

Pathophysiology of Takotsubo syndrome – a joint scientific statement from the Heart Failure Association Takotsubo Syndrome Study Group and Myocardial Function Working Group of the European Society of Cardiology – Part 1: overview and the central role for catecholamines and sympathetic nervous system

Elmir Omerovic^{1*}, Rodolfo Citro², Eduardo Bossone³, Bjorn Redfors¹, Johannes Backs^{4,5}, Bastian Bruns^{4,5,6}, Michele Ciccarelli⁷, Liam S. Couch⁸, Dana Dawson⁹, Guido Grassi¹⁰, Massimo Iacoviello¹¹, Guido Parodi¹², Birke Schneider¹³, Christian Templin¹⁴, Jelena R. Ghadri¹⁴, Thomas Thum¹⁵, Ovidiu Chioncel¹⁶, C. Gabriele Tocchetti¹⁷, Jolanda van der Velden¹⁸, Stephane Heymans^{19,20}, and Alexander R. Lyon^{8,21*}

¹Department of Cardiology, Sahlgrenska University Hospital and Department of Molecular and Clinical Medicine/Wallenberg Laboratory, Institute of Medicine, Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden; ²Heart Department, University Hospital 'San Giovanni di Dio e Ruggi d'Aragona', Salerno, Italy; ³Division of Cardiology, A. Cardarelli Hospital, Naples, Italy; ⁴Institute of Experimental Cardiology, Heidelberg University, Heidelberg, Germany; ⁵DZHK (German Centre for Cardiovascular Research), Partner Site, Heidelberg/Mannheim, Germany; ⁶Department of General Internal Medicine and Psychosomatics, University of Heidelberg, Heidelberg, Germany; ⁷Department of Medicine, Surgery, and Dentistry, University of Salerno, Salerno, Italy; ⁸National Heart and Lung Institute, Imperial College, London, UK; ⁹Aberdeen Cardiovascular and Diabetes Centre, School of Medicine and Dentistry, University of Aberdeen, Aberdeen, UK; ¹⁰Clinica Medica, University of Milano Bicocca, Milan, Italy; ¹¹University Cardiology Unit, Cardiothoracic Department, University Hospital, Bari, Italy; ¹²Clinical and Interventional Cardiology, Sassari University Hospital, Sassari, Italy; ¹³Sana Kliniken, Lübeck, Germany; ¹⁴University Heart Center, Department of Cardiology, University Hospital Zurich, Zurich, Switzerland; ¹⁵Institute of Molecular and Translational Therapeutic Strategies, Hannover Medical School, Hannover, Germany; ¹⁶Emergency Institute for Cardiovascular Diseases 'Prof. C.C. Iliescu', Bucharest, Romania and University of Medicine Carol Davila, Bucharest, Romania; ¹⁷Department of Translational Medical Sciences and Interdepartmental Center for Clinical and Translational Research (CIRCET), Federico II University, Naples, Italy; ¹⁸Department of Physiology, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; ¹⁹Department of Cardiology, CARIM School for Cardiovascular Diseases, Maastricht University Medical Centre, Maastricht, The Netherlands; ²⁰Department of Cardiovascular Sciences, Centre for Molecular and Vascular Biology and Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium; and ²¹Department of Cardiology, Royal Brompton Hospital, London, UK

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*Corresponding authors. Prof. Elmir Omerovic, Department of Cardiology, Sahlgrenska University Hospital, Institute of Medicine, Department of Molecular and Clinical Medicine, Sahlgrenska Academy at University of Gothenburg, Bruna straket 16, 413 45 Gothenburg, Sweden. Tel: +46 31 3427588, Email: elmir@wlab.gu.se
 Dr. Alexander Lyon, Royal Brompton Hospital, Sydney Street, London, SW3 6NP, UK. Tel: +44 207 3528121 ext 82396, Email: a.lyon@ic.ac.uk

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This is the first part of a scientific statement from the Heart Failure Association (HFA) of the European Society of Cardiology focused upon the pathophysiology of Takotsubo syndrome and is complimentary to the previous HFA position statement on Takotsubo syndrome which focused upon clinical management. In part 1 we provide an overview of the pathophysiology of Takotsubo syndrome and fundamental questions to consider. We then review and discuss the central role of catecholamines and the sympathetic nervous system in the pathophysiology, and the direct effects of high surges in catecholamines upon myocardial biology including β -adrenergic receptor signalling, G-protein coupled receptor kinases, cardiomyocyte calcium physiology, myofilament physiology, cardiomyocyte gene expression, myocardial electrophysiology and arrhythmogenicity, myocardial inflammation, metabolism and energetics. The integrated effects upon ventricular haemodynamics are discussed and integrated into the pathophysiological model.

Keywords

Takotsubo syndrome • Pathophysiology • Catecholamine • Beta-adrenergic signalling • G-protein coupled receptor kinase • Electrophysiology • Inflammation • Metabolism

Introduction

Takotsubo syndrome (TTS) is an acute heart failure syndrome which currently lacks evidence-based disease-specific diagnostic and treatment strategies.^{1,2} New diagnostic tests are required to diagnose TTS promptly and differentiate from acute myocardial infarction (AMI) and acute viral or auto-immune myocarditis. New treatments are also required, particularly during the acute phase to prevent and manage the most serious complications and to accelerate recovery.^{1–4} Likewise there is an unmet need for TTS survivors at long-term follow-up to prevent both recurrence and long-term cardiovascular and non-cardiovascular complications associated with TTS.^{3,4} The lack of evidence-based treatments stems from the incomplete understanding of the underlying pathophysiology in TTS syndrome during the different phases of the condition, including predisposing factors and susceptibility, the pathophysiology during the acute episode, and the chronic maladaptive changes in cardiovascular pathophysiology persisting at follow-up. Understanding the pathophysiology of TTS is crucial for the development of appropriate therapeutic interventions.

The Heart Failure Association (HFA) Takotsubo Syndrome Study Group and the Myocardial Function Working Group of the European Society of Cardiology combined to host a workshop dedicated to understanding the pathophysiology of TTS, and the gaps in knowledge requiring further research. The aims of the workshop were to review the current evidence regarding the pathophysiology of TTS from both clinical studies and preclinical models, and to identify the clinical needs at different phases of the syndrome. Where mechanistic evidence exists or is lacking, we provide suggestions to define future laboratory and clinical studies based on current knowledge gaps. In this first part we provide a brief summary of the clinical features of TTS, an overview of the pathophysiology and key questions to consider. We then focus upon the central role of catecholamines and sympathetic nervous system activation and the direct effects upon the ventricular myocardium at multiple levels including receptor signalling, calcium cycling and myofilament physiology. We also discuss the effects upon myocardial electrophysiology, gene expression, inflammation, metabolism and energetics. In part 2 we cover the vascular pathophysiology, the role of the central and peripheral nervous systems, the influence of gender and sex hormones, genetic risk,

the pathophysiology of chronic cardiovascular problems in TTS survivors, current limitations of the preclinical and clinical studies to date and future perspectives and directions for research.⁵

Clinical features of Takotsubo syndrome

Patients with TTS commonly present with chest pain, dyspnoea, diaphoresis and syncope. These symptoms and signs are indistinguishable from those of patients with AMI,³ and 1–2% of patients presenting with suspected AMI are diagnosed with TTS.⁶ TTS also resembles AMI at presentation regarding electrocardiographic (ECG) abnormalities, biomarkers and wall motion abnormalities.³ The clinical details of TTS have been reviewed and described extensively elsewhere.^{1–3} Several diagnostic criteria for TTS have been published including the HFA diagnostic criteria for TTS^{1,2,7–16} and although most of the suggested criteria are similar they have differences which complicate direct comparisons of studies that used different diagnostic criteria for TTS.¹⁷

There are five clinical features which are important to highlight when considering the pathophysiology. First, a preceding emotional or physical stressor is identified in approximately 60–70% of TTS cases.^{3,18} Second is the gender imbalance with women accounting for 90% of TTS cases, with TTS accounting for up to 10% of post-menopausal women presenting with acute chest pain.^{2–4} Gender differences in pathophysiology of TTS remain poorly described and are an area for future research. Third, there is a relatively high mortality during the acute phase, with larger series reporting 4–5% in-hospital mortality which is comparable to AMI.⁴ The main causes of death are cardiovascular secondary to cardiogenic shock (CS), malignant ventricular arrhythmias, ventricular rupture and thromboembolic stroke, and understanding the pathophysiology may lead to new treatment strategies. Given high catecholamine levels contribute to the pathophysiology (see below), catecholaminergic inotropes including dobutamine, epinephrine and milrinone are not scientifically logical, and there are observational data that they are associated with an increased mortality when comparing the rate of inotrope use and mortality between the large registries.^{3,4} Fourth, a significant minority (15–20%) of TTS patients are left with persisting abnormalities of cardiovascular

function leading to reduced quality of life and complications which may include chronic cardiac chest pain, atrial arrhythmias, ventricular arrhythmias, diastolic dysfunction, and reduced exercise capacity and myocardial reserve with exercise.¹⁹ There are also major adverse cardiovascular events (cardiac death, AMI, heart failure, and TTS recurrence), and increased long-term all-cause mortality in TTS patients compared to control cohorts which can be predicted from their acute event,²⁰ and in particular those with severe left ventricular (LV) systolic dysfunction during the acute episode.²¹ Finally, approximately 10% of TTS patients experience recurrent episodes of acute TTS.²² Some patients are asymptomatic between their recurrences, whilst others have persisting cardiac symptoms and abnormalities of cardiovascular physiology. Recurrent acute TTS episodes impart a new acute mortality risk, analogous to recurrent acute coronary syndrome (ACS) episodes for patients with coronary artery disease being associated with the increased risk of CS and sudden cardiac death. To date there is no specific treatment proven to reduce the risk of recurrent TTS.

