levels for the delineation of TTC from other cases of presumed ACS, a comparator group of females presenting with ACS and admitted to CCU. A conventional definition of ACS/MI was applied (Thygesen et al., 2007), requiring (a) a rise and/or fall of cardiac troponin T, with at least one value beyond the 99th percentile of the upper reference limit, together with at least one of the following: (b) a presentation with chest pain or other symptoms compatible with ischaemia, (c) ECG changes indicative of new (new ST-T wave abnormalities or new left bundle branch block (LBBB), (d) development of pathological Q waves on ECG, or imaging evidence of new loss of viable myocardium/new regional wall motion abnormality.

2.2.3 Patient management in hospital

Patients of both groups were monitored for a minimum of 48 hours, during which time intravenous sodium heparin was administered and routine blood chemistry was obtained. Twelve-lead ECG and non-invasive heart rate and BP information was recorded over the first three days of admission. In order to establish baseline characteristics of the two

groups, all patients were evaluated by clinical interview and ongoing review of hospital and physician records. Required information included details of pre-admission risk of CAD, incorporating past history, cardiac risk factors, presenting symptoms and antecedent stressful events (either psychosocial or physiological). The institutional Human Ethics Research Committee approved this study and patients gave written informed consent.

2.2.4 Investigations

2.2.4.1 Biochemical data

(a) Determination of plasma C-reactive protein (CRP) concentrations

CRP was assayed serially to determine the peak in each patient; in cases where C-reactive protein was less than 10 mg/L, the level of high sensitivity C-reactive protein (Olympus au5400, Dallas, Texas, USA) was determined.

(b) Determination of plasma natriuretic peptide levels

Concentrations of NT-proBNP (Elecsys E 170, Roche Diagnostics, Germany) were determined at presentation and serially thereafter to determine the peak for the acute episode.

(c) Evaluation of catecholamine exposure

In order to provide an index of acute catecholamine exposure in TTC, plasma normetanephrine was selected. This non-acidic ortho-methylated derivative of norepinephrine reflects one major pathway of extraneuronal metabolism and has a prolonged plasma elimination half-life (~12-24 hours), thus making it potentially suitable to assess *recent* catecholamine exposure even when the patient was not assessed immediately (Goldstein et al., 2003). As soon as possible in relation to diagnosis, venous blood was drawn into tubes containing potassium ethylene diamine tetra acetic acid and

centrifuged at 1680 relative centrifugal force, for subsequent assay by liquid chromatography/tandem mass spectrometry (Lagerstedt et al., 2004). Where possible, serial measurements were obtained and the peak was recorded.

(d) Markers of cardiac myonecrosis

Both plasma troponin-T and creatine kinase (CK) (ElecSys, Roche, Diagnostics, Mannheim, Germany) were performed at admission. Troponin was not routinely repeated once an abnormal result was observed, whereas CK was repeated at least daily during admission. The peak of each was recorded.

2.2.4.2 Angiographic and invasive haemodynamic assessment

Selective coronary arteriography was preferred in all TTC patients, although allowance was made for physician preference, where invasive procedures were deemed contraindicated (e.g. extremes of age, post-stroke or extra-cardiac critical illness). Incident coronary plaque was categorized according to its probable haemodynamic significance: mild (<50% stenosis), moderate (50-75%) or severe (≥75%). The anatomic location in the coronary tree was recorded and the extent of significant (≥50%) disease was further classified as single, double or triple vessel disease.

At the time of diagnostic angiography, right heart catheterization was performed in 50 patients. Average resting pulmonary capillary wedge pressure (PCWP) was transduced and mixed venous blood oxygen (MVO_2) saturation was determined from pulmonary arterial samples, utilizing a blood gas analyzer (Radiometer Copenhagen NPT 7 Series): these were recorded as indicators of LV filling pressure/pulmonary congestion and cardiac output, respectively.

2.2.4.3 ECG analysis and definitions

The following definitions were applied to standardize ECG assessment:

- ST-elevation: in precordial leads, ≥ 2 mm in ≥ 2 contiguous leads; in limb leads ≥ 1mm STE in ≥ 2 contiguous leads
- ST-depression: ≥ 1mm in ≥2 contiguous leads
- T wave inversion: excluding aVR and V1, (a) negative orientation of peak T wave in ≥ 2 contiguous leads or (b) negative terminal T wave (in biphasic ST/T) ≥2 contiguous leads.
- Multi-regional changes: involving at least 2 inferior leads (i.e., II, III, aVF) and two anterior leads (i.e., V1-V4 for ST-elevation or V2-V4 for T-wave inversion); denoted ΔECG_m.

Left and right bundle branch block patterns were excluded from QT_c, ST-T and regionality analyses. Atrial fibrillation and flutter were excluded from QT_c and regionality analyses, where the T wave was obscured.

2.2.4.4 Clinical imaging

(a) Echocardiographic protocol and analysis

By protocol, imaging studies were obtained acutely and approximately 3 months thereafter. Studies utilized Vivid 7 echocardiography machine with an M5 ultrasound transducer (GE-Vingmed, Horton, Norway). In the acute context of TTC, echocardiography was performed as soon as practically possible, either upon confirmation of the diagnosis or *upstream* of it, if an initial high level of clinical suspicion was present.

Standardised echocardiographic data included two-dimensional grey scale imaging, together with colour flow, pulsed wave and tissue Doppler and in all conventional views, as recommended by the American Society of Echocardiography (Picard et al., 2011). In accordance with these recommendations, LV end-diastolic and end-systolic volumes were recorded by a biplane method, from manual planimetry of the endocardial LV border, in

apical long axis 2-chamber and 4-chamber views. LV ejection fraction was determined automatically from these volumes. The wall motion score index (WMSI) was determined according to a 17-segment model, taking in to account all views of the LV, applying a 4-point scale to describe segmental contraction (1 = normal or hyperkinetic, 2 = hypokinetic, 3 = akinetic and 4 = dyskinetic) (Cerqueira et al., 2002). WMSI was thus the aggregate of segments scored, divided by the number of segments scored.

(b) Multisequential CE-CMR

In the two participating centres, CE-CMR was performed on either a 1.5 T Philips Intera and Achieva systems (Philips Medical Systems, Best, The Netherlands), each with a 5-channel phased array cardiac coil. By protocol, acute-phase imaging was performed as soon as possible, either after angiographic diagnosis or *upstream* of it, but generally pre-discharge (0-7 days). Functional imaging, cine CMR, was performed after initial scout images/setup, utilizing balanced turbo field echo (BTFE) or balanced steady state free precession pulses sequences, in standard cardiac views (horizontal long-axis/4-chamber, vertical horizontal long-axis/2-chamber, LV outflow tracts and short axis). Oedema-sensitive T_2-weighted imaging utilized a triple inversion recovery pre-pulse and homogeneity correction (CLEAR), in accordance with current guidelines of the assessment for myocardial edema in myocarditis (Friedrich et al., 2009b). Contrast-enhanced imaging included standard sensitivity encoded sequences for resting first pass perfusion and inversion-recovery late-gadolinium enhancement (LGE). For the later, inversion time was manually adjusted to 'null' the signal from viable myocardium, with reference to a T_1-scout series (Look Locker sequence (Look and Locker, 1970). Typical imaging parameters are described elsewhere (Section 3.2.6). In terms of tissue characterization, the presence/absence of grossly visible focal/diffuse myocardial oedema or focal/diffuse LGE was recorded.

2.2.5 Clinical event definitions and long term follow-up

All pre-hospital and in-hospital arrhythmias were documented and analyzed. Killip class

(Killip and Kimball, 1967) was assigned retrospectively on the basis of documented clinical/radiographic findings, namely, the presence of crackles, a third heart sound and an elevated JVP and pulmonary crackles (Killip II), frank or radiographically-proven pulmonary oedema (Killip III) or hypotensive shock (Killip IV); patients were designated Killip class I in the absence of these features. Shock was defined as a sustained systolic BP ≤90mmHg, with or without the requirement for positive inotropes and/or balloon counterpulsation therapy (separately recorded). Death was categorized as in-hospital or post-discharge, depending on when it occurred: the likelihood of in-hospital deaths being attributable to a cardiac cause was considered and autopsy performed if possible. Patients were offered clinical follow-up at the participating hospital, with additional telephone contact from a study nurse or physician; median follow-up period was 753 days.

2.2.6 Statistical methodology

Data were analyzed using GraphPad Prism software (version 5.0) and presented as mean and standard deviation (SD), for normally distributed data or median and interquartile range for skewed data, as appropriate. Differences between groups, in categorical variables, were assessed with Fisher's exact test; differences in distributions of non-Gaussian continuous data utilized the Wilcoxon signed rank test. Receiver operating characteristic (ROC) curves were constructed and sensitivity and specificity data were reported with 95% confidence intervals. A value of $P < 0.05$ was considered significant.

2.3 Results

2.3.1 Patient characteristics at admission

Clinical and angiographic characteristics of the study population (both TTC and ACS) are presented in Table 2.1. One hundred and twenty-five patients (119 females, 6 males; mean age 67 ± 14 years) were diagnosed with TTC over a 67-month period, from late 2006 to early 2012. The ACS group consisted of 56 patients (mean age 70 ± 12 years; all female, by design), as defined above (Section 2.2.2). A similar proportion (~18% in both groups)

did not undergo inpatient coronary angiography; reasons for variation in the TTC group included critical illness/recent stroke (50%), extreme age (30%) and physician preference (20%). Eleven (19%) of the ACS patients had a known history of ischaemic heart disease at baseline, including previous angina, myocardial infarction or revascularization.

A clearly defined antecedent stressor was more frequently elicited in TTC patients (82% versus 12%, P < 0.0001). Some patients were diagnosed with TTC in the context of an acute non-cardiac illness, where investigations were prompted by an abnormal ECG and/or cardiac biomarker result. The range of elicited stressors, classified as either primarily emotional or primarily physiological, is described in Table 2.2.

Regarding risk factors for coronary atherosclerosis, current cigarette smoking, systemic hypertension and hyperlipidaemia were more prevalent in the ACS group, as was the presence of multiple coronary risk factors (P < 0.0001). Atherosclerotic plaque was angiographically apparent in 26 (21%) of TTC patients: the extent and severity of coronary disease was generally moderate and in no case explained the acute/reversible LV dysfunction observed (see Table 2.1).

2.3.2 Clinical outcomes in TTC and ACS

Ventricular arrhythmia was documented in 7 (5.6%) TTC patients (5 with ventricular fibrillation, 1 with *torsades de pointes*, and one with sustained VT the evening of presentation), versus 2 (4%) in ACS patients (P = NS). Of the four TTC patients presenting with resuscitated out-of-hospital cardiac arrest, one died during her hospital stay, whilst the remainder made a prompt neurological recovery. Two TTC patients received implantable defibrillators pre-discharge.

Pulmonary oedema was noted less frequently in TTC patients than in the ACS group (7% vs. 23%, P = 0.006). By contrast, shock occurred frequently in TTC patients: of 22 TTC patients with shock, 11 required inotropic support and 4 received counterpulsation therapy and the remainder were managed conservatively. The frequency of severe hypotension was significantly different from that of ACS patients (18% vs. 7%, P = 0.046).

In-hospital death occurred in 6 TTC patients: four of these were due to progressive shock ± multiorgan failure/anoxic brain injury; the remaining two were the result of intracranial haemorrhage. All three deaths occurring in the ACS comparator group were attributable to progressive cardiogenic shock.

2.3.3 Haemodynamic and imaging findings in TTC

Table 2.3 summarizes non-invasive and invasive haemodynamic information, as well as acute phase imaging data: given that neither echocardiography nor CMR was mandated in the ACS patients, only data for TTC patients is presented. Despite the incidence of shock, TTC patients were most frequently normotensive, frequently with erratic blood pressure fluctuations. Mixed venous oxygen saturation (a reflection of cardiac output; MVO_2) and pulmonary capillary wedge pressure (PCWP) varied substantially at cardiac catheterization: mean values were at the lower and upper limits of normal respectively. In the overall cohort, the incidence of RV involvement and pericardial effusion were substantial, in keeping with published data; focal necrosis, demonstrated by LGE-CMR, and thrombus were visualized in only a few patients (see Table 2.3). More detailed imaging data are presented in the following chapters.

2.3.4 Long-term follow-up

Although not the primary purpose of this study, long-term follow-up, with a median of 745 days, was available. Seven definite recurrences were diagnosed (according to our definition), noting that TTC patients were also assessed on multiple other presentations with chest pain, in particular during the first six months, where features were not sufficiently indicative of a reactivation of TTC.

Of the two patients who received implantable cardiac defibrillators, neither experienced a recurrence of TTC, nor any appropriate defibrillator discharges, during follow-up. One of these patients died approximately 2.5 years from implantation, as a result of pacemaker lead endocarditis. A further six patients died during follow-up: two from advanced cancer,

one from the effects of stroke, one from respiratory failure complicating severe emphysema, one from suicide and another from unknown causes. The latter case will be reviewed in the context of a discussion of postmortem findings, in Chapter 4.

2.3.5 Presenting ECG and biomarker findings in TTC, versus ACS

Table 2.4 compares ECG and biochemical findings at presentation, in TTC and ACS. Plasma troponin T levels were similar (and marginally elevated) in both groups. Peak plasma CK levels were lower in the TTC group, whereas CRP elevation and peak plasma NT-proBNP were more pronounced in the TTC group. Significant between-group differences were not observed in plasma metanephrine or normetanephrine levels, acknowledging the smaller number of samples.

In terms of the three broad categories of ST-T wave abnormalities – ST-elevation, ST-depression and T-wave inversion – (without respect to the regional distribution of these findings) the frequency of initial ST-elevation in TTC was 32%, similar to the frequency in the ACS group (40%, P = NS). Of non-ST-elevation patterns, ST-depression and T-wave inversion were more likely to be present on initial ECG. However, TTC was also more likely to be associated with an electrocardiographically 'silent' presentation, without specific ST-T changes, than ACS.

As can be seen in Table 2.5, by 24-hours, the only major differences between TTC and ACS on ECG were the appearance of multiregional T-wave inversion changes (as defined in the Methodology Section 2.2.4.3) and the QT_c prolongation. Given that approximately half of the TTC patients developed multiregional changes, this feature potentially discriminated the two groups. Relevant to the TTC score, the condition of ΔECG_m, was met more frequently in TTC, both at presentation (30% versus 17%, P = 0.05, Fisher's exact test) and 24 hours (62% versus 30%, P < 0.0001): in cases with ΔECG_m, the odds ratio for TTC increased from 2.0 to 3.8, over 24 hours post-presentation.

2.3.6 NT-proBNP in TTC versus ACS

The utility of 24-hour NT-proBNP level, for the differentiation of TTC from ACS, was evaluated. Data from both groups are compared in Figure 2.1A. Despite substantial overlap of levels, the median value from the TTC group was >3-fold higher than that seen in ACS [5,990 (2,780-10,600) pg/mL versus 1,630 (535-5,480) pg/mL, P < 0.0001]. The diagnostic potential of plasma NT-proBNP measurement – in isolation from other elements of clinical evaluation – was assessed by ROC analysis (see Figure 2.1B). In this context, the area under the curve (AUC) was 0.75 (P < 0.0001). Sensitivity and specificity of NT-proBNP for the prediction of TTC, at prespecified NT-proBNP levels were: for 3,000 pg/mL, 70 and 65% and 6,000 pg/mL, 50 and 79% (see Table 2.6).

Further prespecified subgroup analyses, pertinent to the main objectives of the study, are assessed below; sensitivity and specificity values for NT-proBNP, relevant to both the overall cohort and subgroups, are summarized in Table 2.6. In particular, this table outlines the contribution of NT-proBNP elevation to the overall sensitivity and specificity of the TTC score.

2.3.6.1 Diagnostic utility of NT-proBNP: effect of haemodynamic status

As discussed in Section 2.3.3, although haemodynamic compromise in TTC can be profound and lethal, the majority of patients are free of both pulmonary congestion and hypotension. Plasma NT-proBNP concentrations in TTC and ACS were therefore analyzed with respect to haemodynamic status, i.e., the presence or absence of pulmonary congestion/hypotension, as denoted by Killip class II or greater.

When only data from those patients in Killip class I (n = 70 for TTC, n = 40 for ACS) were included in the analysis, a 4-fold difference in medians was apparent [4670 (2560-8990) pg/mL versus 1030 (394-2290), P < 0.0001], (see Figures 2.1C and D). ROC analysis revealed an AUC of 0.84, implying clinical value for NT-ProBNP measurements, in the differentiation of TTC from ACS, in this clinical subgroup.

However, in patients with Killip ≥ II, (N = 28 for TTC, N = 16 for ACS) no between-group difference in plasma NT-proBNP level was apparent (P = 0.20) (see Figure 2.1E). Corresponding ROC analysis (Figure 2.1F) demonstrated no capacity of NT-proBNP to discriminate between conditions in this haemodynamic subgroup, with the ROC being not significantly different from the line of identity (AUC 0.59, P = 0.31).

2.3.6.2 Diagnostic utility of NT-proBNP: effect of ST-elevation

The diagnostic value of NT-proBNP measurements was also evaluated in relation to the presence or absence of initial ST elevation. Twenty-four-hour concentrations of the NT-proBNP were compared between TTC and ACS, for both ST elevation and of non-ST elevation groups (See Figure 2.2). Without reference to haemodynamic status, significant between-group differences in 24-hour NT-proBNP concentrations were observed, regardless of the presence or absence of ST-elevation on initial ECG.

In keeping with these observations, ROC analysis confirmed the discriminatory value of peak/24 hour NT-proBNP, which was similar for patients with and without initial ST-elevation (see Figure 2.2).

2.3.7 Effectiveness of TTC score

Figure 2.3A displays the distribution of TTC score data, in both populations. Although scores in both groups overlapped, scores in TTC were skewed and clustered around a median of 4 (out of 5), versus a median score of 2 for ACS patients (P < 0.0001, non-paired Wilcoxon test). In this context, ROC analysis revealed an AUC of 0.74 (P < 0.0001) (see Figure 2.3B).

The potential impact of differential antecedent stressor exposure was difficult to quantitate. Although stressors were reported by only 12% of patients in the ACS group, we were concerned that this might in part reflect differential emphasis during history taking. We therefore examined the potential impact on TTC scores of imputed rates of stress

exposure of 25 and 50% in the ACS group compared with the actual rate of 82% in the TTC group, as prespecified. Incorporation of these imputed stressor exposures added modestly to the AUC values for TTC scores (see Figures 2.3C and 2.3D).

2.3.7.1 Effectiveness of TTC score: effect of haemodynamic status

TTC score distributions for TTC and ACS patients, according to Killip class, are displayed in Figure 2.4, alongside ROC data. The TTC score separated groups in Killip I (AUC 0.80, P < 0.0001; see Figures 2.4A and 2.4B), whereas no separation was evident in Killip 2 or above (AUC 0.66, P = NS; see Figures 4C and 4D), implying that TTC score is of little value in the presence of pulmonary congestion or haemodynamic compromise.

2.3.7.2 Effectiveness of TTC score: effect of ST-elevation

Figure 2.5 displays TTC score distributions and ROC analyses in the situations of non-ST-elevation presentation (AUC 0.74, P = 0.0001; see Figures 5A and 5B) and with ST-elevation (AUC 0.77, P = 0.0007; see Figures 5C and 5D), indicating that the effectiveness of the TTC score appears to be independent of ST-segment changes.

2.3.8 Optimization of NT-proBNP thresholds: sensitivity and specificity

Table 2.6 displays sensitivity and specificity values for NT-proBNP at the two levels, 3,000 pg/mL and 6,000 pg/mL (as prespecified for use in the TTC score), together with values for the TTC score at ≥ 4 out of 5. As anticipated, the lower 'cut-point' provided greater sensitivity, whereas the higher provided greater specificity, in all of the haemodynamic and ECG conditions tested. NT-proBNP levels influence the TTC score, since both levels are allocated a point on the scale: the sensitivity and specificity of the TTC score are consistently between those of each 'cut-point'.

Optimal selection of the NT-proBNP level, at which the best sensitivity *and* specificity were attained, was performed using ROC data, for all conditions except those with Killip ≥II. A level of NT-proBNP ≥ 2,500 pg/mL conferred 82% and 65% specificity in 'all comers', whereas at the same level in Killip class I patients, these were 79% and 80%, respectively. At this level of NT-proBNP elevation, similar sensitivity and specificity was attained in those without ST-elevation, as with the entire cohort; in ST-elevation, sensitivity was markedly improved to 91%, at the expense of lower specificity (61%). The effect on the TTC score was a modest improvement of the AUC value (0.76), with improvement of sensitivity (65%) and no change in specificity (75%).

2.4 Discussion

This study evaluated the potential of various clinical/investigational indices, in isolation and in combination, to facilitate expedited differentiation of TTC from ACS. In a large and heterogeneous cohort of TTC patients, the effectiveness of a putative "TTC score" was principally driven by differential elevation of plasma NT-proBNP. However, in this study, diagnostic utility was heavily dependent on clinical status: the utility of both 24-hour NT-proBNP and the TTC score was severely diminished in the presence of pulmonary congestion/haemodynamic compromise, but conversely was enhanced in the absence of these. The discriminatory value of both is retained in patients with and without ST-elevation.

2.4.1 Patient population and clinical status

Several key points were observed with respect to the clinical features of TTC in our cohort, some in keeping with current literature, and others, which may represent novel contributions to the understanding of the disorder. With regard to the frequency with which TTC is precipitated by physical or emotional stress, our findings do not differ significantly from those of contemporary series (Sharkey et al., 2010a, Singh et al., 2010); likewise the finding that initial ECG more commonly demonstrates a non-ST-elevation

pattern, although markedly different from the earliest reports, is consistent with more recent data (Dib et al., 2009b).

Two additional aspects of presentation in our series are noteworthy. First, the incidence of atypical chest pain (as defined in the methodology section), was approximately one quarter overall. Such chest pain frequently included a pleuritic component. Second, TTC was frequently diagnosed without preceding chest pain: this has not been previously reported as a commonplace occurrence. Such presentations were generally preceded by a physical illness rather than an emotional event, and were thus diagnosed in the context of an acute non-cardiac illness, in response to ECG and biomarker findings, or as a result of cardiac imaging, obtained for other reasons (e.g. to rule out a source of cardioembolic stroke or to assess cardiac function in ICU); this group was also overrepresented by male patients. The potential for an atypical chest pain description or a 'silent' mode of presentation, complicating a non-cardiac illness, should therefore not be overlooked when considering the "full spectrum" of TTC. Indeed, if TTC is under-diagnosed at all, it is most likely that TTC goes unrecognized in non-classical forms and in non-cardiological settings, for example, general medical, general surgical and intensive care environments.

2.4.2 Additional clinical data

The clinical data compiled in this patient series also provided incidental, but valuable, new insights into clinical aspects of TTC. Ventricular arrhythmias were observed in 6% of our TTC patients: with one exception, none were fatal. Given the prominence of QT_c interval prolongation in TTC, it is surprising that more of the ventricular arrhythmias observed were not definite *torsades de pointes*. Although the occurrence of these ventricular arrhythmias was early (≤24 hours in all cases), QT_c prolongation in fact tended to increase progressively until 48 hours. Thus, on the basis of our data, it is questionable whether QT_c prolongation *per se* is the origin of the arrhythmias documented.

The high incidence of shock (18%) in our series also warrants comment. In contrast to the ACS group, the incidence of shock in TTC patients exceeded that of pulmonary congestion/oedema. However, the majority of patients were normotensive and some 15%

exhibited sustained hypertension (i.e. average BP ≥140/90 mmHg) on day one. The haemodynamic and neurohumoral bases for these varying blood pressure responses to the onset of TTC are currently poorly understood. Indeed, it is not clear whether hypotension is primarily driven by low cardiac output in all cases: further investigation in this area is appropriate.

The incidence of in-patient death in our cohort (5%), exceeds the rate which is generally reported for TTC (~1-2%) (Akashi et al., 2010). Not all of the six deaths were directly cardiac: two were more directly related to primary acute neurological conditions, whereas one patient succumbed to a protracted septic illness, which had likely precipitated TTC early in its course. However, the remainder of the deaths resulted from either of cardiogenic shock (n = 2) or ventricular arrhythmia (n = 1). The lack of specific treatment measures for the management of TTC is therefore particularly regrettable, but highlights current gaps in understanding of the pathogenesis of this condition.

