Implications of catecholamine-related pathophysiology in cardiomyopathy

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Abstract

Although secretion of catecholamines is critical to cardiovascular homeostasis, there is ample evidence that prolonged or marked catecholamine release may engender cardiovascular dysfunction, both in the short and long term. The processes involved include induction of oxidative stress and of inflammation, and the consequences include cell death (apoptosis), resultant fibrosis and both temporary and permanent contractile dysfunction of the heart. Congestive heart failure, both acute and chronic, represents a condition in which catecholamine effects are ultimately deleterious, and indeed many treatments of heart failure target this anomaly.

The subject of this thesis is an examination of two particular aspects of catecholamine-related cardiovascular pathophysiology. The first issue examined is the phenomenon of (autonomic) cardiac denervation, a process which occurs extensively in CHF and leads, via impaired catecholamine re-uptake, to increased tissue exposure to catecholamines. The second is Tako-tsubo cardiomyopathy (TTC), a form of "stress-induced" cardiomyopathy occurring predominantly in post-menopausal women, and apparently precipitated at least in part by bursts of catecholamine hypersecretion.

The study of CHF utilised the technique of ^{123}I-MIBG imaging to quantitate cardiac denervation. The implications of the extent of denervation on (a) evolution of LV dysfunction and (b) late arrhythmogenesis were examined in a cohort of 45 patients. The data showed no significant association between extent of denervation and either of these endpoints. The results therefore cast into question the potential utility of such technique as a means of prognostication and therapeutic decision-making in patients with CHF.

The studies concerning TTC have two major components:

(a) an examination of the release of natriuretic peptides in association with TTC, and the potential for this release to be of diagnostic utility in the disease.

and (b) an evaluation of nitric oxide (NO) signalling in the acute and recovery phase of TTC.

Studies with brain natriuretic peptide (BNP) and its inactive co-product, N-terminal proBNP (NT-proBNP), revealed that plasma levels were markedly elevated in TTC, that extent of elevation correlated both with catecholamine markers and with severity of the individual attack, and the levels remained elevated for at least 3 months. Furthermore, comparison with a cohort of age-matched females who presented with acute myocardial infarction (AMI) suggested that NT-proBNP levels might form part of a diagnostic algorithm to separate TTC from AMI.

Studies with NO signalling were initiated in the expectancy that this would be impaired in TTC. However, it was found that there was "paradoxical" accentuation of NO effects and of biochemical determinants of NO formation in TTC. Despite the apparently paradoxical nature of these findings, it is proposed that the adverse impact of catecholamines on the heart in TTC might be potentiated by products of the NO signalling cascade.

In summary, these studies provide new insights into mechanisms of catecholamine toxicity on the heart, and hint at relationships between catecholamines, natriuretic peptides, and NO as complex modulation of both injury and recovery. On the other hand, the CHF studies suggest that extensive treatment with agents such as angiotensin converting enzyme inhibitors (ACEI) and β-adrenoceptor antagonists may blunt cardiac toxicity of catecholamines.

Signed Statement

I, Thanh Ha Nguyen, certify that this work 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.

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- 2. Neil, C.J., Nguyen, T. H., Sverdlov, A.L., Chirkov, Y.Y., Stansborough, J., Beltrame, J.F., Kucia, A.M., Zeitz, C.J., Frenneaux, M.P., Horowitz, J.D. (2012). "Can we make sense of takotsubo cardiomyopathy? An update on pathogenesis, diagnosis and natural history." Expert Rev Cardiovasc Ther 10(2): 215-21.

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Dedication

This work is dedicated to my husband Trung and to my children, Giang and Hieu. Now I will have more time for you.

List of Abbreviations

- ADMA − Asymmetric DiMethylArginine

- ANP − Atrial Natriuretic Peptide

- AC − Adenylyl Cyclase

- ACEI − Angiotensin Converting Enzyme Inhibitors

- AICD − Automated Implantable Cardio-Defibrillator

- APO − Acute Pulmonary Oedema

- ARBs − Angiotensin Receptor Blockers

- ARDS − Adult Respiratory Distress Syndrome

- AT1 − Angiotensin Receptor Type 1

- AT2 − Angiotensin Receptor Type 2

- ATP − Adenosin Triphospate

- BH4 − tetraHydroBiopterin

- BMI − Body Mass Index

- BNP − Brain-type Natriuretic Peptide

- BP − Blood Pressure

- BRS − Baroreceptor Sensitivity

- CAD − Coronary Artery Disease

- cAMP − Cyclic Adenosine Monophosphate

- ^{11}C-HED − ^{11}C-hydroxyephedrine

- CHF − Chronic Heart Failure

- cGMP − Cyclic Guanosine Monophosphate

- CK − Creatine Kinase

- CMR − Cardiovascular Magnetic Resonance

- CNP − C-type Natriuretic Peptide

- COMT − Catechol-Ortho-Methyl Transferease

- CRP − C-Reactive Protein

- CRT − Cardiac Resynchronisation Therapy

- DDAH − Dimethylarginine DimethylAminoHydrolase

- DCM − Dilated Cardiomyopathy

- DE-MRI − Delayed-enhancement Magnetic Resonance Imaging

- DHPG − DiHydroxyPhenylGlycine

- DM − Diabetes Mellitus

- MR − Mineralocorticoid Receptor

- MRB − Mineralocorticoid Receptor Blockers

- ECG − Electrocardiography

- ECTB − Emory Cardiac Toolbox

- ED − Emergency Departments

- eGFR − estimated Glomerular Filtration Rate

- eNEP − Ectoenzyme Neutral EndoPeptidase

- eNOS − endothelial Nitric Oxide Synthase

- EP − Electrophysiological

- ET1 − Endothelin-1

- GC − Guanylyl Cyclase

- Gi − inhibitory G-protein

- Gs − stimulatory G-protein

- GTP − Guanosine Triphosphate

- HMR − Heart to Mediastinum Ratio

- HR − Heart Rate

- HRT − Heart Rate Turbulence

- HRV − Heart Rate Variability

- hs-CRP − high sensitivity C-Reactive Protein

- HT − Hypertension

- iNOS − inducible Nitric Oxide Synthase

- LAD − Left Anterior Descending Coronary Artery

- LBBB − Left Bundle Branch Block

- LV − Left Ventricular

- LVDd − Left Ventricular Diastolic Dimension

- LVDd − Left Ventricular Systolic Dimension

- LVEF − Left Ventricular Ejection Fraction

- MAO − MonoAmine Oxidase

- MI − Myocardial Infarction

- ^{123}I-MIBG − ^{123}I-metaiodobenzylguanidine

- MPI − Myocardial Perfusion Imaging

- MSNA − Muscle Sympathetic Nervous Activity

- MRI − Magnetic Resonance Imaging

- NET − Noradrenaline Transporter

- NGF − Nerve Growth Factor

- nNOS − neuronal Nitric Oxide Synthase

- NO − Nitric Oxide

- NOS − Nitric Oxide Synthase

- NSTEMI − Non-ST Elevation Myocardial Infarction

- NSVT − Non-sustained Ventricular Tachyarrhythmia

- NT-proBNP − N-terminal proBNP

- NYHA − New York Heart Association

- PARP − Poly(ADP-ribose) Polymerase

- PCWP − Pulmonary Capillary Wedge Pressure

- PET − Positron Emission Tomography

- PKA − Protein Kinase A

- PKG − Protein Kinase G

- RAAS − Renin Angiotensin Aldosterone System

- RCA − Right Coronary Artery

- ROS − Reactive Oxygen Species

- SAECG − Signal-averaged ECG

- SCD − Sudden Cardiac Death

- sGC − soluble Guanylate Cyclase

- SPECT − Single Photon Emission Computed Tomography

- STEMI − ST Elevation Myocardial Infarction

- 4D-MSPECT − 4 Dimensional Single photon Emission Computed Tomography

- Tc − Technetium

- Tl − Thallium

- TTC − Tako-Tsubo Cardiomyopathy

- VASP − Vasodilator-Stimulated Phosphoprotein

- VF − Ventricular Fibrillation

- VT − Ventricular Tachyarrhythmia

- WMSI − Wall Motion Score Index

- WR − Wash-out Rate

Chapter 1

Literature review

1.1 Introduction

Several stressors, including physical, emotional, and chemical stimuli may stimulate the sympathetic nervous system leading to an increase in catecholamine release (Wortsman 2002). Although physiological effects of catecholamines are potentially beneficial as a component of the "fight or flight" response (Wortsman 2002), excessive release of catecholamines both acutely and chronically has been reported to exert a number of harmful effects on the heart. Catecholamines exert their cardiac actions by occupying various adreceptors, including α and β-adrenoceptors. The physiological effects of adrenoceptor stimulation and their downstream mediation are summarised in Table 1.1.1. In general, it has been considered that α-adrenoceptor stimulation exerts predominantly vasocontrictor (including coronary vasocontrictor) effects, while the various β-adrenoceptors exert disparate effects, with variable coupling to nitric oxide synthase (NOS) activation (Moniotte et al. 2001, Brum et al. 2006). The potentially cytotoxic effects of high concentrations of catecholamines on the myocardium have been observed frequently in animal models (Inoue & Zipes 1987, Bonnefont-Rousselot et al. 2002, Okumura et al. 2007) and can be inferred clinically via the production of ischaemia and arrhythmias (Inoue & Zipes 1987, Dhalla et al. 2008). However, it is important to emphasise that these effects may occur both directly and indirectly. Indirect effects might be mediated by regional coronary vasoconstriction in the presence of increased oxygen demand. However, ex-

posure of cardiomyocytes in cell culture to high concentrations of isoproterenol induces cellular apoptosis, in part via increased oxidative stress (Iwai-Kanai et al. 1999). The mechanisms underlie this "direct" cardiotoxicity will be discussed in Section 1.2.1.5.

Adrenoceptor type (Signalling pathway)	Primary location	Predominant effects	Secondary effects
α (Gs)	Blood vessels	Vasocontriction	
β1 (Gs/Gi)	Myocytes	Positive chronotropy Positive inotropy	Increased relaxation
β2 (Gs/Gi/NO)	Myocytes Blood vessels	Positive chronotropy Positive inotropy	Increased relaxation Vasodilatation
β3 (Gi/NO)	Myocytes Blood vessels	Negative chronotropy Negative inotropy	Increased relaxation Vasodilatation

Table 1.1.1: Physiological effects of adrenoceptor stimulation *(Gi = inhibitory G-protein, Gs = stimulatory G-protein, NO = Nitric oxide)*

Chronic effects of catecholamines have been well established in patients with systolic chronic heart failure (CHF). In the absence of myocardial infarction, CHF is a progressive process which involves changes in the structure and geometry of the left ventricle (LV), leading to an increase in LV wall stress, an impairment of myocardial energetics (DeMarco et al. 1988) and decreased contractile performance. Initially, cardiac remodelling is a compensatory process, which then renders the heart incapable of maintaining myocardial function and cardiovascular homeostasis, leading to ongoing CHF progression. Sympathetic nervous system activation plays an important role in disease progression and survival of patients with heart failure (Kaye et al. 1995). Hyperactivity of the sympathetic nervous system results in a rise in plasma noradrenaline concentration in order to increase heart rate, contractility and venous return to preserve cardiac output via chronotropic and inotropic responses. However, chronic elevations of catecholamine concentrations decrease the sensitivity and regulation of myocardial β-adrenoceptors, leading to progression of systolic dysfunction, aggressive remodelling

of the left ventricle and stimulation of myocardial fibrosis (Bristow 1984). Furthermore, there is simultaneous activation of profibrotic pathways (Weber et al. 1995), although the stimuli for this process include not only catecholamines but also aldosterone secretion (Cittadini et al. 2003, Bos et al. 2005): fibrosis results in progressive worsening of both systolic and diastolic cardiac function. This process also results in electrical remodelling of the LV myocardium, leading to develop micro-reentry circuits, which trigger ventricular tachyarrhythmia (VT) or ventricular fibrillation (VF) (Fernandes et al. 2007). In the presence of previous myocardial infarction, all of these processes also take place in non-infarcted myocardium (DeMarco et al. 1988).

In this chapter, effects of catecholamines on the heart, especially the role of catecholamines in the pathophysiology of CHF, are reviewed. The special case of Takotsubo cardiomyopathy (TTC) as a "variant" form of catecholamine cardiomyopathy is discussed. Finally, the scope of the material covered in this thesis is reviewed.

1.2 Effects of catecholamines on the heart

1.2.1 Physiology and kinetics of catecholamines

1.2.1.1 Synthesis of catecholamines

The three major forms of catecholamines: dopamine, noradrenaline, and adrenaline, are physiological products of a common synthetic pathway. These transmitter substances induce different sympathetic effects on the various organs. Noadrenaline is secreted mainly by the sympathetic nerve varicosities through several steps, beginning with the synthesis of DOPA from the amino acid tyrosine via hydroxylation in the axoplasm of the adrenergic nerve endings. Dopamine is synthesised by decarboxylation of DOPA and then transported into the secretory vesicles, where it is hydroxylated to noradrenaline. The final step in this synthetic pathway, that of conversion of noradrenaline to adrenaline, occurs principally in the adrenal medulla via methylation (see Figure 1.2.1).
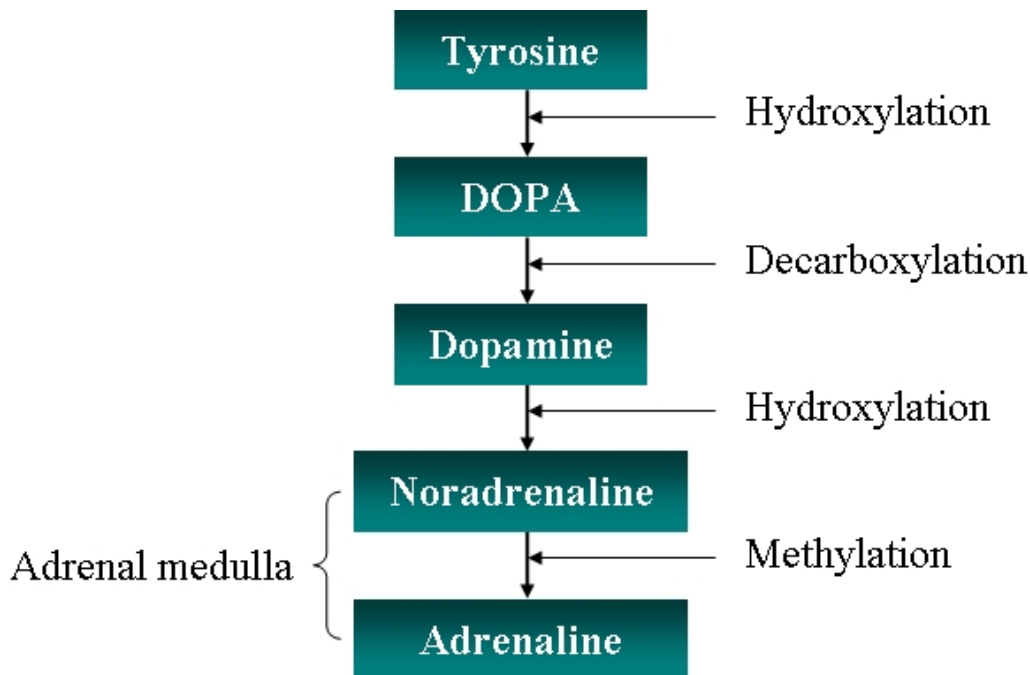
Figure 1.2.1: Synthesis of noradrenaline

1.2.1.2   Release of catecholamines

When stimulated, the noradrenaline and adrenaline stored in the varicosities of the sympathetic fibre are secreted via the depolarisation process of the fibre membrane which allows calcium ions to diffuse into the varicosities and dislodge the transmitters into the exterior environment.

1.2.1.3   Clearance of catecholamines

Once secreted, catecholamines can be cleared from the synaptic cleft by three major mechanisms: (1) 50-80% are subject to reuptake by the active neuronal transporter, a Na^+/Cl^--dependent, high affinity, low capacity and active transport protein (uptake 1) (Leineweber et al. 2002); (2) 20% of noradrenaline produced by sympathetic nerve terminals is cleared by diffusion into the surrounding circulation; (3) the remainder is inactivated by tissue enzymes, including catechol-ortho-methyl transferease in tissues (COMT), and by monoamine oxidase (MAO) in the nerve endings.

These processes of reuptake and diffusion of the released noradrenaline from its site of action are rapid. Therefore, the duration of action of the noradrenaline secreted from

the adrenergic nerve endings is only a few seconds. Circulating catecholamines released by the adrenal medulla tend to exert more prolonged effects because they need time to (1) diffuse into tissues and (2) to be inactivated by COMT.

1.2.1.4 Catecholamines receptors within the myocardium

Noradrenaline and adrenaline bind to the various specific cardiovascular adrenergic receptors (see Table 1.1.1) (Bylund 2007). However, there are a number of subtypes of these receptors. The post-synaptic $\alpha 1$ is the most abundant among the α-adrenoceptors. The proportion of α-adrenoceptors in myocardium, as distinct from blood vessels, may increase if the density of β-adrenoceptors decreases (Elsinga et al. 2004). In healthy subjects, 70-80% of the β-receptors are $\beta 1$-adrenoceptors, 20-30% are $\beta 2$-adrenoceptors, and about 2% are $\beta 3$-adrenoceptors, while in CHF patients, the ratio of $\beta 1$:$\beta 2$ may decrease to 1:1 (Brodde 1988, Bristow et al. 1986). Although the cardiac sympathetic nerve endings are more concentrated at the base of the LV, the density of β-adrenoceptors is greater at the LV apex (Murphree & Saffitz 1987). $\beta 1$ and $\beta 2$-adrenoceptors are coupled to adenylyl cyclase (AC) via stimulator Gs proteins, while $\beta 3$-adrenoceptors are coupled via inhibitory Gi proteins to both adenylyl cyclase and nitric oxide synthase (NOS) activation (Kuschel et al. 1999) (Figure 1.2.2).