The participants at the workshop identified the main clinical priorities where improved understanding of the pathophysiology is required to underpin the development of new diagnostics or treatments which have the potential to improve quality of life, reduce mortality and reduce healthcare costs for TTS patients (Table 1).

Table 1 Major priorities of research and unmet clinical needs for patients with Takotsubo syndrome

Clinical needs	Potential benefits
New diagnostic tests to improve diagnosis of TTS on presentation and during the first 24 h from hospital admission during the acute phase	Earlier diagnosis Reduce time and cost that patients are exposed to inappropriate ACS medication Reduced LoS in hospital
New treatments for TTS patients with cardiogenic shock	Reduce mortality Reduce LoS in ICU, CCU and overall hospital LoS
New treatments for TTS survivors who have long-term cardiovascular complications and symptoms reducing their QoL	Improve QoL and symptom control Reduce hospital readmission ?Improve long-term mortality
New diagnostic strategies to identify TTS survivors at risk of recurrence, and potentially new ways to predict a recurrent episode before it develops	Reduce hospital readmission Improve QoL ?Improve mortality
New treatments to reduce the risk of recurrent TTS in patients with known recurrence or those at higher risk of recurrence	Reduce hospital readmission Improve QoL ?Improve mortality

ACS, acute coronary syndrome; CCU, coronary care unit; ICU, intensive care unit; LoS, length of stay; QoL, quality of life; TTS, Takotsubo syndrome.

Clinically available biomarkers for Takotsubo syndrome

Takotsubo syndrome patients exhibit minor increases in creatine kinase-MB and cardiac troponin, and more significant elevation of brain natriuretic peptide (BNP) or N-terminal pro-BNP (NT-proBNP)^{23–25} when compared to AMI. The lower troponin levels reflect relatively less myocyte necrosis compared to AMI with comparable ventricular dysfunction. In contrast, the significantly higher natriuretic peptide levels in TTS patients are not fully understood. The high natriuretic peptide levels may result from the high stress–strain on the myocardium plus the additional adrenergic stress with further release triggered by inflammation, which are present in TTS. The disproportionately low troponin may result from the anti-apoptotic pathways activated by the β_2 -adrenergic receptor (β_2 AR). Accordingly, the comparative ratio between markers of myocardial necrosis and ventricular strain could be used to differentiate TTS from ACS early in the diagnostic process,²³ with the highest sensitivity and specificity when using the ratio of NT-proBNP to troponin T.²⁴ Indeed, NT-proBNP alone has been included in the HFA diagnostic criteria for TTS,¹ with admission NT-proBNP levels being shown to be an independent predictor of 30-day major adverse cardiac events and long-term mortality.²⁶

Overview of the pathophysiology of Takotsubo syndrome

The exact pathophysiology of TTS is not known. However, important progress has been made over the last decade in our understanding of this syndrome,^{27–32} with an increasing number of publications from experimental and clinical studies that have focused on the pathophysiology of TTS.^{1,2,33} Several hypotheses have been proposed, and it was agreed that currently none of these hypotheses offers a comprehensive and unifying explanation for all the clinical manifestations of TTS.²⁷ Probably, a combination of different triggers together with yet to be discovered mechanisms are causing TTS. In principle, any hypothesis for the pathophysiological basis of TTS needs to provide adequate answers to several questions (Table 2 and Figure 1).

A central role for catecholamines and the sympathetic nervous system in Takotsubo syndrome

Evidence for catecholamines and sympathetic activation in Takotsubo syndrome

A considerable body of evidence supports a role for catecholamines and the sympathetic nervous system in TTS. In addition to being preceded by an identifiable emotional or physical stressor in most cases, TTS is relatively common in conditions of catecholamine excess, such as pheochromocytoma^{34,35} and acute central nervous system injury (e.g. subarachnoid haemorrhage or

Table 2 Key questions regarding the pathophysiology of Takotsubo syndrome

Question	Answer
1. What is the cause of severe mechanical dysfunction (akinesia) and for the characteristic distribution of akinesia in left and right ventricle (apical, midventricular, basal, focal, variants and right ventricular involvement (see Figure 1)?	This is not known and could be related to yet unknown anatomical or physiological characteristics of human heart.
2. Why do some individuals develop TTS while others do not <i>ceteris paribus</i> (all other things being equal)?	This is not known. The susceptibility for TTS could be related to yet unknown genotype.
3. Why do TTS patients generally survive extensive left ventricular dysfunction compared to patients with acute MI since an acute MI involving a similarly sized portion of the left ventricle would usually lead to death?	This is not known. TTS is a cardio-circulatory syndrome. The peripheral component of ventriculo-arterial coupling in TTS could be different (more compensatory to akinesia) than during large MI. It has been shown that cytoprotective Akt/PKB signalling pathways are activated in the akinetic segments during TTS.
4. Why is there such a strong preponderance of postmenopausal females (although not 100%)?	This is not known. Some studies suggest oestrogen and oestrogen receptors may be involved (oestrogen plays permissive role in adrenergic receptor signalling).
5. What are the mechanisms behind the recovery of left ventricular function that typically occurs within a few days?	This is not fully understood. Some studies have shown activation of cytoprotective Akt/PKB signalling pathways in akinetic segments during TTS.
6. What determines why most TTS patients completely recover and are symptom free at follow-up whereas others are left with permanent long-term cardiovascular problems and/or are at risk of recurrent TTS episodes?	This is not fully understood. Some patients may continue to be exposed to negative emotional stress even after the recovery of left ventricular function.
7. Is there a genetic susceptibility to TTS and any homology to known genetic cardiomyopathies?	Some reports suggest existence of genetic susceptibility (e.g. several closest family members developed TTS). The mechanism behind this susceptibility is not known.

MI, myocardial infarction; PKB, protein kinase B; TTS, Takotsubo syndrome.

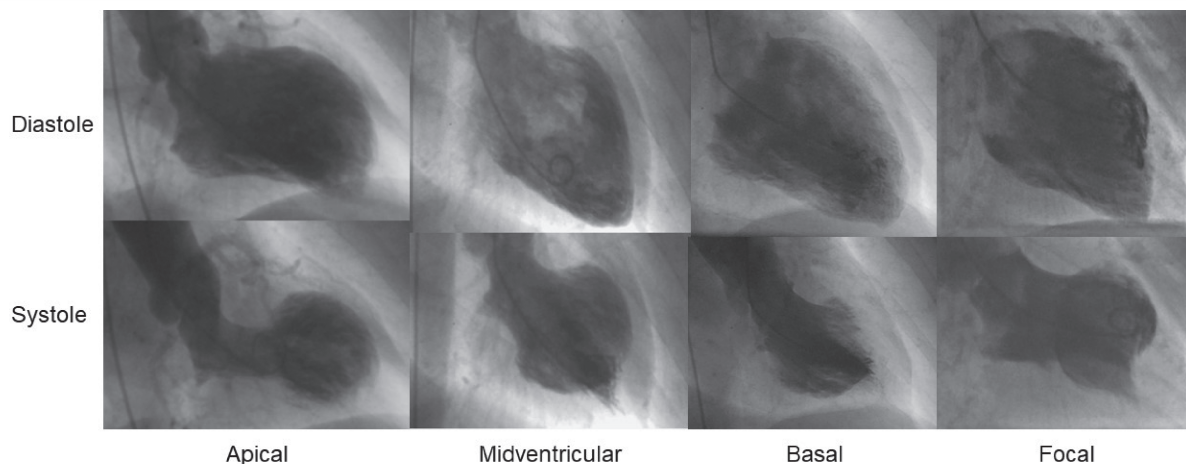


Figure 1 Anatomical variants of Takotsubo syndrome. Representative ventriculograms showing the apical, midventricular, basal and focal Takotsubo syndrome variants during diastole (*upper images*) and systole (*lower images*).