2.4.3 ECG patterns in TTC versus ACS

Although some researchers have confined themselves to TTC patients presenting with ST-elevation (Sharkey et al., 2008, Barker et al., 2009a, Carrillo et al., 2010, Kosuge et al., 2010, Tamura et al., 2011), non-ST-elevation is likely to be the *more common* initial finding (Dib et al., 2009b, Singh et al., 2010), as well the *more challenging* mode of presentation, from a diagnostic point of view. Our data suggest that the heterogeneity of initial ST-T wave findings in TTC resembles that of ACS in females, in general. It is therefore appropriate that an ACS comparator population was enrolled without regard to ST-elevation, in whom the frequency of ST-elevation and its absence were similar (see Table 2.4).

As previously discussed (Section 2.1.2), studies of the utility of ECG characteristics for diagnosis of TTC *at presentation* have been disappointing. In contrast, our findings suggest that, rather than an acute ECG abnormality, the development of changes at 24 hours may more readily distinguish TTC from ACS. Furthermore, rather than a specific ST-T wave abnormality; the presence of *multi-regional* changes may be the ECG feature that is most

widely shared by TTC patients, as against their ACS counterparts. On the other hand, it is unlikely that ECG analysis alone is a sufficient basis of diagnosis/differentiation (see Table 2.5).

2.4.4 NT-proBNP elevation in TTC versus ACS

The demonstration that NT-proBNP plasma concentrations are appreciably elevated in all TTC patients is not in itself novel. At least six previous studies have documented that BNP elevation occurs commonly in TTC (Akashi et al., 2004a, Grabowski et al., 2008, Madhavan et al., 2009, Morel et al., 2009, Wittstein et al., 2005), while one small study obtained analogous data with NT-proBNP (Nef et al., 2008c). Moreover, the extent of such elevation in the current series is similar to that which was previously described.

The extent to which BNP/NT-proBNP plasma concentrations might be diagnostically useful has been partially evaluated (Madhavan et al., 2009): this study extends the available data considerably. In view of our aims concerning the TTC score, we assessed the performance of two predetermined 'cut-points' of NT-proBNP: the first (≥ 3,000 pg/mL) to optimise sensitivity and the second (≥ 6,000 pg/mL) to optimise specificity.

Madhavan *et al* (Madhavan et al., 2009) reported that plasma BNP concentrations were 4-5-fold greater in TTC than in STEMI, nominating an optimal cut-off of 647 pg/mL (sensitivity 81%, specificity 75%) for the diagnosis of TTC. Noting that BNP and NT-proBNP concentrations are not directly comparable, our data suggest a similar degree of differential elevation in TTC (5-fold greater). In "all comers", ROC construction demonstrated an AUC of 0.75; NT-proBNP elevation at the prespecified levels conferred less sensitivity and specificity than anticipated (for ≥3,000 pg/mL, 70 and 65%, respectively; for ≥6,000 pg/mL, 50 and 79%, respectively) (see Figure 2.1 and Table 2.6). An optimised lower "cut point" (2,500 pg/mL), however, increased sensitivity to 80%, while it preserved specificity.

Despite the superficial similarity between the study of Madhavan et al and the current work, differences in the patient populations studied probably explain the seemingly

different diagnostic performance described. Six of the 19 TTC subjects evaluated in the Madhavan et al study had pulmonary congestion or shock, compared to 1 of the 10 STEMI patients. In contrast, in our larger study, rates of Killip class ≥ II were well matched in the TTC and ACS groups (25 and 30%, respectively, P = NS).

The potential modulating effect of haemodynamic status was therefore addressed by a prespecified subgroup analysis, in which it was apparent that NT-proBNP had no capacity to differentiate TTC from ACS, when pulmonary congestion or haemodynamic compromise were present (see Figure 2.1; Table 2.6). In contrast, ROC characteristics were improved when NT-proBNP was compared in Killip class I subjects only (see Figure 2.1D). Early resolution of diagnostic problems, by cardiac catheterisation, is arguably more likely in shock or pulmonary congestion, thus mitigating the poor performance of NT-proBNP in this context.

Although there were no clinical differences between TTC presenting with and without ST-elevation, it is important to establish that any diagnostic strategy is effective in both scenarios. This is especially so, since, as a function of emergent angiography for apparent STEMI, the group which is more likely to benefit from more rapid diagnosis are those with apparent NSTEMI. This was indeed the case (see Figure 2.5).

2.4.5 The TTC score: potential for expedited diagnosis

The intended application of the TTC score is in patients with a working diagnosis of ACS. Importantly, the capacity of this score to expedite diagnosis in the subgroup of these patients who actually have TTC, was tested with readily available clinical data, available within the first 24 hours. Thus, the score aims to highlight the probability of TTC to clinicians, and is neither a means of definitive diagnosis, nor a substitute for full clinical assessment. In selecting the parameters of the score, we sought a balance between sensitivity and specificity. To optimise sensitivity, we selected features common to both TTC and ACS, but which are uncommon or absent when acute myocardial disease is not present: troponin elevation, contiguous ST-T wave change in any region and an NT-proBNP of 3,000 pg/mL. In order to enhance specificity, three components, thought to be more

strongly associated with/suggestive of TTC, relative to ACS: ΔECG_m, NT-proBNP ≥6,000 pg/mL ± presence of an antecedent stressor. The assumptions underlying these score components have been addressed in above sections.

Our data indicate that the TTC score, like NT-proBNP itself, is ineffective in differentiating TTC from ACS in the presence of pulmonary congestion or shock. However, in all other clinical scenarios examined, the TTC score demonstrated characteristics intermediate between the two NT-proBNP cut-points: a score of ≥4 exhibited greater sensitivity than that of an NT-proBNP ≥ 6,000 pg/mL and greater specificity than that of an NT-proBNP of 3,000 pg/mL (see Table 2.6).

Of note, these diagnostic performance characteristics are evident when the TTC score is tested as a five-point version (excluding the factor of exposure to preceding stress). While it is important that the TTC score performs well without assessment of prior stressors, a comparison employing imputed stressor exposure — at conservative rates for ACS — strongly suggests that a six point version of the score would have moderately enhanced diagnostic performance (see Figure 1.1). Furthermore, adjustment of the lower cut-point of NT-proBNP, from 3,000 to 2,500 pg/mL, would marginally improve sensitivity without compromising specificity (see Section 2.3.8).

2.4.6 Limitations

Ideally, the ACS group would have been equal to or larger than the TTC group. This was unfortunately not the case here, due to poorer than expected recruiting. Power calculations were not performed prospectively, given that the SD within the ACS group was not known. In retrospect, with the actual number of patients recruited, the study had 99% power to detect a difference in the TTC score of 1 (equal to 1 standard deviation) compared to the ACS group at $P<0.05$. The control group should also have ideally included about 5% males, although the impact of this omission is likely to be small.

The criteria for diagnosis of TTC in this cohort included 22 patients in whom cardiac catheterisation was not performed. Such individuals would not be classified definitely as

TTC according to the Mayo criteria (Madhavan and Prasad, 2010a). However, in all cases in whom TTC was diagnosed without cardiac catheterisation, reversible segmental LV wall motion abnormalities, disconsonant with the distribution of individual coronary arteries, were present and in most cases regional oedema was confirmed in the absence of confluent myocardial necrosis. This process therefore accords with recently suggested non-invasive methodology for TTC diagnosis (Eitel et al., 2011b). However, it is important to note that the results of these 22 patients were similar to those of the remainder of the TTC cohort. Ideally, also, a blinding procedure should have been employed for the reading of ECG abnormalities between groups.

The lack of utility of NT-proBNP and the TTC score in patients with evidence of LV failure or hypotension is indeed a problem. However, as mentioned, this is likely to be mitigated in clinical practice, due to the practice recommendation of emergent cardiac catheterisation when shock occurs in presumed ACS (Hochman et al., 1999). Similarly, any degree of haemodynamic compromise is likely to stimulate early cardiac catheterisation, effectively limiting the impact of the problem.

Further problems can be envisioned with the use of such a score in critical care environments, for multiple reasons. First, by definition, all such patients have stressor exposure. Second, biomarker strategies are frequently problematic in that many of these patients have a positive troponin result (Thygesen et al., 2008) and NT-proBNP levels are frequently markedly elevated, out of proportion to LV filling pressures (Januzzi et al., 2006a), likely as a result of systemic inflammation (Rudiger et al., 2006). Ironically, the intensive care environment is perhaps the one place in which early management could be most beneficially tailored to TTC — even in the current state of knowledge — based on the avoidance of catecholamine inotropic agents and the utilization of mechanical circulatory support via counter-pulsation therapy. Careful ECG analysis, attention to unexplained hypotension and early recourse to bedside echocardiography are likely to be the mainstay of expedited diagnosis in this important group.

Finally, the TTC score is largely dependant on the results of NT-proBNP assays. These are of uncertain chemical specificity, given the recent comparisons between results of assays

for BNP utilizing commercial kits, compared with mass spectrometric quantification (Miller et al., 2011).

2.5 Conclusions and clinical application

The "TTC score" is a potential adjunct to clinical pattern recognition, most suitable for use 24 hours post-presentation. Although tested across the range of TTC/ACS, the intended use of the score is in apparent NSTEMI, primarily because it is in this group, that TTC is likely to be missed or misdiagnosed. Secondarily, as has been said, cases presenting to hospital with ST-elevation will generally be diagnosed rapidly, without the assistance of biochemical tests, at emergency angiography/ventriculography. The TTC score has the potential to differentiate TTC from ACS and may therefore facilitate earlier diagnosis *in the target group* with apparent NSTEMI. This is especially pertinent in the light of the fact that the majority of TTC patients are now known to present without ST elevation.

This being so, the benefits of earlier diagnosis are not clear at present. The use of clinical information *available at 24 hours* was based on our observation that ECG and biochemical parameters, which were selected for specificity for TTC, develop over that period: although clinical data come to hand piecemeal, we anticipate that the elements of the TTC score would be available synchronously within a 24-hour timeframe. In this respect, it is difficult to envisage authentic *randomised* testing of the TTC score, given that the components of the score are equally available to clinicians in the hospital environment.

Actual implementation of the TTC score, as a routine, could therefore be achieved in a coronary care setting. The ideal situation would see the TTC score coupled with a highly accurate modality for definitive diagnosis – such a test, as yet, does not exist. Nevertheless, an increasing awareness of patients with *probable TTC* would seem an important intermediary step in the development and testing of any strategy of definitive diagnosis. However, as previously discussed (Chapter 1, Section 1.2.3), invasive criteria-based confirmation of the diagnosis is currently to be preferred.

2.6 Tables

Table 2.1 Comparison of acute clinical and angiographic features

	TTC patients n = 125	**ACS patients** n = 56
Age (years) (mean±SD)	67 ± 14	70 ± 12
Sex (male/female)	6/119	0/57
Clinical presentation		
Antecedent stressor	102 (82%)	7 (12%) **
CP ± dyspnoea	92 (74%)	49 (86%)
Dyspnoea alone	3 (2%)	2 (4%)
Acute non-cardiac illness	31 (25%)	1 (2%) **
Cardiovascular risk factors		
BMI (kg/m^2) (mean±SD)	28 ± 6	30 ± 6
Current smoking	9 (7%)	13 (23%) *
Systemic hypertension	58 (46%)	41 (72%) **
Diabetes mellitus	18 (14%)	15 (26%)
Hyperlipidaemia	42 (34%)	37 (67%) **
≥ 3 CV risk factors	10 (8%)	23 (40%) **
Incidence/extent CAD		
Any angiographic plaque	26 (21%)	53 (92%) **
Single vessel disease	14 (11%)	14 (25%) *
Double vessel disease	7 (6%)	10 (18%) *
Triple vessel disease	1 (1%)	15 (26%) **
Features and complications		
Ventricular arrhythmia	7 (6%)	2 (4%)
Pulmonary oedema (Killip II-III)	9 (7%)	13 (23%) *
Hypotensive shock (Killip IV)	23 (18%)	4 (7%) *
In-hospital death	6 (5%)	3 (5%)

All data expressed as number (and percentage), unless otherwise specified; * P < 0.001; ** P < 0.05; Abbreviations: CAD, coronary artery disease; CV, cardiovascular; BMI, body mass index; SBP, systolic blood pressure; OOHA, out of hospital cardiac arrest

Table 2.2 Classification of identified antecedent stressors in TTC group

Psychosocial/Emotional	61 (49%)
Death/impending death of a close other[#]	*16 (13%)*
Interpersonal conflict	*20 (16%)*
Employment/financial pressure/bad news	*6 (5%)*
Other emotional	*20 (16%)*
Physiologic	42 (34%)
Perioperative	*4 (3%)*
Acute respiratory conditions	*17 (14%)*
Acute neurological conditions	*6 (5%)*
Other critical illness/infection	*7 (6%)*
Other physiological	*7 (6%)*
No identifiable stressor	23 (18%)

[#] including loss of pets

Table 2.3 Haemodynamic and imaging characteristics of TTC patients

	TTC patients n = 125[#]
Haemodynamic measurements	
Arrival SBP (mmHg) (mean±SD)	123±24
MVO_2 saturation (%) (mean±SD)	66 ± 9
PCWP (mmHg) (mean±SD)	15 ± 7
Basic imaging findings	
LVEDV (mL) (mean±SD)	82 ± 29
LVESV (mL) (mean±SD)	42 ± 20
LVEF (%) (mean±SD)	49 ± 12
WMSI (mean±SD)	1.9 ± 0.3
RV involvement (%)	9%
Pericardial effusion (%)	7%
Intracardiac thrombus (%)	2%
Focal necrosis (LGE CMR) (%)	3%

[#] N = 52 for invasive haemodynamic measurements

Abbreviations: LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; WMSI, wall motion score index; RV, right ventricle; LGE, late gadolinium enhancement; CMR, cardiac magnetic resonance

Table 2.4 Comparison of acute biochemical and ECG features

	TTC patients n = 125	**ACS patients** n = 57[#]
Acute biochemistry [median (range)]		
Cardiac troponin T (mg/dL)	0.41 (0.23-0.62)	0.46 (0.14-1.14)
Creatine Kinase (IU/L)	197 (120-298)	358 (162-742) *
CRP/hs-CRP (mg/dL)	21 (7-58)	10 (4-36) *
NT-proBNP (pg/mL)	5990 (2780-10600)	1600 (530-5260) **
Metanephrine (pg/mL)	258 (200-381)	210 (200-347)
Normetanephrine (pg/mL)	1110 (793-2350)	980 (613-2510)
Initial ECG changes (%)		
ST-elevation	32%	40%
ST depression	14%	35% **
T-wave inversion	25%	40% *
LBBB/intermittent LBBB	6%	2%
Paced	3%	2%
No specific changes	29%	14% *
QT_c interval (msec) (mean±SD)	457 ± 36	455 ± 30

[#] N = 16 ACS patients for metanephrine/normetanephrine levels.

All data expressed as number (and percentage), unless otherwise specified; * P < 0.001; ** P < 0.05; Abbreviations: CRP, C-reactive protein; NT-proBNP, amino-terminal of prohormone of B-type natriuretic peptide; ECG, Electrocardiography; LBBB, left bundle branch block

Table 2.5 Evolution of selected ECG findings in TTC

		TTC (n = 125)		ACS (n = 56)	
		Presentation	*~24 hours*	*Presentation*	*~24 hours*
ST-Elevation (present/absent)	Regional	21%	1%	33%	5%
	Multi-regional	12%	8%	5%	5%
ST-depression (present/absent)	Regional	10%	0%	30%	2%
	Multi-regional	4%	0%	5%	4%
T-wave inversion (present/absent)	Regional	11%	29%	33%	32%
	Multi-regional	14%	54% *	7%	21%
Other	LBBB/RBBB/paced rhythm	11%	8%	6%	6%
	No ST-T wave changes	29%	0%	14%	14%
	QT_c (msec) [median (IQR)]	459 (424-485)	490 (455-524) *	457 (431-472)	431 (413-461)

* $P < 0.0005$, between group difference, at 24 hours

Table 2.6 Contribution of extent of NT-proBNP elevation to potential discrimination between TTC and ACS.

Data shown include (a) extent of NT-proBNP elevation and (b) TTC score.

		Sensitivity (%)	[95% CI]	Specificity (%)	[95% CI]
'All comers'	NT-pro BNP ≥ 3,000 pg/mL	70	[62 – 80]	65	[51 – 77]
	NT-pro BNP ≥ 6,000 pg/mL	50	[40 – 60]	79	[66 – 89]
	TTC score[#] ≥ 4	62	[51 – 71]	75	[62 – 86]
Killip I only	NT-pro BNP ≥ 3,000 pg/mL	66	[53 – 77]	80	[64 – 91]
	NT-pro BNP ≥ 6,000 pg/mL	41	[30 – 54]	90	[76 – 97]
	TTC score[#] ≥ 4	56	[44 – 68]	87	[73 – 96]
Killip ≥ 2	NT-pro BNP ≥ 3,000 pg/mL	82	[63 – 94]	29	[10 – 56]
	NT-pro BNP ≥ 6,000 pg/mL	71	[51 – 87]	53	[28 – 77]
	TTC score[#] ≥ 4	75	[55 – 89]	47	[23 – 72]
NSTE only	NT-pro BNP ≥ 3,000 pg/mL	66	[53 – 78]	67	[48 – 82]
	NT-pro BNP ≥ 6,000 pg/mL	42	[30 – 55]	76	[58 – 89]
	TTC score[#] ≥ 4	56	[44 – 68]	79	[61 – 91]
STE only	NT-pro BNP ≥ 3,000 pg/mL	80	[62 – 91]	61	[39 – 80]
	NT-pro BNP ≥ 6,000 pg/mL	65	[66 – 80]	83	[61 – 95]
	TTC score[#] ≥ 4	74	[57 – 88]	70	[72 – 99]

[#] TTC score here does not include stressor exposure; Abbreviations: NSTE, non-ST-elevation; STE, ST-elevation

2.7 Figures

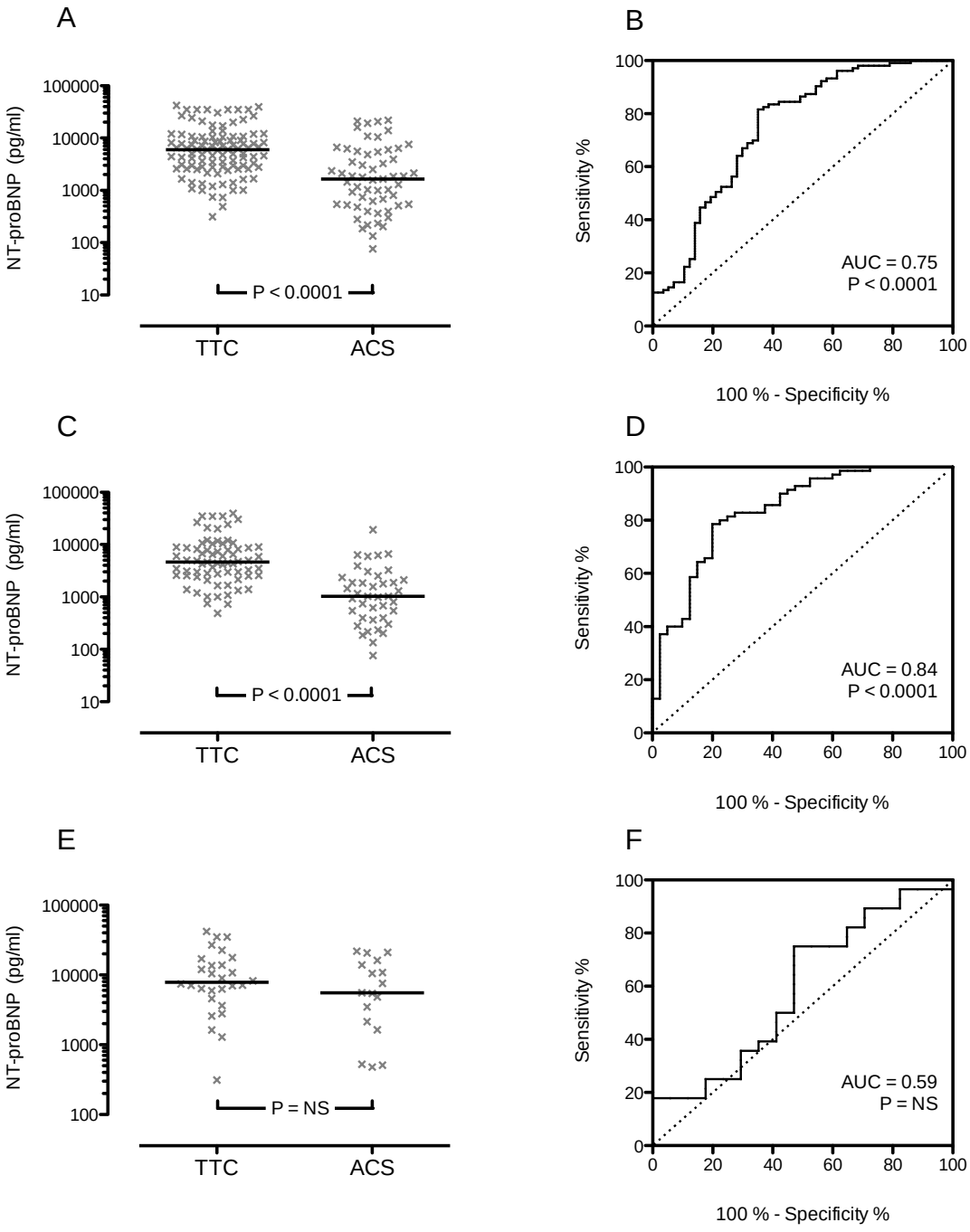

Figure 2.1 Discriminatory value of extent of NT-proBNP elevation in the differentiation of TTC from ACS. Left-hand panels compare concentrations, whereas right-hand side panels display ROC data, for all patients (A and B), Killip class I (C and D) and Killip class ≥ II (E and F).

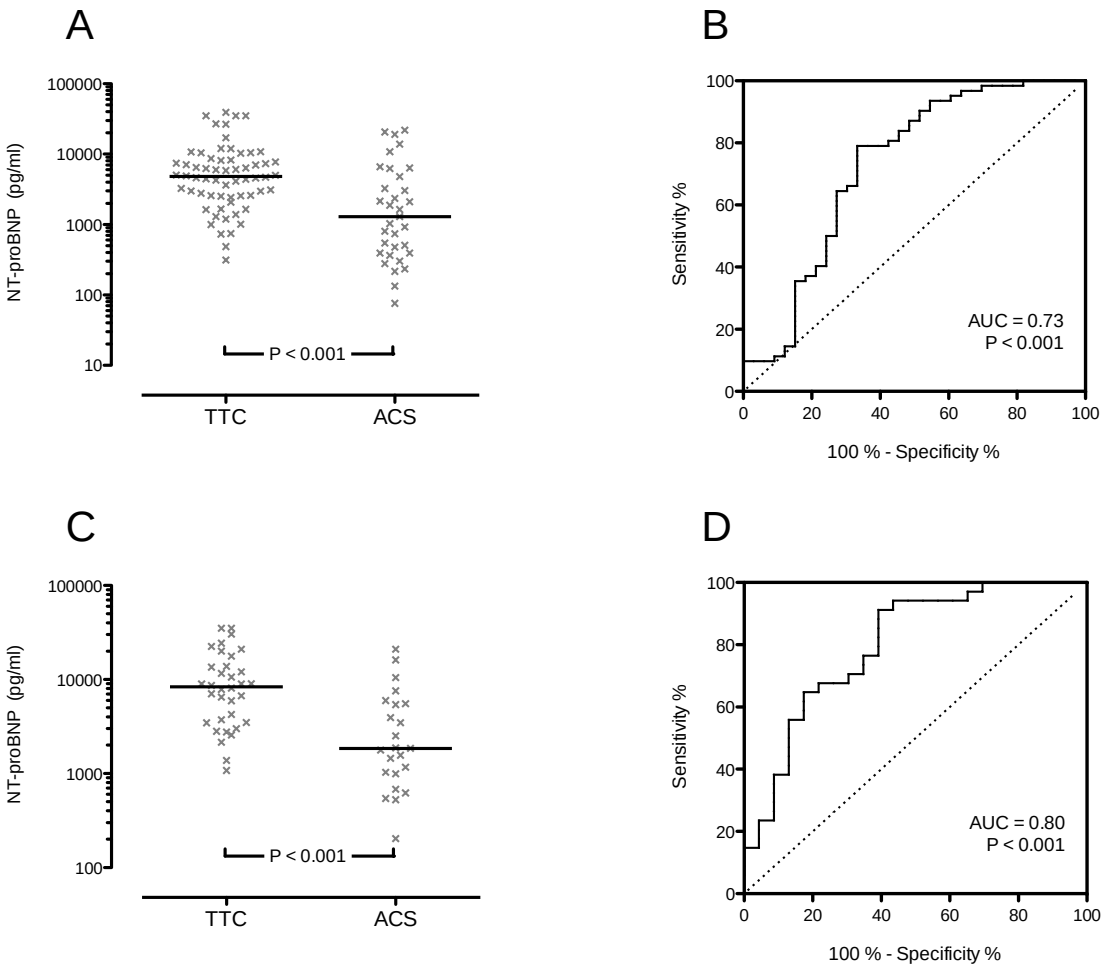

Figure 2.2 Discriminatory value of extent of NT-proBNP elevation in relation to ST-elevation. Comparison NT-proBNP concentrations (TTC versus ACS) and ROC data for patients *without* ST-elevation (A and B) and patients with ST-elevation (C and D).