1.2.1.5 Physiological effects of catecholamines

The binding of catecholamines to adrenoceptors cause several changes in the structure of the affected proteins leading to the excitation or inhibition of the myocytes by (1) changes in membrane permeability to ions, for example Ca^{2+}, or (2) activation or inhibition of several enzymes such as adenylyl cyclase or cyclic guanosine monophosphate (cGMP)-dependent protein kinase. Noradrenaline and adrenaline differ in exiting receptor binding characteristics. While noradrnaline stimulates the β-adrenoceptors to a lesser extent compared to that of the α-adrenoceptors, adrenaline stimulates the α and β receptors equally, with resultant differences in physiological effects.
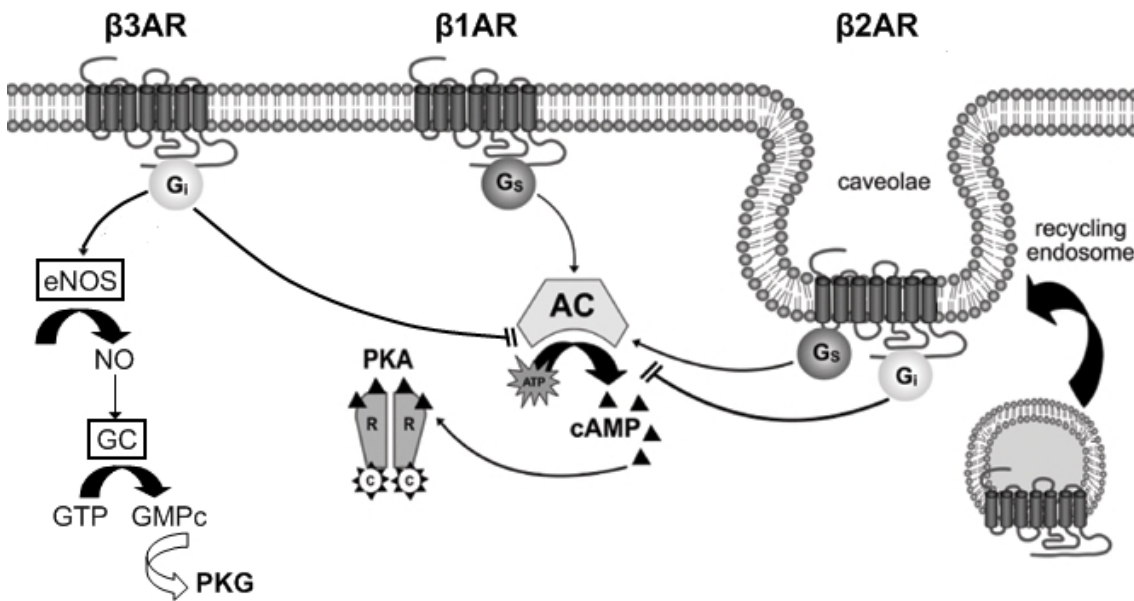
Figure 1.2.2: Intracellular signalling pathways of β-adrenoceptors in cardiomyocytes [AC = adenylyl cyclase, cAMP = cyclic adenosine monophosphate, βAR = β-adrenoceptors, eNOS = endothelial nitric oxide synthase, GC = Guanylyl Cyclase, Gi = inhibitory G-protein, Gs = stimulatory G-protein, GPT = Guanosine Triphosphate, GMPc = Guanosine Monophosphate cyclase, PKA = Protein Kinase A, PKG = Protein Kinase G (modified from Brum et al. (2006)]

In general, stimulation of the sympathetic nervous system results in an increase in blood vessel constriction, arterial pressure, heart rate and myocardial contractility. Other cardiac effects of catecholamines mediated by $\beta 1$ and $\beta 2$-adrenoceptors are positive dromotropic and bathmotropic effects (Takei et al. 1992). Stimulation of $\beta 2$ and $\beta 3$ vascular receptors also causes vasodilatation, in contrast to the constrictor effect of vascular α-adrenoceptors stimulation (Pourageaud et al. 2005).

It has been suggested that $\beta 3$-adrenoceptors are stimulated mainly under conditions of extensive catecholamine release ("catecholamine storms"), leading to opposite effects to those found with $\beta 1$-adrenoceptor stimulation, such as negative inotropy (Varghese et al. 2000, Kohout et al. 2001). Activation of $\beta 3$-adrenoceptors induces more cyclic GMP formation via endothelial nitric oxide synthase (eNOS), which activates the

cGMP-dependent protein kinase and stimulates phosphodiesterase, therefore inducing myocardium relaxation (Lee et al. 2010) (Figure 1.2.2). Stimulation of β 3-adrenoceptor-coupled Gs proteins by catecholamines activate adenylyl cyclase to generate cAMP from ATP, leading to the activation of protein kinase (PKA). As a results, several functional proteins, including L-type Ca channels, phospholamban, and troponin, are phosphorylated (Sulakhe & Vo 1995). The phosphorylation of L-type Ca channels results in an increase in Ca^{2+}-influx into the myocyte, releasing Ca^{2+} from the sarcoplasmic reticulum, thus increasing contractility. In contrast, the phosphorylation of phospholamban and troponin I induces lusitropic effects on the myocardium due to more Ca^{2+}-uptake into the sarcoplasmic reticulum and a reduced affinity of Ca^{2+} to troponin C (Langer 1992).

Stimulation of the adrenergic nerves to the adrenal medulla releases a large amount of adrenaline (about 80%) and noradrenaline (about 20%). Circulating catecholamines produced by the adrenal medulla have similar cardiac effects to direct sympathetic stimulation. However, these indirect effects last longer due to the slow removal duration (about 2-4 minutes) of the hormones from the blood. Moreover, the effects of adrenaline slightly differ from noradrenaline as follows: (1) because adrenaline induces predominantly β-adrenoceptors, it produces substantial positive inotropic effects but exerts weaker effects on blood vessel constriction; and (2) adrenaline exerts greater effects on tissue metabolism (Thomas 2011).

1.2.2 Pathological effects of catecholamines

As already mentioned (Section 1.2.1.5), there is substantial evidence that catecholamines may induce cardiotoxic effects. This evidence arises from (1) the clinical entity of catecholamine cardiomyopathy (Kassim et al. 2008); (2) various experimental studies in intact animals and in cell culture. Rather than summarise the totality of this evidence, examples of each category will be briefly discussed.

1.2.2.1 Historical considerations

Acute effects of catecholamines were first reported in 1942 [as recently reported by Cannon (2002)], who described anecdotal death from fright and called it "voodoo" death.

Cannon (2002) postulated that the cause of death might relate to intense overactivity of the sympathico-adrenal system. His work was continued by his student, Curt Richter, who noticed that there was a very high rate of sudden cardiac death in a rodent colony, in whom the whiskers were clipped. Moreover, rats with trimmed whiskers, a procedure which destroys their proprioceptive mechanism, could swim at a water temperature of 93^0C for much shorter time than control rats (Richter 1957). Moreover, administration of atropine improved survival, while cholinergic agents worsened it, and adrenalectomy was not protective. In 1971, Engel also reported 160 sudden deaths caused by extreme life-threatening events (Engel 1971). The histological changes in those patients include neutrophil infiltration, conection band necrosis, and fibrosis, which are different from the changes in myocardial ischaemic injury (Jiang & Downing 1990).

However, such observations, whether clinical or based on animal studies, are ill-defined both mechanistically and regarding relative risk.

In order to present logically the argument that paroxysmal exposure to high catecholamine concentrations may induce development of a congestive cardiomyopathy, it is appropriate to start with evidence from more specific whole animal and isolated tissue studies.

1.2.2.2 Experimental studies

a. Studies in whole animals

(i) The isoproterenol-treated rat model   This model has been used extensively since its initial description by Rona et al. (1959), who found that isoproterenol induce myocardial necrosis, which was similar to ischaemic injury. The extent of severity of this "infarct-like" necrosis is dose-dependent. Essentially, rats injected with approximately 85mg/kg/day isoproterenol for two consecutive days, showed relatively limited myocardial necrosis in the apical and midventricular areas, but also prominently periapical hypokinesis. Furthermore, these changes are induced, at least in part, by inducing negative β-adrenoceptor-mediated inotropic responses (Brouri et al. 2004) and substantial inflammatory activation (Murray et al. 2000). Finally, the changes are largely reversible

on withdrawal of isoproterenol.

This preparation has therefore been utilised extensively in studies of acute heart failure (Feng & Li 2010).

(ii) Studies in larger animals The isoproterenol-treated model has also been used in larger animals such as dogs and guinea pigs in a number of studies (Uechi et al. 2002, Takei et al. 1992, Soltysinska et al. 2011).

b. Studies in isolated hearts and cell culture Exposure to high concentrations of catecholamines in vitro also results in cardiomyocyte toxicity and serious cell damage. Indeed, histological evidence from isoproterenol treated myocardium reveals that catecholamines induce cardiac hypertrophy, inflammatory processes, and contraction band necrosis (Todd et al. 1985). Administration of oxidised isoproterenol to isolated rat heart was shown to produce intracellular Ca^{2+} overload, an increase in cAMP, activation of α and β-adrenoceptors, and the formation of oxidative catecholamines (Mann et al. 1992). Furthermore, chronic exposure of neonatal and adult rat myocytes to isoproterenol was shown to induce apoptosis by activation of the sympathetic nervous system and the reactive oxygen species-TNF alpha caspase signalling (Communal et al. 1998, Staudt et al. 2003). Finally, it has been shown that in a rat LV myocardium exposed to high adrenaline levels, the $\beta 2$ adrenoreceptor Gs coupling is switched to the β adrenoreceptor Gi coupling leading to a negative inotropic response (Heubach et al. 2004).

1.2.2.3 Evidence in humans

Evidence for the potential cardiotoxic effects of catecholamines in humans is largely inferential and limited. Categories of evidence are as follows:

a. Catecholamine-producing tumours Phaeochromocytoma is usually associated with systemic hypertension rather than CHF (Meune et al. 2006). However, both global (Agarwal et al. 2011) and regional (Kim et al. 2010) patterns of LV systolic dysfunction have been reported in this context, thus establishing that "catecholamine cardiomyopathy" really occurs in humans.

b.   Cardiotoxic effects of agents that increase catecholamine effects   The most clear-cut evidence of this relates to arrhythmogenesis rather than isolated LV dysfunction associated with tricyclic antidepressant overdose (Crome 1986) and also of agents reducing both noradrenaline and serotonin reuptake (Fangio et al. 2007, Vinetti et al. 2011).

c. The special case of tako-tsubo cardiomyopathy (TTC)   While this is closely related to catecholamine release, this condition will be described in more detail in Section 1.4.

d. Heart failure following myocardial infarction   The release of catecholamines post myocardial infarction is both acute and chronic (Slavikova et al. 2007). The possibility that a component of chronic LV dysfunction post myocardial infarction may be catecholamine-induced is supported by studies suggesting that β-adrenoreceptor blockade may preserve LV dysfunction in such patients (Doughty et al. 2004, Fonarow et al. 2007). Nevertheless, it must be conceded that LV dysfunction post infarct is multifactorial, rather than purely catecholamine-mediated.

1.2.3   Mechanisms of catecholamine-induced myocyte damage

While these studies clearly establish the potential for toxic effects of catecholamines to occur both directly and indirectly, they do not delineate the underlying mechanisms. Several mechanisms, including hypoxia and ischaemia, coronary spasm, metabolic alterations, high-energy phosphate depletion, intracellular calcium overload, electrolyte disturbances and oxidative stress, have been suggested to explain the link between myocyte damage and high concentrations of catecholamines (Figure 1.2.3). In this section, these mechanisms are reviewed and the role of oxidative stress as a link between catecholamine release during stressful events and catecholamine-induced cardiomyopathy is highlighted.
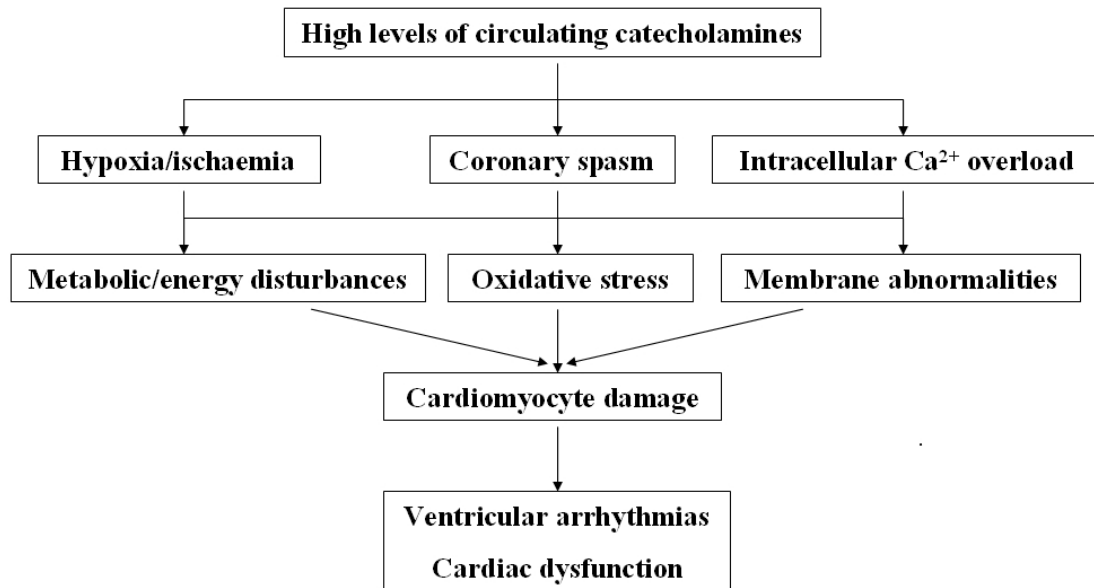
Figure 1.2.3: Postulated mechanisms of catecholamine-induced cardiomyocyte damage

From a purely cellular point of view, there are a number of critical issues involving receptors and their signalling pathways.

1.2.3.1 Which receptors are involved?

It has been reported that high circulating catecholamine levels activate mainly the β-adrenoceptor signalling pathways (Mann et al. 1992). In particular, β2-adrenoreceptor stimulation may switch coupling mechanisms from Gs to Gi, leading to a negative inotropic response (Heubach et al. 2004). Moreover, exposure to extremely high levels of catecholamines may activate the β3-adrenoreceptor signalling, which results in a negative inotropic response (Gauthier et al. 1998), partially mediated by downstream NO release (Kou & Michel 2007).

1.2.3.2 Secondary pathophysiological effects of catecholamines

When the whole heart/whole organism is considered, the increase in oxygen and decrease in coronary flow caused by α-adrenoceptor-mediated constriction of peripheral

and coronary vessels becomes relevant.

High circulating levels of catecholamines have direct effects on adrenoceptors in smooth muscle cells and cardiomyocytes, leading to myocardial hypoxia and ischaemia (Corder et al. 1984). Intravenous administration of isoproterenol at both low and high doses causes increases in heart rate, myocardial contraction, and cardiac work. Moreover, catecholamine toxicity can lead to coronary artery spasm which contributes to more oxygen demand and less oxygen supply (Beamish et al. 1984). Coronary artery spasm leads to decreased coronary flow and reduced myocardial perfusion with a minor change in haemodynamic parameters (Dhalla et al. 2008).

Metabolic disturbance due to high levels of catecholamines results from fat mobilisation and free fatty acid accumulation which lead to mitochrondrial uncoupling. As a consequence, the process of oxidative phosphorylation uncoupling increases oxygen consumption and reduces the amount of high energy phosphate store, which is called the "oxygen wasting effect" of catecholamines (Raab 1943). It has been suggested that the oxidised products of catecholamines but not catecholamines themselves cause coronary insufficiency which can occur in the absence of coronary artery stenosis (Yates et al. 1981, Rump et al. 2001). Furthermore, an increase in oxygen consumption was found to be associated with the "oxygen wasting effect" of catecholamines, but not with an increase in cardiac workload (Raab 1943): a low dose of noradrenaline was shown to induce maximal inotropic effect without increase in oxygen consumption, while a larger dose results in a negative response and an increase in oxygen consumption (Clancy et al. 1967).

1.2.3.3   What are the relevant post-receptor signalling pathways?

a. Cyclic adenosine monophosphate (cAMP)   An excessive increase in levels of catecholamine can induce myocardial toxicity via cAMP mediated calcium overload (Bolli & Marban 1999).

b. Ca^{2+} overload   Increased catecholamine levels are associated with changes in intracellular calcium^{2+} handling. Catecholamine toxicity causes excessive AMP formation

(see above) which is known to alter Ca^{2+} entry and intracellular release and/or uptake. However, it is worthy of note that catecholamine effects on the activation of adenylyl cyclase have been demonstrated to be biphasic. Initially, high levels of catecholamines stimulate Ca^{2+} entry through the sarcolemmal and sarcoplasmic reticulum, while during the process of catecholamine induced cardiomyopathy, this effect was shown to cease (Dhalla et al. 2008). This biphasic effect was also found in an experiment involving cardiomyopathy induced by a high dose of isoproterenol (Tappia et al. 2001). Furthermore, intracellular Ca^{2+} overload was found to activate Ca^{2+} dependent ATPases to impair mitochondrial oxidative phosphorylation and reduce high energy phosphate stores, leading to cardiac dysfunction and mechanical and electrical disturbances (Schaffer & Tan 1985, Minezaki et al. 1994, Vassalle & Lin 2004).

c. Reactive oxygen species (ROS) Catecholamines exert their effects indirectly via formation of oxidised products, including adrenochromes, their metabolised products (aminolutins) and oxyradicals (Dhalla et al. 2008, Costa et al. 2011). The oxidation of catecholamines involves 2 enzymes: COMT and MAO. Adrenochromes have been shown to damage cell membrane and deplete cation channels and Ca^{2+} handling, while oxyradical products result in oxidative stress and further contribute to myocyte Ca^{2+} overload (Adameova et al. 2009). A significant change in Ca^{2+} handling abilities was found when oxidised isoproterenol was infused into isolated rat hearts or adrenochrome was incubated with sarcoplasmic reticular and sarcolemmal vesicles (Takeo et al. 1980, Takeo et al. 1980a, Dhalla et al. 1996). Moreover, adrechrome also directly impairs mitochrondrial function, leading to Ca^{2+} accumulation and a decrease in oxidative phosphorylation (Takeo et al. 1981, Taam et al. 1986).