stroke)³⁶; and can be caused or aggravated by iatrogenic administration of epinephrine or dobutamine.^{37,38} Patients with TTS have been reported to have increased local concentrations of norepinephrine in the coronary sinus,³⁹ and have extremely high plasma levels of catecholamines compared to patients with acute heart

failure due to AMI.⁴⁰ Patients with TTS frequently present with sinus tachycardia, and have on average higher heart rates at presentation than patients with ACS.³ Other clinical observations that support a role for catecholamines in the pathogenesis of TTS include reports of increased sympathetic tone in patients with

TTS⁴¹ and the presence of contraction band necrosis in biopsies obtained from TTS patients.⁴⁰ Further evidence for a role of catecholamines in TTS comes from preclinical models where exogenous catecholamine administration reproduces the typical cardiac wall motion abnormalities observed in clinical TTS.^{17,42–44}

Circulating versus locally released catecholamines in Takotsubo syndrome

The mechanisms through which catecholamines cause TTS are incompletely understood, and the relative importance of systemic blood-borne circulating catecholamines versus local intracardiac catecholamine release or both remains to be established. Circulating catecholamines may be a cause of TTS in some patients. High plasma catecholamine levels in TTS patients have been reported during the acute phase⁴⁰; the well-established association between TTS and pheochromocytoma^{34,35}; the occurrence of TTS in a patient with cervical transection at the level of C5–6, in whom the sympathetic nervous system was not intact³⁸; and the fact that iatrogenously administered catecholamines can cause TTS in both humans³⁷ and rats.^{17,42–44}

A role for excessive local catecholamine release by efferent cardiac sympathetic nerves in TTS is supported by reports of elevated norepinephrine levels in the coronary sinus of patients with TTS³⁹; the high sympathetic neural activity in patients with subarachnoid haemorrhage who develop TTS, and the observation that sympathectomy prevented the occurrence of TTS-like LV wall motion abnormalities after subarachnoid haemorrhage in baboons.⁴⁵ Given the known interaction of autonomic nervous system dysfunction in both acute and chronic heart failure, and the potential for a sympathetic storm and the resulting increased oscillatory autonomic reflex activity in the acute phase, the contribution of acute autonomic nervous system dysfunction and its contribution to the development of TTS remain to be studied.⁴⁶

Another possibility is that peptide hormones that were found to be elevated in TTS such as endothelin-1⁴⁷ can induce local sympathetic activation by inhibiting the neuronal re-uptake of released norepinephrine, resulting in an increased catecholamine net release,⁴⁸ raising the questions whether such factors contribute to the elevated sympathetic activity in TTS.

Takotsubo syndrome in the absence of catecholamine or sympathetic overstimulation

Despite the well-established association between catecholamines and TTS, recent clinical studies have reported near-normal catecholamine levels in some TTS cohorts,^{23,49} although this may reflect delayed sampling hours after the stressful trigger when the serum catecholamine levels would be at their peak. In a recent experimental study, TTS-like cardiac dysfunction could be induced solely by administration of the phosphodiesterase (PDE) inhibitor milrinone.⁵⁰ These studies challenge the notion that excess catecholamine is mandatory for TTS to occur. However, milrinone is a PDE inhibitor which results in elevation of cyclic adenosine

monophosphate (cAMP) and the downstream pathways which are also activated by β ARs. Therefore the downstream effects of milrinone via cAMP-protein kinase A (PKA) activation are similar to the actions catecholamines have on the myocardium. This study therefore demonstrates that mimicking downstream effects of catecholamines is sufficient to induce a TTS phenotype and provides a proof of principle supporting the catecholamine hypothesis. Additional experimental studies are required to establish whether experimental TTS can be induced independently of the cAMP-PKA pathway, or whether the milrinone study demonstrates that TTS can also occur when there is amplification of the β AR–cAMP-PKA pathway. This study, and the models using isoproterenol suggest TTS can be initiated by mechanisms independent of vascular dysfunction.

Myocardial catecholamine signalling during the acute phase of Takotsubo syndrome

Myocardial β 1- and β 2-adrenergic receptor activation and signalling during acute Takotsubo syndrome

Epinephrine and norepinephrine exert their effects on cardiomyocytes via β 1ARs and β 2ARs, which in acute situations typically lead to increased contractility. In the normal healthy human heart, the ratio of β 1AR: β 2AR in the left ventricle is approximately 80:20. At normal physiological levels during exercise or minor stress, the endogenous levels of epinephrine and norepinephrine bind to the β 1ARs and β 2ARs and activate coupling to the intracellular $G\alpha_s$ protein signalling pathway. $G\alpha_s$ protein activation, predominantly by β 1ARs but also by β 2ARs, leads to activation of the enzyme adenylyl cyclase (AC) and increases intracellular cAMP.^{51,52} Previous studies have shown that the intracellular cAMP compartmentalization differs in healthy ventricular cardiomyocytes between β 1ARs (cell wide) and β 2ARs (localized). cAMP activates PKA which in turn phosphorylates a number of downstream targets (see above) ultimately resulting in increased contractile activities.⁵³

Pharmacological studies have identified important differences between epinephrine and norepinephrine regarding their effects upon β 2ARs activation at highest doses, which may be similar to the high serum levels reported in TTS patients. Binding of epinephrine, but not norepinephrine, to β 2ARs at supra-physiological epinephrine concentrations leads to coupling to the $G\alpha_i$ rather than the $G\alpha_s$ protein family, known as stimulus trafficking.⁵⁴ Increased PKA activity as a result of β 1AR– $G\alpha_s$ signalling by either epinephrine and/or norepinephrine may phosphorylate the β 2AR and is necessary to prime the receptor for further G-protein coupled receptor kinase (GRK) phosphorylation. This occurs at higher agonist concentrations and GRK is recruited by $G\beta\gamma$. This results in internalization of the β 2AR and stimulus trafficking to β 2AR– $G\alpha_i$ ⁵⁵ in a two-step process. The $G\alpha_i$ protein pathway is called ‘inhibitory’ as $G\alpha_i$ activation leads to reduced AC activity, reduced cAMP concentration and reduced contractile function (negative inotropy).⁵⁴ Excess epinephrine released by severe stress

in TTS may cause acute contractile dysfunction via the β_2 AR and the $G_{\alpha i}$ protein family,⁵⁶ with high initial surges in epinephrine and norepinephrine causing PKA- and GRK-mediated β_2 AR phosphorylation, and subsequent epinephrine activating β_2 AR– $G_{\alpha i}$ signalling and the negative inotropic effect. This could also be triggered by PDE inhibitors such as milrinone, which cause an increase in intracellular cAMP and PKA-dependent β_2 AR phosphorylation.

Another component of the β AR– $G_{\alpha i}$ hypothesis is that across all mammalian hearts studied there is a β AR gradient with a higher density of β_1 ARs and β_2 ARs in the apical ventricular cardiomyocytes compared to those in the basal LV myocardium. This apical-basal β_1 AR and β_2 AR gradient counterbalances the opposite gradient of sympathetic nerve terminal density, which is highest in the basal myocardium and lowest in the apical myocardium. Normal

levels of sympathetic activation lead to catecholamine-mediated LV stimulation, which is relatively balanced, with the neuronal-released norepinephrine highest in the basal myocardium and circulating adrenal gland-released epinephrine having maximal effect in the apical myocardium dictated by the receptor density (Figure 2). Following a stressful episode, the apical myocardium is more sensitive to the higher levels of circulating epinephrine, the region most commonly affected in TTS.²⁷ This was supported by *in silico* modelling studies which introduced apical-basal β_1 AR and β_2 AR gradients and could recreate a Takotsubo-like dysfunction with apical dysfunction following intense receptor activation (Figure 2).⁵⁷

Recently, detailed studies comparing the subcellular signalling after β_1 ARs or β_2 AR activation in apical versus basal ventricular cardiomyocytes identified differences in subcellular signalling and