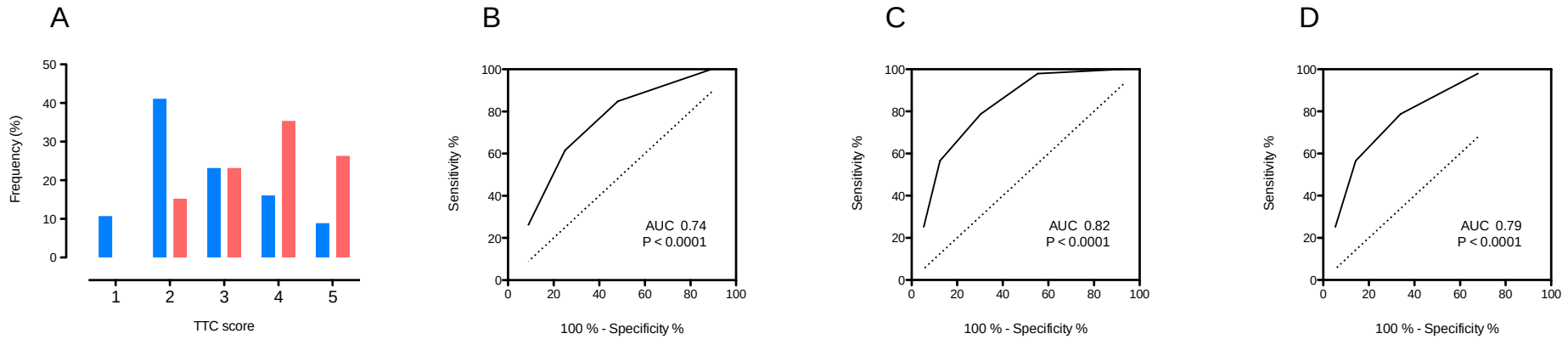

Figure 2.3 Discriminatory value of TTC scores with and without the putative factor of an antecedent stressor. Distribution of TTC scores (without accounting for a stressor) in TTC (red) versus ACS (blue; $P < 0.0001$, Wilcoxon signed rank test) (Panel A), with accompanying ROC data (Panel B). Panels C and D depict ROC data where preceding stress in TTC has been included in the TTC score and two stressor rates, 25% and 50%, have been applied to the ACS group.

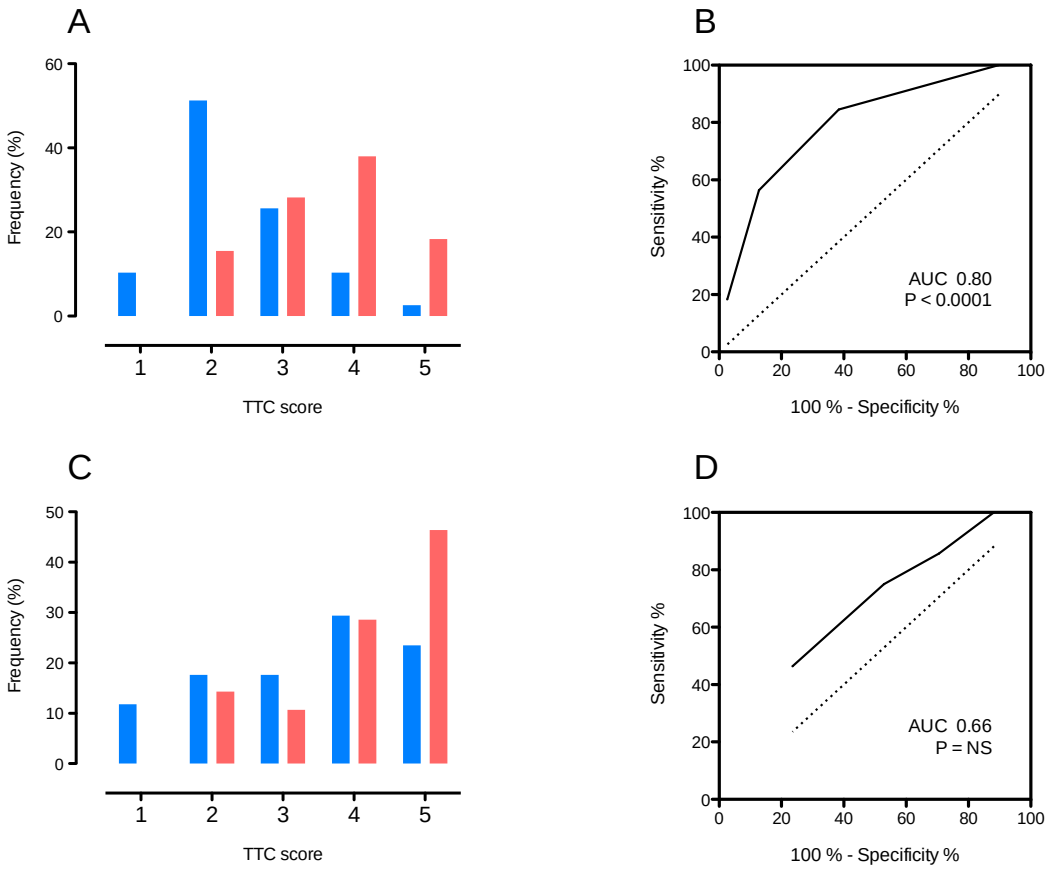

Figure 2.4 Discriminatory value of TTC scores with respect to haemodynamic status. Distributions of TTC scores and accompanying ROC data are presented for cases in Killip I (Panels A and B) and Killip II or greater (Panels C and D) (TTC = red; ACS = blue). Median TTC scores were significantly different in Killip class I ($P < 0.0001$), but not in Killip ≥ II ($P = 0.07$, Wilcoxon signed rank test).

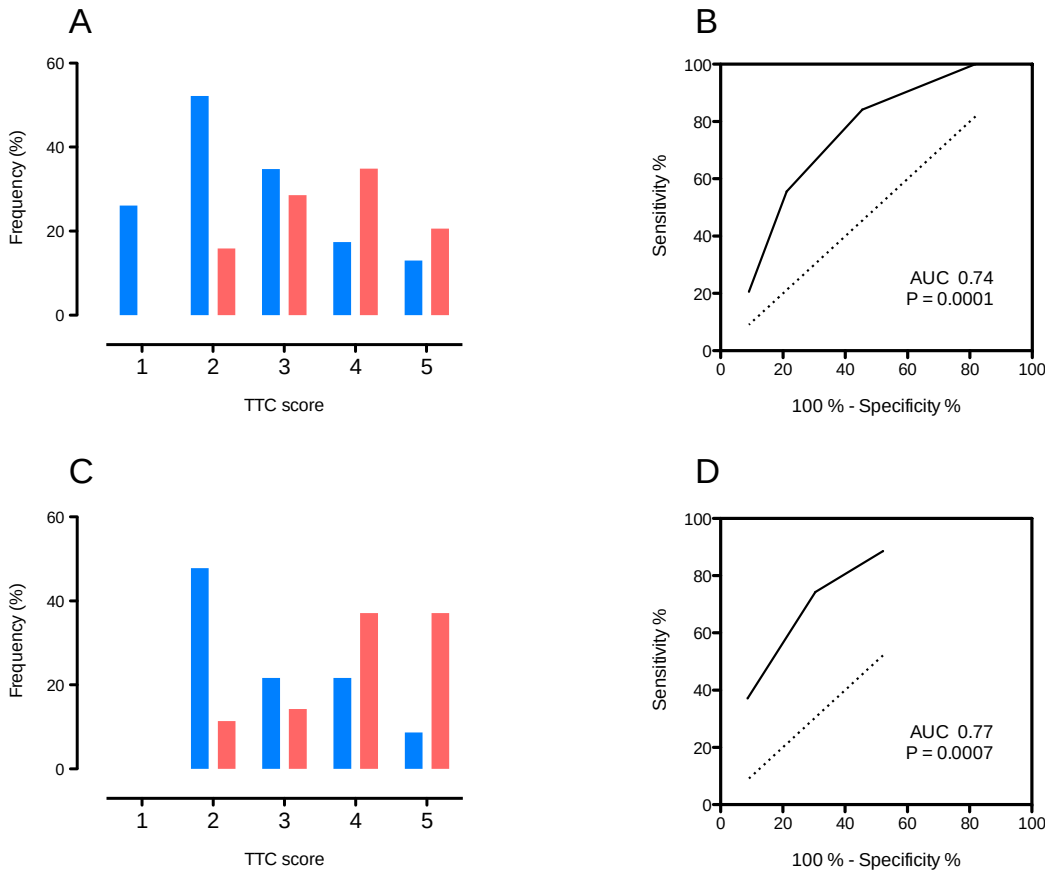

Figure 2.5 Discriminatory value of TTC scores with respect to ST-elevation status. Distributions of TTC scores and accompanying ROC data are presented for cases *without* ST-elevation (Panels A and B) and *with* ST-elevation (Panels C and D) (TTC = red; ACS = blue). Median TTC scores were significantly different in both situations ($P < 0.001$, Wilcoxon signed rank test).

Chapter 3

Inflammatory activation during the acute phase of Tako-Tsubo cardiomyopathy: information from cardiac magnetic resonance

3.1 Introduction

As is implicit in the rapid recovery of global systolic function in the majority of cases, TTC is associated with a minor amount of myocardial necrosis, with modest elevation of cardiac biomarker levels (Bybee et al., 2004a, Gianni et al., 2006b) and generally with the absence of late gadolinium enhancement on cardiac magnetic resonance (CMR) imaging (Rolf et al., 2009, Haghi et al., 2006). However, this does not necessarily mean that there is no cardiomyocyte loss, for instance, via apoptosis.

Inflammatory activation, both systemic and intra-myocardial, has been documented in TTC. Most studies have revealed modest elevation of plasma C-reactive protein concentrations (Madhavan et al., 2009, Morel et al., 2009, Lee et al., 2009a), while intra-myocardial inflammatory activation has been documented directly via myocardial biopsy studies (Nef et al., 2007b) as well as via technetium pyrophosphate imaging (Ito et al., 2003). Analogously, a number of investigators have documented the presence of intramyocardial oedema, presumably of inflammatory origin, via CMR (Rolf et al., 2009, Otsuka et al., 2008, Abdel-Aty et al., 2009, Eitel et al., 2010).

An investigation was therefore undertaken, regarding the regional distribution and intensity of intra-myocardial oedema in a cohort of patients with TTC. All analyses were structured according to the following hypotheses: -

1. The distribution of oedema, during the acute phase of TTC, would parallel that of regional hypokinesis and that oedema would resolve synchronously with recovery of left ventricular wall motion

2. The extent of oedema would be correlated with plasma normetanephrine concentrations, as an index of individual catecholamine exposure and a putatively causative factor

3. The extent of oedema, presumably a consequence of intramyocardial inflammation, would be correlated with markers of

a. myocardial injury [N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) (Masters et al., 1999, Ogawa et al., 2008, Jensen et al., 2010) and cardiac troponin T release] and

b. systemic inflammatory activation [C-reactive protein elevation (CRP) and impairment of circulating platelet responsiveness to nitric oxide (Chirkov et al., 1999, Anderson et al., 2004a).

3.2 Methodology

3.2.1 Patient selection

Patients with a diagnosis of acute TTC (onset within the preceding 48 hours), inclusive of confirmatory diagnostic cardiac catheterization, were eligible for study. Over a period from March 2009 to October May 2012, 70 such patients were evaluated at two tertiary referral hospitals. The diagnosis of TTC was made if the following criteria were met: (i) a presentation with abnormalities of ST segments/T waves, with or without chest pain or dyspnoea (ii) a sustained elevation of cardiac troponin T levels, (iii) demonstration of a characteristic periapical wall motion abnormality (or an accepted variant), not consistent with a single epicardial coronary territory and (iv) angiographic exclusion of obstructive coronary disease in the territory subserving the regional wall motion abnormality (Bybee et al., 2004a, Madhavan and Prasad, 2010b), as well as elimination of viral myocarditis by clinical and CMR criteria (Friedrich et al., 2009b). Of these 70 patients, 21 were excluded from the MRI protocol because of claustrophobia (7 patients), prior device implantation (3 patients), critical illness/death (7 patients), inability to perform CMR within the required timeframe (2 patients); one patient was not referred because of extreme age and another because of the degree of acute bereavement. Thus, 49 patients participated in the investigation. Follow-up CMR was performed on 30 patients at a median of 105 (interquartile range, 90-116) days.

3.2.2 Clinical protocol

As previously described (see Chapter 2, section 2.2.3), patients were monitored for a minimum of 48 hours, during which time intravenous sodium heparin was administered and routine blood chemistry was obtained.

3.2.3 Biochemical investigations

Biochemical investigations were also performed as previously discussed (Chapter 2, Section 2.3.1):

- Plasma normetanephrine, as an index of acute catecholamine exposure (Goldstein et al., 2003). Within 24 hours of diagnosis, venous blood was drawn into tubes containing potassium ethylene diamine tetra acetic acid and centrifuged at 1680 relative centrifugal force, for subsequent assay by liquid chromatography/tandem mass spectrometry (Lagerstedt et al., 2004).

- Cardiac troponin T and levels of NT-proBNP (Elecsys E 170, Roche Diagnostics, Germany) were determined at presentation and serially thereafter to determine the peak for the acute episode. NT-proBNP concentration was re-evaluated 3 months after the index event.

- C-reactive protein (CRP) was assayed serially to determine the peak in each patient; in cases where CRP was less than 10 mg/L, the level of high sensitivity CRP (Olympus au5400, Dallas, Texas, USA) was determined.

3.2.4 Platelet responsiveness to nitric oxide

Platelet responsiveness to nitric oxide was of interest both as a physiological marker of systemic redox stress (Chirkov et al., 1999, Anderson et al., 2004a) and in view of the reported thrombogenicity associated with TTC (Kurisu et al., 2009). For platelet

aggregation studies, blood samples were drawn by venesection from an antecubital vein (0-24 hours from diagnosis). Blood was collected in plastic tubes containing 1:10 volume of acid citrate anticoagulant (2 parts of 0.1 mol/L citric acid to 3 parts of 0.1 mol/L trisodium citrate); acidified citrate was utilized in order to minimize deterioration of platelet function during experiments (Kinlough-Rathbone et al., 1983). Aggregation in whole blood was examined utilizing a dual-channel impedance aggregometer (Model 560, Chrono-Log, Havertown, PA, USA) as described previously (Anderson et al., 2004a). Aggregation was induced with ADP (final concentration of 2.5 μmol/L). In order to determine platelet responsiveness to nitric oxide, inhibition of aggregation by the nitric oxide donor sodium nitroprusside was utilized (Chirkov et al., 1999, Anderson et al., 2004a).

3.2.5 Control subject selection

Female age-matched controls were utilized to determine physiologically normal ranges of T_2-weighted signal intensity (T_2-w SI, as described in the Appendix), in the absence of oedema. They were sought by advertisement and were eligible in the absence of a contraindication to CMR or known cardiac or renal disease. The institutional human research ethics committee approved the study and all patients and controls provided written informed consent.

3.2.6 Cardiac magnetic resonance imaging

Imaging protocol

For acute, follow-up and control imaging, CMR was performed on 1.5 T Philips Intera and Achieva systems (Philips Medical Systems, Best, The Netherlands), at the respective hospital sites, each with a 5-channel phased array coil and electrocardiographic gating. After initial scout images, cine CMR was performed in standard short and long axis cardiac views, utilizing Balanced Turbo Field Echo sequences. All short axis imaging (cine CMR, T_2-

weighted and late gadolinium enhancement) employed the same slice thickness (8 mm) and interval space (no gap), to allow consistency of regional comparison between image sets, via a seventeen-segment model (Cerqueira et al., 2002). A triple inversion recovery pre-pulse (double inversion dark blood suppression, followed by selective fat saturation – SPAIR) was applied for imaging of myocardial oedema in short axis, in accordance with current guidelines of the assessment for myocardial oedema (Friedrich et al., 2009b). Typical imaging parameters were: field of view 360 mm, TR 2 x RR interval, TE 80 milliseconds, turbo factor 35, matrix 240/560, EPI factor 1, NSA 1, a fat inversion pulse time 150 milliseconds and signal homogeneity correction (CLEAR). Gating was prospective and timed to mid-diastole. Late gadolinium enhancement studies were obtained with parallel imaging, utilizing a field of view of 340 mm, TR of 3.6 milliseconds, TE of 1.1 milliseconds, and an inversion time manually adjusted to null the signal from viable myocardium, following bolus administration of 0.2 mmol/kg Gadobutrol (Bayer Schering Pharma, Switzerland). A panel of experienced CMR readers reviewed all imaging. Methodology for T_2-weighted signal quantification is described in the Appendix.

3.2.7 T_2-weighted signal quantitation methodology

(a) Principles and validation

The quantitative potential of T_2-weighted cardiac imaging rests on the ability to correct myocardial signal intensity against an extracardiac tissue reference. Such correction is necessary because the magnitude of the MR signal is proportional to voxel volume and is also dependent on factors such as the gain settings used during the scan, all of which will vary from patient to patient and possibly from scan to scan. It is therefore necessary to reference T_2-weighted signal intensity (T_2-w SI) against a suitable baseline. Furthermore, between-slice variation should be minimized via signal homogeneity correction (see methods). The comparison of T_2-w SI between a myocardial region of interest (ROI) and a skeletal muscle SI reference, has been advocated for the categorical diagnosis of myocardial inflammation (Friedrich et al., 2009a), for example in suspected cases of

suspected myocarditis (Jeserich et al., 2010) and cardiac allograft rejection (Taylor et al., 2010).

For the purposes of this case-control study, we evaluated a similar approach, in order to obtain a measure of the magnitude of T_2-w SI, i.e. as a continuous, rather than categorical, variable. The upper pole of the spleen (UPS) was evaluated as a possible alternative to a skeletal muscle tissue reference. In each of 10 normal volunteers, T_2-w SI was determined within left ventricular myocardium (MC), UPS and latissimus dorsi (LD). The coefficient of variability of the T_2-w SI ratio for MC:UPS was 12%, versus 19% for MC:LD. Furthermore, the UPS, which would be expected to have consistent T_2 properties in the absence of hypersplenism or infiltrative disease, was consistently identifiable within short axis stack series.

There was no significant variability of T_2-w SI within the UPS: consistency of signal within the spleen was demonstrated by a coefficient of variation of 2.9% (n = 10). Therefore, UPS was selected as the extracardiac reference, for this study: all expressions of myocardial T_2-w SI, therefore, denote a unitless quantity derived from this ratio.

(b) Regional and Global quantitation of T_2-w SI

The above concept was applied to both regional and global quantification. For analysis of variation in base-to-apex signal, mean T_2-w SI was determined from whole slice cardiac ROI's, avoiding subendocardium (frequently enhanced due to low flow (Edwards et al., 2009)). For regional assessment, a 10-20mm^2 ROI was placed in the mid-wall, in the geometric centre of each segment, according to a 17-segment model (Cerqueira et al., 2002) excluding the extreme apex, where signal can be diminished due to partial volume averaging. In normal subjects, within-segment coefficient of variability for T_2-w signal, sampling adjacent mid-wall areas, was 5.2% (n = 10).

The reproducibility of the entire method, taking into account splenic and myocardial sampling, was assessed via two observers in 10 normal subjects: the coefficient of

variability was 4.5%, with no significant difference between sets of observations, nor with increasing T_2-w SI (Bland Altman analysis).

Quantification of norms for regional T_2-w SI was performed in 10 healthy age-matched female subjects (see figure 1A, main text) and mean normal values for each region were derived from these data. In order to quantitate local oedema in patients, the difference between regional patient T_2-w SI and mean normal values was derived for each component ROI. In order to obtain a single expression of total left ventricular oedema, T_2-w SI values from each of the seven standard slices were integrated, relative to these population norms. For this purpose, global myocardial T_2-w signal was expressed in arbitrary units and is denoted as "corrected T_2-w SI".

3.2.8 Global and regional wall motion quantitation

Offline analysis was undertaken using Philips ViewForum software (release 2.5.3.0). Endocardial and epicardial borders were traced in short-axis cine CMR images, throughout the cardiac cycle, allowing calculation of left ventricular volumes, ejection fraction and wall thickness. Presystolic and peak systolic thicknesses were derived automatically: from these, peak radial strain was calculated for each left ventricular segment, as a percentage of deformation. Subjective wall motion scoring and the derivation of a wall motion score index (WMSI) was also undertaken (Cerqueira et al., 2002).

3.2.9 Statistics

Differences between groups, in categorical and continuous variables, were assessed with Fisher's exact test or Student's *t* test, respectively, as appropriate. The degree of relationship between acute global T_2-w SI and various systemic markers were assessed by Pearson's r correlation, or Spearman's rank correlation in the case of non-Gaussian data. Differences in myocardial T_2-w signal distribution in the acute and recovery (3 months) phases were examined utilizing two-way ANOVA with repeat measures. GraphPad Prism

software (version 5.0) was utilized for all analyses and statistical significance was inferred with a P value of <0.05.

3.3 Results

3.3.1 Patient/control characteristics

All but one of the TTC subjects were female, whilst of the female subjects, one was premenopausal; patients' ages ranged from 41 to 86 years. Other demographics and comorbid conditions are summarized in Table 3.1. In all but 5 of the TTC patients, clear precipitant stressors were identified (65% psychological, 25% physical stress). Thirteen patients presented with ST-elevation on electrocardiography, three with a bundle branch block pattern and the remaining 31 without ST elevation (all of whom exhibited T-wave inversion). Twenty seven demonstrated typical apical ballooning, whereas 21 were found to have a mid-ventricular variant; two patients had findings consistent with the basal variant, or so called "inverted TTC" (Ennezat et al., 2005). In all cases, routine serial echocardiography demonstrated partial or total resolution of left ventricular regional hypokinesis within 10-14 days.

Patients and control subjects did not vary significantly as regards age, body mass index or clinical history (Table 3.1). Thirteen patients were diagnosed with TTC in the presence of atherosclerotic coronary disease. In all cases, the coronary disease location was not consonant with the site of wall motion anomaly (Madhavan and Prasad, 2010b). Two experienced CMR readers reviewed multisequential imaging, examples of which are provided (see Figure 3.1). All patients exhibited wall motion scores of ≥1.125, but global LV ejection fraction was frequently normal acutely, despite segmental dysfunction; LV ejection fraction was reduced ≤ 40% in one quarter of patients. Focal mid-anterolateral late gadolinium enhancement was evident acutely in two patients, but was noted in each case to be not sub-endocardially based, to be of low intensity and to have resolved by the time of follow-up: myocardial necrosis due to infarction was deemed unlikely.

3.3.2 CMR findings in TTC: T_2-w SI quantification

(a) Control values

Individual data from the 10 control subjects are shown in Figure 2A. Across all slices, the mean ratio of LV myocardium:spleen T_2-w SI values was 0.47 ± 0.04. As can be seen, Data were homogeneous without evidence of a base-to-apex gradient. Radial inter-segment coefficient of variability was 7.9%.