Intravenous administration of adrenaline in rats has been shown to induce cardiac remodelling, myocardial fibrosis, and ventricular arrhythmias (Bonnefont-Rousselot et al. 2002, Sethi et al. 2009): this was reversed by antioxidants such as N-acetyl-L-cysteine, vitamin E, and vitamin C, providing incremental evidence that ROS generation plays an important role in acute catecholamine toxicity (Singal et al. 1981, Sethi et al. 2009).

d. Potential for peroxynitrite generation   A number of studies in experimental models have indicated that the formation of peroxynitrite ($ONOO^-$) in circumstances of increased ROS formation is associated with persistent NO generation (Munzel et al. 2005). The importance of $ONOO^-$ generation is its potential to increase apoptotic cell death via activation of poly(ADP-ribose) polymerase (PARP) receptors (Ungvari et al. 2005). While presence of $ONOO^-$ is readily detected via tyrosine nitration (Ungvari et al. 2005), pathophysiological effects can only be reduced by interruption of signalling pathways, for example with PARP antagonists (Szabo et al. 2006) or $ONOO^-$ scavengers (McCarty et al. 2009).

To date, the potential involvement of $ONOO^-$ in catecholamine-induced cell injury may be inferred, but no detailed studies are available.

1.3   Roles of catecholamines in chronic heart failure

Systolic chronic heart failure (CHF) is a complex, multifactoral syndrome, which is characterised by diminution in inotropic reserve capacity of the left ventricle. Several factors can exacerbate the progression of CHF. Among these, neuroendocrine systems, including catecholamines and the sympathetic nervous system, the renin-angiotensin-aldosterone system, vasopressin, endothelin-1, natriuretic peptides, nitric oxide, and cytokines, all play important roles. In this section, chronic heart failure, its complications, diagnostic methods and management are briefly reviewed, followed by a detailed discussion of the role of sympathetic nervous function, the implications of regional denervation, and techniques for the assessment of sympathetic nervous activity in CHF.

1.3.1   Definition of chronic heart failure (CHF)

Many definitions of chronic heart failure have been used over the last 50 years, referring to several complicated features of this syndrome such as haemodynamics, oxygen consumption, and exercise capacity. Recently, the European Society of Cardiology has proposed a definition of CHF which highlights the importance of clinical symptoms and physical signs of CHF (Dickstein et al. 2008). CHF is a complex syndrome, which in-

volves (1) clinical symptoms, such as dyspnoea and/or fatigue leading to a decreased exercise ability; (2) fluid retention; and (3) objective evidence of structural abnormalities or an impairment of cardiac function at rest. In past decades, CHF had been more intensively studied in terms of pathophysiology, diagnosis and management. Diastolic CHF, which is present in more than half of CHF patients, is often considered as a separate disease (Abhayaratna et al. 2006), although diastolic and systolic CHF may frequently co-exist (Dickstein et al. 2008). The question of whether there is a distinct pathophysiology in diastolic heart failure is clinically important [see (Borlaug & Paulus 2011) for review], but from the point of view of the research summarised in this thesis, diastolic CHF remains a peripheral issue.

1.3.2 Incidence of CHF

The aging population in developed countries and the decrease in mortality of all causes of cardiovascular diseases has resulted in an increase in the incidence of patients living with CHF (Hunt 2005). In Europe, about 15 million patients have CHF. The overall prevalence of symptomatic and asymptomatic CHF is approximately 4% (Mosterd & Hoes 2007). In Australia, approximately 300 000 patients suffer from CHF with 30 000 new CHF patients each year (Australian Institute of Health and Welfare 2004). Moreover, the incidence of CHF increases dramatically in people over 65 years of age. About 80% of patients admitted to a hospital due to CHF progression are elderly (Masoudi et al. 2002). A similar trend has been reported in the Australian population, with an incidence of 1% in people aged 50 to 59 years old, rising to over 10% in those over 65 years, and 50% in those over 85 years old (Krum et al. 2006).

1.3.3 Aetiology of CHF

Causes of systolic CHF include ischaemic and non-ischaemic heart disease. Coronary artery disease (CAD) is the leading cause of CHF, and accounts for approximately 50% of CHF patients who are under 75 years old (Fox et al. 2001, Maggioni et al. 2010). The prevalence of CHF due to in the population over 75 years is even higher. A pooled

study of more than 20 000 subjects in 13 randomised, multicenter heart failure trials suggested that CAD is the major cause of CHF (about 70% of patients) (Gheorghiade & Bonow 1998). It has been shown in many studies that the presence of CAD in patients with CHF is related to a poor outcome (Smith et al. 2001). Patients with previous myocardial infarction (MI) had a 2-fold higher chance of progressive CHF hospitalisation and a 4-fold higher rate of cardiac death than patients without a history of MI (Konstam et al. 1993). Ischaemic cardiomyopathy has been reported to have a worse prognosis than non-ischaemic heart failure in a study of 1230 patients (Felker et al. 2000).

The most common causes of non-ischaemic heart disease leading to LV dysfunction are non-ischaemic dilated cardiomyopathy (DCM), hypertensive heart disease, and valvular heart disease. Evidence from the Framingham study indicates that, in the last few decades, the proportion of CHF cases due to ischaemic heart disease has risen because the survival rate has improved significantly in patients post myocardial infarction (Lloyd-Jones et al. 2002). The incidence of new patients with CHF due to previous MI has increased by 26% per decade in men and 48% per decade in women, while the number of CHF cases resulting from hypertension and valvular heart disease has been reduced by 13% and 24% in men, and by 25% and 17% in women, respectively (Lloyd-Jones et al. 2002).

1.3.4   Mortality and morbidity in CHF

Mortality and morbidity in CHF have been reported to be very high with a mortality of 30% to 40% in the first year of CHF diagnosis (McMurray & Pfeffer 2005). Despite recent advances in CHF management, 5 year cardiac death rates are still as high as 59% for men and 45% for women (Levy et al. 2002). Rosamond et al. (2008) has reported that more than 250 000 die each year in the USA alone due to either progressive CHF or sudden cardiac death (SCD) which is usually defined as unexpected death within an hour from the onset of acute symptoms due to a cardiac cause (Zipes & Wellens 1998). In Australia, CHF accounted for about 2700 deaths in 2008 (Krum et al. 2011). The cause of SCD in patients with CHF is usually a ventricular tachyarrhythmia (Myerburg et al. 1993).

1.3.4.1 Progressive CHF

Progressive CHF, rather than sudden death, accounts for the majority of deaths in patients with a new diagnosis of CHF which about 10% died by 30 days and 52% died within 6 months of diagnosis (Levy et al. 2002, Mehta et al. 2008). The rate of readmissions to the hospital because of progressive and unstable CHF remains very high. In a multi-centre investigation from 12 European countries, the ESC-HF Pilot, 75% of the patients were hospitalised due to decompensated CHF (Maggioni et al. 2010). An increasing rate of hospitalisation due to CHF (up about 7%) has been reported in the Australian national database over the last decade (Krum et al. 2011).

1.3.4.2 Sudden cardiac death (SCD)

Approximately 50% of deaths resulting from cardiovascular diseases are SCD (Myerburg et al. 1997), which accounts for 456 076 deaths a year in America (Zheng et al. 2001). Ischaemic cardiomyopathy accounts for 80% of SCD, while 10-15% of SCD are consequences of non-ischaemic dilated cardiomyopathy and the remaining 5-10% are due to other cardiac disorders (Huikuri et al. 2001).

SCD in patients with CHF often results from a fatal cardiac arrhythmia (Myerburg et al. 1993). Ventricular tachycardia (VT) and ventricular fibrillation (VF) occur more frequently than brady-arrhythmias and pulseless electrical activity which are normally observed in more severe heart diseases. A number of predisposing factors are thought to provoke malignant arrhythmias in CHF. In ischaemic cardiomyopathy, although cardiac arrest might be triggered by acute ischaemia at any time, an admixture of scar tissue and viable myocytes in the peri-infarcted region which is thought to develop micro-reentry circuits, can also trigger VT or VF (Fernandes et al. 2007). Congestive cardiomyopathies of genetic basis also vary from one to another as regards to the risk of tachyarrhythmias (Myerburg 2001). Therefore, any reliable test to identify patients at high risk of developing malignant arrhythmias will assist in the decision-making regarding automated implantable cardio-defibrillator (AICD) insertion.

1.3.5   Pathophysiology of CHF

Pathophysiology of CHF is complicated, involving activation of several systems leading to progression of clinical symptoms, from compensated CHF to refractory disease. In most cases, CHF results from an abnormality in the structure of the heart. While the initial injury is usually obvious, for example MI or myocarditis, it is sometimes unknown (idiopathic dilated cardiomyopathy). In the compensatory stage, in order to maintain cardiac output, there is an increase in pre-load resulting from raising volumes, ventricular dilatation, peripheral vasoconstriction, and renal sodium and water retention, which are controlled by various neurohormonal vasoconstrictor systems. Among these, the neuroendocrine systems, including catecholamines and the sympathetic nervous system, the renin-angiotensin-aldosterone system, vasopressin, endothelin-1, natriuretic peptides, nitric oxide, and cytokines play fundamental roles in pathophysiology of CHF. It is important to review a number of components of the neurohormonal response to CHF which are relevant to this thesis.

1.3.5.1   Catecholamines and the sympathetic nervous function in CHF

The sympathetic nervous system, including afferent, efferent, and intercommunicating neurons, has a number of effects on heart rate, cardiac conduction, myocardial contractility, venous capacitance, and vessel resistance. It is activated in several clinical scenarios, including response to various stressful stimuli. The cardiac sympathetic fibres travel along the main coronary arteries, are located in the subepicardium, and are distributed predominantly near the base of the ventricle (Pierpont et al. 1985). They are more concentrated in the anterior compared to the inferoposterior wall (Janes et al. 1986, Matsuo et al. 2009). The sympathetic nervous system, which is regulated by cardio-cardiac reflexes, acts through 4 principal chemomechanistic pathways: (1) Noradrenaline synthesised by decarboxylation and hydroxylation of the aminoacid tyrosine and released by the right and left stellate ganglions resulting in a rise in heart rate, contraction, and blood pressure, as well as a shortening in atrioventricular conduction; (2) adrenaline released by the adrenal cortex affecting peripheral vessels and myocardium; (3) local adrenaline and noradrenaline released by peripheral vessels; and (4) circulat-

ing noradrenaline affecting multiple locations (Van Stee 1978). These disparate forms of catecholamine release result in different pharmacokinetic characteristics, and different durations of action. The purpose of this section is to review the mechanisms of the neurohormonal system activation in CHF and its consequences, focusing on cardiac denervation with respect to diagnostic and prognostic perspectives.

The neurohormonal systems, including the adrenergic nervous system and renin-angiotensin aldosterone system, are the main cardiovascular system controllers and play a crucial role in cardiac remodelling, leading to progressive CHF. Packer (1992a) was the first investigator to establish the role of neurohormonal system in the progression of CHF independent of haemodynamic disturbance. Hyperactivity of the sympathetic nervous system is reflected by a rise in plasma noradrenaline, increased central sympathetic outflow, and increased noradrenaline spillover from myocardial nervous fibres. Activation of the sympathetic nervous system at first aims to increase heart rate, contractility and venous return in order to maintain cardiac output via chronotropic and inotropic effects (Pepper & Lee 1999). However, noradrenaline concentration in the myocytes may be reduced, due to a decrease in neuronal density and function (Regitz et al. 1991). On the other hand, this sustained hyperactivity may lead to decreased sensitivity and down-regulation of myocardial β 1-adrenoceptors, which leads to impairment of the contractile capacity of the heart (Bristow et al. 1982, Bristow et al. 1986). It also results in increases in vasoconstriction, myocardial oxygen consumption, and the rate of spontaneous myocardial depolarisation via $\beta2$-mediated prodysrhythmic effects (Billman et al. 1997). Furthermore, stimulation of $\alpha1$ and $\beta2$-receptors can cause LV hypertrophy and cell death (Mann 1998). As a consequence, the development and progression of CHF are accelerated by both haemodynamic and directly harmful hormonal effects on the myocytes, leading to ongoing dysfunction and poor outcome.

1.3.5.2 The renin-angiotensin-aldosterone system (RAAS in CHF)

The RAAS plays a pivotal role in the pathogenesis of CHF and its progression, acting via angiotensin II, angiotensin receptors, and aldosterone. Figure 1.3.4 describes the complex regulation of the RAAS.
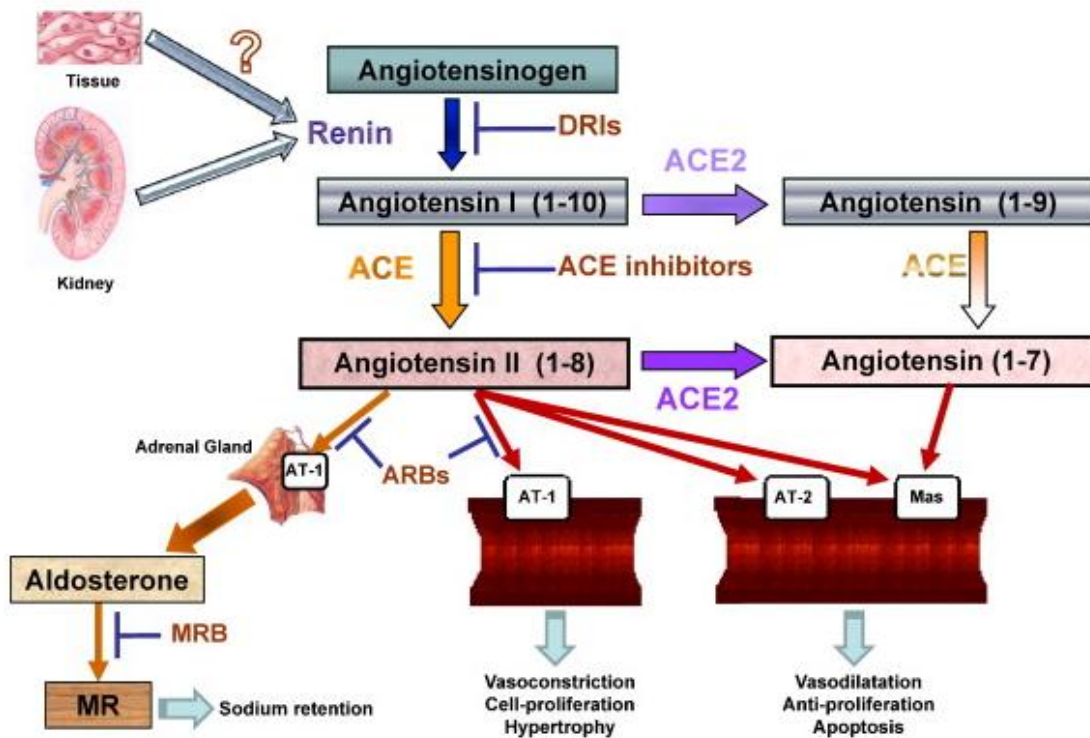
Figure 1.3.4: The renin-angiotensin-aldosterone system (RAAS)

(ACE = angiotensin converting enzyme, ACE 2 = angiotensin converting enzyme 2, ARBs = angiotensin receptor blockers, AT 1 = angiotensin receptor type 1, AT 2 = angiotensin receptor type 2, MR = mineralocorticoid receptor, MRB = mineralocorticoid receptor blockers)

In the liver, angiotensinogen is converted to angiotensin I through the effects of renin, a proteolytic enzyme synthesised by the kidneys. Angiotensin II is then produced from angiotensin I by angiotensin converting enzyme (ACE). Angiotensin II is the main biologically active component, which has direct effects on hypertrophy, remodelling and fibrosis processes (Crawford et al. 1994, Brilla et al. 1994a). Moreover, it can lead to vasoconstriction and an increase in aldosterone release through two receptors (AT1 and AT2). It has been suggested that in addition to the effects on vasoconstriction and water and sodium retention, AT1 receptor activation also promotes oxidative stress, leading to cardiac hypertrophy and fibrosis (Kabour et al. 1994, Nickenig & Harrison 2002).

The AT2 receptor mainly affects apoptotic processes (Yamada et al. 1996). Because the AT1 receptor is down-regulated, leading to an increase in the AT2 receptor density, the AT2 receptors may play an important role in CHF (Asano et al. 1997).

The pathophysiological effects of aldosterone have been demonstrated not only on electrolyte and water homeostasis but also on cardiac fibroblasts, cardiomyocytes and endothelial cells which can lead to cardiac hypertrophy and fibrosis (Stockand & Meszaros 2003, Rude et al. 2005, Leopold et al. 2007). Aldosterone exerts its harmful effects by increasing superoxide anion production secreted by endothelial and vascular smooth muscle cells (Iglarz et al. 2004, Leopold et al. 2007). High levels of aldosterone have been detected in patients with CHF and it has been suggested that aldosterone elevation is associated with worsening vascular dysfunction and increased mortality rate (Guder et al. 2007).