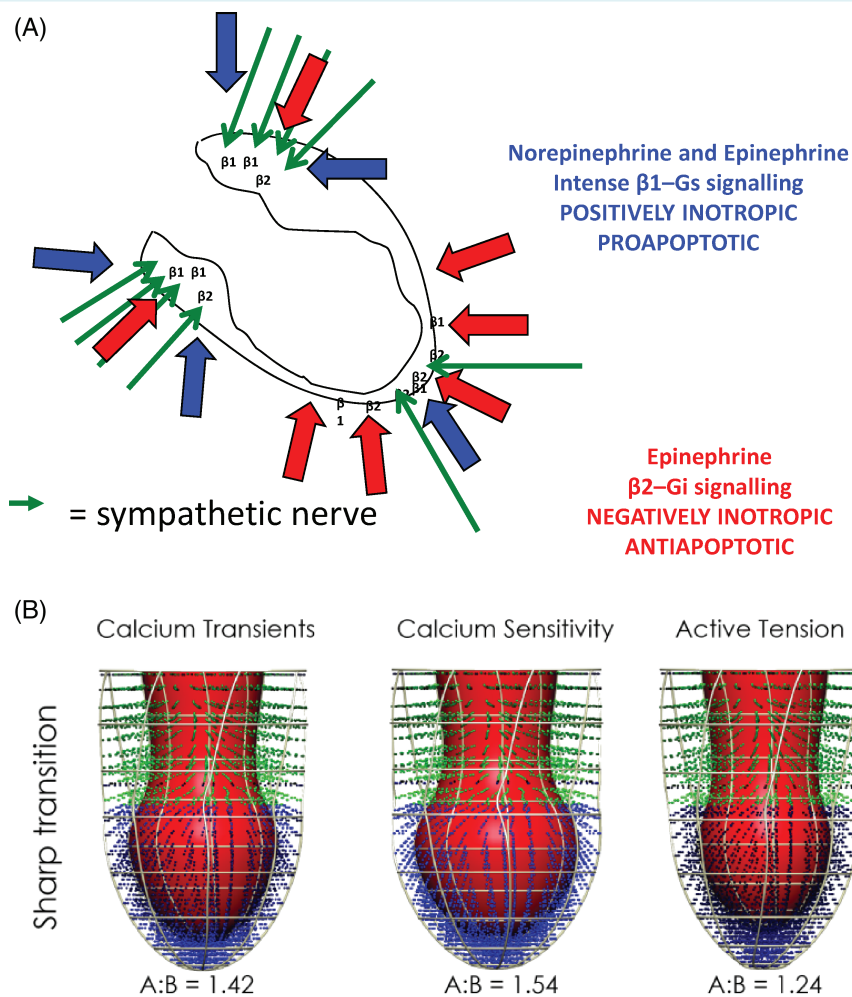


Figure 2 Differential responses of the apical and basal left ventricular myocardium to high epinephrine levels with activation of β_2 -adrenergic receptor– G_i signalling with negatively inotropic and anti-apoptotic signaling predominant in the apical myocardium. (A) Schematic of the opposing β AR and sympathetic innervation gradients in the left ventricle with differential responses to epinephrine released from the adrenal glands and delivered via the coronary circulation versus norepinephrine released by the local sympathetic nerve terminals (adapted from Lyon et al.⁵⁶ with permission). (B) Computer modelling demonstrating the impact of apical-basal β -adrenergic receptor gradients on myocardial calcium transients, myofilament calcium sensitivity and active tension in an *in silico* model of the left ventricle model with the generation of apical dysfunction with sharp transition of the apical-basal β -adrenergic receptor gradients (from Land et al.⁵⁷ with permission).

cAMP responses to the same receptor activation protocol.⁵⁸ These studies showed that apical ventricular cardiomyocytes have greater β 2AR-mediated cAMP responses due to fewer caveolae (sequestration) and lower cAMP buffering by PDEs compared to basal ventricular cardiomyocytes isolated from the same heart (Figure 3).

In the rat epinephrine-induced TTS model, systemic pre-treatment of rats with the selective $G\alpha_i$ inhibitor pertussis toxin prevented the development of TTS, and epinephrine-induced negative inotropic responses in isolated ventricular cardiomyocytes could be blocked by pertussis toxin *in vitro*, converting the epinephrine response from negative to a positive inotropic response.⁴⁴ Recent use of this model with upregulation of TTS-associated microRNAs demonstrated predisposition to TTS generation following epinephrine with reductions in regulator of G-protein signalling 4 and G-protein subunit $G\beta$ as underlying the effect.⁵⁹ Further, the reduction in contractility of apical, but not basal, ventricular cardiomyocytes observed *in vitro* was reversed by inhibition of $G\alpha_i$ with pertussis toxin.⁵⁹

The final component of the β AR- $G\alpha_i$ hypothesis is that increased cardiomyocyte $G\alpha_i$ signalling results in the activation of antiapoptotic pathways which are cardioprotective, particularly the PI3K-Akt and p38MAP kinase pathways. Endomyocardial biopsies from TTS patients during the acute phase demonstrate acute activation of the PI3K-Akt pathway which is believed to be beneficial,⁶⁰ and although PI3K-Akt is not solely activated by β 2AR- $G\alpha_i$, it would be consistent with intense β 2AR- $G\alpha_i$ pathway activation (Figure 3). Inhibiting the β 2AR- $G\alpha_i$ -p38MAP kinase pathway in TTS models resulted in an increased mortality. Two independent groups, with different preclinical TTS models, report that pre-treatment with the selective β 2AR blocker ICI 118551 pre-catecholamine administration resulted in increased mortality in TTS models.^{42,44} This was also observed with pre-treatment with the p38MAP kinase inhibitor SB203580.⁴⁴ We therefore emphasize caution when targeting a pathophysiological substrate in acute TTS as certain pathomechanisms which cause negative inotropic effects may concomitantly induce both beneficial and cardioprotective effects.

Whilst the β AR- $G\alpha_i$ hypothesis may contribute to the pathophysiology of the most common apical TTS variant, it is difficult to reconcile with atypical morphological TTS types where the apex is unaffected.²⁷ It is conceivable that previous stressors may lead to a redistribution of β 1AR and β 2AR expression and density across different regions of the heart. Some patients report chronic stresses preceding their main TTS triggering event, and prior elevated norepinephrine release from sympathetic nerve endings, mainly concentrated in the basal LV myocardium, could alter local β 1AR and β 2AR expression leading to a reverse receptor gradient from that commonly observed, and predisposing an individual to the basal or inverted variant in the context of a subsequent high surge in epinephrine. However, this hypothesis remains to be confirmed.

Although epinephrine but not norepinephrine induced TTS-like cardiac dysfunction in one rat model,⁴⁴ norepinephrine as well as other catecholamines that do not act via the β 2-Gi pathway directly induced TTS-like cardiac dysfunction in rats.¹⁷ In all these studies, endogenous catecholamines may also contribute to TTS pathophysiology. In summary intense activation of β 1AR-Gs and β 2AR-Gs, phosphorylation of β 2AR and the subsequent

shift to negative inotropy, whilst concomitantly activating the anti-apoptotic β 2AR- $G\alpha_i$ pathway, may contribute to the pathophysiology in concert with other mechanisms discussed below.

Abnormalities in G-protein coupled receptor kinase activity and Takotsubo syndrome

The role of catecholamine stress and modification of β ARs signalling pose the basis for the possible role of GRKs in the pathophysiology of TTS, and their contribution to both development and patterns of the disease. GRK 2 and 5 are the isoforms predominant in the heart, where, upon catecholamine binding to β ARs, GRK 2 and 5 are activated and initiate β AR phosphorylation and increased affinity to β -arrestins of the receptors with their consequent desensitization and downregulation.⁶¹ Besides this canonical role, they also present some peculiarities regarding subcellular localization and regulatory roles. GRK2 is a highly dynamic protein, able to quickly accumulate within the cytosol and shuttle to the plasma membrane or other cellular compartments,⁶² such as mitochondria. GRK2 is activated in response to acute stimuli and plays a protective role in cellular function and fate,⁶³ by attenuating the effects of catecholamine toxicity⁶³ and/or preserving mitochondrial and metabolic functions.^{64,65} The accumulation and subcellular compartmentalization of GRK2 has recently been observed in the LV myocardium during the acute phase of TTS.⁶⁶ GRK2 and β -arrestin2 expression at the plasma membrane is higher in TTS than in dilated cardiomyopathy. These observations support the concept that β ARs on the cell membrane are modified by GRK2 and β -arrestin2, and support their role in the development of LV dysfunction in TTS, which presumably prevents catecholamine-induced cardiac tissue damage via a trade-off between cell survival and reduced wall motion.

GRK5 is localized at the plasma and nuclear membrane and GRK5 regulates long-term β AR expression and signalling.^{67,68} GRK5 also effectively phosphorylates inactive G-protein coupled receptor structures and activates the β -arrestin recruitment and the Gs to Gi switch of the β 2AR discussed above. These molecular properties of GRK5 became particularly relevant in the context of increased susceptibility to TTS of those patients bearing GRK5 polymorphisms. Notably, in isolated cells and transgenic mice, the L41Q GRK5 polymorphism, a 'gain-of-function' polymorphism known to increase cardiac GRK5 activity and β AR phosphorylation, enhances β AR desensitization and causes a negative inotropic effect under conditions of acute massive catecholamine release.⁶⁹ Moreover, the same polymorphism was found significantly more frequent in two Italian TTS groups^{70,71} but not confirmed in a larger Australian cohort of 92 TTS.⁷² The role of GRKs and their genetic variants in GRK5 in susceptibility of an individual to TTS are a focus for further research and provide an insight into the mechanism of TTS, particularly concerning GRK modulation and the expression of β ARs as a possible cardioprotective mechanism during TTS. GRK subtype specific inhibitors are under development and will help to dissect the adaptive versus maladaptive roles of these kinases in TTS as well as other heart diseases.