(b) Acute TTC

Individual uncorrected T_2-w data for TTC patients during acute presentation are shown in Figure 1B. The data were not normally distributed: hence no mean values are shown. In acute TTC, but not in control subjects, mean mid-anterior T_2-w SI was approximately 17% greater than corresponding posterior wall signal ($p < 0.005$). In all cases, the distribution of T_2-w signal was neither confined to the territory of a single coronary artery, nor concentrated in a distribution suggestive of viral myocarditis (Friedrich et al., 2009b). However, T_2-w SI values exhibited a significant gradient from apex to base (for example, comparing slice 1 to 7, the mean gradient was 0.13 ± 0.10 arbitrary units; $p < 0.0001$). Furthermore, even at the base, T_2-w SI values were significantly greater than for comparable control data ($p < 0.0001$). Given the high proportion of patients with the mid-ventricular variant, we compared apical:mid-zone T_2-w SI for apical TTC [median ratio 1.06 (interquartile range 1.01-1.20)] and mid-ventricular TTC [median ratio 1.01 (0.97 – 1.05), P = 0.02].

(c) Three months post-onset

At 3 months, oedema had decreased substantially, with loss of the apex-to-base gradient, but with overall incomplete resolution, as depicted in bull's-eye format in Figure 1C. Comparison of mean corrected regional T_2-w SI values at presentation and at 3 months is shown in Figure 2A. There was a substantial decrease in T_2-w SI, with abolition of the

regional T_2-w SI gradient (for difference in T_2-w SI, F = 40, (< 0.0001; for regional heterogeneity of resolution, F = 28, p < 0.0001 on 2-way ANOVA). Although there was abolition of a regional gradient of T_2-w SI at 3 months, the data suggested incomplete resolution. Comparison of global T_2-w SI for TTC patients at 3 months with that of the control subjects confirmed that T_2-w SI was abnormally elevated (p = 0.02).

3.3.3 Correlates of corrected T_2-w values

(a) Corrected regional T_2-w SI versus wall motion (Hypothesis 1)

The expected inverse relationship between average peak radial strain and T_2-w intensity was present at the mid-ventricular (r = -0.49, p < 0.001) and apical apex (r = -0.39, p = 0.04), but was not significant at the base (p = 0.08). Figure 2B summarizes these regional data for individual patients.

(b) Corrected global T_2-w SI versus normetanephrine level (Hypothesis 2)

Plasma normetanephrine concentration correlated directly (r = 0.33, p = 0.028) with global T_2-w signal (Figure 3A).

(c) T_2-w SI versus markers of myocardial injury (Hypothesis 3a)

A number of investigators have shown that TTC is associated with acute release of NT-proBNP (Nef et al., 2008c), as well as BNP (Madhavan et al., 2009, Lee et al., 2009a, Akashi et al., 2004a, Grabowski et al., 2008, Morel et al., 2009). We recently demonstrated that this anomaly persists for at least 3 months after the onset of symptoms (Nguyen et al., 2011b) and now sought to evaluate potential correlations with the extent of oedema. Significant correlations were observed between T_2-w SI and NT-proBNP (r = 0.40, P = 0.0045; see Figure 3B) and plasma troponin T (r = 0.29, P = 0.045).

(d) T_2-w SI versus markers of systemic inflammation (Hypothesis 3b)

The degree of elevation of C-RP was similar to that seen in other studies (median peak value 16.5 mg/L) (Lee et al., 2009a, Madhavan et al., 2009). There was a modest correlation between C-reactive protein and global corrected T_2-w SI (r = 0.34, P = 0.023). Mean inhibition of platelet aggregation, with the nitric oxide donor sodium nitroprusside, was 40 ± 28%. There was no correlation between platelet response to sodium nitroprusside and global T_2-w SI.

3.4 Discussion

The current study has evaluated the correlates of initial distribution and subsequent evolution of intracardiac oedema in a group of 49 patients presenting with TTC. As hypothesized, the distribution of oedema paralleled that of hypokinesis (Hypothesis 1), but with the important qualifying observations that (i) basal segments were also (more mildly) affected and that (ii) residual oedema was evident at 3 months. Also as postulated, LV oedema increased in proportion to catecholamine exposure (Hypothesis 2), indicators of cardiac injury (Hypothesis 3a) and partially with parameters of systemic inflammation (Hypothesis 3b). These findings suggest a central role of inflammation in TTC, certainly in the acute phase and possibly in recovery. It is important to emphasize that the subjects studied were representative of the clinical spectrum of TTC, as delineated in Chapter 2: specifically, while only 30% of patients had initial ST elevation or left bundle branch block, 8% had presented in a shocked state.

3.4.1 Implications of global oedema in TTC

In this context, utilizing T_2-weighted CMR imaging, it was demonstrated that one measurable component of the myocardial injury associated with acute TTC is oedema. The

presence of oedema *per se* has been described in previous MR series (Rolf et al., 2009, Otsuka et al., 2008, Abdel-Aty et al., 2009, Eitel et al., 2010), in qualitative terms. Notwithstanding the expected base-to-apex gradient of oedema (Hypothesis 1), abnormal T_2-w SI was seen in the basal myocardium, which has tended to be regarded as being "spared" (Lee et al., 2002, Prasad et al., 2008). As TTC has generally been conceptualized as a regional disorder, owing to the obvious differences on cardiac function, these findings strongly suggest that TTC is, rather, a global myocardial process, typically with differential regional manifestation.

The heterogeneity of the TTC sample studied also allowed this hypothesis to be tested by an alternative means. In a comparison between variant subgroups, a higher apical:mid-ventricular T_2-w SI was observed ($P = 0.02$) in the apical, compared to the mid-ventricular, variant. These results show that in the mid-ventricular form of TTC, there is essentially as much oedema at the apex as in the mid-zone. The reason for preservation of apical movement is therefore uncertain. In the two patients with basal TTC, there was no intraventricular T_2-w SI gradient.

Consistent with the proposition that the base is not completely spared in acute TTC, data from the study of Mansencal *et al* demonstrate substantial impairment of systolic peak velocity, strain and strain rate in basal segments of patients with acute apical ballooning (Mansencal et al., 2009). Adding further weight to idea of a global process in TTC, a recent series documented 3 cases of reversible global LV dysfunction, resembling TTC in terms of its classical elements: precipitation by stress, the expected electrocardiographic and biomarker changes and the absence of coronary disease or alternative causes (Win et al., 2011).

The current data do not permit determination of the extent to which fluid accumulation, detected by T_2-weighted imaging, is interstitial (vasogenic), as distinct from intracellular (cytogenic) (Edwards et al., 2009). However, in keeping with the initial presence of oedema, TTC was also associated with reduction of LV mass during follow-up (see Table 3.2). In addition, a serial reduction of LV end-diastolic volume was noted. This may suggest an acute response of mild diastolic LV dilatation. Whilst the mechanistic or pathophysiologic significance of this finding is not known, in the context of myocardial

inflammation, it is possible that there is a shared feature across several acute/reversible disorders, such as viral myocarditis and sepsis cardiomyopathy (Finkel, 1996).

3.4.2 Implications of persistent/slowly resolving oedema in TTC

Likewise, the persistence of oedema is perhaps surprising, given that TTC is usually portrayed as a rapidly reversible and benign process (Gianni et al., 2006b{Bybee, 2004 #912). However, in other pathological processes, such as myocardial infarction (Thompson et al., 1991), persistent foci of abnormal T_2-weighted signal have been described at 3 months. The documented presence of T-cells and macrophages in the cardiac interstitium in biopsies from TTC subjects as late as 3 months post-diagnosis (Nef et al., 2007b) supports this and, in fact, led to our selection of 3 months as a follow-up time-point. Thus, whilst a degree of residual T_2-weighted signal, alongside normalized LV systolic function, may seem a mere curiosity, chronic cardiac edema itself may be a substrate for the development of interstitial fibrosis {Laine, 1991 #1631} and, thus, this possibility in TTC deserves prospective consideration.

3.4.3 Implications for pathogenesis: demonstration of catecholamine effect

A growing body of associative data [although not so in all studies (Madhavan et al., 2009) links the injury of TTC with both endogenous (Wittstein et al., 2005, Akashi et al., 2003) and exogenous (Abraham et al., 2009) catecholamines. However, the precise mechanism by which myocardial damage occurs is not known. As discussed in Chapter 1 (Section 1.5), possibilities include both a direct interaction with cardiomyocyte function, energetics and integrity, as well as an indirect impact on the myocardium, secondary to coronary vasoconstriction and/or microvascular obstruction. Accurate determination of the magnitude of this putative catecholamine stimulus is inherently difficult, due to instability of concentrations: the study therefore utilized plasma normetanephrine, in view of its longer plasma elimination half-life (Goldstein et al., 2003, Lagerstedt et al., 2004),

compared with norepinephrine. As regards the widely assumed causal relationship with catecholamines, this work is, the first confirmation of a relationship between catecholamines and the measured severity of TTC in humans. Notwithstanding a possible underestimation of the effect (for example via sampling after peak catecholamine release), these data suggest that a "dose-response" relationship exists between greater norepinephrine exposure and greater acute myocardial injury (Hypothesis 2, see Figure 3.6A). At the very least, then, the finding underscores the potential significance of neurogenic catecholamine release in the development of TTC, without clarifying the mechanism further, nor excluding the possibility that patients with TTC may constitute a "hyper-responsive" population (Barletta et al., 2009a). Furthermore, the possibility that a component of catecholamine release in TTC may represent a consequence, rather than a cause of the disorder, cannot be excluded.

3.4.4 NT-proBNP and troponin elevation in relation to underlying oedema

In order to explore the implications of intramyocardial inflammation and associated oedema, global T_2-w SI was examined in relation to indicators of myocardial injury (Hypothesis 3). Marked elevation of both BNP (Wittstein et al., 2005, Madhavan et al., 2009, Morel et al., 2009, Lee et al., 2009a, Akashi et al., 2004a) and NT-proBNP (Nef et al., 2008c) have previously been observed in TTC series. Whilst the classical stimulus for the release of BNP/NT-proBNP is left ventricular wall stress [either systolic or diastolic (Liang and Gardner, 1999), as discussed in Chapter 1, Section 1.4.2.2], previous investigators, including our group, have suggested that this is not the case in TTC (Akashi et al., 2004a, Madhavan et al., 2009, Nguyen et al., 2011a). Remarkable elevation of BNP/NTproBNP is also known to occur in response to cardiac inflammation (Masters et al., 1999, Ogawa et al., 2008), although it appears that the resultant elevation is proportionally greater in the case of NT-proBNP (Jensen et al., 2010) versus BNP. Thus, the demonstrated relationship between oedema and peak NT-proBNP concentration during the acute phase of TTC is consistent with the hypothesis that the primary stimulus for NT-proBNP elevation may also be that which promotes oedema, i.e. inflammation (see Figure 3.6B).

There was a direct relationship between extent of elevation of troponin T levels and SI (Figure 3.6C). The association between inflammation and cellular injury/death tends to suggest equilibrium between these related processes in TTC and is consistent with a spectrum of responses to a common injurious stimulus.

3.4.5 Markers of systemic inflammation in relation to cardiac oedema

A moderate relationship between extent of CRP elevation and intramyocardial oedema was observed. However, caution is again necessary with interpretation: whilst the majority of patients with TTC exhibited only minor systemic inflammatory activation, whereas some patients within the sample had a primarily non-cardiac cause of CRP elevation, such as infection. Nevertheless, it can be concluded that extent of CRP elevation is a direct reflection of TTC severity. On the other hand, the extent of oedema did not correlate with platelet responsiveness to nitric oxide, a measure of tissue nitric oxide signaling. As this parameter is modulated largely by oxidative stress (Anderson et al., 2004b), these observations raise the possibility that systemic oxidative stress is not a substantial component of TTC. However, oxidative stress was not specifically measured in the current study.

3.4.6 Limitations

As CMR was performed only once during the acute presentation period, it is possible that, in some cases, scanning might have preceded the development of maximal extent of intracardiac oedema. The utility of the spleen as a reference point is also subject to question, but if splenic inflammation were to occur in TTC, this would tend to decrease, rather than increase, the observed myocardial anomalies. In the current series, there was also a significant anterior-to-posterior T_2-w SI difference in acute TTC. It is possible, in the light of previous reports (Keegan et al., 2006, Kellman et al., 2007), that this was partially modulated by coil sensitivity. On the other hand, the anterior wall of the human left

ventricle is more densely innervated by sympathetic neurons, compared with the inferoposterior wall (Janes et al., 1986b, Matsuo et al., 2009a). Therefore we cannot exclude the possibility that the difference in T_2-w SI observed in acute TTC (but not controls) reflects the local impact on oedema generation of differential neurogenic catecholamine exposure, within the left ventricle. In either case, no marked effect on the conclusions of the current study is likely. It may be pointed out that the use of a T_2-mapping sequence (Giri et al., 2009) would have allowed derivation of T_2 times and, thus, directly quantitative appreciation of oedema: this technique was not available at the time of this study. Nevertheless, this method may also be inherently unsuitable for current purposes, given that it yields consistently higher T_2 times in periapical myocardium in normal subjects, when applied to short axis studies. In calculating global T_2-w SI by subtraction of patient versus control T_2-w SI, we acknowledge that factors other than myocardial oedema may have differed between the two groups. In the entire methodology, blinding was not possible, due to the fact that an experienced reader could readily distinguish TTC from normal images, by eye. Despite these factors, it is likely that these effects are small in comparison with the effect due to increased free water content.

3.5 Conclusions

In conclusion, the current study supports the understanding of TTC as a state of intramyocardial oedema secondary to a global left ventricular inflammatory response, early after the index event and persisting well beyond the resolution of segmental left ventricular contractile dysfunction. Furthermore, heterogeneity in the severity of oedema is correlated with catecholamine release. These findings are of significance in our evolving understanding of TTC and present a partial basis for development of therapeutic intervention strategies for the disorder.

3.6 Tables

Table 3.1 Patient and control characteristics

	Patients (n = 49)	Controls (n = 10)
Demographics		
Age (mean±SD) years	67 ± 11	60 ± 8
Gender: female/male	48/1	10/0
BMI (mean±SD) (kg/m^2)	26.2 ± 6.0	26.4 ± 3.7
Patient condition [no. (%)]		
Hypertension	25 (51%)	4 (40%)
Diabetes mellitus	8 (6%)	2 (20%)
Hypercholesterolaemia	22 (45%)	3 (30%)
Current cigarette smoking	3 (6%)	0 (0%)
Coronary artery disease		
Single vessel	6 (12%)	0 (0%)
Double vessel	6 (12%)	0 (0%)
Triple vessel	1 (2%)	0 (0%)
Blood chemistry [median (interquartile range)]		
Normetanephrine (pg/mL)	1105 (867 – 2310)	N/A
Metanephrine (pg/mL)	252 (200 – 350)	N/A
NT-proBNP (pg/mL)	6260 (2590 – 9750)	N/A
Cardiac troponin T (ng/L)	347 (219 – 576)	N/A
Creatine kinase (IU/L)	183 (118 – 273)	N/A
C-reactive protein (mg/dL)	16.5 (6.85 – 83.5)	N/A

Abbreviations: BMI, body mass index; NT-proBNP, amino-terminal of prohormone of B-type natriuretic peptide

Table 3.2 CMR findings: LV volumes and functional indices

			Serial data (N = 30)	
	Acute	Controls	Acute	3-months
LVEDVI (mean±SD)	67 ± 15	60 ± 10	68 ± 15	60 ± 14**
LVESVI (mean±SD)	32 ± 14	25 ± 7	32 ± 14	20 ± 8**
WMSI (mean±SD)	1.73 ± 0.3	1**	1.70 ± 0.3	1.05 ± 0.1**
LVEF (%) (mean±SD) (normal range ≥ 57%)	50 ± 15	60 ± 6	50 ± 16	66 ± 8**
LV mass (g) (mean±SD)	101 ± 29	76 ± 15*	96 ± 29	78 ± 21*

*: $p < 0.05$ versus acute patient data;

**: $p < 0.005$ versus acute patient data

3.7 Figures

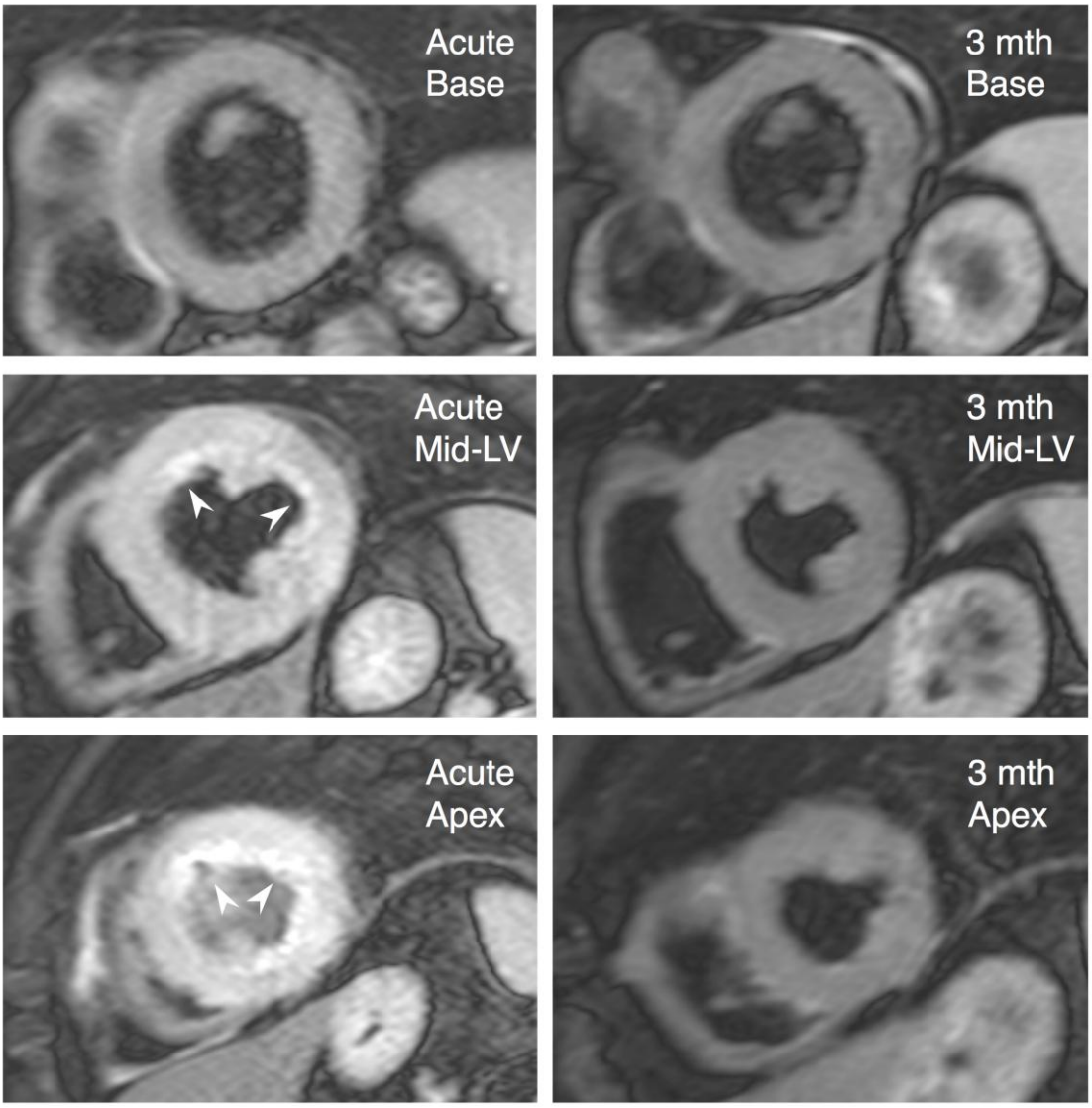

Figure 3.1 Example T_2-weighted short axis images from the same patient, at three levels of the LV. The acute phase is represented in the left hand column, whereas 3 month follow-up imaging is shown in the right hand column. Low-flow artifact, present acutely at the blood-subendocardial interface, at the akinetic/hypokinetic apical and mid-LV levels, is indicated (arrows). Hence, for the purposes of the analysis, artifactually enhanced signal was avoided by excluding them from the region of interest (see Methodology).

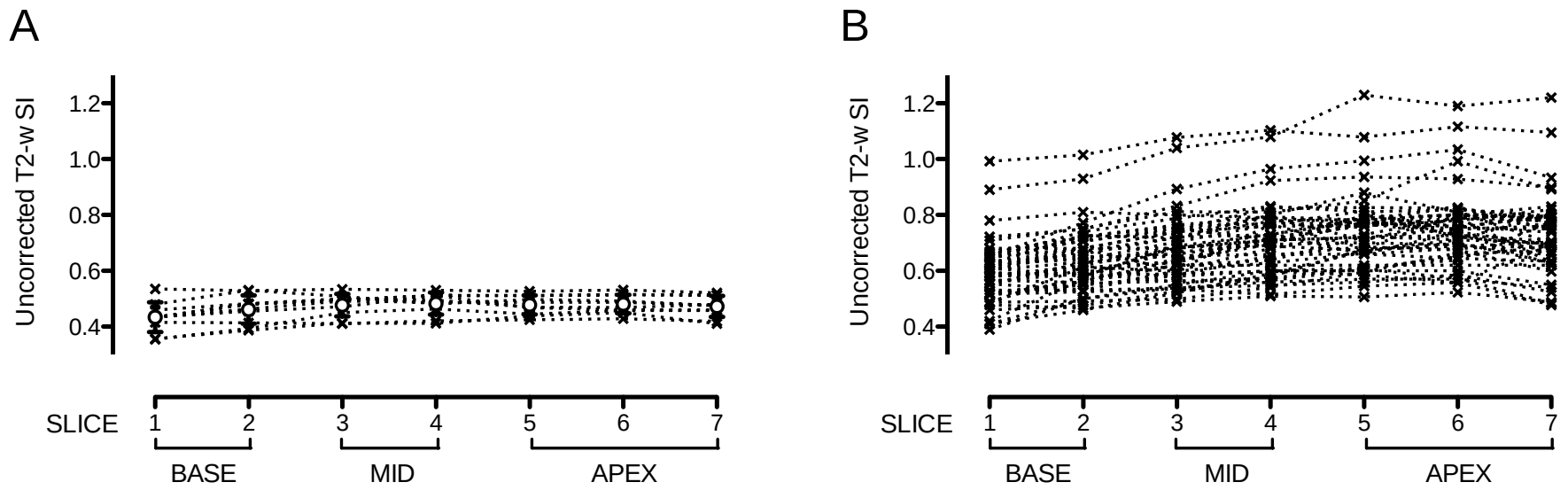

Figure 3.2 Whole slice T_2-w SI data from normal controls (1A) and acute TTC patients (1B). Slices 1 through 7 extend from base to apex, here and throughout the thesis. Mean (open circles) and SD are indicated only for the normal subjects, as the TTC data were skewed (see text).

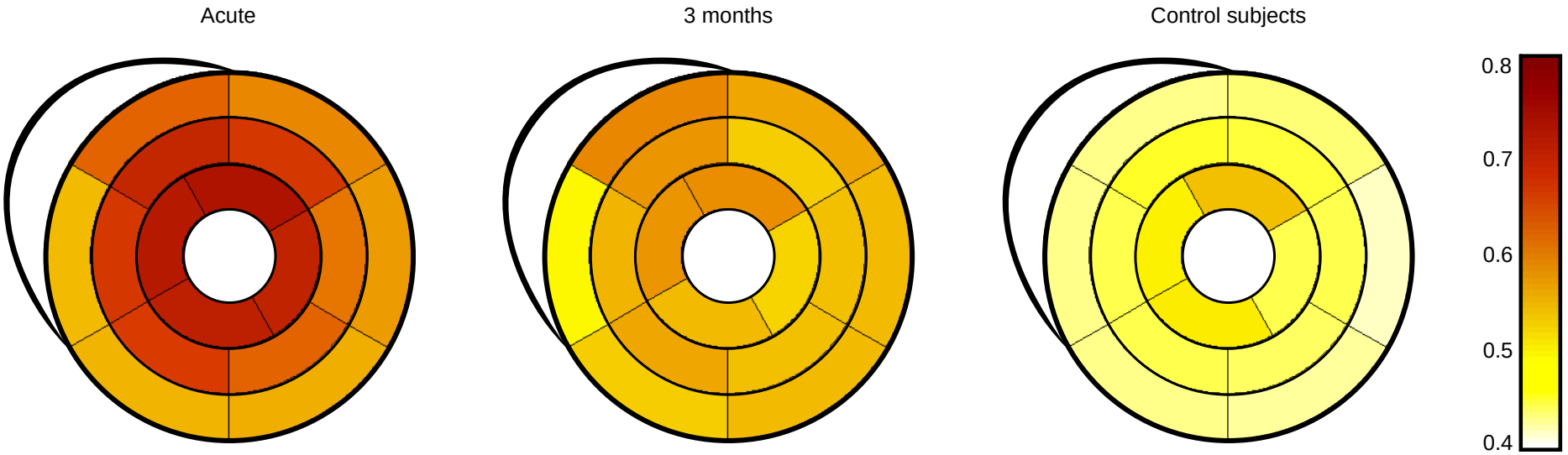

Figure 3.3 Colour-coded bull's-eye plots (as labeled) compare uncorrected T_2-w SI (median) in acute and recovery phases of TTC, to that of controls. T_2-w SI data was not collected from the extreme apex (see methods).