1.3.5.3 Vasopressin

Arginine vasopressin is an antidiuretic hormone which controls urinary water excretion and also has vasoconstrictor effects. Since the first study in 1983 showing that vasopressin levels are progressively elevated in CHF, treatment with several vasopressin antagonists such as conivaptan, lixivaptan, and tolvaptan have been investigated in both animals and humans. However, all have failed to improve survival rates in heart failure (Ghali & Tam 2010).

1.3.5.4 Endothelin-1 (ET1)

Endothelin-1, a potential vasoconstrictor, plays an important role in the pathogenesis of CHF. Since its discovery in 1988 by Yanagisawa et al. (1988), the role of ET1 has been well established and pharmacological agents such as ET1 inhibitors have been developed. Due to its long half-life and powerful vasoconstrictor effect, ET1 is thought to be a major blood pressure regulator (Yanagisawa et al. 1988, Clarke et al. 1989). However, ET1 receptor blockade fails to inhibit both systemic and local vasoconstrictor effects of ET1. For example, no systemic or pulmonary haemodynamic changes were found after administration of bosentan, a dual ETA and ETB receptor blocker, in anaesthetised dogs

during both normal (Teerlink et al. 1995) and hypoxic conditions (Hubloue et al. 2003). This has been explained by the effect of endogenous NO release which counteracts and/or inhibits ET1 (Gellai et al. 1997, Hubloue et al. 2003). ET1 also appears to have effects on renal flow and glomerular filtration (Bohm et al. 2003, Vuurmans et al. 2004).

Elevations of plasma ET1 concentrations have been reported in animal models of CHF as well as in CHF patients (Teerlink et al. 1994, Pacher et al. 1993). Moreover, a significant correlation between the elevation of circulating ET1 and both cardiac dysfunction and worse outcomes has been shown in a number of investigations (Pacher et al. 1996, Aronson & Burger 2003, Van Beneden et al. 2004). The activation of the ET1 system in CHF contributes to CHF progression through its haemodynamic and renal effects, vascular resistance, cardiac remodelling, and interactions between ET1 and other neurohormones.

1.3.5.5   Natriuretic peptides

The "natriuretic peptides" are a group of three peptides: atrial natriuretic peptide (ANP), brain-type natriuretic peptide (BNP), and C-type NP, which share a 17-amino-acid ring structure. These peptides act as protectors of the cardiovascular system from volume overload.

The existence of natriuretic peptides was suspected in the 1950s, when the heart was found to function as an endocrine organ. 30 years later, de Bold and investigators observed an increase in sodium and chloride excretion (up to 30 fold) and urine volume when myocardial homogenates were intravenously injected into nondiuretic rats (de Bold et al. 1981). In 1984, the structure of ANP was published and ANP was reported to be increased in both CHF and asymptomatic LV dysfunction (Seidah et al. 1984). BNP was found in 1988 and the primary site of synthesis and release of BNP was established in the early 1990s (Sudoh et al. 1988). In 1990, the third NP, C type NP, was identified (Sudoh et al. 1990).

ANP is released from the atria, BNP from the ventricular myocardium, and C-type NP from the endothelium. Unlike ANP, which is stored in granules and released following minor triggers, pre-pro BNP is synthesised in the ventricular myocardium and secreted in

bursts (Yoshimura et al. 1993). A number of investigations have demonstrated that CHF is associated with an increase in hormone production and hormone deficiency/resistance. In this section, the biological production of BNP, its stimuli and the utility of BNP in clinical practice are the main focus.

a. Biological production of BNP Unlike ANP, BNP is synthesised and secreted in bursts rather than stored in granules (Yoshimura et al. 1993). BNP is formed within ventricular myocardium by progressive cleavage of a larger precursor peptide, pre-pro-BNP1-134 to proBNP1-108. The critical step is the cleavage of the pro-BNP1-108 molecule into an active moiety, BNP1-32, and a larger inactive moiety, N-terminal-proBNP1-76 (NT-proBNP) (Figure 1.3.5).

b. Known stimuli for BNP release Natriuretic peptides are synthesised and secreted from the myocardium under conditions of pressure and volume overload, reflecting both systolic and diastolic function. Although ventricular distension is the classical stimulus, BNP release also may occur with ischaemia, inflammation, redox stress and the local effects of catecholamines, angiotensin II and endothelin-1 (Zhang et al. 2004, Mehra et al. 2006, de Bold 2009). This will be discussed further in Chapter 3.

c. Clearance of BNPs Although the performance characteristics of BNP and NT-proBNP are similar, BNP has a half-life of 20 min and is quickly cleared while NT-proBNP has a longer half-life of 1-2h which results in higher circulating levels and slower fluctuation (Pemberton et al. 2000). BNP binds to the membrane-bound NPR-A (natriuretic peptide A receptor) mediating natriuresis, vasodilatation, renin-inhibition, antimitogenesis, anti-ischaemic effects, and positive lusitropism via cGMP signalling (Chen et al. 1999). BNP is degraded by the ectoenzyme neutral endopeptidase (eNEP), and cleared by NPR-C receptor mainly via the kidneys (Figure 1.3.6). In normal subjects, there is a reasonable correlation between BNP and NT-proBNP levels. In CHF, the NT-proBNP plasma level is 2-10 times higher than the BNP concentration (Noveanu

Natriuretic Peptides

ANP (atrium)   **BNP (ventricle)**   **CNP (endothelial cells)**

Mechanical:
Ventricular distension (myocyte stretch)
Non-mechanical:
Ischaemia, Inflammation, Redox stress
Catecholamines (α/β), Angiotensin II, Endothelin-1

Pre-proBNP $_{1-134}$

proBNP $_{1-108}$ 26AA signal sequence

NTpro-BNP $_{1-76}$ **BNP** $_{1-32}$
$t_{1/2}$ **= 1-2 hr** $t_{1/2}$ **= 18 min**

Particulate Guanylyl Cyclase

Natriuresis
Positive lusitropy
Vasodilatation
↓ RAAS

Figure 1.3.5: Synthesis and release of B-type natriuretic peptide
(ANP = atrial natriuretic peptide , BNP = brain-type natriuretic peptide, NT-proBNP
= N-terminal-proBNP, RAAS = the renin angiotensin aldosterone system)

et al. 2011). Despite high levels of "BNP" as determined by standard assay methodologies in circulation, CHF is associated practically with BNP insufficiency which results from depletion of the authentic bioactive BNP1-32 (Chen 2007, Niederkofler et al. 2008). This could be explained by the fact that the standard commercial BNP immunoassays widely used in clinical settings recognise both inactive and active BNPs. In addition, abnormal proBNP processing into the active form may contribute to the BNP deficiency (Lam et al. 2007). Furthermore, CHF patients also may manifest a state of BNP
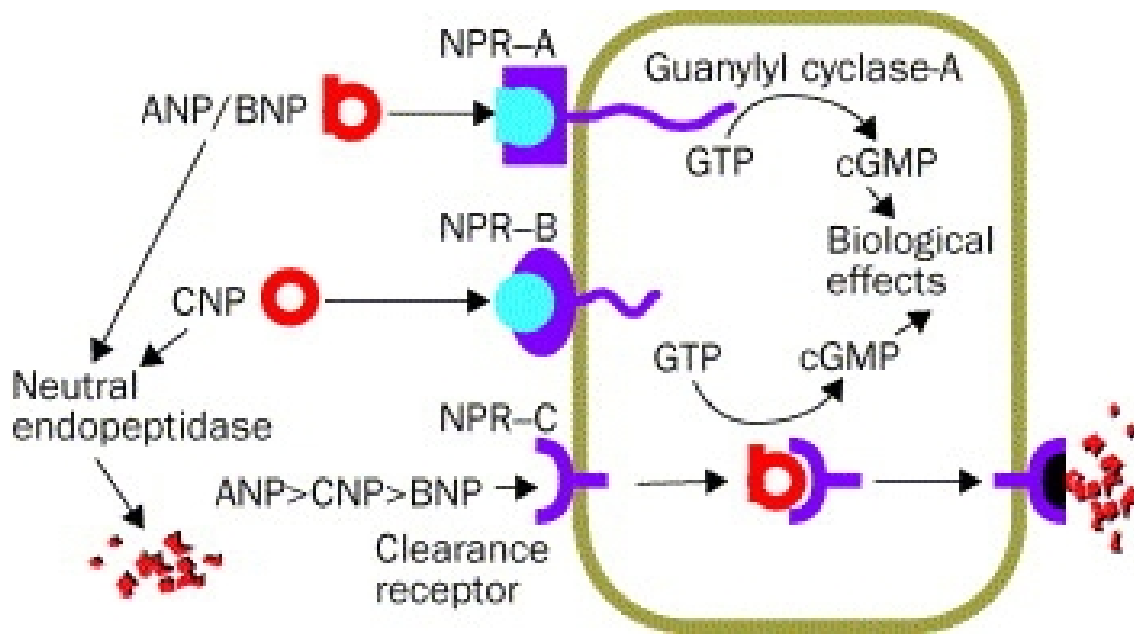
Figure 1.3.6: Natriuretic peptide hormone binding and clearance

[ANP = atrial natriuretic peptide , BNP = brain-type natriuretic peptide, CNP = C-type natriuretic peptide, GTP = guanosine triphosphate, cGMP = cyclic guanosine monophosphate , NPR = natriuretic peptide A receptor (Modified from deLemos et al. (2003)]

resistance at the level of signal transduction post NPR-A binding (Forfia et al. 2007).

d. BNP cleavage It has been suggested that corin and furin are two prohormone convertases responsible for natriuretic peptide processing (Sawada et al. 1997, Wu et al. 2002). These enzymes have different cleavage sites and are responsible for distinct BNP forms (Semenov et al. 2010) (see Figure 1.3.7). Mass spectrometric analysis has revealed that the furin-mediated cleavage of proBNP results in BNP 1-32, whereas corin-mediated cleavage leads to the production of BNP 4-32 (Semenov et al. 2010).

Discovered in 1999 (Yan et al. 1999), corin, a trypsine-like transmembrane serine protease, is present both in myocardium and plasma. Corin mRNA and protein were
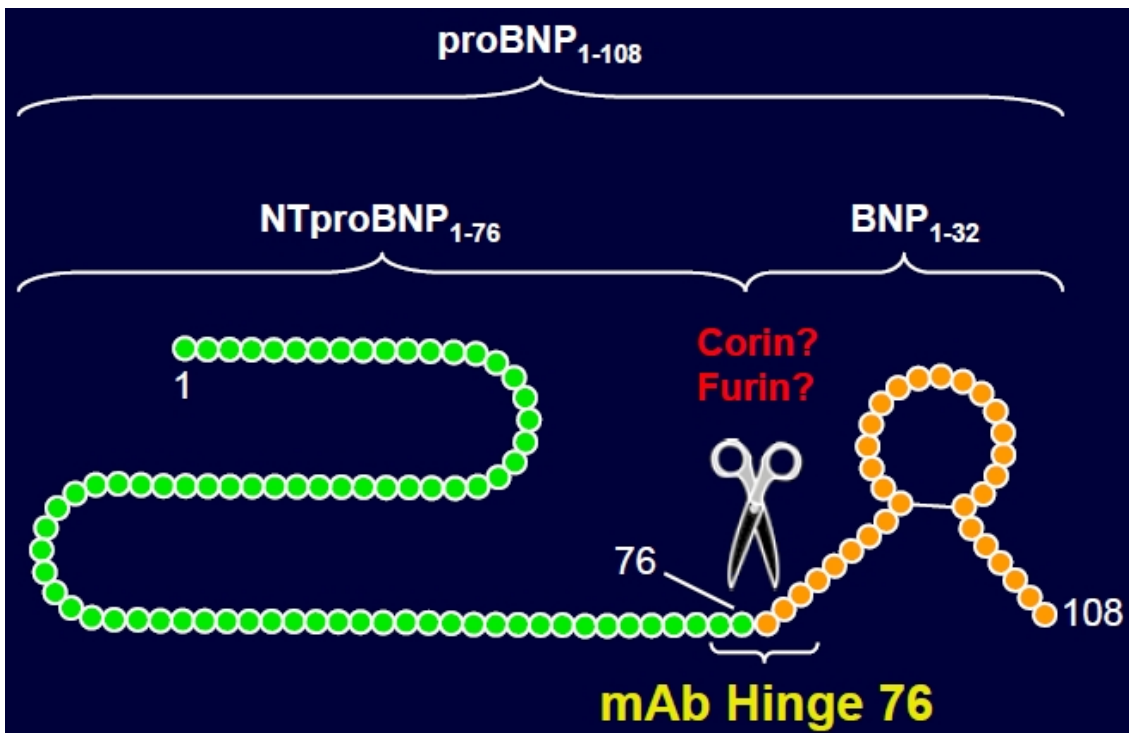
Figure 1.3.7: Cleavage sites of corin and furin in the processing of natriuretic peptides

observed in foetal and adult cardiomyocytes and in scar myofibroblasts in rats (Yan et al. 1999). Corin levels have been demonstrated to increase in patients with myocardial infarction and cardiac hypertrophy, and decrease in cases of heart failure (Mair 2009). Catecholamine exposure (Jiang et al. 2005) and activation of intramyocardial inflammatory processes (Mair 2009) have both been shown to increase myocardial corin expression.

Furin is a membrane-associated calcium-dependent serine endopeptidase in the yeast Kex2 family which is localised in the trans-Golgi networks and recycles between the trans-Golgi networks and plasma membranes. Unlike corin, which is uniquely distributed in the myocardium, furin is found in various tissues but becomes highly expressed in hypertrophic cardiomyocytes (Mair 2009).

1.3.5.6   Nitric oxide (NO)

The first evidence that endothelial cells produce an endothelial-derived relaxing factor, was provided by Furchgott & Zawadzki (1980). Subsequent investigations have indi-

cated that the major source of endothelial-derived relaxing factor activity is NO. NO is synthesised from L-arginine by NO synthases (NOS) through 2 monooxygenation steps, which require the involvements of O_2^- and NAD(P)H (Dudzinski et al. 2006). There are 3 isoforms of NOS: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (Dudzinski et al. 2006). A number of studies suggest that increased oxidative stress and inflammatory processes in CHF result in impaired NO formation and signalling, leading to "endothelial dysfunction" (Landmesser & Drexler 2005).

The biochemical pathways implicated in the pathogenesis of "endothelial dysfunction" are summarised in Figure 1.3.8.

The critical components of the process are:

a. Formation of NO from arginine   This is potentially subject to:

- Impairment due to lack of the critical co-factor tetrahydrobiopterin (BH4).

- Inhibition by endogenous arginine analogues, notably asymmetric dimethylarginine (ADMA), which is discused below.

b. "Scavenging" of NO by reactive oxygen species such as superoxide (O_2^-)
Increases in O_2^- generation within tissues may occur as a result of activation of a number of enzymes, notably NAD(P)H oxidase and xanthine oxidase. Importantly, eNOS may become "uncoupled" in the absence of BH4 and/or with high concentrations of ADMA, resulting in formation of O_2^- rather than NO (Mangoni 2009, Anderssohn et al. 2010).

The reaction of $NO^.$ with O_2^- generates peroxynitrite anion $(ONOO^-)$ which is capable of activating soluble guanylate cyclase, but which may also damage DNA via the PARP1 and 2 receptors.

c. Dysfunction of soluble guanylate cyclase (sGC)   sGC readily undergoes oxidative change, with or without heme moiety depletion (Schmidt et al. 2009), rendering it relatively unresponsive to NO.
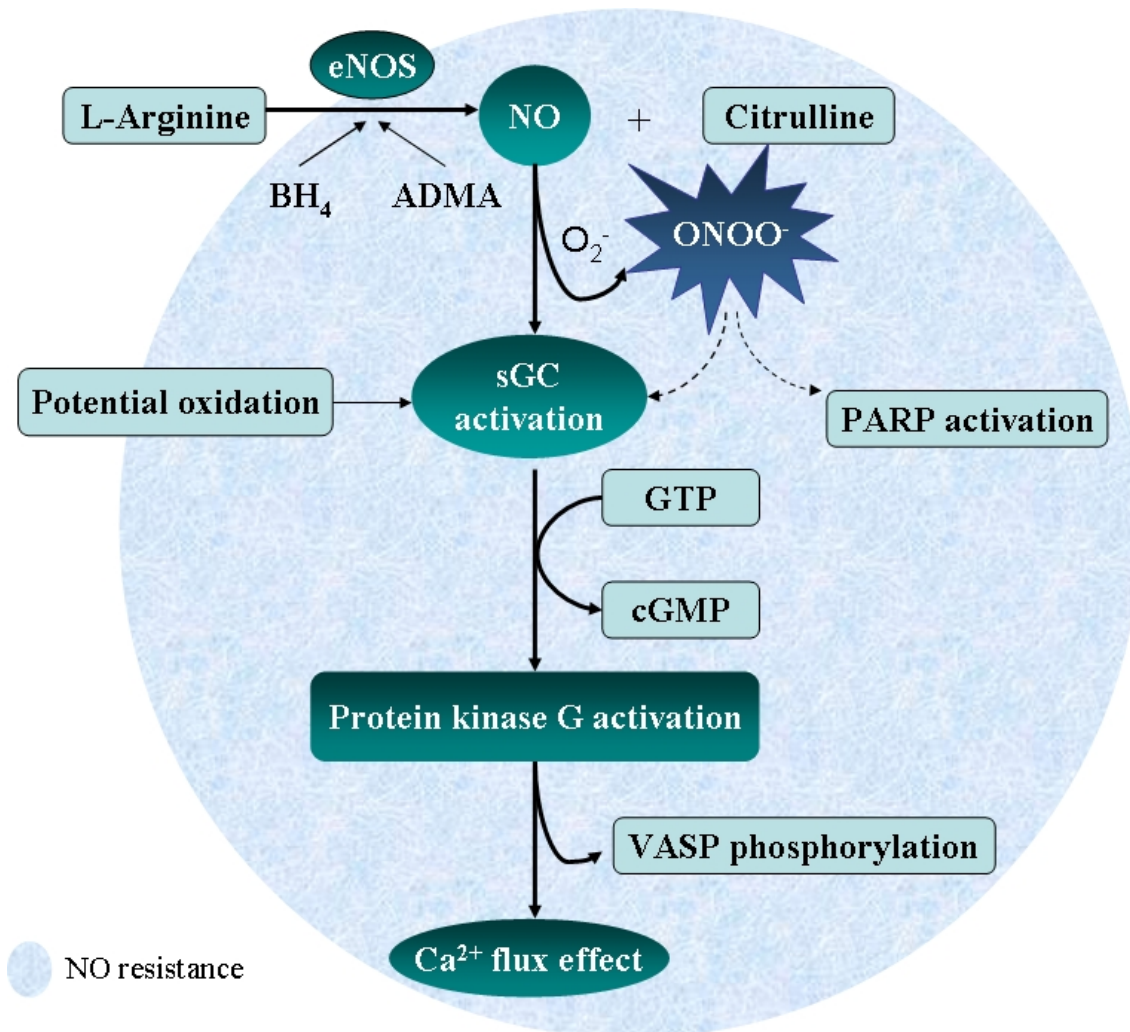
Figure 1.3.8: Biochemical NO signalling pathways

(ADMA = asymmetric dimethylarginine, BH4 = tetrahydrobiopterin, cGMP = cyclic guanosine monophosphate, eNOS = endothelial nitric oxide synthases, GTP = guanosine triphosphate, NO = nitric oxide, sGC = soluble guanylate cyclase, ONOO⁻ = peroxynitrite, PARP = poly(ADP-ribose) polymerase, VASP = vasodilator-stimulated phosphoprotein)

The various components of endothelial dysfunction may be assessed physiologically or biochemically. ADMA, which is generated via protein catabolism and cleared by the redox-dependent enzyme, dimethylarginine dimethylaminohydrolase (DDAH), can be assayed in plasma or tissue (Murray-Rust et al. 2001), and it is also possible to evaluate tissue sGC activity (Sakurada et al. 2008). Physiologically, responses to acetylcholine

(Schachinger et al. 2000, Halcox et al. 2002) or salbutamol (Rambaran et al. 2008, Tahvanainen et al. 2009) are commonly used as measures of "endothelial function", while responses to NOS-independent sources of NO such as nitroglycerine or sodium nitroprusside are used to evaluate integrity of NO-based signalling pathways (Kasprzak et al. 2006).