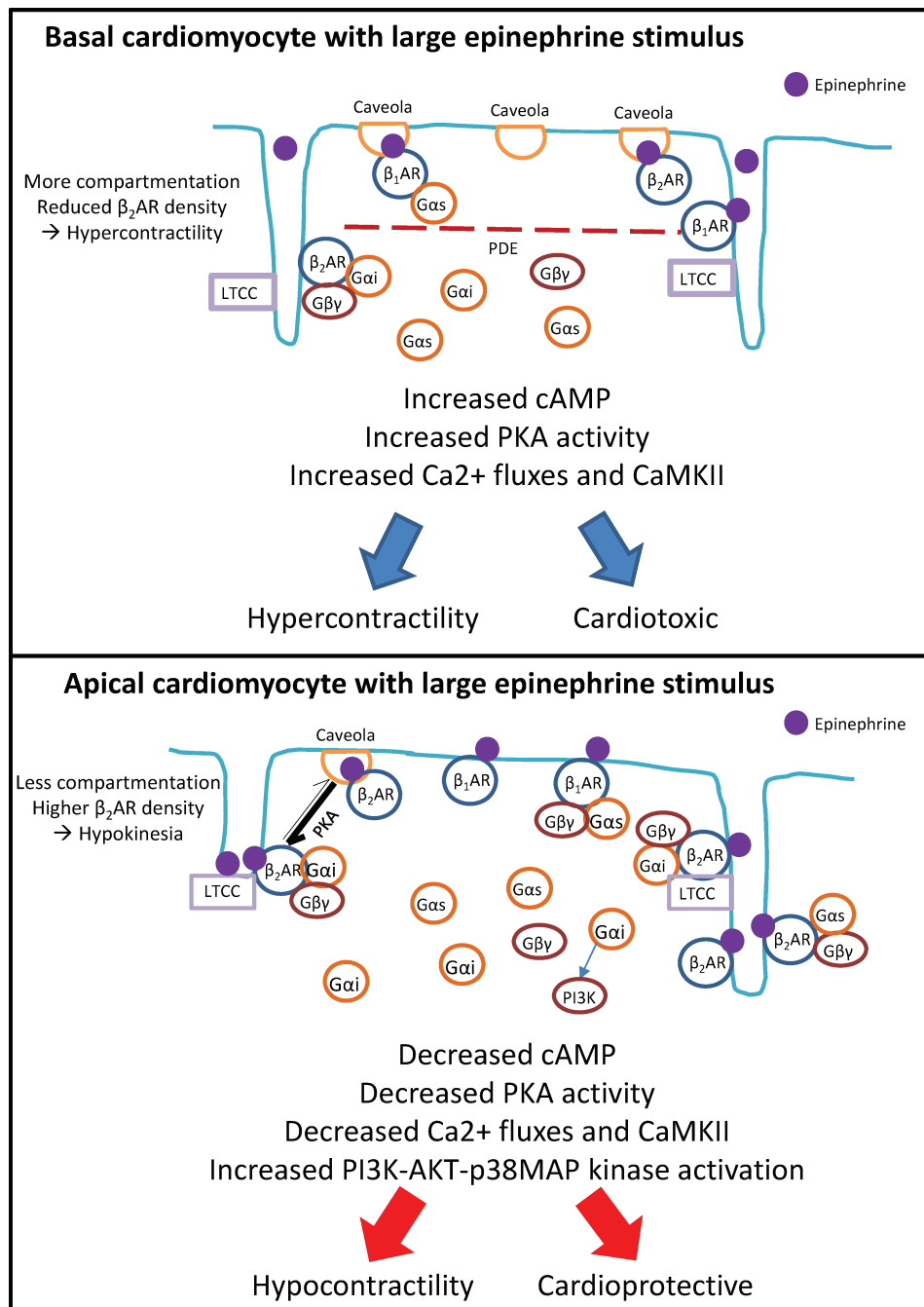


Figure 3 Overview of differences in response of apical and basal ventricular cardiomyocytes to intense β_1 -adrenergic receptor (β_1 AR) and β_2 AR activation on ventricular cardiomyocytes. (*Upper panel*) Surface membrane and secondary messenger signalling in a basal ventricular cardiomyocyte in response to high epinephrine levels. Both β_1 ARs and β_2 ARs are coupled to Gs with the β_2 AR sequestered within caveolae limiting interaction with Gi. The integrated result is increased cAMP, protein kinase A (PKA) activity and PKA-mediated phosphorylation of downstream targets leading to hypercontractility and increased apoptosis. (*Lower panel*) Surface membrane and secondary messenger signaling in an apical ventricular cardiomyocyte in response to high epinephrine levels. Both β_1 ARs and β_2 ARs are coupled to Gs with the β_2 AR on the sarcolemma and following activation and G-protein coupled receptor kinase- and PKA-mediated β_2 AR phosphorylation there is reduced Gs and increased Gi signalling. The integrated result is reduced cAMP, PKA activity and PKA-mediated phosphorylation of downstream targets plus increased PI3K–p38MAP kinase signalling leading to hypocontractility and reduced apoptosis through activation of antiapoptotic pathways. Adapted from Wright et al.⁵⁸ with permission.

Downstream effects of excessive β 1- and β 2-adrenergic receptor activation

Cardiomyocyte calcium cycling and contractility

Catecholamines activate G α s protein via β 1ARs and β 2ARs, thereby increasing intracellular cAMP-AC-PKA activity, ultimately leading to enhanced calcium influx which enhances contractility.⁷³ PKA also phosphorylates the β 2AR in a negative feedback loop which may be relevant as discussed above. Whilst the exact role for altered calcium signalling in TTS is not clear, the effect of catecholaminergic stimulation on cardiomyocyte calcium handling is well understood. Excessive β 1AR and β 2AR activation by high levels of circulating catecholamines results in exaggerated calcium influx into the ventricular cardiomyocytes in experimental studies, resulting in downregulation of sarcoplasmic Ca²⁺-adenosine-triphosphatase 2a (SERCA2a) expression and activity, partly from increased inhibition by phospholamban. An increased phospholamban/SERCA2a ratio reduces Ca²⁺ affinity and could lead to contractile dysfunction. Elevated cytoplasmic Ca²⁺ levels also cause increased ryanodine receptor phosphorylation via calmodulin kinase II (CaMKII), and possibly PKA, leading to increased sarcoplasmic reticulum (SR) calcium release and leak.

A recent induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) cell line from TTS patients showed increased calcium transients and kinetics.⁷⁴ Alterations in calcium handling were recently observed in a preclinical model of TTS when TTS-associated microRNAs were upregulated, with reduced calcium transient amplitude, SR calcium content and peak calcium current amplitude in apical cardiomyocytes, found to result from downregulation of CACNB1 (L-type calcium channel Cav β subunit).⁵⁹ This disparity could reflect the differences in β 1AR and β 2AR which result in temporospatial differences in contractility in TTS with an initial hypercontraction and subsequent apical depression.

Excessive calcium influx can also lead to contraction band necrosis, which has been observed on endomyocardial biopsies from patients with TTS.¹⁴ Excessive calcium influx and SR calcium leak also causes mitochondrial Ca²⁺ overload, initially stimulating but ultimately reducing mitochondrial metabolism and increasing mitochondrial reactive oxygen species (ROS) generation. Administration of high-dose catecholamines to rats results in apical contractile dysfunction and intracellular lipid deposition which also supports acute mitochondrial dysfunction,⁴² as well as apical apoptosis⁷⁵ and fibrosis.⁷⁶ Similar findings have been observed in clinical TTS.^{77,78}

Catecholamines and myofilament function during acute Takotsubo syndrome

β 1ARs and β 2AR stimulation results in PKA-mediated phosphorylation of the myofilament proteins troponin I, titin and myosin binding protein C, which decreases myofilament Ca²⁺ sensitivity and passive force development, and increases kinetics of cross-bridge cycling.⁷⁹ These PKA-mediated myofilament changes exert a positive lusitropic effect. In addition, PKA-mediated myofilament protein phosphorylation enhances length-dependent activation which underlies the Frank–Starling mechanism.

The biological changes in myofilament function during TTS have not been studied and are an area for future research. The initial catecholamine surge in TTS is expected to result in hyperphosphorylation of these proteins, while hypophosphorylation of the PKA myofilament targets is expected upon predominance of β 2AR–G α i signalling. Both hyper- and hypophosphorylation would impair the important myofilament regulatory mechanisms that underlie proper activation and relaxation of cardiomyocytes. Accordingly, iPSC-CMs from TTS patients showed a higher sensitivity and response to catecholamine-stimulation compared to controls.⁷⁴ Troponin I phosphorylation tended to be higher in TTS iPSC-CMs compared to controls upon catecholamine stimulation. It is tempting to speculate that the bi-phasic acute changes in PKA signalling initially result in hyperphosphorylated troponin I with reduced myofilament Ca²⁺ sensitivity, and subsequent hypophosphorylated myofilaments with increased Ca²⁺ sensitivity. These opposite changes in myofilament Ca²⁺ sensitivity are observed in dilated and hypertrophic cardiomyopathy, respectively, and have been shown to trigger diverse cardiac remodelling.⁸⁰ Moreover, the opposite changes in myofilament behaviour are target for novel therapies.^{81–83} Future studies in TTS rat models and human iPSC-CMs are needed to define the acute, bi-phasic changes in myofilament function, and test promising new therapies targeting myofilament function.