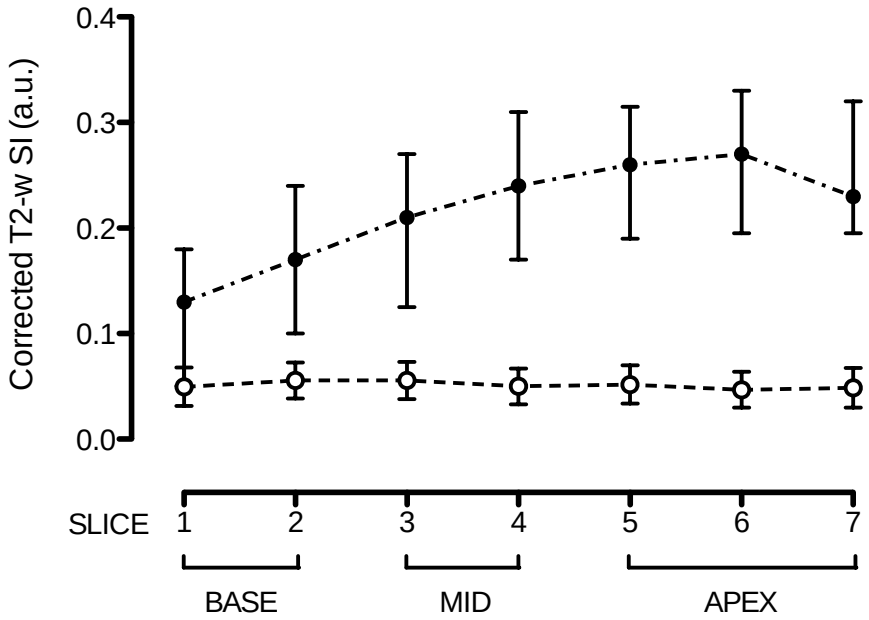

Figure 3.4 Acute (closed symbols) and 3 month (open symbols) values for corrected T_2-w SI are displayed according to region. Acute data were non-Gaussian and are therefore plotted as median and interquartile range, whereas data in recovery were normally distributed and are plotted as mean and standard error. T_2-w SI was markedly different between time points ($p < 0.0001$, 2-way ANOVA).

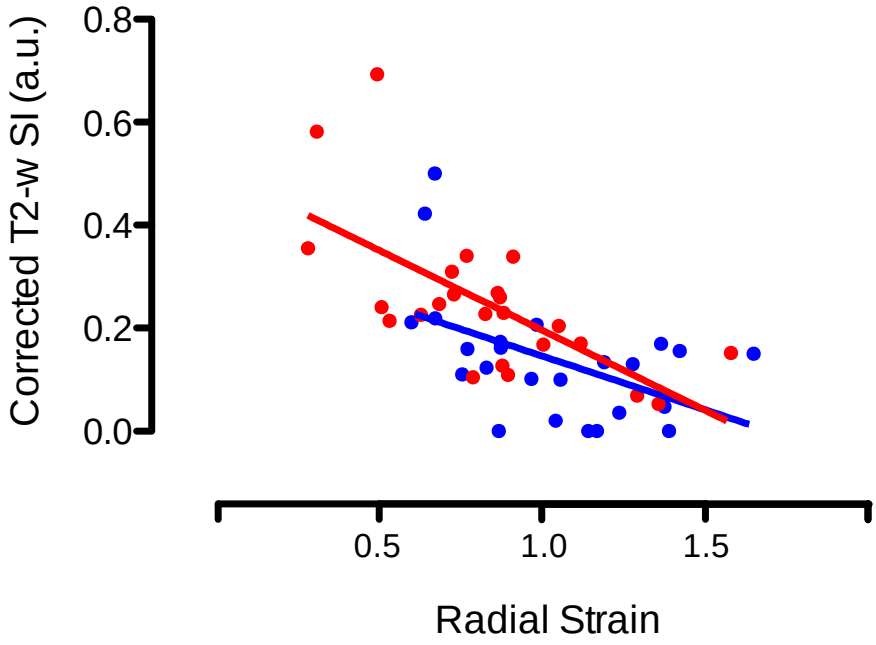

Figure 3.5 Peak radial strain versus corrected T_2-w SI correlations, shown for both mid-ventricular ($r = -0.49$, $p < 0.001$; in blue) and apical ($r = -0.39$, $p = 0.04$; in red) levels. N=

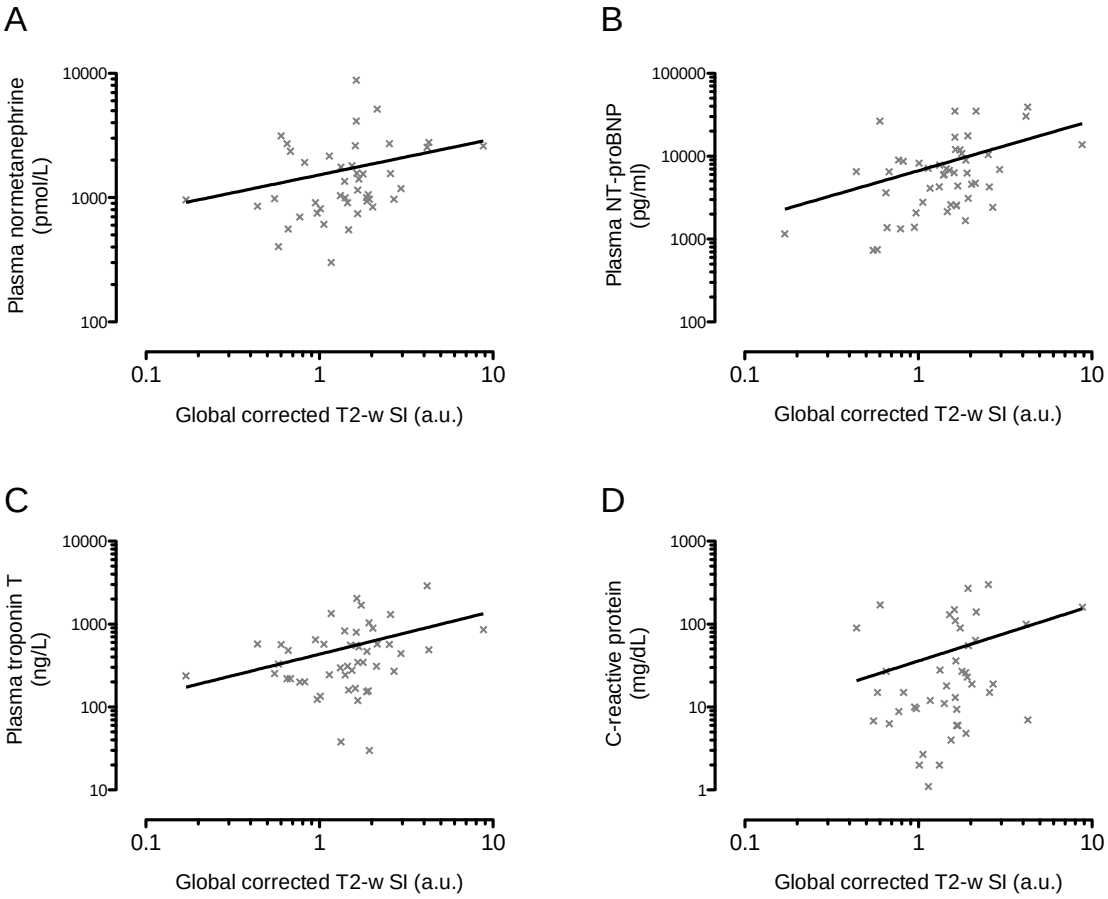

Figure 3.6 Acute phase correlations between global T_2-w SI and (A) plasma concentrations of both normetanephrine (r = 0.33, P = 0.028), (B) NT-proBNP (r = 0.40, P = 0.003), (C) troponin T (r = 0.29, P = 0.045) and (D) C-reactive protein (r = 0.34, P = 0.023.

Chapter 4

Recovery from TTC: short and long-term aspects.

4.1 Introduction

Tako-Tsubo cardiomyopathy, although it mimics myocardial infarction in its acute phase, is associated with a low but appreciable incidence of mortality and complications. Despite the presence of intense myocardial oedema (Eitel et al., 2011b, Nef et al., 2007b, Neil et al., 2012a), most, but not all (Naruse et al., 2011), studies report a low incidence of myocardial necrosis. This, together with the normalization of overt wall motion abnormalities, has tended to reinforce an optimistic view of TTC, in which the potential for "permanent" cardiac damage – even if subtle – has rarely been considered.

As has been stated in Chapter 1, a 'benign' view of TTC tends to prevail. The characterization of TTC as 'neurogenic stunning' (Wittstein et al., 2005), may serve to promote the assumption that no lasting cardiovascular damage is sustained: this assumption has received no critical evaluation. The kinetics of recovery of TTC have been addressed only partially and have not to date been the specific focus of an examination. While most studies report "full" recovery at follow-up (Gianni et al., 2006b, Sharkey et al., 2010a), relatively insensitive measures, such as LV ejection fraction (LVEF), have largely been relied upon.

Furthermore, certain published findings run contrary to this expectation. In view of the presence of significant acute-phase intramyocardial inflammation, which has now been documented by both histology (Nef et al., 2007b) and imaging modalities (Eitel et al., 2011b, Neil et al., 2012a), it would be surprising if *all* residual effects were to be averted. Several lines of evidence suggest that microscopic myocardial fibrosis might be a long-term consequence of TTC. Both acute inflammation and the documented presence of oxidative stress within the myocardium in TTC (Nef et al., 2008b) may stimulate the development of fibrosis. The local expression of angiotensin II and osteopontin, which have also been demonstrated acutely (Nef et al., 2008b, Izumi et al., 2009b), are likewise potential mediators of myofibroblast activation and fibrosis. Given that at ~3 months, mild cellular infiltrate (Nef et al., 2007b) and residual oedema (Neil et al., 2012a) can be demonstrated, such a pro-fibrotic *milieu* may persist beyond the acute phase; associated subtle cardiac dysfunction may also explain our previous observation of abnormal elevation of natriuretic peptide concentrations, 3 months from the index event (Nguyen et al., 2011a).

On the other hand, neither an excess of long-term arrhythmias, nor incident heart failure – events that might plausibly result from myocardial fibrosis – have yet been associated with TTC. In particular, it may equally be that the acute activation of cardioprotective signaling, such as components of the RISK pathway [also observed in TTC (Nef et al., 2009a)] mitigates deleterious effects on cardiac function and fibrosis, aiding recovery and preventing long-term structural and functional impairment.

4.1.1 Evaluation of subtle LV dysfunction and fibrosis: imaging tools

(a) Echocardiography

Two-dimensional speckle-tracking (2DS) echocardiography facilitates a detailed and multidimensional assessment of LV function. Radial deformation, or strain, and the rate of deformation, strain rate (SR), reflect transmural function, whereas circumferential strain/SR reflects the action of circumferentially oriented mid-wall fibers. Longitudinal strain/SR are particularly dependent on subendocardial myofibers function, have excellent reproducibility characteristics and proven utility in detecting subclinical abnormalities in other conditions, such as diabetes (Ng et al., 2011). Of additional relevance to this predominantly apical disorder, the complex rotational movements of the LV apex can be quantitated via 2DS (Helle-Valle et al., 2005).

Preliminary data from our laboratory, collected from normal individuals, considering global longitudinal strain, longitudinal SR and apical twist, suggested that global longitudinal strain exhibited the greatest consistency in such individuals. Pertinent reproducibility findings are presented in Section 4.2.6, Figure 4.1. Therefore, it was proposed that global longitudinal strain would represent an appropriate primary basis for comparison between normal and TTC subjects, given the issue of detection of relatively small differences between groups.

(b) CMR quantitation of myocardial fibrosis

All contrast-enhanced CMR techniques for the imaging of myocardial fibrosis rely on the extracellular volume of distribution of chelated gadolinium-based compounds. Focal scar and necrosis can be readily appreciated with the widely available late gadolinium enhancement (LGE) method, post-contrast myocardial T_1-mapping is a more recently developed approach, which offers a validated approach to the assessment of diffuse myocardial fibrosis (Messroghli et al., 2004, Iles et al., 2008, Flett et al., 2010) (Messroghli et al., 2011a). Given that diffuse fibrosis expands extracellular volume (V_e), permitting a greater concentration of intramyocardial gadolinium, quantitative assessment of post-inversion-recovery longitudinal relaxation (which occurs more rapidly in the presence of Gadolinium), via T_1-mapping, can provide an estimate of diffuse myocardial fibrosis.

4.1.2 Aims and hypotheses

The overall aim of this study was to comprehensively evaluate cardiac structure and function in patients after TTC. To accomplish this, two separate case-control studies were performed: the first, an evaluation of myocardial function by 2D speckle tracking analysis, acutely and at two subsequent time points (Study 1) and, the second, a CE-CMR/T_1-mapping study in patients ≥12 months from an episode of TTC (Study 2).

For Study 1, the primary null hypothesis to be tested was:

- 3-month values of global longitudinal strain would not differ from those of age-matched normal controls (Hypothesis 1a). Longitudinal SR and peak apical twist were nominated as variables of secondary interest for this comparison.

Further hypotheses for study 1, addressing the determinants of potential deficit at 3 months, were prespecified as follows:

- Three month global longitudinal strain would be independent of measures of acute-phase severity of the TTC episode: catecholamine exposure, release of troponin T/NT-proBNP, degree of LV wall motion impairment or the extent of myocardial oedema in patients eligible for T_2-weighted CMR (Hypothesis 1b).

- Any residual impairment of global longitudinal strain residual at 3 months would correlate with residual elevation of plasma NT-proBNP, high sensitivity C-reactive protein (hs-CRP) and plasma markers of collagen turnover (Hypothesis 1c).

For study 2, the primary (null) hypothesis was that:

- Extent of myocardial fibrosis (V_e) as measured by CE-CMR would not vary between groups

The secondary hypothesis was that:

- Extent of fibrosis would be inversely correlated with LV global longitudinal strain (Hypothesis 2b).

4.2 Methodology

4.2.1 Patient/subject selection

Female TTC patients, in both study 1 and study 2, were eligible for inclusion on the basis of the acute diagnosis, according to the Mayo Clinic criteria (Madhavan and Prasad, 2010a), accounting for the following: (i) a presentation with abnormalities of ST segments/T waves, with or without chest pain or dyspnoea, (ii) a sustained elevation of cardiac troponin levels (iii) demonstration of a characteristic wall motion abnormality, not confined to a single epicardial coronary territory and (iv) the exclusion of obstructive coronary disease, via selective coronary angiography, in the territory subserving the regional wall motion abnormality. Given that the hypotheses under evaluation related to potential residual LV dysfunction, exclusion criteria included known pre-existing LV or valvular disease, phaeochromocytoma and myocarditis, each of which was prospectively considered.

(a) TTC patients

Patients for Study 1 were enrolled in the acute phase at two participating hospitals. Over a period from January 2009 to November 2011, 55 female patients fulfilled the Mayo

criteria, as above. Management and investigations during the acute presentation were identical to those described in Chapter 3. Two patients died prior to final follow-up. One patient was excluded on the basis of phaeochromocytoma and another on the basis of a mechanical mitral prosthesis. Of the remainder, 19 were excluded because of failure to attend follow-up evaluation (3-month) because of medical incapacitation (4 patients), rural location (3 patients) or refusal (3 patients); others were excluded on the basis uninterpretable image quality (3 patients) or data loss (2 patients). Thus, a total of 36 patients were included for study.

For Study 2, sixteen female subjects, with an acute diagnosis fulfilling the Mayo criteria, were enrolled from a registry of TTC patients, ≥12 months since the last documented episode. In addition to the above exclusion criteria, patients were excluded in the presence of a contraindication to MR (e.g., claustrophobia), and/or glomerular filtration rate of ≤60 mL/min/1.73m^2 (estimated via the Cockcroft-Gault method). One patient was excluded from analysis on the basis of frequent ventricular extrasystoles, which interfered with image quality and therefore quantitative T_1 mapping.

(b) Control subjects

17 female age-matched controls were recruited by advertisement and were eligible in the absence of known cardiac or renal disease, together with the absence of claustrophobia or renal disease. All underwent echocardiography and 10 also underwent CE-CMR. The institutional human research ethics committee approved the study and participants in each group provided written informed consent.

4.2.2 Echocardiographic methodology (Study 1 and 2)

4.2.2.1 Serial protocol and analysis

All echocardiography (studies 1 and 2) was performed according to a predetermined protocol, utilizing a Vivid 7 echocardiography machine (GE-Vingmed, Horton, Norway) with

an M5 ultrasound transducer. In the acute context (Study 1), echocardiography was performed (i) as soon as practicable, (ii) at 10-14 days and (iii) 3 months thereafter. Thus, the median time of acquisition of studies, at each of the three time points, were 1, 11 and 97 days from the day of presentation.

Standardised echocardiographic data included pulse wave Doppler, tissue Doppler and two-dimensional grey scale imaging in all conventional views, as recommended by the American Society of Echocardiography (Picard et al., 2011). Doppler and tissue Doppler parameters included the peak early (E) and late (A) diastolic transmitral flow velocities, the deceleration time of E (DcT) and the average of septal and lateral mitral annular velocities in early diastole (Ea). The ratios of E/A and E/Ea were also derived.

To facilitate multidirectional 2DS analysis, short axis views (at three levels of the LV) and long axis views, were obtained from parasternal and apical positions, respectively. Short-axis images conformed to the following definitions: the cardiac base was defined by the presence of mitral leaflet tips; the mid-ventricular, or equatorial, section, required the presence of papillary muscles in cross section; the apex was taken as the cross-section immediately above that at which systolic cavity obliteration was seen (Helle-Valle et al., 2005). The protocol allowed breath holding at end-expiration and placement of the transducer 1-2 intercostal spaces caudally, as previously described (van Dalen et al., 2008), in order to optimize the apical view. Loops of three consecutive cardiac cycles, with a minimum frame rate of 50 s^{-1} were stored offline, for subsequent analysis with a dedicated software package (EchoPAC version BT11, GE-Vingmed, Horton, Norway).

4.2.2.2 Multidirectional deformation and rotational parameters

For all analyses, the endocardium was manually traced at end-systole to generate a segmented region of interest, the width of which was adjusted to include the thickness of the LV myocardium. Segmental motion was then automatically tracked throughout a cardiac cycle. The software indicated whether segments were tracked adequately or not, while allowing the user the final decision to accept or reject a segment, based on visual assessment of tracking quality.

Strain was defined as the change in the length of the segment studied, as a percentage of its final (end-diastolic) length, with respect to the three orthogonal directions of tissue deformation (longitudinal, radial and circumferential, in relation to the cardiac axis); SR was defined as the rate at which this deformation occurs. Peak values were derived from strain-time and SR-time curves, respectively. Mean global longitudinal strain/SR were derived from the three individual apical views and thus represent triplanar averages. Radial and circumferential function was studied as strain and SR (averaged from all segments) at the base, mid-ventricle and apex. Twist mechanics were studied at the LV base and apex and the average peak rotation and rotation rate were determined from automatically generated curves. Counter-clockwise rotation, as observed at the apex, was expressed as a positive value, whilst basal clockwise rotation was expressed as negative (Helle-Valle et al., 2005). LV twist was defined as the net difference between peak apical and peak basal rotation, whereas LV torsion was defined as LV twist, normalized for LV diastolic length, as elsewhere described (Helle-Valle et al., 2005).

Coefficients of variation for repeat measures of the parameters global longitudinal strain, longitudinal strain rate and apical twist were 7.0%, 11.1% and 17.1%, respectively. Bland-Altman analyses are depicted, with 95% limits of agreement, in Figure 4.2 (Panels A, B, C): no inter-observer bias was noted.

4.2.2.3 Non-2DS echocardiographic parameters

LV end-diastolic and end-systolic volumes were determined by a biplane method, from manual planimetry of the endocardial LV border, in apical long axis 2-chamber and 4-chamber views (Stamm et al., 1982). LV ejection fraction was determined automatically from these volumes. The end-systolic left atrial volume was similarly determined (Kircher et al., 1991) and LV mass (g) was derived by a previously described area-length method (Park et al., 1996). All cardiac volumes and mass were indexed to body surface area. The wall motion score index (WMSI) was determined according to a 17-segment model, only in the acute phase. WMSI took into account all views of the LV, applying a 4-point scale to describe segmental contraction (1 = normal or hyperkinetic, 2 = hypokinetic, 3 = akinetic

and 4 = dyskinetic) and was thus the aggregate of segments scored, divided by the number of segments scored (Cerqueira et al., 2002).

4.2.3 Additional biological investigations (Study 1)

All other acute-phase investigations pertinent to testing Hypothesis 1b, were obtained as previously described (see Section 2.2.4). These included plasma metanephrines, troponin T, NT-proBNP and T_2w-SI (see also Section 4.2.4.1, below).

Specifically in regards to Hypothesis 1c, additional peripheral venous blood samples for plasma NT-proBNP, hs-CRP and collagen biomarkers were drawn into EDTA tubes before being centrifuged (10 minutes at 4°C) and then separated into aliquots for storage at 80°C. Accordingly, analysis of samples was later performed with commercially available reagents and a radioimmunoassay analyzer (ElecSys, Roche Diagnostics, Mannheim, Germany). With regard to the selection of plasma collagen markers, amino-terminal propeptides of procollagen type I (PINP), which circulate in the blood after having been cleaved in the extracellular processing of type I procollagen was taken as a measure of collagen synthesis; this marker was selected the light of previous work demonstrating type I collagen fibrils in the myocardium, early after TTC (Nef et al., 2007b); a plasma marker type-III collagen synthesis was not utilized for this study. Plasma carboxy terminal telopeptide of collagen type I (CITP), which has been shown to be elevated in hypertensive heart disease (Collier et al., 2011), was measured as a reflection of collagen degradation. Finally, the ratio of PINP/CITP was also considered as an index of nett collagen turnover.

4.2.4 CMR methodology

4.2.4.1 Scanning protocol

In the acute phase (Study 1), CMR was obtained according to the protocol in detailed in Chapter 3 (Section 3.2.6), utilizing either an Achieva or Intera 1.5 Tesla machine (Philips, Best, Netherlands), according to the site of enrollment. An assessment of acute

intramyocardial oedema, relevant for the testing of Hypothesis 1b, was available in 24 of 36 patients: the parameter utilized for this comparison was global corrected T_2-w SI. Reasons for failure to undergo acute CMR included claustrophobia (n = 5), the presence of an implanted device (n = 3); CMR data from four patients were excluded from the analysis, 2 because images were not obtained within the 7 day timeframe and a further 2 because the T_2-weighted black blood sequence utilized was different from that described and validated in Chapter 2.

All CMR studies for Study 2 were performed with subjects in the supine position using a 1.5 Tesla MRI scanner (Magnetom Avanto, Siemens, Germany) and a phased array surface coil. After determination of cardiac axis, Cine-CMR images (25 phases) were obtained during end-expiratory breath-hold with retrospectively ECG-gated True-FISP (Fast Imaging with Steady-State Precession) sequences with the following typical scan parameters: image matrix 256 × 150, field of view 380 mm, repetition time 52.05 ms, echo time 1.74 ms and flip angle 70°. Sequential short axis slices of the LV, covering base to apex, were obtained with reference to long-axis views, utilizing a section thickness of 6 mm with intersection gaps of 4 mm (Lorenz et al., 1999).