CHF is often associated with both endothelial dysfunction and with NO resistance. In a rat model of CHF, a decrease in eNOS expression and NO synthesis in the endothelium has been demonstrated (Comini et al. 1996). In patients with CHF, a reduction in nitrate excretion by the kidneys at rest and during exercise after L-^{15}N-arginine infusion, which reflects impairment of NO generation, has been shown by Katz et al. (1999). Moreover, an increase in plasma concentrations of ADMA has been found in CHF patients and this elevation is associated with poor cardiovascular outcomes (Duckelmann et al. 2007). NO resistance at the level of platelet aggregation has also been described in CHF (Anderson et al. 2004).

1.3.6 Diagnosis of CHF

Diagnosis of CHF is based on a combination of clinical history, examination, and assessment of cardiac dysfunction. Patients with CHF can present with a variety of different clinical scenarios and a wide range of patterns of LV dysfunction: from a normal LV size and preserved EF to severe LV dilatation and reduced EF. Systolic and diastolic LV dysfunction usually coexist in most patients. Several tests can be used to confirm the diagnosis of CHF. Most tests are more sensitive for the identification of systolic CHF than for diastolic CHF.

1.3.6.1 Clinical assessment

Patients with CHF often exhibit symptoms of cardiac dysfunction such as dyspnoea, fatigue with exertion or at rest and fluid retention (pulmonary or systemic congestion and peripheral oedema). Persistent cough, nausea and anorexia are less specific symptoms. Signs of CHF, including cardiac murmur, jugular venous pressure elevation, lung crepitations, and peripheral or sacral oedema, may be helpful in identifying the aetiology

and assessing the severity of CHF.

Symptoms and signs of CHF play a fundamental role in early detection of the disease. However, the diagnosis of CHF remains difficult because the clinical picture of CHF is variable and the symptoms are non-specific. Whereas exercise intolerance may be the principle symptom in some patients, others only experience fluid retention. The degree of functional limitation imposed by CHF can be divided into 4 classes according to the New York Heart Association (NYHA) classification. NYHA class I refers to patients with minimal exercise intolerance. Symptoms during ordinary and less than ordinary exertion refer to NYHA class II and class III, respectively. Patients with CHF symptoms at rest are assigned to class IV. Although it is a crude assessment of CHF symptoms and based only on symptoms, not on disease progression, NYHA class has been shown a strong predictor of cardiac death in CHF (Bart et al. 1997). Since 2001, a new disease staging system has been introduced by American Heart Association committee which highlights the importance of both development and progression of HF. Four stages of HF are classified as stage A, B, c, and D. Stages A and B identify patients at high risk of HF development, while stage C is referred to patients with history of current HF symptoms and stage D denotes decompensated HF. This approach focuses on identifying patients at high risk who may benefit from early preventive intervention (Hunt et al. 2001). Symptomatic severity of CHF is also reflected in other criteria such as quality of life score and daily activity levels, which provide independent prognostic information (Parissis et al. 2009, Parissis et al. 2009a). The presence of peripheral or pulmonary oedema and the prolonged duration of symptoms in patients with CHF also indicates a poor prognosis (Komajda et al. 1990, Rickenbacher et al. 1996).

1.3.6.2   Laboratory assessments

Several blood tests, for example blood count, thyroid function, and autoimmune screening may help to diagnose causes of CHF and determine exacerbating factors. Recently, it has been suggested that measurement of plasma BNP levels should play an important role in diagnosis and long-term management of CHF. The use of BNP testing as a diagnostic tool in CHF is the focus of this section.

Incremental utility of BNP for diagnosis of acute HF Acute elevation of plasma concentrations of BNP has high sensitivity and specificity for identifying HF in emergency departments (ED). In the "Breathing Not Properly" Multinational Study of 1586 patients presenting with dyspnoea in ED, the sensitivity and specificity of BNP levels were 90% and 76%, respectively. The negative predictive value was 96% (BNP<50 pg/ml) (Maisel et al. 2001). In another study, the PRIDE study, NT-proBNP has been suggested as an accurate test to detect CHF (Januzzi et al. 2005). Therefore, in conjunction with clinical information, BNP level may be useful in confirming or refuting a diagnosis of HF in patients with acute dyspnoea. A cut-off of BNP<100pg/ml or NT-proBNP<300pg/ml was proposed to rule-out HF. In patients with asymptomatic left ventricular systolic dysfunction, although there is a close correlation between BNP and both LV end-diastolic pressure and pulmonary capillary wedge pressure (PCWP) (Omland et al. 1996), BNP is less accurate than for symptomatic patients. In patients with diastolic dysfunction, BNP > 100 pg/ml had a sensitivity of 86%, a negative predictive value of 96%, and an accuracy of 75% (Maisel 2003).

The situation with CHF is less clear-cut. The issue of whether serial BNP monitoring should be utilised to guide therapy with CHF remains controversial, with both supporting and non-supporting data for this concept. On the one hand, a significant reduction in cardiovascular death or hospitalisation and CHF decompensation, primarily in patients with NYHA class II CHF, using BNP target < 200pg/ml, has been reported (Troughton et al. 2000). Moreover, in a randomised multicentre trial, the STARS-BNP, using BNP target < 100pg/ml, further increases in doses of ACEI and β-blockers were associated with a decrease in hospitalisation and death (Jourdain et al. 2007). Improved outcomes with BNP-guided therapy have been shown in patients < 75 years old in the BATTLESCARRED trial (Lainchbury et al. 2009). However, the STARBRITE trial of 130 patients failed to show a significant improvement in primary outcome. In another trial, the TIME-CHF study of 499 patients aged > 60 years, including 289 patients > 75 years old, no significant improvement in 18-month survival free of all-cause hospitalisation was found between BNP-guided (<2 times the normal limit) and symptom-guided therapy groups (Pfisterer et al. 2009). A similar result has been con-

firmed in the SIGNAL-HF trial (Persson et al. 2010). Moreover, in patients > 75 years old, more adverse effects from up-titration have been reported (Pfisterer et al. 2009). However, difficulties in the interpretation of BNP levels in these trials should be taken into account. Firstly, β-blockers affect secretion and clearance of BNP levels independent of haemodynamic changes. It has been reported that serial BNP levels did not correlate with left ventricular ejection fraction (LVEF) improvement in NYHA class II-IV patients treated with metoprolol, but did with other drugs (Yoshizawa et al. 2004). Because the plasma half-life of BNP is prolonged by β-blockers, it has been found that BNP and NT-proBNP levels increase significantly with the introduction of metoprolol (Davis et al. 2006). Moreover, exercise-induced BNP release is higher in patients with CAD who are being treated with β-blockers (Marie et al. 2004).

The utilisation of BNP for the diagnosis and management of CHF has several limitations. Firstly, several factors which influence BNP concentration should be considered when interpreting BNP results. In a population-based study of 2042 patients, in which "age-related" diastolic dysfunction was excluded, advanced age was found to be associated with elevated BNP level (Redfield et al. 2002). Gender is another factor, for example women have a higher BNP and NT-pro BNP level than men at any age (Redfield et al. 2002). Sex hormones can also impact on BNP regulation. For example, there is an inverse relationship between free testosterone and BNP levels. In contrast, oestrogen up-regulates BNP. An inverse relation between BNP levels and body mass index has also been reported. In a multicentre trial of 1586 acute dyspnoea patients, a lower cut-point (BNP $\geq$ 54 pg/ml) for severely obese patients was found (Daniels et al. 2006). Other factors such as a low haemoglobin level or renal dysfunction, also influence BNP levels (Tsuji et al. 2004). In the Dallas Heart study, a multiethnic population-based trial of 2784 patients, NT-proBNP and BNP were inversely and independently associated with renal function (Das et al. 2008).

Secondly, in patients with concomitant disorders, a very high BNP level does not exclude the presence of other diseases. Furthermore, patients with CHF may have persistently high BNP levels despite adequate treatment, in which case serial BNP tests and base-line comparisons are required. Finally, in asymptomatic patients, a small

increase in BNP level is not specific for diagnosis of HF.

Elevations in plasma BNP levels can be regarded categorically as well as quantitatively. Many disorders of cardiac structure and function are thought to be associated with BNP elevations: a partial list as proposed by Daniels & Maisel (2007) is provided in Table 1.3.2.

1.3.6.3 Imaging techniques in the assessment of cardiac dysfunction

The measurement of cardiac function plays an important role in the diagnostic and therapeutic decision making in patients with CHF. Several tests can be used to determine the cause of CHF, e.g. ischaemia, DCM, or valvular disease. The most widely used objective measures of the global and regional systolic function of the heart are the LVEF and wall motion scores. The value of LVEF assessment has been well established for risk stratification in CHF. Several methods can be utilised for measurement of LVEF, including radionuclide or radiographic contrast ventriculography, gated single photon emission computed tomography (SPECT) myocardial perfusion scintigraphy, cardiovascular magnetic resonance (CMR), and 2-D echocardiography. The accuracy and reproducibility of LVEF measurements vary between techniques. In this section, methods for the assessment of LV systolic function are reviewed and the advantages and disadvantages of each technique are discussed in detail.

a. Echocardiography (ECG) Echocardiography is the most widely used technique for the assessment of LV systolic function. It is relatively cheap, widely available, safe, quick to perform and can be repeated with no short-term or long-term side effects and no radiation risk to patients. Assessment of systolic function by echocardiography includes LV fractional shortening, LV end-diastolic and end-systolic internal dimensions, end-systolic wall thickness, wall motion, and mitral E-point septal separation. The presence of regional rather than global systolic dysfunction might indicate an ischaemic basis for the CHF. LVEF which expresses LV contractile performance, has been utilised as an entry criterion in most large, randomised, controlled studies, as a basis for patient risk stratification. The accuracy of this measurement with echocardiography still remains

Heart muscle disease	
	Hypertrophic heart muscle disease
	Infiltrative myocardiopathies such as amyloidosis
	Acute cardiomyopathies
	Inflammatory: myocarditis and chemotherapy
Acute coronary syndrome	
Valvular heart disease	
	Aortic and mitral stenosis
Arrhythmia	
	AF and flutter
Critical illness	
	Bacterial sepsis
	(induction of endotoxin or other inflammatory mediators)
	Burns
	Adult respiratory distress syndrome (ARDS)
Stroke	
Pulmonary heart disease	
	Sleep apnoea
	Pulmonary embolism
	Pulmonary hypertension
	Congenital heart disease
Renal dysfunction	
Cirrhosis	
Hyperthyroidism	

Table 1.3.2: Differential diagnosis of an elevated BNP

Adapted from Daniels & Maisel (2007)

problematic in terms of its predictive value in multivariate analysis (Pernenkil et al. 1997, Cowburn et al. 1998), which is about $\pm\ 10\%$ with both visualisation and Simpsons rule calculation approaches (McGowan & Cleland 2003). Especially in subjects with obesity, excessive motion, and lung diseases, the technique is potentially inaccurate due to poor "acoustic windows".

In addition to measures of systolic function, echo Doppler measurements can also be used to quantitate valvular stenosis or regurgitation, and to provide measures of diastolic function, such as the ratio of flow velocity through the mitral valve during early and late diastole (the E/A ratio)(Luers & Maisch 2011).

b. Cardiac catheterisation   While cardiac catheterisation can be utilised to document the presence/absence of coronary disease and to determine left ventricular EF, it can also provide more detailed information about haemodynamic status. However, construction of pressure volume loops (Kass 1992) and detailed evaluation of diastolic LV function via catheterisation (Penicka et al. 2010) is of limited value beyond acute intervention studies and the risk of radiation exposure to patients, given the complexity of the equipment used and the unsuitability of the technique for the evaluation of cardiac reserve.

c. Cardiac computed tomography (CT)   Cardiac CT utilising multidetector (MDCT) not only provides information related to ventricular function, perfusion and scar, but also coronary artery anatomy and structure. It also can be utilised for evaluating cardiac dyssynchrony, cardiomyopathies, and assessing post-transplant patients (Butler 2007). With the advantages of newer MDCT scanners which allows faster image acquisition time, lower radiation exposure to patients (about 5-20 mSv), cardiac MDCT has been widely used in clinical setting. However, in patients with renal dysfunction or with low blood pressure, the use of MDCT may be limited due to requirement of intravenous iodinated contrast and β-blockers prior to MDCT (Mangalat et al. 2009).

d. Cardiovascular magnetic resonance (CMR)   Another imaging modality currently under development for the evaluation of heart diseases is CMR. Introduced in

1987 by Paulin et al. (1987), CMR offers excellent spatial resolution and produces unsurpassed images of the heart and its function (Schwitter & Arai 2011, Deshpande et al. 2012, Schuster et al. 2012). Like echocardiography, CMR is a safe modality and can be repeated with no short-term or long-term side effects for the patients. It involves neither ionising radiation nor iodinated contrast material exposure. Similarly, CMR does not have problems with attenuation artefacts. However, several contraindications that are often found in patients with CAD limit the application of cardiac MRI. The exclusion of ferromagnetic objects from the scanner area is imperative because these will become projectiles. Therefore, the performance of cardiac MRI is traditionally precluded in patients with implanted permanent pacemakers or defibrillators. The presence of basic life-support and physiologic-monitoring equipment in the MRI room is also a safety issue. Finally, over 2% of 400 consecutive patients in a retrospective study by Kuijpers were unable to undergo CMR due to claustrophobia, although this problem can be reduced with anxiolytic therapy (Kuijpers et al. 2004). Moreover, severe kidney dysfunction is also a contraindication for the use of gadolinium contrast during CMR because this can cause nephrogenic fibrosis in such patients. Other issues limiting the application of cardiac MRI are the complicated and varied acquisition techniques, the lack of experienced clinicians and institutions performing CMR, and the substantial expense of CMR equipment and software.

CMR has been recently developed as an accurate non-invasive test for prognostic assessment in patients with CHF. Gated CMR is known as a golden standard for quantitative measurement of global and regional wall motion, ventricular function, volumes, and mass and can be repeated to assess functional recovery and evaluate serial changes during therapy (Hundley et al. 2010). Measurement of size of myocardial infarction and area at risk utilising CMR has been reported as a good predictor of cardiovascular events. In ischaemic cardiomyopathy, the size of the scar volume and the scar percentage were better predictors of cardiac events than standard measures (LVEF and left ventricular volumes) (Yokota et al. 2008). In non-ischaemic dilated cardiomyopathy, patchy scar tissue (late enhancement post-Gadolinium) has also been seen in many patients using CMR. In a prospective study of 101 patients, the presence of mid-wall fibrosis was

a strong predictor of the combined end-point of all-cause mortality and cardiovascular events (Assomull et al. 2006).

In recent years, substantial attention has been directed to the utility of CMR for the evaluation of the location and extent of cardiac fibrosis (Ugander et al. 2012). This may provide incremental prognostic information, with prognosis generally impaired as fibrosis increases (Assomull et al. 2006). Finally, it should be mentioned that magnetic resonance spectroscopy with ^{32}P has the ability to evaluate regional myocardial energetic depletion in CHF (Neubauer 2007), but to date has only been utilised for research purposes.

e. Nuclear medicine techniques

(i) Radionuclide ventriculography Radionuclide ventriculography has been used for over 30 years. After radiolabelling autologous red blood cells with ^{99}Technetium, either first-pass or equilibrium ECG-gated images which allows cardiac phase correlation during image reconstruction, are acquired in the left anterior oblique plane (to separate left from right ventricular activity) using a planar gamma camera, which enables an accurate determination of the ventricular wall motion and ejection fraction. The accuracy of this method largely relies on the endocardial definition and the utility of the algorithms used. It has been utilised as a reference method with good reproducibility in many studies (van Royen et al. 1996).