Catecholamines and gene expression during acute Takotsubo syndrome

Gene expression changes are classically thought to be a critical step towards cardiac remodelling consisting of cardiac hypertrophy, fibrosis and cardiomyocyte death, eventually leading to chronic heart failure. The historical perception is that changes in gene expression take some time and are the cause of chronic rather than acute heart failure such as TTS. However, this has been challenged because the classical steps of cardiac remodelling were shown to be dissociated from cardiac dysfunction in genetic mouse models.⁸⁴ Moreover, a recent study showed that mice lacking an epigenetic factor, named histone deacetylase 4 (HDAC4), develop cardiac fatigue (reduced systolic ejection fraction) during exercise, which recovers within 10 min after exercise.⁸⁵ HDAC4 is uniquely responsive to catecholamines because it is inactivated by a direct interaction with one downstream kinase of β ARs, CaMKII.^{86,87} PKA, another downstream kinase of β ARs, which is typically activated in acute situations, partly and specifically antagonizes CaMKII–HDAC4 interaction and subsequent gene expression changes.^{86,88} Changes in gene expression may cause cardiac fatigue (= transient heart failure) under stress.⁸⁵ Catecholamine-induced PKA activation led to proteolysis of HDAC4, resulting in an N-terminal fragment that inhibits the transcription factor myocyte enhancer factor 2 (MEF2). MEF2 in turn inhibits another transcription factor, Nr4a1, that activates the hexosamine biosynthetic pathway with consequent O-linked glycosylation and inhibition of the calcium handling protein STIM1. This study strongly suggested that abnormal calcium handling is mediated by metabolic processes downstream of a rapid epigenetic signalling pathway rather than by classical signalling events that ‘directly’ connect the receptor

level to a calcium handling effector protein. Moreover, acute cardiac dysfunction was induced within a 1 h time course strongly suggesting that gene expression changes clearly account for the maintenance of cardiac function.

Interestingly, Nr4a1 was also recently found to be highly regulated in the iPSC-CM model for TTS described above,⁷⁴ suggesting that at a given genetic basis (that still needs to be elucidated) for this pathway might be sensitized and contributes to the pathophysiology of TTS upon catecholaminergic stress through the epigenetic mechanisms described by Lehmann et al.⁸⁵ These recent data call for the combination of TTS mouse models with genetic models to investigate the potential causative roles of single molecules of the suggested pathway in the pathogenesis of TTS. One model which studied the neuronal pathogenesis of TTS (stroke-induced TTS)⁸⁹ was characterized by elevated Nr4a1 levels, and functional experimental validation of this pathway is required with identification of potentially druggable targets.

Catecholamines, cardiac arrhythmias and electrophysiology during the acute phase of Takotsubo syndrome

Arrhythmias are common in the acute phase of TTS, occurring in 20–26% of patients.^{90–92} They are associated with a substantial risk of severe complications and short as well as long-term mortality.^{93–95} The range of cardiac arrhythmias during the acute phase of TTS are summarized in Table 3.

The most frequent arrhythmia is atrial fibrillation (AF) of new onset affecting 7–26% of this elderly patient population with a high risk profile for AF.^{3,91–93,95} In the majority of patients, new onset of AF is observed within the first 3 days after symptom

onset.⁹² The recurrence rate of AF in TTS during follow-up is low (11%).⁹⁵ Acute LV dysfunction with elevated end-diastolic pressure and/or mitral regurgitation results in left atrial pressure and volume overload which may trigger the onset of AF. High catecholamine levels leading to electrical instability⁹⁶ and transient left atrial dysfunction even in sinus rhythm⁹⁷ are additional factors contributing to the development of AF. Moreover, inflammation has been associated with the initiation and perpetuation of atrial arrhythmias.⁹⁵

A striking feature of TTS are repolarization abnormalities with transient T-wave inversion and QT interval prolongation developing over the first 72 h following presentation.^{92,98–101} Both coronary microvascular dysfunction¹⁰² and high levels of epinephrine are known to prolong the QT interval.¹⁰³ The dynamic ECG changes coincide and correlate with the apico-basal gradient of myocardial oedema assessed by cardiac magnetic resonance imaging. The interstitial oedema creates repolarization inhomogeneities either regional or transmural which result in T-wave inversion and prolongation of the QT interval.^{99,100} The repolarization abnormalities slowly resolve over a period of several months in parallel to the slowly resolving myocardial oedema as evidenced by cardiac magnetic resonance imaging.^{99,100,104}

Life-threatening ventricular arrhythmias have been reported in 4–9% of patients with TTS.^{90,92,94,98,105} They typically develop within the first 3 days of presentation⁹² frequently associated with a QRS duration >105 ms,⁹⁴ hyperacute J waves¹⁰⁶ and prolongation of the QTc interval >500 ms.^{98,100} Pause-dependent polymorphic ventricular tachycardia (torsades de pointes, TdP) with degeneration into ventricular fibrillation is the most common manifestation.^{98,100} Monomorphic ventricular tachycardia due to a

Table 3 Cardiac arrhythmias secondary to Takotsubo syndrome

Arrhythmia type	Prevalence acute phase	Time frame after symptom onset	Long-term follow-up	Mechanisms
Atrial fibrillation of new onset	7–26%	Median 2 (range 1–15) days	Low recurrence rate of atrial fibrillation in TTS (11%) related to age and comorbidities	<ul style="list-style-type: none"> - Catecholamine induced electrical instability - LA pressure and volume overload due to LV dysfunction ± mitral regurgitation - Transient catecholamine induced LA dysfunction - Inflammation
QT interval prolongation	54–82%	Maximal QT-interval on day 2–3	Normalization of repolarization changes mostly within 3 months	<ul style="list-style-type: none"> - Coronary microvascular dysfunction - Catecholamine induced QT prolongation - Apico-basal gradient of myocardial oedema
Polymorphic VT/TdP Monomorphic VT	4–9%	Within 72 h QRS duration >105 ms prolonged QTc interval	No TdP or VT during follow-up	<ul style="list-style-type: none"> - Repolarization inhomogeneities due to myocardial oedema - Pause-dependent TdP - Re-entry mechanism
AV block (complete AV block, 2:1 AV block)	2.8–4.5%	On presentation or within 3 days	Transient for days Persistent for months Permanent	<ul style="list-style-type: none"> - Catecholamine excess, reflex bradycardia - Functional 2:1 block due to pronounced QT interval prolongation - Myocardial oedema - Pre-existing AV conduction abnormality - Persistent structural changes due to TTS

AV, atrioventricular; LA, left atrial; LV, left ventricular; TdP, torsades de pointes; TTS, Takotsubo syndrome; VT, ventricular tachycardia.

reentry mechanism has also been observed in a substantial number of patients.^{100,105} Since male patients with TTS may have a more pronounced QT interval prolongation due to a higher LV mass¹⁰⁷ with an early peak of the QT interval 3 to 6 h after symptom onset,¹⁰⁸ they may be more susceptible to develop TdP than women early in the course of TTS.¹⁰⁹ The catecholamine surge increases automaticity, action potential duration, QT interval and QT dispersion and induces early and delayed afterdepolarization. Myocardial oedema and inflammation increase the transmural dispersion of repolarization. All these mechanisms may result in abnormal automaticity, triggered activity and cardiac re-entry contributing to the onset of ventricular arrhythmias in TTS.¹¹⁰

Ventricular arrhythmias appear to be less common in TTS than in AMI (given the more extensive segmental dysfunction in TTS with up to 60% of ventricular myocardium affected). Whether the cardioprotective pathways activated also serve to preserve myocardial conduction and reduce arrhythmic risk is not known and is worthy of investigation. Indeed, in a recent study from Sweden based on 215 TTS patients, it has been shown that inverted T waves (but no other ECG pattern) were associated with a significantly lower risk of ventricular tachycardia/fibrillation (adjusted odds ratio 0.27, 95% confidence interval 0.10–0.76, $p = 0.010$).¹¹¹

The general effects of low-dose catecholamine stimulation on cardiac electrophysiology are well studied. β AR stimulation by isoproterenol is associated with elevated heart rate, lability in T-wave morphology and QT prolongation. While heart rate increases in a steady dose-dependent manner after isoproterenol infusion, an early rise in heart rate is observed due to dual agonism of α ARs and β ARs via epinephrine without further progress at higher doses¹⁰³ or even reflex bradycardia and atrioventricular (AV) node block.¹¹²