Pre- and post-contrast T_1 mapping was performed utilizing a modified Lock Locker (MOLLI) technique, based on the original description by Messroghli and colleagues (Messroghli et al., 2004). Briefly, three inversion-recovery pulses are applied over a single breath-hold, to yield total of 11 readouts with different inversion times, i.e. one image for each of 11 consecutively longer inversion times. Acquisitions were obtained at end-expiration for each of the three standard short axis slices (basal, mid-ventricular, and apical), with a 10 mm section thickness and the following parameters: Imaging parameters were repetition time 4.0 ms, echo time 1.8 ms, flip angle 45°, acquisition matrix 256 x 160, and 38 cm field of view. The standardised contrast protocol employed dimeglumine gadopentetate (Magnevist; Schering, Germany), administered as a weight-adjusted intravenous bolus (0.2 mmol/kg; injection rate, 3.5 mL/s), followed by a 30 mL saline flush (Lyne et al., 2007). Post-contrast MOLLI were obtained exactly 15 minutes after contrast bolus. Delayed enhancement images were obtained in the same predetermined short axis slices, utilizing a phase-sensitive inversion-recovery segmented gradient echo T_1 weighted sequence (typical parameters: TR = 2 heart beats, TE = 3.32 ms, flip angle = 25°, FOV = 38 cm, matrix

size = 176 9 256), 12 minutes post-contrast. Per protocol, venous blood was drawn prior to CMR, for the determination haematocrit, to facilitate calculation of V_e.

4.2.4.2 Derivation of T_1 and V_e values

In an inversion-recovery experiment, longitudinal recovery of protons occurs in response to an inversion-recovery pulse, along the main magnetic field in the z-axis. T_1 describes the exponential rate of this recovery, according to the formula, $M_Z(t) = M_{Z(t=\infty)}[1 - e^{-t/T1}]$, where $M_Z(t)$ is the magnetization of the sample at time t and $M_{Z(t=\infty)}$ denotes the equilibrium magnetization in the z-axis. To quantify whole-slice average pre- and post-contrast myocardial T_1 times, regions of interest (ROI) were manually drawn offline, utilizing Osirix software (version 4.0, Pixmeo Sari) in each of the 11 images generated by the MOLLI sequence, for each short axis slice. To accurately determine the average myocardial signal intensity (SI) of each ROI, care was taken to include the whole slice and exclude both blood pool and extramural/epicardial fat. Phase-corrected data was fit by a least squares method to an exponential decay curve, with Prism software (version 5.0, GraphPad), utilizing the average signal intensity (in arbitrary units) for each inversion time (see Figure 4.2). Phase correction was achieved by inverting the SI values from initial phases up to the inversion time corresponding to the zero crossing, in order to optimize the goodness of fit.

Pre- and post-contrast T_1 times were thus derived for basal, mid-ventricular and apical levels and the average of each was denoted 'global T_1'. The parameter of primary interest, V_e, was then determined from global T_1 and the haematocrit, as described by Jerosch-Herold et al (Jerosch-Herold et al., 2008), according to the equation below:

$$V_e = [T_1 \times 1.05 \times (1\text{-haematocrit})] - 0.045$$

This method assumes equilibrium of contrast concentrations between blood and myocardium: a bolus-washout method was used in preference to the original bolus-infusion protocols (Jerosch-Herold et al., 2008, Flett et al., 2010), in view of the excellent concordance between these methods (Schelbert et al., 2011), noting that derivation of V_e via a bolus-washout method was recently validated against histologically quantified

fibrosis (Messroghli et al., 2011b). Given that it combines data from each slice, V_e is considered a global expression of LV extracellular volume fraction, and therefore intramyocardial fibrosis, throughout the paper.

Coefficient of variation for repeat measurements of the parameter V_e was 11%. Bland-Altman analysis, presented in Figure 4.2D, revealed no significant inter-observer bias.

4.2.4.3 Phantom studies

In order to test the accuracy of the MOLLI sequence, agarose gel phantoms were manufactured, with differing quantities of nickel chloride. By utilizing a mixture of two compounds, objects with specific T_1/T_2 properties can be designed. For manipulation of T_1, nickel chloride, copper sulfate and gadolinium chloride have similar relaxivities: nickel was selected due to temperature independence for T_1. Following a previously validated formula (Tofts et al., 1993), differing mixtures of nickel and agarose were employed in eight phantoms, the properties of which are detailed in Table 4.1. Phantoms were made in duplicate and set in 40mL containers.

Phantoms were then scanned with MOLLI sequence, as described above (see section 1.2.5.2). The accuracy of MOLLI-derived T_1 was then assessed by plotting the *estimate* for each phantom against the corresponding *predicted* T_1 times for each (see Figure 4.1). As can be seen in Figure 4.1, the estimated T_1, derived via MOLLI/ROI-based curve fitting technique described in the previous sections, showed poor agreement with predicted T_1 times, tending to underestimate higher T_1 times.

Although accuracy was arguably less of a concern than precision (reproducibility), and may not mask between-group differences, we nevertheless opted to correct MOLLI-derived T_1 times, via the formula shown in Figure 4.1. The latter was done to allow comparison of our results with those of other studies utilizing the parameter V_e, in other conditions.

4.2.4.4 Other CMR data analysis

LV end-diastolic and end-systolic volumes, LVEF and mass were derived from cine images, using open source software (Segment v1.9 R1852, http://segment.heiberg.se), as previously described (Heiberg et al., 2010). All volumes and mass values were indexed to body surface area. The presence or absence of focal LGE was assessed visually.

4.2.5 Statistics

Reproducibility analyses utilized coefficient of variation and Bland-Altman analysis, as above (Section 4.2.2.2 and 4.2.4.2). Patient and control data are expressed as mean and standard deviation. Recovery of functional indices was assessed by one-way ANOVA with repeat measures. For testing of the main hypotheses, between-group differences were assessed with Student's *t* test and correlations, with Pearson's test. Curve-fitting procedures, specific to Study 2, are described above (Section 4.2.4.2). All analyses were undertaken in Prism software (version 5.0, GraphPad) and statistical significance was inferred with a P value < 0.05.

4.3 Results

4.3.1 Patient and control characteristics

Patient and control characteristics, for the two study components, are summarized in Table 4.2. For Study 1, the TTC sample comprised 24 with typical apical involvement and 12 with the mid-ventricular variant; two of these patients required acute circulatory support. Of note, groups were not matched for hypertension in Study 1 (see Table 4.2), in contrast to the samples in Study 2.

4.3.2 Results of Study 1

4.3.2.1 Serial echocardiographic indices and time-course of recovery

Serial echo parameters for TTC patients are presented alongside those of normal controls, in tabular form: for LV and left atrial volumes and global function, see Table 4.2; for longitudinal and rotational parameters, see Table 4.3; for parameters of radial and circumferential deformation, see Table 4.4.

Recovery data for the three 2DS parameters of interest (Hypothesis 1a), global longitudinal strain, longitudinal strain rate and apical twist, are displayed in Figure 4.3. Each of these parameters showed improvement over the three-month period ($P < 0.001$, one-way ANOVA, repeat measures).

4.3.2.2 3-month values of nominated 2DS indices, versus controls (Hypothesis 1a)

Between-group comparison of selected 2DS parameters, in 3-month post-TTC versus normal controls, is presented in Figure 4.2. Global longitudinal strain was significantly reduced in post-TTC patients, versus normal controls ($P = 0.0057$) (Hypothesis 1a, see Figure 4.2a). Similar comparisons with longitudinal strain rate and apical twist are presented in Figures 4.2b and 4.2c: while similar trends were present, differences between TTC and control group did not reach statistical significance.

In regards to the above univariate comparison of global longitudinal strain, the potential impact of the differential prevalence of hypertension in the TTC group was addressed in a *post hoc* multivariate analysis. To control for an effect of long-term hypertension, distinct from the TTC process, the relationship between LV mass (as a reflection of long term hypertrophic response to hypertension) and global longitudinal strain, in both groups, was examined using the analysis of covariates method. LV mass was distributed in a non-Gaussian fashion and, hence, log-converted for this purpose. There was a relationship between LV mass and global longitudinal strain ($F = 4.28$, $p = 0.04$; see also Figure 4.5), such that for every 10g increment of LV mass, a 0.23% decrease was observed. The two relationships were separated ($F = 9.8$, $p = 0.0029$), with a ~2.5% difference in global

longitudinal strain by linear regression, across the range of LV mass, i.e. there was no interaction (F = 0.13, P = 0.72).

4.3.2.3 Acute-phase determinants of residual deficit in global longitudinal strain (Hypothesis 1b)

Global longitudinal strain at 3-months was not correlated with acute severity of the TTC episode, as indexed by any of the following: (i) plasma normetanephrine levels (r = 0.020, P = 0.92), (ii) troponin T (r = 0.068, P = 0.71), (iii) NT-proBNP levels (r = 0.15, P = 0.40), (iv) echo-derived WMSI (r = 0.17, P = 0.33) or myocardial oedema (r = 0.23, P = 0.33). The latter comparison was assessed in 24 patients, utilizing the parameter global corrected T_2-w SI, as discussed in Chapter 3 (Section 3.2.7).

4.3.2.4 Determinants of residual deficit in global longitudinal strain (Hypothesis 1c)

In the light of our previous observation of elevated mean plasma NT-proBNP at 3-months (compared to age-matched females) (Nguyen et al., 2011a), we evaluated the potential relationship between this and residual functional impairment. As can be seen in Figure 4.6, a modest but significant correlation between simultaneous 3-month plasma NT-proBNP and global longitudinal strain was observed.

In contrast, 3-month global longitudinal strain did not correlate significantly with simultaneous hs-CRP (r = 0.02, P = 0.88) or collagen turnover, as indexed by plasma PINP (r = -0.02, P = 0.89), CITP (r = 0.09, P = 0.62) and PINP/CITP (r = -0.19, P = 0.32).

4.3.3 Results of Study 2

Parameters of global LV function are presented in Table 4.6. Of note, no *focal* LGE was identified in any of the patients or controls.

4.3.3.1 Evidence of myocardial fibrosis at ≥ 1 year (Hypothesis 2a)

Figure 4.7 compares TTC patients (≥1 year from the last TTC episode) with age-matched female controls, in terms of V_e, as an index of extent of myocardial fibrosis (see Methodology, section 4.2.4.2). Mean values of V_e in TTC patients and normals were 0.24 (± 0.025) and 0.21 (± 0.032), respectively (P = 0.0013), thus implying greater long-term *diffuse* interstitial fibrosis in the myocardium of TTC patients.

4.3.4 Correlates of extent of myocardial fibrosis at ≥ 1 year (Hypothesis 2b)

At one year, no relationship between intramyocardial fibrosis, as reflected by V_e, and simultaneous global longitudinal strain, was observed (r = 0.17, P =0.62).

4.4 Discussion

The two components of this study provide complimentary information with regards to cardiac structure and function, after TTC. In sum, our data indicate for the first time that TTC may result in long-term detrimental effects in the left ventricle, without clarifying the nature of the impact on individual patients, in terms of symptoms or prognosis. Study 1 demonstrated incomplete recovery of global longitudinal strain at three months, thereby disproving the primary null hypothesis and challenging conventional thinking with regard to TTC recovery. Study 2 demonstrated greater cardiac fibrosis (as inferred by increased V_e) in TTC patients, as a late complication of TTC, again contrary to the null hypothesis. The latter finding was supported by those of an individual with a previous history of recurrent TTC, studied post-mortem, with evidence of substantial interstitial fibrosis. Although fibrosis was postulated to be a correlate of LV functional impairment, as has been demonstrated in other conditions (Jellis et al., 2011, Ng et al., 2012), the current data do not support such an association in the context of late (≥ 1 year) after TTC. Thus, these findings form an incomplete and essentially subclinical picture of the post-TTC state, for which the relevant clinical correlates and pathogenetic bases remain to be elucidated.

4.4.1 Non-deformational parameters in acute and recovery phases (Study 1)

Consistent with the CMR finding in Chapter 3 (discussed in Section 3.4.1), LVEDVi was reduced at 3 months, implying some extent of dilatation in the acute phase (see Table 4.3). Interestingly, the same trend of recovery was noted in serial reduction of left atrial volumes: LAVi did not appear to fully normalize (see Table 4.3) in the TTC group. Although the age difference relative to controls may be a confounder, it is noted that chronic expansion of LA volume (in the *absence* of atrial fibrillation) is considered an indicator of long-term LV diastolic dysfunction (Paulus et al., 2007), and relates to prognosis, for instance in the post-MI context (Beinart et al., 2004). Although a detailed assessment of LV dysfunction was beyond the scope of this study, of the three parameters of resting diastolic function documented, E/A was lower in post-TTC subjects versus controls (see Table 4.3). This may signify overall reduced efficiency of early diastolic relaxation after TTC. However, this was not borne out in E/Ea, suggesting LV filling pressures equivalent to those of the control group (Paulus et al 2007). Gross LV systolic function, as assessed by LVESVi and LVEF, normalized, as expected.

4.4.2 Deformational parameters in acute and recovery phases (Study 1)

Acute impairment, and subsequent recovery, was evident in most parameters of LV deformation (see Tables 4.3 an 4.4). Radial and circumferential contractile recovery was evident at the mid-LV and apical levels, although this tended to be evident with strain, rather than strain rate, parameters. Basal function was generally spared acutely, however, *circumferential* strain rate exhibited a significant trend to reduced contractile function with time, consistent with a hypercontractile state acutely. This contrasts somewhat with findings of Mansencal and colleagues, who described reduced *radial* contraction in basal segments, acutely, without quantifying *circumferential* wall motion (Mansencal et al., 2009). The findings are thus distinct, but it is unlikely that both can be true.

As this Chapter primarily concerns the completeness, or otherwise, of recovery, no attempt has been made to relate acute myocardial mechanics with other aspects of the acute TTC severity. Overall, however, the current findings pertaining to myocardial mechanics in acute TTC concur with studies, which have previously employed 2DS or tissue Doppler in TTC (Mansencal et al., 2009; Meimoun et al., 2011). In particular, in 3 patients, apical rotation was found to be occurring in the reverse direction (clockwise when viewed from the apex), an observation also made in 3 out of 17 patients studied by Meimoun and colleagues (Meimoun et al., 2011). The relatively reduced frequency of this interesting finding in the current study may be explained by the greater inclusion of mid-LV variant cases, with 'apical sparing', in contrast to the exclusion of these subjects in the aforementioned work. In both studies, the direction of apical twist recovered in the subgroup with acutely reversed apical rotation.

Overall, therefore, apical rotation is mostly reduced and occasionally reversed in acute TTC. Subepicardial fibres play a dominant role in cardiac rotation (due to a longer arm of movement over that of their subendocardial counterparts) (Helle-Valle et al., 2005), whereas longitudinal LV contraction is largely a function of subendocardial fibres (Henein et al., 1993). Hence, it is possible to say that acute cardiac dysfunction in TTC is transmural, at least at the apex, despite the frequent absence of ST-elevation in TTC.

4.4.3 Subtotal LV recovery in TTC and its correlates (Hypotheses 1a, 1b and 1c)

As can be seen in Figure 4.4a, the magnitude of each of the three parameters tended to be markedly heterogeneous at 3 months: only global longitudinal strain demonstrated a clear difference from control values (Hypothesis 1a). Global longitudinal strain was selected on primarily on the basis of consistency in normal subjects and reproducibility, however, this particular parameter has been associated with prognosis in other disease contexts (Antoni et al., 2010). In addition, two previous studies have demonstrated correlations between non-invasively estimated diffuse myocardial fibrosis and longitudinal dysfunction (Jellis et al., 2011, Ng et al., 2012).

Of note, correlations between global longitudinal strain and variables associated with acute severity of TTC (Hypothesis 1b), including catecholamine exposure, acute LV wall motion deficit and intramyocardial oedema, were universally negative. Thus, it appears that no acute variable tested, is capable of predicting the degree of residual LV deficit observed at 3 months.

Biological correlates of global longitudinal strain were also tested to gain insight into residual dysfunction at 3-months (Results Section 4.3.2.4). There was no correlation with hs-CRP, as a marker of systemic inflammation, or with collagen biomarkers; however, a modest relationship with NT-proBNP was observed (Hypothesis 1c; see Figure 4.5). This tends to support the biological plausibility of the finding of LV deficit at three months, without explaining the mechanistic nature of the association.

4.4.4 Myocardial fibrosis in TTC and its correlates (Study 2)

The evidence here presented regarding the occurrence of late diffuse myocardial fibrosis after TTC, comprises a CMR-based case-control study and will be supplemented by a single post-mortem pathological description.

Study 2 indicates that expansion of the extracellular volume (V_e) is in fact common after TTC (Hypothesis 2a); in the absence of focal replacement fibrosis (i.e., focal LGE), this suggests that reactive fibrosis has occurred. This represents an important finding in TTC and raises the possibility that TTC might leave a 'permanent scar', albeit a diffuse one, after all overt LV dysfunction has recovered.

Although V_e did not correlate with simultaneous global longitudinal strain measurement (Hypothesis 2b), this may be a result of an inadequate sample size. No mechanistic insights as to the origin of fibrosis in TTC are therefore available, although a post-inflammatory process remains possible: this should be the focus of future work. Furthermore, as stated, the functional impact of LV fibrosis on TTC patients remains to be determined.

4.4.5 Post-mortem findings in support of post-TTC myocardial fibrosis

The following brief case report is provided to corroborate the above results, especially with regard to Hypothesis 2a and the presence of intramyocardial fibrosis post-TTC.

A 59-year-old woman presented to a peripheral hospital, with acute shortness of breath. On initial assessment, she was found to be in rapid atrial fibrillation (rate 138 beats per minute), without documented evidence of cardiac failure. ECG showed a right bundle branch block pattern, similar to that of previous ECGs. Although not apyrexial, she had been diagnosed with a mild chest infection within the previous week and had received oral antibiotics; the presumptive diagnosis of infection was otherwise only supported by an elevated white cell count. Initial treatment consisted of intravenous amiodarone and antibiotics.

However, hypotension, attributed to sepsis, ensued over the next two hours; the potential of intravenous amiodarone to induce hypotension was not recognized and this treatment continued. Although hypotension appeared to be tolerated, multiple doses of metaraminol were administered, with good initial effect. Cardioversion of AF was considered but was not pursued due to lack of anticoagulation and risk of stroke. Profound hypotension ensued, together with ventricular tachycardia, requiring DC shock and doses of adrenaline and noradrenaline, followed by intubation/ventilation. Attempts at fluid resuscitation were limited and totaled < 1 litre. The patient was refractory to inotropic support and died at 0400 hours.

This patient had previously suffered two definite episodes of TTC. The first of these was 26 months prior and the second was 14 months prior to death. In keeping with the Mayo clinic criteria, angiography had demonstrated normal coronary arteries; her risk factor profile did not indicate high risk of atherosclerosis. Of note, the second TTC presentation was associated with hypotension for which dopamine had initially been prescribed, before TTC was recognized. Recovery had been documented on both occasions, on the second utilizing CMR, in which no focal LGE was noted.

Family members requested post-mortem examination, primarily to elucidate the unknown cause of death. A secondary aim was to provide an opportunity to study cardiac structure

in a patient with a remote history of TTC, as a means of gauging long term histological effects of the disorder. Extracardiac findings, briefly, included mild alveolar oedema in the lung bases, without evidence of acute pneumonia. No evidence of bacterial sepsis was gained, either from macroscopic or microscopic examination, or from post-mortem culture. The adrenal glands did not demonstrate pheochromocytoma.

Coronary arteries did not demonstrate thrombotic occlusion or obstructive atherosclerotic plaque. Myocardium was macroscopically normal. Photomicrographs are shown in Figures 4.7 and 4.8. Scattered lymphocytes were seen in the myocardium, but neutrophils, monocytes and macrophages were absent. Prominent intercellular and perivascular fibrous tissue was visualized, particularly using the Masson Trichrome stain. Fibrosis was present in all levels sampled (basal, mid-LV and apical regions): although not formally quantified, distribution of fibrosis was roughly uniform between these levels and between anterior, lateral, posterior and septal quadrants.

In summary, with respect to the cause of death, the post mortem was inconclusive. Combining clinical and pathological information, it was not possible to determine whether a recurrence of acute TTC had contributed to the patients demise, however, it will be apparent that certain clinical features could have contributed to a reactivation of that process (e.g. administration of catecholamines).

These uncertainties aside, post-mortem examination provided a unique opportunity. In sum, the findings in this patient support the hypothesis that myocardial fibrosis is a long-term consequence of TTC. Of note, the fact that this patient had experienced previous (definite) episodes of TTC may mean that her microscopic findings are more dramatic than might be expected after only a single TTC episode. On the other hand, it is not possible to completely exclude an independent cardiac fibrotic process.

4.4.6 Limitations

Certain limitations apply to the current study and must be borne in mind in the interpretation of the results. The choice of the primary echocardiographic outcome

measure for Study 1, global longitudinal strain, was made on the basis of the consistency of measurements in normal individuals; this variable was prospectively nominated as the principal measure for testing Hypothesis 1a, alongside other 2DS variables (longitudinal strain rate and apical twist); the reasons for selecting these were noted (see Section 4.1.1). The positive finding in regards to Hypothesis 1a would therefore have been more robust if all three parameters had shown significant between-group differences, rather than simply trends. It is also acknowledged that a blinding procedure was not possible and would have been preferable. The fact that only global longitudinal strain was different may reflect the greater reproducibility of this measure, but may equally arise from the underlying myocardial mechanics, perhaps with selective dysfunction of the longitudinally oriented subendocardial myofibers.

Also, with regard to Hypothesis 1a, the lack of matching for comorbidity between patients and controls, specifically for hypertension, is a limitation. Prospective enrolment of controls had anticipated age- and sex- matching; subjects' cardiovascular risk factors were documented, but not actively matched. None of the patients exhibited uncontrolled hypertension or a phenotype of hypertensive heart disease, with concentric hypertrophy. Given the direct relationship between LV mass and BP elevation, an attempt was made to control for a differentiated effect of hypertension on global longitudinal strain. Although the ANCOVA suggested that the between group differences in global longitudinal strain were not dependent on LV mass, it is acknowledged that a potential pre-existing end-organ effect of hypertension remains a possible confounding factor, which only could be resolved be specific matching for hypertension. With regard to the determinants of 3 months global longitudinal strain, (Hypotheses 1b and 1c), it was not possible to relate the degree of residual deficit to any of the acute-phase measurements assessed. The only significant correlate was simultaneous (3 month) NT-proBNP, which, although it confers biological plausibility of the observation of residual functional impairment, does not elucidate the mechanism of the finding further. Of relevance to Study 2, collagen biomarkers did not correlate with longitudinal function.

Study 2 drew on a separate cohort of patients in order to provide complementary information, relevant to the question of long term recovery, in the form of non-invasive quantification of potential diffuse fibrosis. This arose, chiefly, in the light of the

unexpected finding of persistent inflammatory oedema, a situation that could theoretically engender fibrosis. It should be acknowledged that although the V_e technique has received histological validation in humans, since its inception in 2007 (Jerosch-Herold et al., 2008), these studies utilized multiple breath-hold T_1 mapping sequences (Flett et al., 2010), as distinct from the single breath-hold MOLLI sequence. In the context of V_e-derivation, MOLLI has been validated against histologic fibrosis quantification only in small animals (Messroghli et al., 2011b). For the purposes of this study, MOLLI-derived measurements of T_1 were compared to known values of pre-prepared phantoms. It is therefore acknowledged that comparison against human myocardial fibrosis would have been ideal; nevertheless, the case control methodology employed for the testing of Hypothesis 2a, would arguably have negated any inaccuracies in V_e/fibrosis estimation, as these would have applied to both groups equally. Although cardiac fibrosis would appear to have been increased in those with a previous history of TTC, a relationship between V_e and global longitudinal strain was not apparent at this late time point (Hypothesis 2b): superficially, these two findings are discrepant, although the persistence of fibrosis and the normalization of resting global longitudinal strain could feasibly coexist in recovery.