(ii) Gated myocardial perfusion Single Photon Emission Computed Tomography (SPECT) Gated SPECT imaging is a highly reproducible, fully automated method to assess LVEF, LV volumes, and wall motion (Abidov et al. 2004). In a multicentre study, gated SPECT was shown to be a reliable method to assess LVEF with a SD less than 3.6% (Nakajima & Nishimura 2006). Measurement of stress-induced ischaemia and LVEF poststress utilising gated myocardial SPECT imaging are useful for risk stratification in patients with nonfatal MI. In combination with sizes of inducible ischaemia, LVEF measured by SPECT imaging is sufficient for separating patients into low to high risk groups (Sharir et al. 2001). However, the main purpose of the evaluation of myocardial perfusion in CHF is the delineation of a reversible ischaemic component

underlying the clinical presentation, to provide a basis for either anti-ischaemic pharmacotherapy or revascularisation. While the original use of ^{201}Thallium has now been largely supplanted by ^{99}Technetium tracers, there is substantial uncertainty about (a) the optimal "provocative" test to elicit ischaemia and (b) the value of invasive intervention based on its detection. Recently, the STICH study (Bonow et al. 2011) found no substantial value in viability-guided decision-making on cardiac surgery in CHF, contradicting results of some earlier studies (Chan et al. 1996).

(iii) Positron emission tomography (PET)   Although PET imaging was introduced in the late 1970s to study the cardiovascular system (Hoffman et al. 1977, Weiss et al. 1977), the advantages of quantitative PET imaging in clinical applications, particularly in aetiology and treatment assessments at various stages of CHF, have been recently established. Compared to SPECT imaging, PET has higher temporal and spatial resolution, less artefacts due to attenuation correction (Bateman 2004). Measurement of myocardial blood flow and both glucose and fatty acid metabolism by PET may be useful for the diagnosis of early stage of CHF and asymptomatic patients (Chacko 2005). However, this remains essentially a research tool, the utility of which reflects the extensive change in myocardial metabolism and energetics underlying CHF (Knuuti et al. 2004).

1.3.7   Risk stratification techniques in patients with CHF

Stratification methods to identify CHF patients at high risk of cardiac death are crucial for optimal patient management and for reducing the economic burden associated with CHF. Several techniques have been proposed to identify patients with a high risk of cardiac events such as symptomatically worsening heart failure and SCD. In this section, the advantages and disadvantages of these methods are discussed.

1.3.7.1   Utility of BNP for prognosis assessment in patients with CHF

A number of studies have established the role of BNP measurement as a prognostic tool in patients with CHF. In emergency departments, the REDHOT trial found

that increased BNP levels were associated with a higher rate of mortality and future CHF hospitalisation (Maisel et al. 2004). CHF patients with elevated BNP levels in spite of optimal medical therapy have a higher rate of mortality and morbidity (Anand et al. 2003). Furthermore, BNP measurements may be helpful in predicting SCD. In a study of 452 patients with LVEF < 35%, BNP levels > 130 pm/ml were associated with a significantly higher rate of SCD (Berger et al. 2002). In patients undergoing AICD implantation, BNP levels have been shown to be independently predictive of an appropriate AICD discharge (Verma et al. 2006). In patients with NYHA class III-IV undergoing cardiac resyncronisation therapy (CRT), BNP levels are also useful in predicting the efficacy of CRT and in assessing the likelihood of CRT response (Fruhwald et al. 2007, Lellouche et al. 2007).

1.3.7.2   Resting haemodynamics: LVEF

LVEF has been widely used as a predictor not only of progressive CHF but also of SCD in patients with CHF. In patients with non-ischaemic dilated cardiomyopathy, a significant correlation between low LVEF and an increase in overall mortality has been reported. In patients with acute MI (AMI), LVEF = 30% is utilised widely for distinguishing "high" risk from "low" risk patients (Bigger et al. 1984, Shiga et al. 2009). In a meta-analysis of 7294 patients with previous MI, Bailey and investigators found a 4.3 fold increased risk for arrhythmic events in patients with LVEF from 30-40%, compared to those with LVEF < 30% (Bailey et al. 2001). Moreover, a number of randomised trials have shown the benefit of AICDs in patients with LVEF < 35% for SCD prevention compared to optimal medical therapy (MADIT I, MADIT II, and SCD-HeFT) (Moss 1997, Moss et al. 2002, Bardy et al. 2005). Based on the results of these studies, AICD implantation is widely considered to be indicated for patients with LVEF < 35% with no need of further risk stratification (Zipes et al. 2006, Epstein et al. 2008).

There is also substantial evidence that LVEF should be considered in combination with the extent of LV dilatation in all patients with CHF. The incremental prognostic impact of increased LV end-systolic dimensions was demonstrated by Hina et al. (1993).

However, the limited sensitivity and specificity of quantitation of LV systolic dysfunc-

tion for identification of SCD (59.1% and 77.8%, respectively for LVEF < 35%) suggests that a combination of tests may help improve risk stratification (Bailey et al. 2001). In a study of 1248 post MI patients after 21 months follow-up, a low sensitivity of LVEF for the prediction of SCD has been reported and this was only improved when cardiac autonomic function tests such as heart rate variability and baroreflex sensitivity were taken into account (La Rovere et al. 1998). Another study of 492 individuals during 4 years follow-up showed no difference in the incidence of SCD between patients with severe and moderate cardiac dysfunction, even in the subgroup of patients with CAD (Gorgels et al. 2003). The specificity of LVEF in identifying patients at high risk of SCD is also suboptimal. Buxton et al. (2002) in the MUSTT study of 1791 patients with CAD found that LVEF reduction failed to predict SCD, although a higher total mortality rate was demonstrated in patients with lower LVEF. Subsequently, in a sub-study from the MUSTT authors, LVEF alone did not predict mode of death in CAD patients who did not receive antiarrhythmic drugs (Buxton et al. 2007). In another study, the Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI), there was no difference in risk of SCD between patients with LVEF < 35% or > 35% (La Rovere et al. 1998). Furthermore, two large studies (DINAMIT and CABG-Patch) found no benefit of AICDs in patients with LV dysfunction early after an MI or in patients undergoing coronary artery bypass surgery (Bigger 1997, Hohnloser et al. 2004). The results of these trials have supported the idea that LVEF might be a better predictor of progressive CHF rather than arrhythmic events. It is worth noting that indications for AICD implantation in current clinical practice are mainly based on LVEF, which may result in an inappropriate AICD implantation (Santangeli et al. 2011). Indeed, only 23% of patients in the MADIT II study had an appropriate AICD discharge on follow-up and approximately 50% of the patients never received defibrillator shocks (i.e. no benefit from the therapy) (Daubert et al. 2008). The fact that CHF patients with LVEF > 30% are at higher risk of SCD (Buxton 2009) highlights the importance of establishing better methods for risk stratification. The finding that > 50% of SCD occurred in patients with LVEF > 35% has also been confirmed by the ATRAMI and the Maastricht prospective registry studies (La Rovere et al. 1998, Gorgels et al. 2003).

1.3.7.3 Electrocardiographic (ECG) parameters

ECG (12-lead ECG and signal averaged ECG) is a traditional method for identifying CHF patients at high risk of SCD. It is cheap, easy to perform, and can be done serially. Several ECG parameters, including QRS duration, QT dispersion, and fragmented QRS have been reported to have prognostic value in CHF patients. However, low positive and negative prediction values limit the application of ECG parameters for risk stratification. Moreover, there is substantial overlap in ECG parameters between CHF patients and healthy controls.

a. QRS duration The duration of ventricular activation on ECG has been used to detect intra- or inter-ventricular conduction delay or blockage with high reproducibility ($< 5\%$ variation). Ventricular conduction delay is associated with poor prognosis in patients with reduced LVEF, particularly in ischaemic cardiomyopathy (Shamim et al. 1999). Prolonged QRS duration may result from LV dysfunction leading to an increase in mortality (Park et al. 1985). Moreover, slow ventricular conduction may trigger ventricular arrhythmias (Akar et al. 2004). In the CASS study of 15609 patients with chronic CAD, it was found that bundle branch block was related to more severe CAD, lower LVEF, and higher cardiac event rate. In particular, a significant increase in SCD was found in patients with left bundle branch block (LBBB) in a study by Freedman et al. (1987). In contrast, no relationship between LBBB and inducible VT has been reported in the MUSTT trial despite the significantly higher total mortality rate in this group (Zimetbaum et al. 2004).

In AICD trials, QRS duration has been investigated to see whether there are any relationships between QRS prolongation and either mortality rate or arrhythmic events in patients at high risk of SCD. In the MADIT II study, QRS duration > 120ms was a strong predictor of SCD in the medically treated arm, but not in patients with an AICD implantation (Dhar et al. 2008). Moreover, in 431 CAD patients who underwent AICD implantation for secondary or primary prevention, QRS duration was not associated with appropriate AICD discharge (Buxton et al. 2005).

b. QT interval and QT dispersion   QT interval and QT dispersion has been found to be widely variable between cardiac patients. In addition, there is significant overlap between normal subjects and patients, even between patients who experienced ventricular arrhythmias or not (VanHuysduynen et al. 2005). Finally, it caries no prognostic value in patients with severe CHF (Brendorp et al. 2001).

c.   Fragmented QRS   A fragmented QRS, including various RSR patterns and S wave notching morphologies, probably represents conduction delay during ventricular depolarisation. Recently, it has been suggested that fragmented QRS may have prognostic value in predicting cardiac events such as MI, need for revascularisation, and cardiac death in CAD patients (Das et al. 2007). However, because this trial was a retrospective study and because it was not specifically designed to evaluate SCD events, a prospective trial is required to confirm this conclusion.

Early QRS repolarisation, defined as QRS-ST junction elevation (Hlaing et al. 2005), has also been reported to provide incremental value in predicting ventricular arrhythmias. In a study of 10864 community-based subjects over a 30 year follow-up period, early QRS repolarisation in the inferior leads was shown to predict cardiac deaths (Tikkanen et al. 2009). However, in a study of 70 CHF patients who underwent Biventricular AICD implantation, QRS repolarisation failed to predict appropriate AICD therapy during 1 year of follow-up (Dilaveris et al. 2009). The prognostic value of QRS repolarisation therefore remains uncertain.

d. Signal-averaged ECG (SAECG)   SAECG has been used to determine the likelihood of ventricular arrhythmias by detecting the presence of late potentials within the QRS complex. The late potentials, which are defined as low-amplitude high-frequency waveforms in the QRS complex, reflect slow ventricular myocardial conduction. This delayed conduction may be due to the presence of infarcted regions interspersed with viable myocardium. There are a number of studies investigating the prognostic value of SAECG (Hartikainen et al. 1996, Huikuri et al. 2009). However, its variable sensitivity, ranging from 15% up to 75%, with a low positive predictive value, limits its application in clinical practice. Furthermore, because the normal values of SAECG in patients

with LBBB and ventricular pacing is unknown, these patients are usually excluded from studies. Finally, in a large randomised study (the Coronary Artery Bypass Graft Patch Trial), which was performed in 900 patients with LVEF < 36%, SAECG was shown to be unhelpful in differentiating patients with high risk of AICD discharge during 3 years of follow-up (Bigger 1997). Because of its high negative predictive value, this test might be useful in identifying low risk patients.

1.3.7.4   Ambulatory ECG (Holter) monitoring

24h Holter ECG monitoring is primarily used to detect ventricular arrhythmias (non-sustained VT or premature ventricular complexes). This standard and easy to perform test can be done on patients with AF or a pace-maker. However, its application in clinical practice is limited by low sensitivity and specificity (Connolly & Cairns 1992). In a prospective randomised study of 1080 patients with NYHA class III or IV, ventricular arrhythmias on ambulatory ECG does not pridict risk of SCD (Teerlink et al. 2000). Recently, additional parameters which can be obtained from Holter recordings, such as heart rate variability and heart rate turbulence, have been reported to improve its applicability in identifying patients at high risk of SCD.

a. Ventricular ectopy and NSVT   The presence of ventricular ectopy and NSVT on 24 h Holter monitoring has been reported to be predictive for fatal arrhythmias and death in CHF patients (Meinertz et al. 1984, Doval et al. 1996). In another trial of 2130 post-MI patients, NSVT was an independent predictor of SCD in patients with LVEF > 35%, but not in patients with LVEF < 35% (Makikallio et al. 2005). However, the value of ventricular ectopy and NSVT on ambulatory ECG-recording for SCD prediction is limited by low specificity and sensitivity.

b. Short-term and long-term heart rate variability (HRV)   It is believed that a variable heart rate, which is better able to respond to demands, may be associated with better cardiovascular outcomes. Heart rate variability can be measured non-invasively by recording the beat to beat variation of the RR interval on the standard ECG, which

represents cardiac autonomic modulation (Task Force of ESC and NASPE 1996). It was first described by Hon & Lee (1965), who observed that HRV was altered in foetal distressed hearts, before any change in heart rate was detectable. This is a practical and reproducible technique, which can be obtained from a normal 12-lead ECG or standard Holter monitor using additional software. Several parameters (time domain, frequency domain, spectral indexes, and nonlinear indexes) and methods of assessment such as short-term (2 to 30 mins) and long-term HRV (24 hours or continuous HRV from an implanted device) have been utilised by different groups. It has been suggested that abnormal HRV indices are associated with worse outcomes in CHF patients (Galinier et al. 2000, La Rovere et al. 2003, Aronson et al. 2004). However, the fact that HRV is affected by several variables, including age, gender, and some medications such as thrombolysics, β-blockers, ACEI, and antiarrhythmic drugs, and the fact that HRV cannot be performed in patients with AF or frequent premature ventricular complex, limits its clinical use. Moreover, no consensus agreement on either the method of assessment or parameters to be used has yet been proposed.

c. Heart rate turbulence (HRT)   HRT is defined as a transient tachycardic or bradycardic response to a fall in blood pressure (BP) resulting from a premature ectopic beat. In CHF, HRT is reduced and strongly correlated with baroreceptor sensitivity (Davies et al. 2001, Roach et al. 2002). It has been reported in several large-scale prospective studies that blunted HRT is associated with poor prognosis in patients with CHF (Koyama et al. 2002, Grimm et al. 2003). However, HRT can only be obtained for sinus rhythm patients with a markedly premature ectopic beat. Furthermore, it is unable to predict fatal ventricular arrhythmias in such patients (Koyama et al. 2002).

1.3.7.5   Exercise ECG test

Exercise ECG, which is usually used for the assessment of ischeamia, can also be utilised as a simple test for evaluating autonomous nervous system function. For example, exercise capacity, chronotropic incompetence, heart rate recovery, and the occurrence of premature ventricular beats represent pathophysiological changes of sympathetic tone

in patients with CHF.

a. Exercise capacity and chronotropic incompetence Reduced exercise capacity and maximal heart rate during exercise are usually observed in CHF patients. An inability to reach at least 85% of target heart rate during exercise may indicate a reduced sympathetic reaction to a rise in LV wall stress in response to peak exercise (the Bezold-Jarisch reflex) (Mark 1983). Patients with CHF have increased sympathetic nervous activity at rest, but a blunted sympathetic reaction in response to exercise (or chronotropic incompetence) (Francis et al. 1985). Heart rate at peak exercise and the ability to increase heart rate during exercise, reflecting responsiveness to sympathetic nervous stimulation, are reduced in CHF (Colucci et al. 1989). A smaller rise in heart rate in relation to the level of noradrenaline increase during exercise has been reported (Colucci et al. 1989). It has been suggested that chronotropic incompetence provides important prognostic information. In a study of 470 CHF patients, using multivariate analysis, a low chronotropic response, (but not the maximal oxygen consumption level) was found to be an independent predictor for heart failure mortality (Robbins et al. 1999).

b. Heart rate recovery and recovery ventricular ectopy Heart rate recovery after exercise represents a physiological response of vagal activity. Reduced autonomous modulation and vagal tone have been reported to associate with an increase in all-cause mortality in CHF patients (Arai et al. 1989, Imai et al. 1994, Watanabe et al. 2001).

The role of premature ventricular ectopy during and after exercise was reported in a study of 2123 patients with LVEF < 35%. After adjustment for other factors, recovery ventricular ectopy during the first 3 min after exercise, but not during exercise, remained a predictive indication of mortality (O' Neill et al. 2004).

c. Microvolt T-wave alternans Microvolt T-wave alternans is defined as a measure of the beat to beat fluctuation in the morphology of the T-wave reflecting cellular repolarisation alternans (Cutler & Rosenbaum 2009a). The measurement of T-wave alternans during exercise, while the stimulated myocardium is more proarrhythmic, may offer

an advantage over signal-averaged ECG and QT dispersion (Armoundas et al. 2002). T-wave alternans was first reported to be a potential risk predictor of arrhythmic events by Rosenbaum et al. (1994). This test yields a high negative value for SCD stratification in both ischaemic and dilated cardiomyopathy populations (Hohnloser et al. 2003, Chow et al. 2006, Calo et al. 2011). However, the problem with this test is that a high proportion of patients are in the grey-zone or "indeterminate" category, these are usually patients who are unable to attain an adequate heart rate during exercise. Furthermore, Chow et al. (2006) reported that even patients with "indeterminate" results, who had ischaemic cardiomyopathy and LVEF < 35%, still had a high risk of arrhythmia. Therefore, microvolt T-wave alternans may play an important role in identifying CHF patients who are unlikely to have AICD discharge. However, there are several disadvantages of this test. It requires a clean ECG and can be used only in patients with sinus rhythm. Moreover, its low positive predictive value requires it to be used in conjunction with other tests in order to improve the accuracy of risk stratification.