Epinephrine also facilitates lability in T-wave morphology as well as QTc prolongation in healthy humans. Taken together, T-wave alterations and QTc prolongation present a common finding of catecholamine stimulation. In a review of case reports of drug-induced TTS, epinephrine was the most commonly reported agent, followed by dobutamine.¹¹³ High-dose epinephrine with subsequent development of TTS is associated with ST-segment elevation (40%), T-wave abnormality (22.5%) and ST-segment depression (12.5%).¹¹⁴ Experimentally, vagal reflex bradycardia due to initial hypertension has been elaborated.⁴⁴ Low-dose epinephrine infusions have been used as a QT stress test to identify channelopathies in the electrophysiology laboratory¹¹⁵ and the prolonging effect of epinephrine on QT time in healthy individuals is well documented.¹⁰³

In dobutamine stress test-induced TTS, the predominant ECG finding was ST-segment elevation.^{116,117} In TTS after accidental injection of norepinephrine, ST-segment and T-wave changes were observed in the precordial leads.¹¹⁸ Also, left bundle branch block has been observed after norepinephrine injection for suspected anaphylaxis.¹¹⁹ Norepinephrine in the treatment of sepsis was associated with the development of TTS, ventricular tachycardia and ST-segment alterations.^{120,121} Experimentally, rats with norepinephrine-induced TTS showed a lower heart rate than rats with TTS after isoproterenol.¹⁷

On a cellular level, epinephrine stimulation of ventricular cardiomyocytes facilitates an increase in action potential duration by increasing intracellular Ca^{2+} .¹²² Treatment of cardiomyocytes with isoproterenol caused a dose-dependent increase in field potential duration whereas beating rate and signal amplitude were decreased. Since the QT interval reflects the durations of action potential depolarization and repolarization of ventricular myocytes,¹²³ this finding is consistent with QT prolongation in TTS.¹²⁴ Also, the observed loss of electrical signal amplitude in cardiomyocytes exposed to isoproterenol is mirrored by the clinical finding of QRS complex attenuation in TTS patients.¹²⁵

The initial electrophysiological studies from the iPSC-CM model of TTS also suggest abnormal electrophysiological responses to catecholamines in TTS iPSC-CMs compared to controls.⁷⁴ Isoproterenol application resulted in electrical silence in more than half the TTS iPSC-CMs studied, but rarely in control iPSC-CMs, and the mechanism(s) for these differences remain to be determined but may involve abnormal metabolic responses and mitochondrial function.⁷⁴

Sinoatrial block in the context of TTS is rare occurring in 0.9–1.4% of the patients, with almost half of them receiving a permanent pacemaker.^{94,105} New onset of complete AV block occurs in 2.8–4.5% of patients in the acute and subacute phase of TTS and may be transient for a few days, persistent for months, or permanent.^{90,92,94,105,126} Catecholamine excess with increased vagal tone and reflex bradycardia may explain the occurrence of an AV block in the acute setting.¹¹² In the context of severe prolongation of the QTc interval, a functional 2:1 AV block may be present, which is fully reversible after regression of the QTc interval.¹⁰⁰ In a substantial proportion of patients, the AV block may persist for months despite recovery of LV ejection fraction or proves to be permanent requiring pacemaker implantation.^{94,100,105} Preexisting AV conduction disturbance in patients of advanced age¹⁰⁰ as well as persistent long-term structural changes as a consequence of TTS¹⁹ may be responsible for a permanent AV block in these patients and points to a particular sensitivity of the conduction fibres to oedema, inflammation and ischaemia.⁹⁴ Recent experimental data suggest that tachycardia may play a 'permissive role' for development of akinesia in apical segments. In the rat model of isoprenaline-induced Takotsubo-like phenotype, no rat with third-degree AV block at baseline developed apical akinesia after administration of isoprenaline.¹²⁷

Myocardial inflammation during and following acute Takotsubo syndrome

Influx of inflammatory cells, coupled with increased levels of ROS and nitrosative stress, and deposition of collagen and fibrosis, have also been observed in clinical TTS, as well as in rat models of Takotsubo-like dysfunction.^{28,75,76,128–130} However, despite repeated demonstration of oedema and inflammation in affected myocardial segments in TTS patients, it is not clear whether inflammation plays a causative role in the pathogenesis of TTS or whether it is merely a consequence of tissue injury.^{40,60,131–135}

A recent multicentre study¹³⁶ showed that compared with control subjects, patients with TTS had greater ultra-small iron

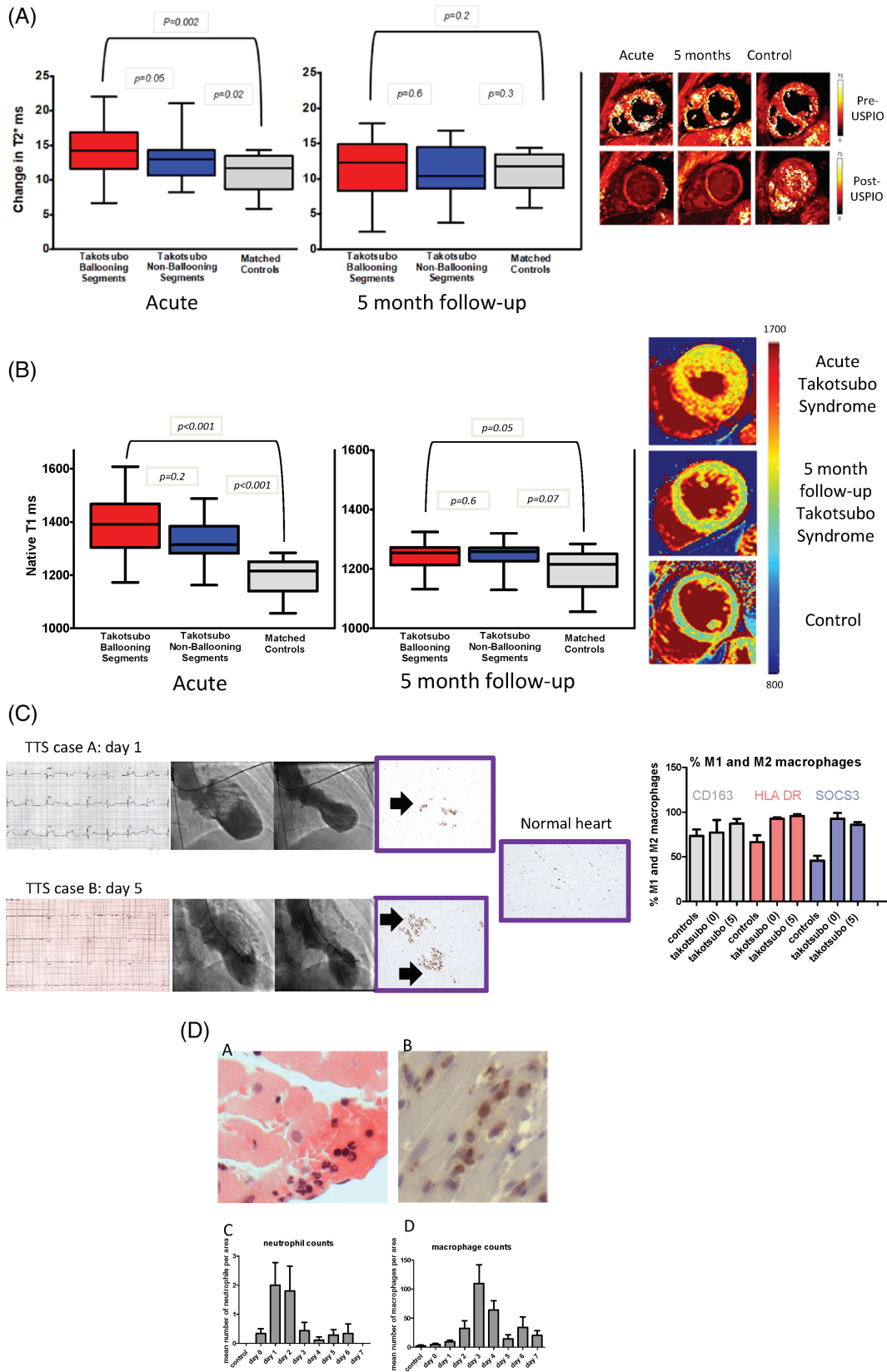


Figure 4 Continued

oxide superparamagnetic particle (USPIO) retention in both ballooning and non-ballooning left ventricle during the acute phase (Figure 4). As USPIOs are phagocytosed exclusively by activated tissue macrophages and not by any other inflammatory cells, this study demonstrated conclusively that macrophages are the main cellular protagonists of myocardial cellular inflammation in acute TTS (in contradistinction with acute myocarditis which is lymphocyte-mediated). Additionally, serum interleukin-6 and chemokine (C-X-C motif) ligand 1 concentrations as well as classic CD14++CD16 monocytes were increased, whereas intermediate CD14++CD16+ and non-classic monocytes were reduced in patients with TTS. At 5-month follow-up, USPIO enhancement was no longer detectable in the LV myocardium, although persistent elevations in serum interleukin-6 concentrations and reductions in intermediate CD14++CD16+ monocytes remained. Post-mortem examination of human hearts from patients who demised during the acute phase of the condition demonstrated that these macrophages are predominantly of M1, pro-inflammatory type, as opposed to the reparative M2 type.¹³⁰ The presence of M1 macrophages and the persistence of the intermediate (CD14++CD16+) monocyte subset at 5-month follow-up are strongly indicative of lesser repair and more pro-inflammatory state compared to similar stages of patients with AMI.