The other limitation imposed by the small size of the current data set is that it is not practicable to assess the determinants of either residual contractile disturbance at 3 months or extent of fibrosis beyond 12 months. A major priority for further studies is to investigate these putative heterogeneities of recovery.

4.5 Summary and conclusions

This two-part study represents an unprecedented body of work in two important aspects of TTC, namely, the kinetics of recovery and the potential for residual cardiac pathology. Although recovery clearly occurs, the finding that LV function has failed to completely normalize by three months, joins several similar observations made at approximately the same time point: the presence of T cells and macrophages in the cardiac interstitium, elevated plasma NT-proBNP and residually enhanced T_2-w SI, indicative of mild oedema.

The idea of post-TTC myocardial fibrosis has the potential to cause a minor paradigm shift. Whilst the observations at 3-months could be argued to be temporary phenomena within a prolonged period of (eventually complete) recovery, the demonstration of myocardial fibrosis ≥1 year after TTC argues much more strongly for a permanent and detrimental consequence of TTC. The question, however, is *how significant this consequence* is for individual patients, in terms of symptomatology, cardiopulmonary exercise function, or incident heart failure? Even in the absence of corollary data, the presence of fibrosis in patients with remote TTC should prompt renewed focus on the mechanisms at work in this unique disorder, both acutely and in recovery; indeed, the association with permanent fibrosis highlights the need for the investigation of therapeutic strategies, even in this 'self-limiting' disorder.

4.6 Tables and Figures

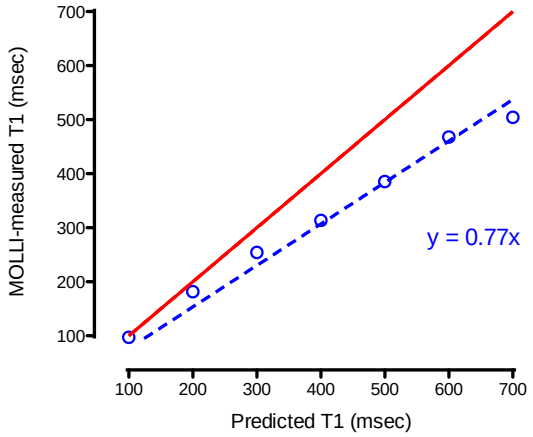

Figure 4.1 Phantom validation of MOLLI-derived T_1 values (blue; line of identity in red), showing systematic underestimation by MOLLI. Linear regression demonstrated a slope of 0.77 (95% CI 0.72-0.81).

Table 4.1 Phantom manufacture according to specified T_1 and T_2 times

	Phantoms						
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
Predicted T_1 (msec)	100	200	300	400	500	600	700
Nickel chloride (mM)	15.1	7.92	5.52	4.31	3.59	3.11	2.77

* Equations from *Quantitative MRI of the Brain: Measuring changes caused by disease*., Ed. Tofts, P. John Wiley and Sons 2003

Table 4.2 Clinical and laboratory features in patients and controls

Study 1

	TTC patients n = 36	**Controls** n = 16
Age (years) (mean±SD)	67 ± 11	64 ± 11
Cardiovascular risk factors		
BMI (kg/m^2) (mean±SD)	28 ± 6	26 ± 4
Current smoking	6 (17%)	2 (13%)
Systemic hypertension	20 (56%)	3 (19%) *
Diabetes mellitus	8 (22%)	2 (13%)
Hyperlipidaemia	18 (50%)	4 (25%)

Study 2

	TTC patients n = 15	**Controls** n = 10
Age (years) (mean±SD)	61 ± 12	67 ± 12
Haematocrit (mean±SD)	0.40 ± 0.05	0.42 ± 0.03
Cardiovascular risk factors		
BMI (kg/m^2) (mean±SD)	28 ± 7	26 ± 4
Current smoking	2 (13%)	0 (0%)
Systemic hypertension	4 (27%)	1 (6%)
Diabetes mellitus	1 (7%)	1 (6%)
Hyperlipidaemia	5 (33%)	2 (13%)

All data expressed as number (and percentage), unless otherwise specified;

* $P < 0.05$; Abbreviations: BMI, body mass index

Table 4.3 Non-deformational parameters of LV function (Study 1)

		Normal controls (n = 16)	**TTC patients** (n = 36)		
			Acute	**10-14 days**	**3 months**
	Heart rate (min^{-1})	67 ± 5	76 ± 18	70 ± 13	63 ± 13 #
Cardiac volumes and mass	LVEDVi (mL/m^2)	34.2 ± 2.8	45.1 ± 15.9	38.1 ± 8.6	34.8 ± 9.6 #
	LVESVi (mL/m^2)	13.0 ± 2.6	22.7 ± 11.5	16.0 ± 4.4	14.1 ± 4.7 #
	LVEF (%)	62 ± 6	52 ± 14	59 ± 8	60 ± 7 #
	LAVi (mL/m^2)	26.5 ± 8.7	35.4 ± 9.5	31.9 ± 7.5	31.6 ± 8. 9 # *
	LVMI (g/m^2)	65 ± 8	78 ± 16	76 ± 12	71 ± 15
Diastolic function	Transmitral E/A ratio	1.04 ± 0.27	1.09 ± 0.52	0.98 ± 0.32	0.89 ± 0.40 *
	Transmitral DT (msec)	216 ± 40	211 ± 137	194 ± 44	236 ± 127
	E/Ea	9.3 ± 3.9	12.7 ± 10	10. 7 ± 3.5	10.3 ±3.5

P ≤ 0.005, for trend of recovery (ANOVA with repeated measures); * P ≤ 0.05, for comparison with normal (unpaired t-test).

Abbreviations: LVEDVi, left ventricular (LV) end diastolic volume index; LVESVi, LV end systolic volume index; LVEF, LV ejection fraction; LAVi, left atrial volume index; LVMI, LV mass index; E/A, ratio of peak early to peak late transmitral diastolic flow; DT, deceleration time; E/Ea, see text.

Table 4.4 Longitudinal and rotational parameters of LV function (Study 1)

		Normal controls (n = 16)	**TTC patients** (n = 36)		
			Acute	**10-14 days**	**3 months**
Global longitudinal function	strain (%)	-20.3 ± 1.6	-12.7 ± 3.9	-16.0 ± 3.3	-17.9 ± 3.1 ## *
	strain rate (s^{-1})	-1.05 ± 0.09	-0.88 ± 0.22	-1.03 ± 0.24	-1.00 ± 0.15 #
Rotational indices	Basal twist (°)	-4.5 ± 2.3	-6.2 ± 4.7	-6.9 ± 4.9	-6.6 ± 3.3 *
	Apical twist (°)	11.3 ± 5.0	4.9 ± 3.9	8.8 ± 5.1	9.3 ± 4.0 ##
	LV twist (°)	14.8 ± 6.6	11.1 ± 6.8	15.7 ± 6.0	15.9 ± 5.9 #
	LV torsion (°cm^{-1})	2.05 ± 0.73	1.44 ± 0.89	2.03 ± 0.80	2.07 ± 0.75 #

P ≤ 0.05, for trend of recovery (ANOVA with repeated measures), ## P ≤ 0.005, for trend of recovery (ANOVA with repeated measures), * P ≤ 0.01, for comparison with normal (unpaired t-test); Abbreviations: LV, left ventricular.

Table 4.5 Radial and circumferential deformational parameters of LV function, by region (Study 1)			**Normal controls (N = 16)**	**TTC patients (N = 36)**		
				Acute	**10-14 days**	**3 months**
Basal	Radial	strain (%)	50 ± 22	40 ± 18	49 ± 18	42 ± 18
		strain rate (s^{-1})	1.6 ± 1.1	1.9 ± 0.7	2.0 ± 0.5	1.9 ± 0.6
	Circumferential	strain (%)	-19.3 ± 4.9	-17.3 ± 6.0	-19.8 ± 5.9	-18.7 ± 5.1
		strain rate (s^{-1})	-1.3 ± 0.3	-1.5 ± 0.5	-1.6 ± 0.4	-1.3 ± 0.3 #
Mid-LV	Radial	strain (%)	51 ± 19	25 ± 13	39 ± 19.3	43 ± 18 ##
		strain rate (s^{-1})	1.6 ± 0.3	1.5 ± 0.4	2.0 ± 0.5	1.7 ± 0.5
	Circumferential	strain (%)	-21.7 ± 3.9	-14.8 ± 7.0	-19.8 ± 5.9	-21.4 ± 5.3 #
		strain rate (s^{-1})	-1.1 ± 0.4	-1.2 ± 0.4	-1.3 ± 0.7	-1.3 ± 0.3
Apical	Radial	strain (%)	34.4 ± 22.	13.8 ± 10.3	21.5 ± 13.0	29.2 ± 20.6 ##
		strain rate (s^{-1})	1.57 ± 0.51	1.17 ± 0.44	1.25 ± 0.38	1.38 ± 0.57
	Circumferential	strain (%)	-26.3 ± 6.4	-13.6 ± 8.0	-21.0 ± 8.5	-24.8 ± 5.3 ##
		strain rate (s^{-1})	-1.25 ± 0.91	-0.97 ± 0.53	-1.61 ± 0.66	-1.45 ± 0.30 ##

P ≤ 0.05, for trend of recovery (ANOVA with repeated measures); ## P ≤ 0.005, for trend of recovery (ANOVA with repeated measures)
* P ≤ 0.05, for comparison with normal (unpaired t-test); Abbreviations: LV, left ventricular.

Table 4.6 CMR indices in Study 2

	TTC (n = 15)	**NC** (n = 10)
LV EDVi (mL/m2)	67.3 ± 9.8	61.2 ± 7.8
LV ESVi (mL/m2)	25.9 ± 7.3	26.2 ± 4.6
LVEF (%)	62.1 ± 7.1	57.3 ± 5.0
LVMi (g/m2)	79.9 ± 7.6	71.9 ± 14.1

* P ≤ 0.05; Abbreviations: LV EDVi: left ventricular end-diastolic volume index; LV ESVi: left ventricular end-systolic; LVEF: left ventricular ejection fraction; LVMi: left ventricular mass index

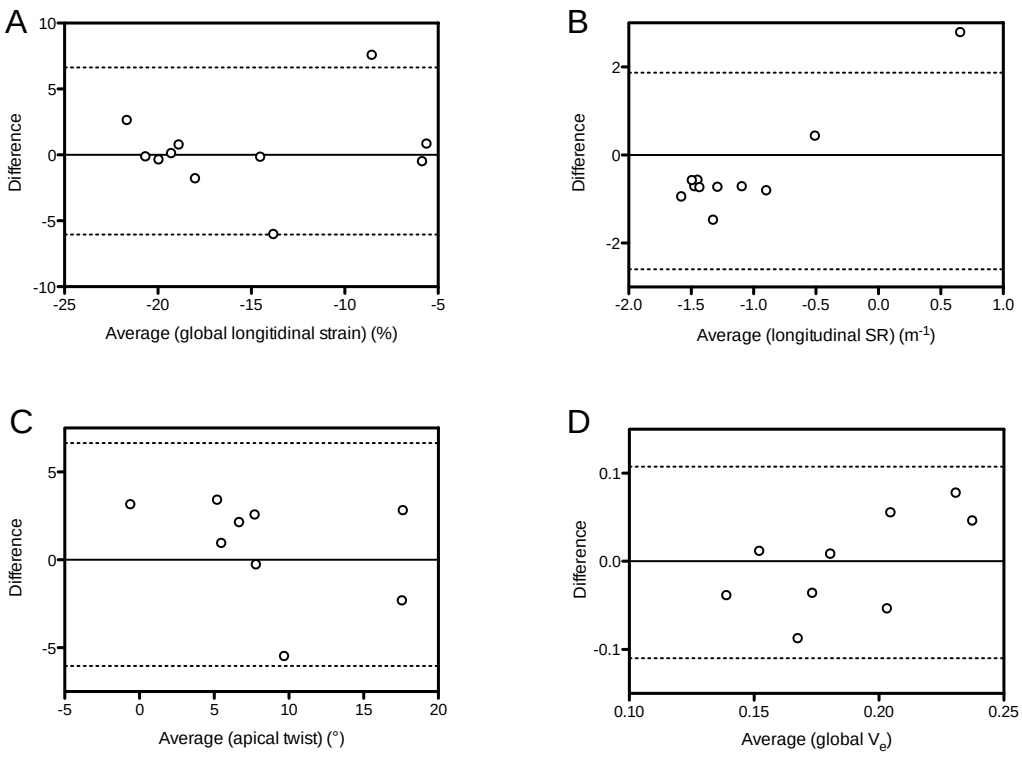

Figure 4.2 Measurement and reproducibility characteristics of the main indices for studies 1 and 2. Bland-Altman plots, with 95% limits of agreement (broken lines), are shown for global longitudinal strain (A), longitudinal strain rate (SR) (B), apical twist (C) and global V_e (D).

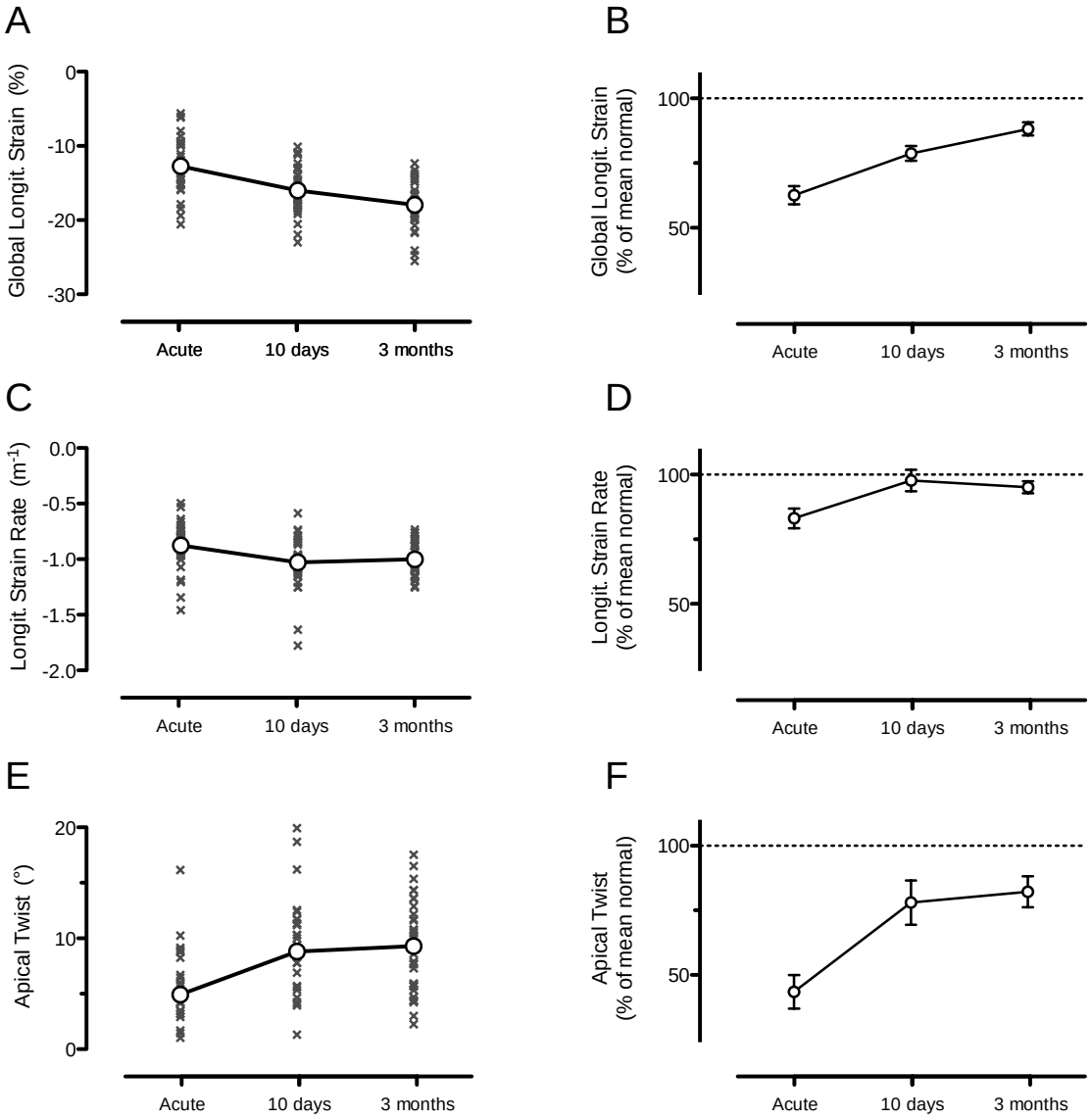

Figure 4.3 Recovery of LV function, expressed in terms of mean absolute values (left hand side) and as percentage of control group-derived normal values (right hand side; mean ± SEM), for global longitudinal strain (A and B), longitudinal strain rate (C and D) and apical twist (E and F). Recovery of each index was significant ($P < 0.005$; ANOVA with repeated measures).

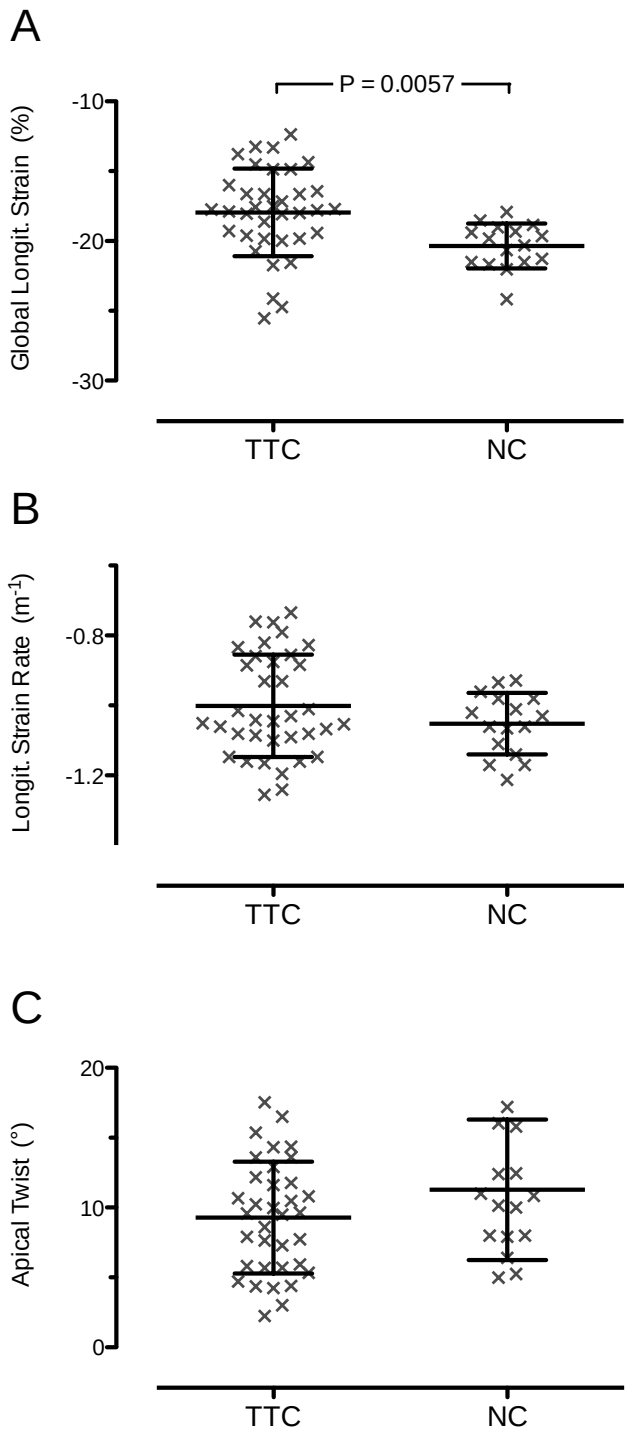

Figure 4.4 Comparison of LV function 3-month post-TTC, versus normal controls (NC), in terms of global longitudinal strain (A), longitudinal strain rate (B) and apical twist (C).

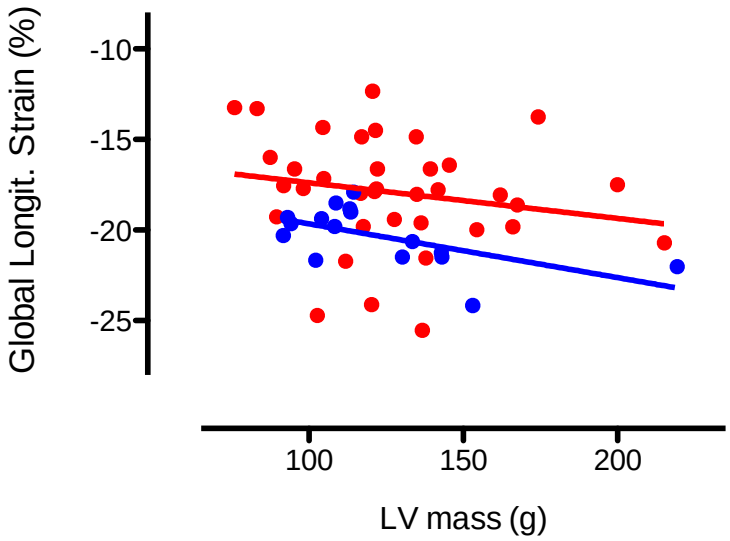

Figure 4.5 Relationship between 3-month global longitudinal strain with simultaneous LV mass, in TTC (red circles), compared with normal controls (blue circles).

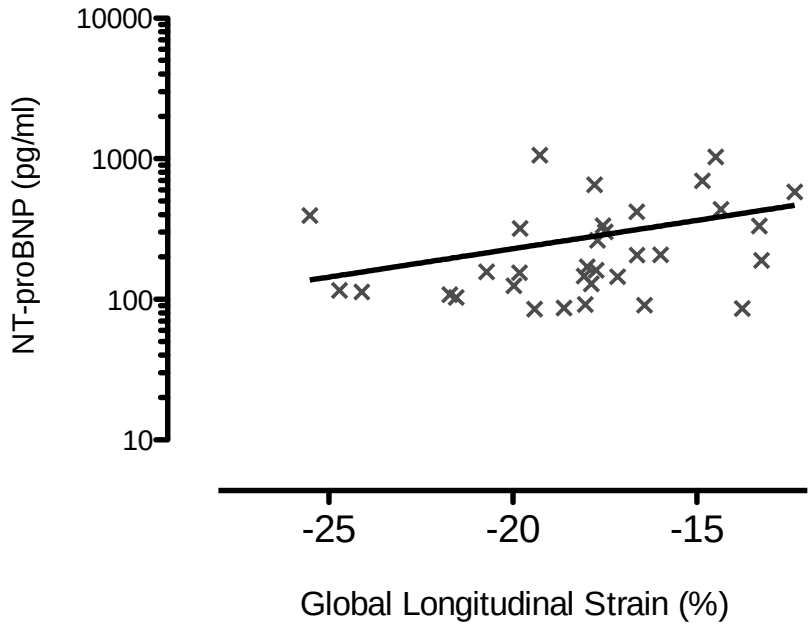

Figure 4.6 Relationship between global longitudinal strain and NT-proBNP concentration at 3 months (r = 0.38, P = 0.027).

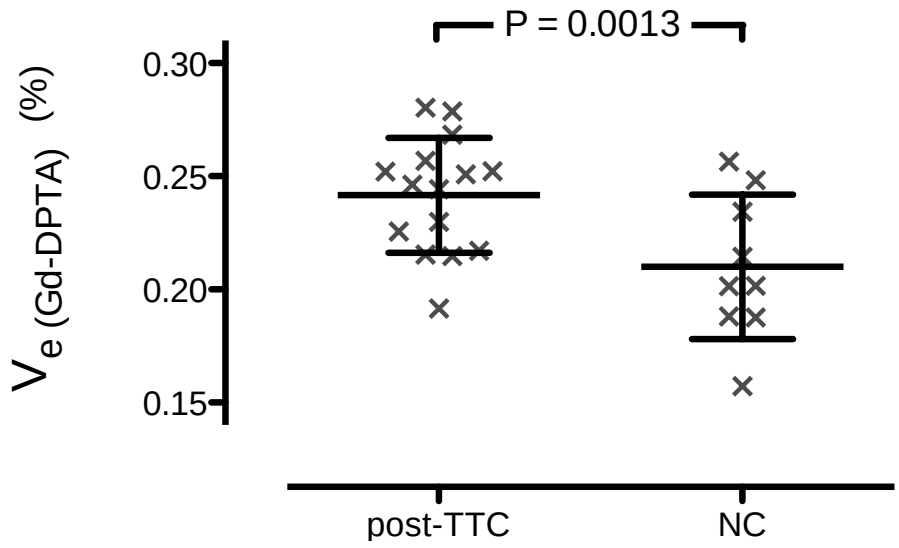

Figure 4.7 Comparison of Ve in TTC patients (≥ 1 year from index event) versus age-matched female normal controls (NC).