1.3.7.6   Baroreceptor sensitivity (BRS)

BRS is a method to measure BP response to a change in heart rate (HR) after an infusion of either pressor or vasodilator agents. For example, an administration of phenylephrine results in an increase in systolic BP leading to a decrease in HR and cardiac output, which is controlled by the parasympathetic nervous system, while infusion of nitroprusside leads to a reduction in BP, and an increase in HR, which is mediated by the sympathetic nervous system. These responses are markedly blunted in CHF (Amorim et al. 1981). Moreover, depressed baroreceptor sensitivity is associated with more severe clinical symptoms, LV dysfunction and poor survival (Mortara et al. 1997).

1.3.7.7   Invasive electrophysiological (EP) testing

The role of electrophysiological (EP) testing for SCD risk assessment in CHF patients is conflicting. In early AICD studies (MADIT I and MUSTT), inducible EP results were associated with a higher rate of sustained VT or VF and a worse outcome (Moss et al. 1996, Buxton et al. 2000). However, in patients with a positive EP test and a

LVEF < 30%, inducible EP tests did not predict SCD (Schmitt et al. 2001, Buxton et al. 2002). Therefore, EP testing is unreliable for the prediction of the mode of death in patients with severe cardiac dysfunction [with a wide range of sensitivity from 28% to 80% (Buxton 2009)] and is no longer performed routinely in AICD studies (MADIT II, COMPANION, and SCD-HeFT) (Moss et al. 2002a, Bristow et al. 2004, Bardy et al. 2005). Recently, the only recommendation of EP testing in CHF patients is to investigate unknown-cause syncope (Zipes et al. 2006). Furthermore, the invasive nature, cost, and the requirement of specialised staff and equipment limit the application of the test as a screening tool.

1.3.7.8 Imaging techniques

a. Quantitation of infarct size and fibrosis via cardiovascular magnetic resonance (CMR) In ischaemic cardiomyopathy, sizes of the scar volume and scar percentage were better predictors of cardiac events than standard measures (LVEF and left ventricular volumes) (Yokota et al. 2008). In non-ischaemic dilated cardiomyopathy, patchy fibrosis (late enhancement post-Gadolinium) has also been seen in many patients using CMR imaging. In a prospective study of 101 patients, the presence of mid-wall fibrosis was a strong predictor of the combined end-point of all-cause mortality and cardiovascular events (Assomull et al. 2006).

b. Nuclear Medicine Techniques: evaluations of viability and denervation Nuclear medicine techniques have been utilised predominantly in two distinct ways, to evaluate cardiac risk in patients with CHF:

(i) Assessment of regional cardiac viability In patients with CHF of ischaemic origin, there is greater theoretical potential for reversibility of haemodynamic impairment if dysfunction is engendered by reversible contractile dysfunction due to either acute oxidative stress (a process usually termed "stunning") or chronic down-regulation of contractile processes together with energetic depletion ("hibernation") (Partington et al. 2011).

Tests for assessment of degree of myocardial viability tend to utilise either FDG PET or SPECT perfusion imaging. In particular, PET tracers for integrity of myocardial metabolism in non-contractile areas (Yoshinaga et al. 2004, Slart et al. 2005) or SPECT perfusion imaging utilising either ^{201}Thallium or ^{99}Technetium-labelled myocardial tracers (Leoncini et al. 2001, Shimoni et al. 2003) have been validated as markers of regional viability.

However, no conclusive evidence that such viability assessments affect outcomes in any subset of patients with CHF, irrespective of treatment strategies was recently reported in the STICH trial (Bonow et al. 2011). However, the most important limitation of the study is that viability testing was performed in only 601 of the total 1212 recruited patients which were nonrandomised and nonblinded, therefore introducing selection biases.

(ii) Assessment of cardiac denervation   This is a central theme of this thesis as discussed in Section 1.3.8.2.

1.3.8   Cardiac denervation in CHF

1.3.8.1   Occurrence and implications of cardiac denervation in CHF

There is considerable evidence that CHF, both in animal models (Higgins et al. 1972, Nakamura et al. 2000) and in humans (Mancia 1990, Kaye & Esler 2005), is associated with variable degree of localised or generalised cardiac denervation. For example, in a dog model of coronary embolisation of a diagonal branch, it has been shown that cardiac denervation occurred not only in the infarcted areas, but also in the apex, which is perfused by the LAD (Inoue et al. 1988). In MI rats, extensive denervation of the LV myocardium below the infarct has been found, while sympathetic nerve fibres were retained in the base of the heart. In humans, direct evidence for the existence of cardiac denervation in CHF comes from both biopsy and imaging studies. In patients post myocardial infarction, cardiac denervation occurred and was followed by reinnervation within the LV (Minardo et al. 1988, Hartikainen et al. 1996a). Cardiac denervation is also found in diabetic cardiomyopathy (Sacre et al. 2010). Reduced ^{123}I-metaiodobenzylguanidine

(^{123}I-MIBG) uptake has also been reported to correlate with autonomic dysfunction and increased mortality in patients with diabetes mellitus (Kim et al. 1996).

The mechanisms leading to cardiac denervation in CHF, and pharmacological agents which might manipulate the process are still controversial. For example, the patho-physiological explanations for sympathetic nervous dysfunction in myocardial dysfunction induced by ischaemia include: (1) transient cardiac sympathetic dysfunction due to myocardial stunning or (2) complete cardiac denervation following an infarction (Zipes 1990). Furthermore, there is also a considerable body of evidence that the kinetics of catecholamines within the heart are disturbed in CHF, although it is not clear to what extent this disturbance reflects the denervation process. In this section, the evidence for changes in functional sympathetic activity in CHF, and the factors which affect the regulation of sympathetic tone, release and reuptake of noradrenaline are discussed.

a. Physiological context of cardiac denervation in CHF   A number of studies have documented that myocardial infarction is associated with irreversible sympathetic nervous dysfunction (Stanton et al. 1989). Moreover, cardiac denervation has been seen not only in the infarcted regions, but also the area surrounding the infarction. This results from down-stream denervation of irreversibly injured sympathetic nerve fibres. In nontransmural MI, cardiac sympathetic function may be preserved in the viable subepicardium (Inoue et al. 1988). More importantly, Elvan & Zipes (1998) have documented that a region with denervated, but viable myocardium post myocardial infarction, may be more arrhythmogenic: these areas have been shown to be hypersensitive to programmed electrical stimulation and to catecholamine infusion. This hypersensitivity was diminished by intravenous administration of propranolol (Inoue & Zipes 1987). It has been suggested that denervated but viable myocardium may exhibit upregulation of β-adrenoceptors and increased sympathetic nerve sprouting leading to increased responses to sympathetic stimulation (Oh et al. 2006).

b. Changes in cardiac sympathetic function in CHF   It has been reported that disordered sympathetic nervous activation in CHF is a very early characteristic of the

neurohormonal modulation of this process (Rundqvist et al. 1997). CHF is associated with high plasma levels of catecholamines and these correlate with adverse outcomes (Cohn et al. 1984). More specifically, systemic noradrenaline spill-over rate from the sympathetic nervous system to plasma was shown to be significantly increased in CHF (Kaye et al. 1994). Elevations of neuropeptide Y have also been documented in CHF patients (Kaye et al. 1994, Feng et al. 1999). Furthermore, although direct electro-physiological methods for the measurement of cardiac nerve activity are only available in animal models (Jardine et al. 2005), in humans, muscle sympathetic nervous activity measured by microneurography was found to correlate with cardiac spill-over rate (Persson et al. 1989). Tyrosine hydroxylase, an enzyme involved in the first step of the noradrenaline synthetic pathway (see Figure 1.2.1), was shown to be reduced in myocardial biopsies from CHF patients (Kaye et al. 2000) and also in animal models of CHF (Himura et al. 1993, Kimura et al. 2010). Moreover, noradrenaline content in the myocardium of CHF patients was also found to be reduced (Chidsey et al. 1965).

c. Disordered regulation of cardiac sympathetic tone   It has been suggested in previous studies that high pressure baroreceptors and low pressure cardiopulmonary receptors play a role in controlling the activity of the sympathetic and parasympathetic nervous systems. Pulmonary capillary wedge pressure and pulmonary artery pressure were found to correlate with cardiac noradrenaline spill-over rate (Kaye et al. 1994). Similarly, a significant correlation between LV filling pressure and muscle sympathetic nervous activity was also reported (Ferguson et al. 1990). This findings are supported by evidence that infusion of sodium nitroprusside so as to reduce LV filling pressure was associated with a reduction in cardiac sympathetic activity (Kaye et al. 1998). ACEI therapy has been shown to decrease cardiac sympathetic activity, increase noradrenaline neuronal uptake, and myocardial β-adrenoceptor density (Gilbert et al. 1993, Kawai et al. 1999). Moreover, lowering of the filling pressure by lower-body negative pressure reduced cardiac noradrenaline spill-over rate in CHF patients, but not in control subjects (Azevedo et al. 2000).

The role of the central nervous system in controlling sympathetic activity has also

been investigated. Subcortical noradrenaline spill-over rate was directly correlated with whole-body noradrenaline spill-over rate and was significantly higher in patients with CHF, compared to controls (Aggarwal et al. 2002). Furthermore, brainstem angiotensin II was shown to regulate sympathetic activity. Cardiac dysfunction and sympathetic nervous activation post myocardial infarction were not observed in rats with brainstem angiotensinogen deficiency (Wang et al. 2004). Recently, it has been suggested that superoxide may play a role in the modulation of angiotensin II-mediated sympathetic nervous system activation (Francis et al. 2004).

d. Local regulation of sympathetic function in the myocardium A number of studies have reported that the cardiac noradrenaline spill-over rate is increased in CHF (Kaye et al. 1994), despite the reduced noradrenaline content in the LV myocardium, which is typically half that of normal subjects (Chidsey et al. 1963). Moreover, the cardiac uptake rate of noradrenaline measured by radiolabel and nuclear imaging methods is reduced in CHF (Eisenhofer et al. 1996, Gerson et al. 2003). The mechanisms for these findings are uncertain. Although a significant decrease in myocardial noradrenaline uptake1-transporter (NET) binding sites was observed in animal models of CHF and patients with CHF (Haider et al. 2010), the expression of NET mRNA was unchanged (Backs et al. 2001). This was explained by post-transcriptional down-regulation of NET activity (Backs et al. 2001, Narula & Sarkar 2003). Loss of NET may contribute to the reduced noradrenaline stored in the synaptic vesicles and the increased plasma noradrenaline levels in CHF.

Nerve growth factor (NGF) in the myocardium may also take part in the regulation of innervation density in CHF. In both human and animal experiments of CHF, the myocardial content and the rate of release of NGF were decreased (Kaye et al. 2000, Kimura et al. 2010). Moreover, the myocardial expression of NET and its function were increased by NGF (Lockhart et al. 1997, Kreusser et al. 2006).

1.3.8.2   Assessment of sympathetic nerve activity in CHF

As outlined in the previous section, the extent of sympathetic activation in CHF is potentially variable, for example with specific treatments. Furthermore, sympathetic activation ultimately affects outcomes in CHF. Techniques to measure sympathetic nervous activity therefore play an important role in assessing CHF processes and their outcomes. In the following section, methods to measure sympathetic nervous system activity in CHF are reviewed.

a.   Plasma noradrenaline concentration   It has been established in numerous studies that plasma noradrenaline concentrations are increased substantially in CHF (Ferguson et al. 1990, Francis et al. 1990, Francis et al. 1993). Elevated plasma noradrenaline concentration has been associated with an increase in mortality rate in CHF (Francis et al. 1993, Benedict et al. 1996). Using multivariate analysis, plasma noradrenaline has been reported to be the most powerful independent predictor of all-cause and cardiovascular mortalities, when heart rate, stroke volume index, LVEF, plasma ANP, and plasma renin were taken into account (Cohn et al. 1984, Francis et al. 1990, Francis et al. 1993). However, this method for assessment of sympathetic nervous system activity has several limitations. Firstly, plasma noradrenaline concentration depends not only on sympathetic tone and noradrenaline release, but also on the clearance of neurotransmitters from plasma, which is slowed in CHF patients because of a decrease in cardiac output and regional blood flow (Esler et al. 1990). Secondly, global sympathetic nervous function does not always reflect the activity of regional sympathetic nervous function (Esler et al. 1990). Moreover, because it is variable during the day with a peak in the morning and lower levels at night, a single measurement is not representative of overall sympathetic nervous activity (Bleske et al. 1999). Finally, many drugs utilised for CHF therapy can affect noradrenaline plasma clearance and neurotransmitter concentration. Thus, methods to assess regional sympathetic nervous function are needed.

b. Regional sympathetic nervous function assessments   It is believed that the washout of noradrenaline from an organ reflects the level of sympathetic nervous activity in that organ. Methods for regional sympathetic nervous function assessments include clinical microneurography and organ-specific noradrenaline "spill-over" rate assessment. However, these have been only used to assess different aspects of sympathetic nervous function for experimental rather than clinical purposes.

(i) Clinical microneurography   Microneurography was first utilised for the direct measurement of sympathetic nervous function in the late 1960s (Vallbo et al. 2004). The most common measurement is the muscle sympathetic nervous activity (MSNA), which provides information on sympathetic nerve firing rates to skin and skeletal muscle. Nerve activity, synchronised with the heart beat is recorded using fine tungsten electrodes inserted through the skin and positioned in sympathetic fibres of peroneal nerve (Vallbo et al. 2004). Multi-unit neuron recordings of bursts of nerve activity are commonly used, while single neuron recordings are technically challenging (Macefield et al. 2002). MSNA is significantly increased in CHF (to about double that of healthy controls) (Grassi et al. 2001) and was shown to have prognostic value in patients with CHF (Barretto et al. 2009). In heart transplant patients, MSNA was found to be consistently decreased, 1 year after transplantation (Rundqvist et al. 1996). Moreover, it has been suggested that in CHF patients, exercise training is associated with a significant reduction in MSNA (Roveda et al. 2003).

(ii) Organ-specific noradrenaline "spill-over" rate assessment   In 1984, Esler et al. (1984) pioneered a method to measure the organ-specific noradrenaline "spill-over" rate which can be used to assess transmitter removal from plasma. The technique involves intravenous infusion of tritiated noradrenaline and coronary sinus blood sampling to determine the gradient of noradrenaline across the heart. It has been shown that patients with CHF have a higher noradrenaline spill-over rate across the heart and kidneys, but not the lungs, than controls: this might be due to increased sympathetic nervous activity or decreased reuptake (Hasking et al. 1986). Cardiac noradrenaline spill-over rate is directly correlated with the amount of sympathetic neural activity in muscle,

which is measured by microneurography (Kingwell et al. 1994, Rundqvist et al. 1996). In untreated CHF patients, the amount of cardiac noradrenaline "spill-over" may be up to 50 times than that of normal subjects (Morris et al. 1997).

c. Imaging techniques to assess sympathetic nerve activity in CHF   Assessment of cardiac sympathetic neurons can be achieved by non-invasive nuclear medicine imaging techniques such as PET and SPECT by using several radio-labelled tracers, which can be used to assess several aspects of synaptic neurotransmission. While most of these agents have been utilised to assess presynaptic function, new agents which bind to postsynaptic α and β receptors are now under investigation (Merlet et al. 1993, Ueki et al. 1993, Gaemperli et al. 2010).

(i) Positron emission tomography imaging (PET)   Assessment of sympathetic nerve activity and cardiac denervation has been extensively evaluated utilising PET imaging. Because of its high spatial and temporal resolution relative to SPECT imaging, PET provides an excellent tool to quantitate neurocardiological processes at the tissue level.

A number of radiotracers for the assessment cardiac synaptic neurotransmission have been developed (Travin 2009). They can be divided into 2 groups: (1) radio-labelled catecholamines, including 6-[^{18}F]-fluorodopamine, 6-[^{18}F]-fluoronoradrenaline and ^{11}C-adrenaline; and (2) radio-labelled catecholamine analogues (also called false adrenergic neurotransmitters) such as ^{11}C-hydroxyephedrine (HED), ^{11}C-neuronal tracers (^{11}C-adrenaline, ^{11}C-phenylephrine), ^{18}F-flurometaraminol, and meta^{76}Br-bromobenzylguanidine (Figure 1.3.9).

^{11}C-HED is the most common neurohormonal PET tracer. It has more highly selective uptake 1 concentration than ^{123}I-MIBG and the ^{11}C-HED uptake seems to be more homogeneous in normal subjects than ^{123}I-MIBG, leading to a better differentiation between cardiac innervation and denervation (Matsunari et al. 2010). Reduced ^{11}C-HED uptake on PET imaging correlates with reduced LVEF (Bengel et al. 2001). Reduction in ^{11}C-HED uptake, peak oxygen uptake, and LV end-diastolic volume were reported to be associated with poor outcome in 46 patients with CHF during 55 ± 19 month

follow-up (Pietila et al. 2001). Furthermore, ^{11}C-HED has shown a potential benefit in the evaluation of hibernating myocardium (Luisi et al. 2005).

[11]C-neuronal tracers, which have recently been investigated, may have an advantage in the evaluation of vesicular storage function due to their rapid metabolism through MAO (Tipre et al. 2008). PET-based techniques for measuring myocardial β-adrenoceptor density, a further variable in CHF, are increasingly investigated. For example, down-regulation of β-adrenoceptors measured by PET using ^{11}C-CGP-12177 has been found in patients after AMI, hypertrophic cardiomyopathy as well as dilated cardiomyopathy. Low myocardial 11C-CGP-12177 uptake was shown to be associated with poor LV function at follow-up in these patients (Merlet et al. 1993, Ueki et al. 1993, Spyrou et al. 2002, Gaemperli et al. 2010). Furthermore, 11C-CGP-12177 PET imaging has been utilised to investigate the beneficial effects of carvedilol in patients with dilated cardiomyopathy (Naya et al. 2009).