The temporal changes in inflammatory cell infiltrate have also been studied in the rat preclinical model of TTS induced by isoproterenol injection, demonstrating that these inflammatory changes are triggered specifically by catecholamines (Figure 4). There is an initial increase in neutrophil infiltration in the affected hypokinetic segments during the first 48 h followed by an increase in macrophage infiltration peaking at 72 h following isoproterenol injection. Both M1 and M2 macrophage populations were present, and there was a corresponding increase in myocardial and

circulating levels of the anti-inflammatory cytokines interleukin-6 and interleukin-10.

Therefore, TTS is characterized by a myocardial macrophage inflammatory infiltrate, changes in the distribution of monocyte subsets, and an increase in systemic pro-inflammatory cytokines. Many of these changes persisted for at least 5 months, suggesting a low-grade, chronic inflammatory state.¹³⁶ This offers the explanation for the low-grade, chronic inflammatory substrate and the evolution of acute Takotsubo into a long-term heart failure phenotype.¹⁹

Increased cardiac inflammation could result from the direct myocyte injury, and/or myocardial ischaemia, caused by the extreme adrenergic stimulation. ROS production by over-activated cardiomyocytes may also attract cytotoxic neutrophils, T cells and macrophages, aggravating cardiomyocyte dysfunction (stunning) or even necrosis. Factors released by the neurons during intense adrenergic activation in the heart may also attract inflammatory cells which induce cardiomyocyte and endothelial cell dysfunction, as well as fibrosis. Whereas increased activation of the cholinergic parasympathetic system has proven anti-inflammatory properties in the myocardium, (ortho)sympathetic activation causes cytotoxic T cells and monocytes to invade the heart and cause injury (as previously reviewed¹³⁷).

One area of future research therefore is to evaluate the development and termination of the inflammatory response during and following an acute episode of TTS. One hypothesis is whether the subgroup of TTS survivors, which long-term cardiac problems including diastolic impairment, reduced contractile reserve, arrhythmias and/or chest pain, have persisting inflammation and, in these cases, TTS evolves from an acute to a chronic inflammatory condition. Another interesting hypothesis is whether pre-existing inflammatory diseases predispose to TTS. An interesting observation is the TTS cases triggered by immune checkpoint inhibitors

Figure 4 Evidence of myocardial inflammation from clinical and preclinical studies of Takotsubo syndrome (TTS). (A) Myocardial inflammation detected by ultra-small superparamagnetic iron oxide particle (USPIO) enhanced T2* cardiac magnetic resonance imaging. T2* values were highest in affected left ventricular segments during the acute phase of TTS compared to unaffected segments and matched controls (left panel) consistent with higher uptake of USPIO in myocardial macrophages during the acute presentation phase. The T2* signal had returned to normal at 5-month follow-up consistent with attenuation of the macrophage inflammatory signal (central panel). The right panel shows an example of T2* maps before and after USPIO administration in a Takotsubo patient compared with a matched control subject at presentation and after 5-month follow-up (from Scally et al.¹³⁶ with permission). USPIO uptake into the myocardium in ballooning (red) and non-ballooning (blue) segments versus matched control subjects (grey) measured with T2* cardiac magnetic resonance imaging. (B) Increased myocardial oedema on native T1 mapping. Cardiac Magnetic Resonance –native T1 mapping of ballooning (red) and non-ballooning (blue) left ventricular myocardial segments versus matched control (grey) demonstrating intense myocardial oedema in Takotsubo patients during the acute phase (left panel) and significant resolution (albeit not complete) of myocardial oedema at 5-month follow-up (central panel). On the right panel exemplary T1 maps showing intense myocardial oedema (and increased wall thickness/mass) during the acute phase, with significant resolution of the oedema and restoration of normal wall thickness at 5-month follow-up, compared with a matched healthy control. (C) Characterization of the inflammatory response from post-mortem examinations. Electrocardiogram, end-diastolic and end-systolic frames from left ventriculograms and myocardial tissue samples from two patients with TTS who died during the acute presentation. The thick arrows point towards clusters of CD68 positive cells (macrophage staining) in comparison to the rare, scattered tissue resident macrophages in a healthy control. On the right, the graph shows higher percentage of CD68 positive macrophages staining for M1, pro-inflammatory markers (HLA-DR and SOCS3) in Takotsubo patients compared to matched controls, whereas staining for M2 (reparative) marker CD163 is not different (adapted from Wilson et al.¹³⁰ with permission). (D). Characterization of the inflammatory response in the rat model of TTS demonstrating an initial influx of neutrophils in days 1–2 (A and C) followed by a macrophage infiltration of the left ventricular myocardium (B and D) in days 3–4 (adapted from Wilson et al.¹³⁰ with permission).

which activate T lymphocytes and increase inflammation,¹³⁸ implying that inflammation itself may play a causative role.

An increased incidence of TTS has been reported with acute COVID-19 infection.¹³⁹ This may partly reflect higher emotional anxiety in individuals aware of the risk of acute COVID-19, along with the cytokine storm, increased inflammation, endothelial dysfunction and increased sympathetic responses that occurs during acute COVID-19 infection.

Abnormal cardiac metabolism and energetics during the acute phase of Takotsubo syndrome

Cardiac energetics (the phosphocreatinine/adenosine triphosphate ratio obtained non-invasively at³¹ P-magnetic resonance spectroscopy) is severely reduced acutely in TTS and only partly recovers by 4 months.¹⁴⁰ Several groups have demonstrated abnormalities in uptake of myocardial substrates, although some of these reports remain at variance, with some showing increased or decreased myocardial glucose uptake and impaired fatty acid metabolism.¹⁴¹ The precise nature of the metabolic abnormalities in the hearts of TTS patients remains unclear, and whether they are contributory and causative, or the consequence of TTS, is not known. Clinical trials of metabolic therapies could be designed to evaluate if they could offer benefit.

Ventricular haemodynamics during the acute phase of Takotsubo syndrome

Intracardiac systolic pressure has been proposed to play a role in the initiation of TTS.¹⁴² High LV end-systolic pressure increases myocardial wall tension. Increased wall tension has been suggested to cause TTS either via direct effects on the contractile apparatus¹⁴² or by inducing relative myocardial ischaemia (i.e. demand:supply mismatch).¹⁴³ Conditions leading to high myocardial wall tension include LV outflow tract obstruction (LVOTO), and during acute LVOTO wall tension is particularly high in the cardiac apex. LVOTO can be detected in up to one third of all TTS cases with the apical variant at the time of echocardiography,¹⁴⁴ and it is not known how many patients have LVOTO prior to presentation.^{142,145} How alterations in intra-cardiac pressure and wall tension could explain other non-apical variants of TTS is more difficult to explain. It is possible that alterations in electrical conduction and deformation patterns lead to development of wall tension in other cardiac regions^{146,147} or alter β AR distribution and expression.

Another possible result of acutely elevated LV intracavity pressure is an acute endocardial injury. The endocardium is a source of important paracrine factors including nitric oxide for the adjacent myocardium, and acute disturbance or disruption of LV endocardial function has been proposed as a contributing factor in TTS.¹⁴⁸

Relationship between Takotsubo syndrome and acute myocardial stunning

The sudden occurrence of temporary myocardial mechanical dysfunction with the absence of irreversible myocardial damage is

known as myocardial stunning. Myocardial ischaemic stunning is defined as temporary mechanical dysfunction that occurs in the setting of myocardial ischaemia and that persists after resolution of ischaemia, with the absence of irreversible histological damage.¹¹ Myocardial stunning is closely related to myocardial conditioning, a term used to describe short-term alterations in protein synthesis and post-translation modification that occur in response to brief episodes of ischaemia, and that renders cardiomyocytes resistant to subsequent ischaemic insults.¹⁶ Many of the same cardiac genes and intracellular signalling pathways are activated in ischaemic stunning and TTS.¹⁴⁹ TTS has been suggested to be a form of catecholaminergic stunning.^{143,149}

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