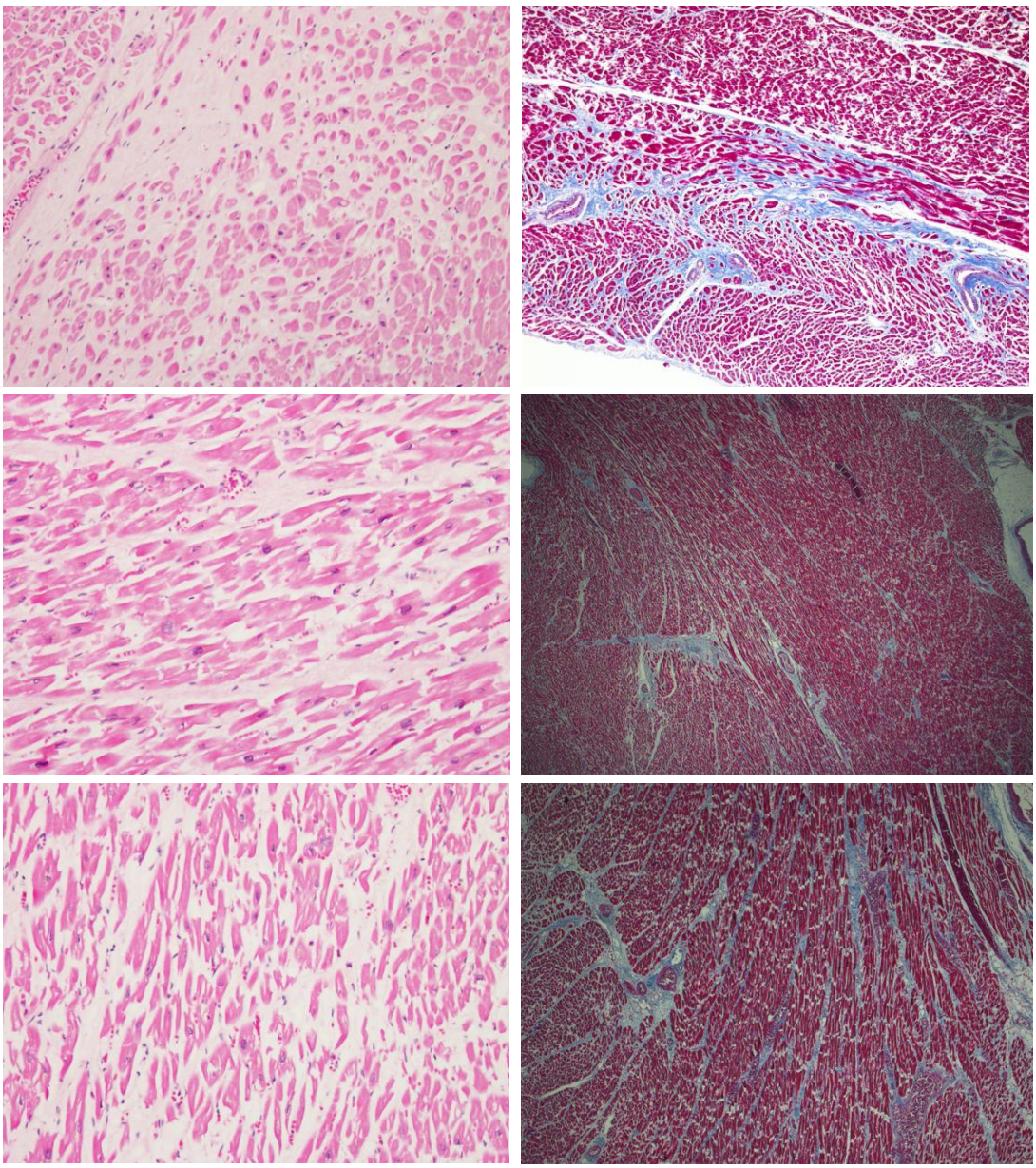

Figure 4.8 Representative images of haemotoxylin and eosin (left hand column) and masson trichrome (right hand column), in basal (first row), mid-ventricular (second row) and apical (third row) samples. Intercellular and perivascular patterns of fibrosis are appreciated in all three levels. Quantitative comparison between regions was not undertaken.

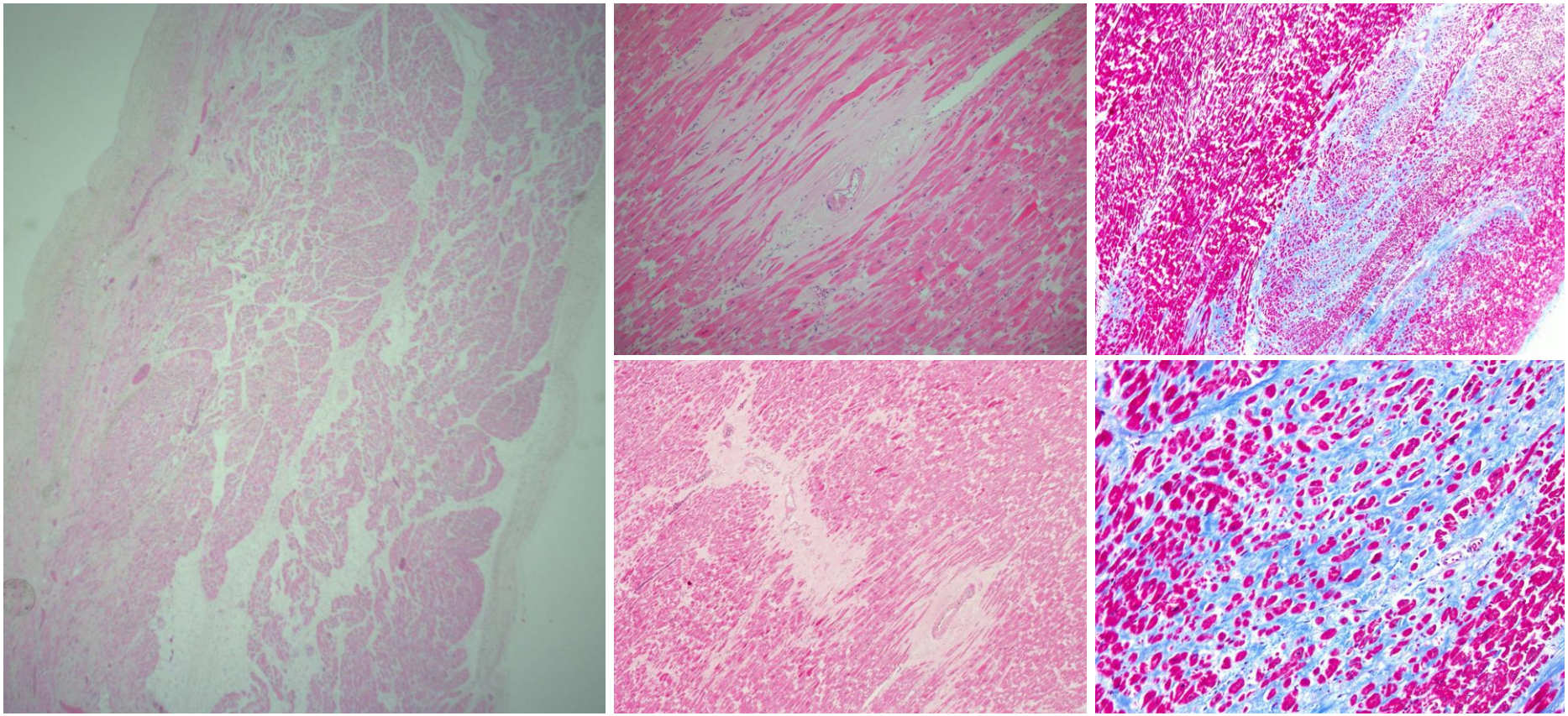

Figure 4.9 Low power haematoxylin and eosin (H&E) staining (left panel) showing foci of fibrosis in the subendocardium and mid-wall regions. Examples of perivascular fibrotic foci (H&E) are shown in the middle panels. Examples of fibrous expansion of extracellular space (blue pigment), demonstrated with Masson Trichrome stain (right hand panels), with Low power (upper right, perivascular and intercellular) and high power (lower right, intercellular fibrotic pattern only).

Chapter 5

Conclusions and future perspectives

5.1 Summary: major findings

This thesis has been structured around three major foci, with the aim of exploring the three following aspects of TTC, in each of three experimental chapters:

(i) the clinical spectrum and facilitated diagnosis (Chapter 2),

(ii) the magnitude, duration and correlations of the acute inflammatory process (Chapter 3), and

(iii) aspects of intermediate- and long-term recovery (Chapter 4).

In the context of current cardiological practice, prospective consideration of TTC in patients presenting with apparent ACS would seem to be rare. Partly in response to evidence that TTC is *not rare.* the aim of Chapter 2 was to develop tools to facilitate *a priori* consideration of this diagnosis, utilizing a large cohort of TTC patients, together with a matched female patients with ACS. The biomarker NT-proBNP, as well as a putative "TTC score", incorporating simple clinical and ECG criteria, appeared to be clinically useful in general.

In relation to the second aim, although inflammation has been recognized to occur in TTC, characterization has been limited and qualitative. Thus, in Chapter 3, acute intramyocardial inflammation was quantified via T_2-weighted CMR and related to other aspects of acute disease severity, in a cohort of 49 TTC patients. As postulated, the distribution of oedema paralleled that of hypokinesis, exhibiting a base-to-apex gradient, whilst not completely sparing the basal LV myocardium. Plasma concentration of normetanephrine, as an index of noradrenaline exposure, exhibited a "dose-dependent" relationship with the severity of global intramyocardial oedema, whereas metanephrine concentrations did not. Univariate comparisons revealed further direct relationships between oedema and markers of myocardial injury and systemic inflammation. Two unexpected pieces of information also emerged: (i) T_2-w SI was abnormal even in the cardiac base and (ii) was persistently abnormal at 3 months, implying slow resolution of oedema.

The third objective was to evaluate recovery from TTC, employing two case-control methodologies, as described in Chapter 4. The aims of the first study were to document the kinetics of LV functional recovery, its completeness at 3 months and to evaluate potential determinants of incomplete resolution. This revealed recovery of overt wall motion abnormalities, but subnormal longitudinal function in the TTC group, which varied according to the magnitude of persistent elevation of plasma NT-proBNP. Although the latter underscores the validity of the main findings, further correlations, aimed at clarifying mechanisms for residual dysfunction, were negative. Specifically, residual intramyocardial oedema and markers of systemic collagen I turnover at three months did not correlate with long-axis function.

The potential for the formation of long-term diffuse myocardial fibrosis was addressed in the second study. Utilizing a novel "T_1-mapping" CMR methodology, a globally increased extracellular volume, highly suggestive of interstitial fibrosis, was demonstrated ≥ 1 year post-TTC.

5.2 Implications of these findings

Although retrospective in nature, the study in Chapter 2 makes novel contributions to the diagnosis of TTC, which can be facilitated by consideration of NT-proBNP release and the TTC score. As acknowledged, in the hospital setting, such a strategy would translate to clinical benefit only in those patients *without* ST-elevation and who are haemodynamically stable. However, in this majority, the approaches tested were purposefully simple and emerged as highly predictive: clinical use of NT-proBNP and the TTC score is therefore feasible in emergency and CCU settings, ahead of further confirmatory investigations, *in patients without ST-elevation*. Although the flow-on effects of such earlier diagnosis are not yet clear, it is likely that they would include at least (i) reduced misclassification of NSTE-TTC and ACS (and subsequent underestimation of TTC incidence), (ii) inclusion of patients in prospective research programs, in the acute-phase and (iii) earlier tailoring of treatment strategies, even if disease-specific therapy is not currently available.

From the study in Chapter 3, it is apparent that intense myocardial inflammation is present in TTC. The fact that this parallels simultaneous contractile dysfunction suggests that inflammation plays a role in the *maintenance* of abnormalities in the acute phase, as a *response* to injury and raises the issue of *sequelae* to inflammatory activation. This study provides a novel index of myocardial injury in TTC. In terms of pathogenesis, it is noted that noradrenaline exposure (as reflected by plasma normetanephrine), but probably not adrenaline exposure (as reflected by plasma metanephrine), exhibited a dose-dependent relationship with acute severity of oedema. These findings are somewhat at odds with the weight of evidence regarding the role of β-adrenoceptor stimulation in the pathogenesis of TTC, and raise the possibility that much of the elevation of catecholamine metabolite levels detected in such cases reflects a sympathetic discharge response rather than the actual cause of the disorder.

The above observation of late persistence of abnormal T_2-w SI (Chapter 3) adds to existing evidence that recovery from TTC is not as rapid as generally thought; the novel findings of Chapter 4 extend and reinforce this notion. Whilst the LV dysfunction observed has yet to be correlated with cardiac reserve, functional capacity or susceptibility to future clinical events, these data nonetheless denote a prolonged myocardial recovery phase and/or an element of 'permanent' structural damage, as suggested by the CE-CMR data. As such, these findings (i) suggest that a departure from the characterization of TTC as completely reversible/benign is appropriate, (ii) emphasize the need for further understanding TTC-related injury, with a view to preventative treatment in TTC. The latter could reasonably be aimed at preserving myocardial function and preventing late interstitial fibrosis.

5.3 Key unresolved issues related to the current thesis

Unresolved issues with regard to the diagnostic study (Chapter 2) primarily relate to (a) the validity of incorporating antecedent stress-exposure into the TTC score and to (b) the lack of utility of NT-proBNP/TTC score in certain clinical contexts, namely hypotension or pulmonary congestion. With regard to the former, preliminary analysis employing imputed frequencies of prior stress in ACS indicate that prospective incorporation of this component into the TTC score would substantially enhance its diagnostic performance. On

the other hand, the *ineffectiveness* of the TTC score, in the presence of haemodynamic compromise, is a substantial concern, given the prevalence of TTC in critical care environments; it would appear that alternative strategies for early identification of TTC are required. Finally, it is implicit in the TTC score that definitive diagnosis must wait suitable confirmatory testing.

At present, invasive exclusion of obstructive fixed coronary lesions is the generally accepted standard for definitive diagnosis of TTC: the prospect of non-invasive imaging-based diagnosis of TTC has yet to be fully tested, although candidate techniques have been reviewed (see Chapter 2, Section 2.1). In this regard, the potential exists for the TTC score to be coupled with appropriately validated, timely, non-invasive investigations.

The intramyocardial oedema documented in Chapter 3 is likely a response to the initiating injury of TTC *and* a key part of the continuation of LV dysfunction in the acute phase. Due to the difficulty of performing *serial* CMR immediately post-TTC, it was not possible to describe the early time-course of oedema development in this study. Pathogenetic mechanisms can only be partially addressed by this methodology: although a modest correlation with the systemic inflammatory marker, C-reactive protein, was observed, other potential mediators were not measured. These include cytokines, which, as negatively inotropic and proinflammatory agents, may play a significant pathogenetic role in TTC. In this regard, the notion that TTC is part of a wider spectrum of reversible non-ischaemic and stress-related myocardial disorders with *diverse initiating causes* but shared mechanisms (e.g., cytokine-induced NO elaboration) is intriguing. In addition, the current findings raise the issue of whether an *anti-inflammatory* therapeutic strategy could be applied in TTC, perhaps with the aim of accelerating recovery or preventing post-inflammatory fibrosis. Finally, although the diagnostic potential of multisequential CMR for TTC (as against ACS) have been heralded by other authors (Eitel et al., 2011b), the quantitative T_2-weighted CMR techniques utilized in this study have yet to be prospectively explored in the context of acute diagnosis.

Although the findings of Chapter 4 are novel and provocative, the key issue with regard to the demonstration of (i) delayed recovery of parameters of LV function and (ii) interstitial fibrosis after TTC, is the present inability to relate these to the clinical syndrome, i.e. symptomatology, functional capacity, or aspects of prognosis (e.g., recurrence of TTC or

the development of atrial fibrillation/arrhythmias or de-novo heart failure, etc.). In the absence of such correlation, these findings remain "subclinical" and of uncertain significance. At present, whether impaired LV long-axis function at 3 months is a *transitory* or *permanent* feature cannot be known; ≥1 year data suggest that, whilst the pathological presence of fibrosis is indicated, recovery of resting long axis function is apparently complete at this time point. It remains to be seen whether post-TTC hearts can be stimulated to the same degree as normal hearts (e.g. by inotropic challenge) or whether a diminished cardiac reserve applies. Beyond this, questions as to the specific mechanism(s) by which fibrosis is promoted in TTC and whether the process can be beneficially modulated either in the acute, sub-acute or late phases, is not known.

5.4 Potential means addressing these unresolved issues

Chapter 2

Prospective evaluation of the TTC score is necessary for clinical validation. A study applying the score in female patients with an infarct-like presentation would have certain practical distinctions from the retrospective analysis provided here. These would include (i) restriction of the score to those with a more relevant ECG presentation (i.e. apparent NSTEMI, excluding STEMI), (ii) incorporation of an optimized lower cut-off for NT-proBNP (i.e. 2,500 pg/mL – one point; see Section 2.3.8) and (iii) incorporation of scoring for the presence of an antecedent stressor. In a prospective study, it may be possible to evaluate the performance of the TTC score at an earlier time-point (e.g., admission versus 24 hours). However, the available data on the kinetics of NT-proBNP in acute TTC suggest a progressive rise up to ~24 hours (Nguyen et al., 2011a) and, together with the evolution of ECG changes during the first day of admission (see Chapter 2, Section 2.3), this indicates that delineation with this tool is highly time-dependent, in the majority of cases. The development of novel means of differentiating TTC *at initial presentation* therefore remains a priority.

Chapter 3

As regards the unresolved issues relating to the place of inflammation in TTC, the opportunity exists for further biochemical correlation with T_2-w SI. The determination of (i) circulating cytokine concentrations or levels of nitrosative stress (in view of both direct and indirect negative inotropic effects, via myocardial iNOS induction/NO formation), or (ii) pro-inflammatory myocardial signaling pathways in human cardiac biopsy studies, would be highly informative. However, there is arguably greater promise for the study of TTC pathogenesis in appropriately derived animal models, given the current state of biochemical understanding of TTC and the complexity of immune-regulation. This is especially true, given the salutary history of anti-inflammatory or anti-cytokine strategies in cardiac disease: unfavourable outcomes have been associated with such treatments in both MI [both steroidal (Bulkley and Roberts, 1974) and non-steroidal (Boden and Sadaniantz, 1985) anti-inflammatory agents] and heart failure [anti-TNF-α (Gupta and Tripathi, 2005)]. In the light of these examples, although any future disease-specific therapies for TTC are likely to be administered during a phase of inflammation, animal models should anticipate the possibility that inflammation is, to some extent, a *protective* response to the initiating injury. It is, in any case, appropriate that animal models demonstrate applicability to human TTC by the incorporation of measurements of the cardiac inflammatory reaction.

Chapter 4

Regarding the trajectory of recovery from TTC, the present documentation of serial LV function should be corroborated with other aspects of patients' well-being and functional capacity. Appropriate means of addressing these issues would include (i) serial administration of standardised questionnaires (to quantify symptoms, severity of fatigue or global quality of life) or (ii) objective assessment of exercise capacity and/or inotropic cardiac reserve. Such approaches would delineate the clinical significance of the present "subclinical" findings of subnormal *resting* LV function and late myocardial fibrosis. However, even if it is not corroborated in this way, the concept of "permanent" myocardial fibrosis as a pathological complication of TTC, is highly challenging, in isolation. This is in part because of the prominent role which diffuse myocardial fibrosis plays in other conditions [e.g., dilated cardiomyopathy (Iles et al., 2008)], in which such fibrosis is

considered an indicator of disease severity and substrate for malignant arrhythmias. Furthermore, the potential for *cumulative* fibrosis post-TTC is raised by the possibility of *recurrence* and the interaction of other pro-fibrotic influences in individual patients (e.g. diabetes or hypertension). In view of these concerns, the prevention of post-TTC fibrosis *as a therapeutic target* becomes a relevant consideration for future studies in animal-models and, ultimately, human TTC. A treatment that is effective in reducing the degree of acute injury/inflammation, may translate into less late fibrosis; it is also possible that the therapeutic window in which a fibrotic response could be modified has an extended duration.

5.5 Priorities in advancing the understanding of TTC:

Finally, the following research priorities are highlighted, as they correspond to the objectives of this thesis.

I. Diagnosis

a. Identification of high-risk subsets of ageing females

TTC presents via diverse routes, including, on the one hand, the more or less typical emergency department presentation with chest pain and, on the other hand, a complications of medical and surgical disease, frequently in the absence of chest pain. The true incidences of TTC-like abnormalities in general medical, acute neurological, perioperative or critical care settings have yet to be established: such information is vital for understanding the multidisciplinary "spectrum" of TTC.

b. Characterizing pre-hospital course and risk

Another unknown element of the epidemiology of TTC is the extent to which it is responsible for sudden cardiac death. Certainly common histological features are shared between survivors of TTC and patients suffering either sudden accidental death (e.g., drowning, etc) *or* sudden death following severe emotional distress. However, such histological findings are non-specific. If, as a result of finer delineation of the *specific* delineation of the biochemistry of TTC, the potential

contribution of a TTC-like process to sudden unexplained cardiac death could be assessed.

c. TTC prophylaxis in at-risk patients

No agreement exists with regard to the medical prevention of TTC. However, this is a relevant consideration in two contexts: (i) the risk of recurrence in patients with previous TTC and (following on from (a) above) (ii) anticipating the possibility of primary TTC in groups identified as being at substantial risk. With regard to recurrence, actual determinants of higher risk are not known: such information might inform appropriate tailoring of prophylactic therapy. With regard to the second context, depending on the incidence and risk determinants in specific groups (e.g. post-stroke or perioperative patients), it is possible to envisage specific prophylaxis against stress-cardiomyopathy/TTC.

II. Inflammation: understanding the process

Very little research has focused on the transition from catecholamine-induced myocardial injury to local inflammatory activation. Several groups have demonstrated the expression of mitogen activated protein kinases, in the signaling cascade downstream of stress/catecholamine exposure [p44/p42 (Ueyama, 2004) and p38 (Chen et al., 2009, Paur et al., 2012)]: pro-inflammatory as well as cardio-protective pathways may be activated by these permissive kinases.

A number of rodent models for TTC have recently been developed and offer the potential to examine the role of various adrenoceptor-related signaling pathways in initiating inflammation and oxidative stress, as well as convenient testing of the relevant “Koch’s postulates”, prior to these being evaluated in humans. Importantly, none of the currently reported animal studies have revealed precisely why ageing women are at particular risk of TTC.

III. Recovery

The two critical issues relevant to recovery are the bases for prolonged intramyocardial inflammation and the factors controlling the development (or non-development) of permanent fibrosis. Perpetuation of inflammatory response suggests the presence of a source of potential redox stress within the myocardium post TTC, rather than ongoing catecholamine-induced damage. For example, presence of a stimulated population of myofibroblasts within the myocardium should be sought as a potential source of ongoing inflammatory activation.

Similarly, pathological fibrosis may also reflect the conversion of fibroblasts into myofibroblasts. Stimuli for this differentiation are diverse, but include redox stress, pro-inflammatory cytokines, growth factors (pre-eminently TGF-β) and matricellular proteins (e.g. osteopontin); the local and systemic renin-angiotensin system (including aldosterone) also modulates myofibroblast function and this pro-fibrotic effect is augmented under conditions of local inflammation.

Whilst these factors can initiate and sustain fibrosis, the pathological outcome is also dependent on anti-fibrotic factors, such as NO, natriuretic peptides and anti-inflammatory cytokines (e.g. IL-10). Thus, the elucidation of the time-course of activation of the above elements in TTC is necessary to understanding the therapeutic window, which may exist for the prevention of fibrosis. Such information would also be a critical prelude to interventional studies.

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