However, molecular imaging techniques utilising PET are not widely utilised due to its relatively high cost, limited availability of cyclotron, and short half-life of current radiotracers (Shah et al. 2011). Therefore, alternative approaches using SPECT imaging to evaluate sympathetic nervous activity have been extensively investigated, and can be used in the clinical setting.

(ii) ^{123}I-metaiodobenzylguanidine imaging (^{123}I-MIBG) The widespread availability of MIBG imaging has been associated with proliferation of studies directed towards the utilisation of this technique for clinical risk indexation in CHF. In 1981, ^{131}I-MIBG imaging (utilising MIBG labelled with [131]iodine and standard nuclear medicine gamma cameras) was first established to assess cardiac sympathetic nervous innervation and denervation in humans (Kline et al. 1981). Because of the high energy emission of 364KeV (γ and β photons) and very long half life (about 8 days) of the [131]Iodine isotope, it has been replaced by ^{123}I-MIBG tracer (McGhie et al. 1991). [123]I has a shorter half life of 13.2 hours and produces mainly γ photons with energies of 159KeV, which are more favourably imaged for gamma camera imaging. ^{123}I-MIBG cardiac imaging has
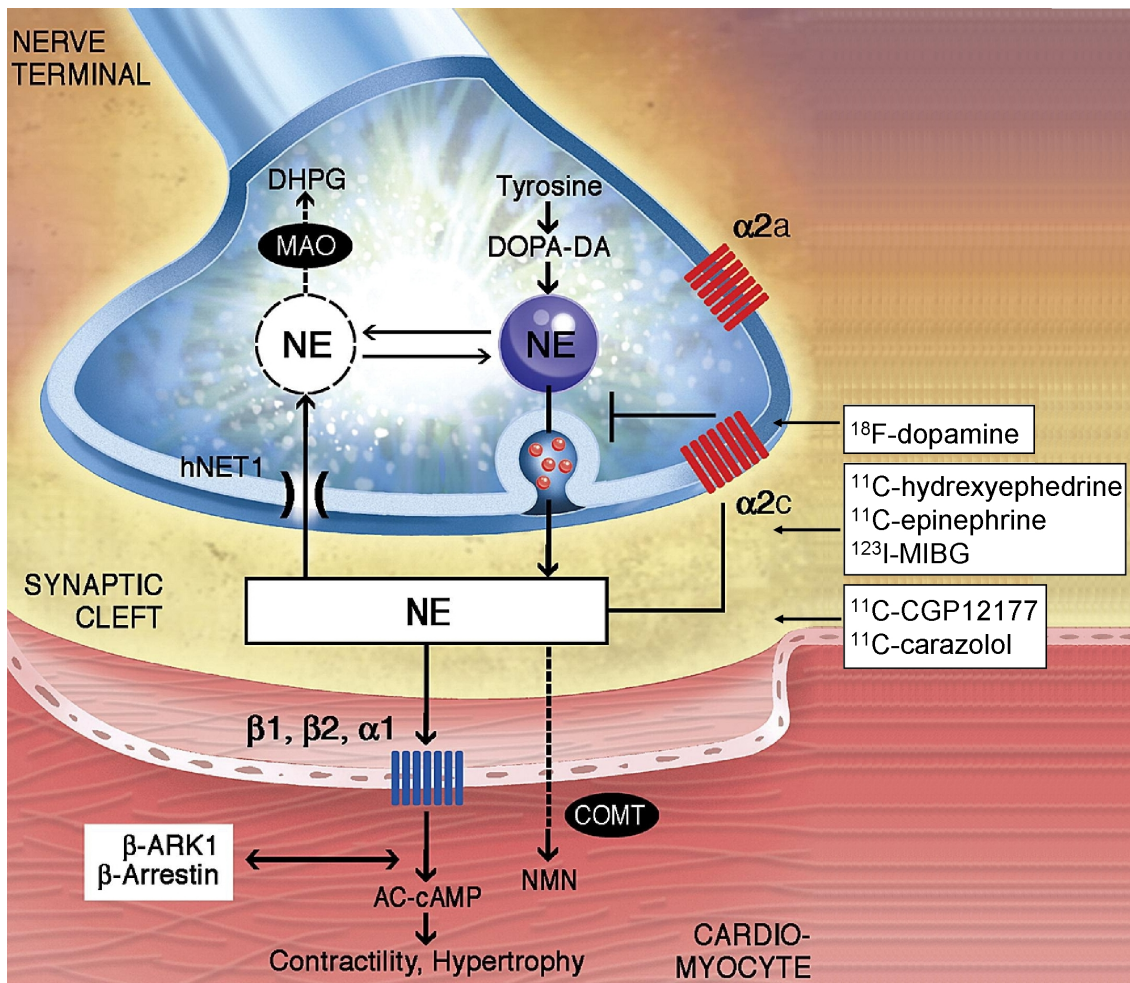
Figure 1.3.9: Diagram of adrenergic neurotransmitter synthesis, receptors, noradrenaline transport in the cardiac pre-synaptic nerve endings and binding sites of radio-labelled PET and I-MIBG tracers

[AC = adenylyl cyclase, β-ARK = β-adrenoceptor kinase, cAMP = cyclic adenosine monophosphate, COMT = catechol-ortho-methyl transferease, DHPG = dihydroxyphenylglycine, NE = noradrenaline, NET = noradrenaline transporter, MAO = monoamine oxidase (Modified from Haider et al. (2010)]

been used in Japan and Europe for many years, while it is currently under FDA review in the US (Ji & Travin 2010).

The development of MIBG imaging was established via investigation of the selective sympathetic neuron blocking agent, guanethidine. It was first utilised to image adrenal tumours and was found to have stable concentration in tissues with high adrenergic in-

nervation. Peak myocardial uptake of MIBG is obtained at 1 hour post injection which is about as much as 20 times than plasma concentration, and gradually decreasing to 18% at 24 hours (Schanker & Morrison 1965). In an experiment in dogs to determine myocardial concentration of different analogs of guanethidine, Wieland et al. (1980) demonstrated that the metaoido-radiolable compound provides the most stable myocardial uptake with an optimal heart to blood and heart to lung ratio, compared to the ortho- and para-iodobenzylguanidine. The tissue uptake of MIBG is mainly in the liver and is lower in the myocardium, lungs, spleen, skeletal muscles, and thyroid, while it is not taken up by normal adrenal glands.

It is appropriate to discuss briefly how ^{123}I-MIBG imaging "works". Considerable evidence has been accumulated from both animal and human experiments that regional cardiac denervation is associated both with a propensity towards arrhythmogenesis and with increased cardiac effects of catecholamines. Presumably, this reflects in part the failure of catecholamine uptake into sympathetic nerve endings. Noradrenaline is produced and stored in high concentrations in presynaptic vesicles through a complicated biochemical process (see Section 1.2.1.1). When a stimulus occurs, noradrenaline is released into the synaptic cleft and binds to post-synaptic receptors, leading to several cardiovascular stimulatory effects (Borowsky & Hoffman 1995). In order to terminate the action of sympathetic stimulation, neurotransmitters are taken up from the synaptic space into the cytoplasm mainly by neuronal noradrenaline transporter, uptake 1 (Borowsky & Hoffman 1995, Brownstein & Hoffman 1994). MIBG is a noradrenaline analogue which is taken up, stored by the myocardium and then retained in sympathetic nerve endings, similar to noradrenaline but without further metabolism because MIBG is not metabolised by MAO or COMT (Figure 1.3.9) (Wieland et al. 1981). The majority of MIBG is cleared un-altered by the kidneys within 24 h, while only 1% of the MIBG dose is excreted via the faeces. Therefore, ^{123}I-MIBG imaging may be sub-optimal for diagnostic purposes in patients with severe kidney dysfunction, due to poor image quality resulting from slow clearance of the tracer. However, in a recent study by Verberne et al. (2011), it has been suggested that it is not necessary to correct for renal function and blood pool uptake of the tracer because the heart and mediastinal counts

are not interfered by residual vascular activity.

In an animal model of HF, it has been found that MIBG washout rate was accelereted during the early HF stage (Takatsu et al. 1995). In order to differentiate the neuronal or nonneuronal uptake, pretreatment of desipramine, a specific blocker of neuronal noradrenaline reuptake, was used in this study. The investigators demonstrated that increase in ^{125}I-MIBG washout rate was due to increased noradrenaline release from the neuronal sympathetic endings. In addition, reduced myocardial MIBG uptake in autotransplanted dog hearts was found to be associated with a significant decrease in myocardial noradrenaline stores (Rabinovitch et al. 1987). This was confirmed by immunohistochemistry that myocardial MIBG uptake corresponded with the presence or absence of sympathetic nerve in the ventricle (Gaudino et al. 2002). In humans, a significant decrease in myocardial MIBG uptake has been found in patients with congestive cardiomyopathy, compared to healthy controls (Henderson et al. 1988). Reduced myocardial MIBG uptake has also reported in acute and chronic ischaemia, as well as in myocardial infarcted regions (McGhie et al. 1991, Nakata et al. 1996, Estorch et al. 2000).

Given that MIBG imaging reflects cardiac catecholamine kinetics, there are several potential sources of perturbation in this measure. Medications such as tricyclic antidepressants, antipsychotics, tramadol, cocaine, and opioids are known to interfere with the uptake of catecholamines, including MIBG, and these medications should be withdrawn 7 to 14 days before an MIBG scan. Other cardiovascular/antihypertensive agents, including ACEI, ARB, and β-blockers have been demonstrated to have no effects on ^{123}I-MIBG imaging (Yamashina & Yamazaki 2007, Agostini et al. 2009, Carrio et al. 2010).

Imaging methods

To date, there is no consensus establishment of ^{123}I-MIBG dosage. Intravenous administration of 111-370 MBq of ^{123}I-MIBG injected over a one minute interval, has been suggested, followed by planar and SPECT imaging at 15-30 min (early scan), and 4 hours post IV (delayed scan). These time points were demonstrated by Nakajo et al. (1986) to be the optimum to evaluate myocardial MIBG uptake and retention. A larger

dose of 370 MBq has been proposed for patients with severe LV dysfunction in order to obtain sufficient tracer uptake for SPECT imaging (Flotats & Carrio 2004). ^{123}I-MIBG should be avoided in patients with a known history of allergy to MIBG, MIBG sulphate, or iodine (Ishibashi et al. 2009). In general, ^{123}I-MIBG imaging is safe with only some rare side effects such as dizziness, flushing, pruritus, and infection/haemorrhage at the injection site (Merlet et al. 1995).

<u>Image acquisition</u>

Because ^{123}I also emits high energy photons with energies of more than 400 keV, it has recently been sugested that medium energy collimators, rather than the conventional low energy collimators which have lower contrast, should be used in order to reduce noise from scattered photons (Bax et al. 2008). This may be very important because there are substantial overlap in the heart to mediastinum ratio (HMR) between low and high-risk patients. Moreover, the use of a cut-off of a HMR to distinguish those patients in most studies highlights the importance of providing high quality images in clinical practice. However, most studies in the literature have used low energy collimators, as was the use in the current studies.

Planar imaging, which provides global cardiac sympathetic innervation and denervation information, has been used in most of the published studies (Agostini et al. 2008), while only a few recent studies have used single photon emission computed tomography (SPECT) imaging to localise areas of denervation (Flotats & Carrio 2004). Planar imaging, which acquires two-dimensional images of the chest in anterior, left lateral and left anterior oblique views, provides global cardiac sympathetic innervation and denervation information. The early and late MIBG activity can be quantified as the early and late HMR, which is the ratio of the mean counts from the heart to the mean counts from the upper third of the mediastinum, measured on the planar anterior view. High reproducibility of both early and late HMR has been recently reported by Okuda et al. (2011). The HMR and wash-out rate (WR) can be calculated as:

$$HMR = \frac{\text{Heart counts per pixel}}{\text{Mediastinum counts per pixel}} \qquad (1.3.1)$$

$$\text{WR} = \frac{\text{Early MIBG activity} - \text{Late MIBG activity}}{\text{Early activity}} \times 100\%. \qquad (1.3.2)$$

The HMR represents myocardial uptake of MIBG, while the WR may be a parameter of myocardial sympathetic function, as it has been shown to correlate with CHF severity in a multi-centre study (Agostini et al. 2008).

In contrast, with SPECT imaging, three-dimensional images are achieved using a multiheaded camera rotating around the patients' chests. SPECT reconstruction provides transaxial slices showing the amounts of radioactivity detected in the imaged three-dimensional volume. The final images are oriented in a consistent manner after the reconstruction process, providing short-axis, horizontal long-axis, and vertical long-axis tomograms, and allow generation of polar maps. In comparison to the planar technique, SPECT imaging has a relative lack of myocardial territory overlap, which is a limitation of the planar technique. Quantitative analysis has been developed to make the interpretation and analysis process objective and standardised. This is particularly useful as a research tool to compare changes in uptake during serial imaging of the same patient and compare with myocardial perfusion SPECT imaging.

Recently, there have been several attempts to quantitate regional myocardial denervation on ^{123}I-MIBG SPECT imaging by using either a point scale for visual evaluation, or commercially available software (Boogers et al. 2011). Most of the ^{123}I-MIBG studies have used visual scoring systems, in which each segment on the polar map is given a score (on a scale of 0-4) according to segmental myocardial tracer activity. However, in a recent study of 961 patients with CHF in North America and Europe, it has been suggested that quantitation of SPECT ^{123}I-MIBG using a defect scoring system on the polar map is not reproducible (Jacobson et al. 2010). Furthermore, due to the low uptake of MIBG in patients with severe cardiac dysfunction, it is very difficult to reconstruct SPECT images in such patients (Agostini et al. 2009, Ji & Travin 2010). Finally, the lack of an available normal data base limits the utility of the available commercial quantitative soft ware packages. Recently, Japanese investigators have developed a normal data-base from a multicentre study in order to assess SPECT ^{123}I-MIBG (Matsuo et al. 2009). However, this may be specific to the Japanese population.

Validation of ^{123}I-MIBG imaging

Standardisation and validation of ^{123}I-MIBG images is fundamental to improving its implementation clinically. There has been a lack of standardisation of this technique, there is variation between institutions in the acquisition parameters used, and other technical aspects of procedure. The HM ratio and washout rate generated from planar images are commonly used as quantitative methods. However, it has been reported that these indices show significant variation particularly between different institutions (Verberne et al. 2008). For example, a wide variation in the mean normal value of the HM ratio from 1.4 to 2.8 has been reported by an investigation into 49 institutions across Japan (Nishimura et al. 1997). The European Society of Nuclear Medicine has proposed a standard method to quantitate ^{123}I-MIBG images in order to minimise this problem (Flotats et al. 2011).

Image interpretation

Although ^{123}I-MIBG SPECT imaging has many advantages compared to planar imaging, this is a challenging technique requiring careful steps of acquisition and processing, with several potential technical errors which can lead to false positive results. For optimal image interpretation, a review of patients body habitus, planar projection images, and accuracy of left ventricular long axis selection should be carried out. SPECT image interpretation requires a systematic evaluation for extent, severity, and location of a myocardial defect. A substantial overlap between the heart and the liver and/or the lungs, often seen in MIBG scans, is a potential hazard for interpretation.

Utility of ^{123}I-MIBG imaging in patients with CHF

Given that cardiac denervation represents increased intracardiac sympathetic response, impaired ^{123}I-MIBG uptake/increased washout rate could theoretically predict:

(1) haemodynamic deterioration

and/or (2) arrhythmogenesis in patients with CHF.

The key issue is under which circumstances this information is likely to affect clinical decision making. I will therefore discuss:

(1) Evidence for utility of ^{123}I-MIBG imaging

(2) Clinical implications

<u>Evidence for utility</u>

Correlates of ^{123}I-MIBG parameters and haemodynamic deterioration

A significant decrease in late HMR and increase in washout rate of myocardial ^{123}I-MIBG has been shown in patients with CHF, compared to normal healthy controls (see Table 1.3.3) (Merlet et al. 1992, Bengel et al. 1999, Cohen-Solal et al. 1999, Zhao et al. 2001, Matsuo et al. 2003). In patients with ischaemic heart disease, MIBG uptake is decreased in areas of ischaemia and MI (acute and chronic) (McGhie et al. 1991). A number of investigations have demonstrated that decreased ^{123}I-MIBG uptake is associated with extent of LV systolic dysfunction at that time. Low myocardial MIBG activity and increased "washout" have been reported to correlate with the presence of low LVEF (Zhao et al. 2001). In an investigation of 93 patients with CHF, a close correlation was found between low late HMR (4 hours) and LVEF, cardiac index, pulmonary wedge pressure, and peak oxygen uptake (Cohen-Solal et al. 1999).

Investigators	MIBG parameters	Controls	CHF patients	p
Matsuo et al 2003	LVEF	73±7	31±1	< 0.05
	Delayed HMR	2.6±0.3	1.8±0.9	< 0.05
	Washout rate	28±3	38±3	< 0.05
Zhao et al 2001	LVEF	71±11.2	22±8.4	-
	Delayed HMR	2.66±0.21	1.73±0.39	-
	Washout rate	11.88±7.23	31.58±11.18	-
Cohen-Solal et al 1999	LVEF	-	25±10	-
	Delayed HMR	-	1.31±0.2	-
	Washout rate	-	34.8±6	-
Bengel et al 1999	Delayed HMR	2.8±0.55	2.36±0.66	0.04
	Washout rate	0.2±10.2	11.6±7.9	0.002

Table 1.3.3: Global ^{123}I-MIBG quantitative parameters in CHF, compared to controls from previous studies

On the other hand, it might be assumed that cardiac denervation would also pre-

dict deterioration of LV function, via down-regulation of myocardial β-adrenoceptor signalling. However, this possibility has not been widely investigated to date. While this is partially understandable, given the complex interplay of potential CRT insertion, the absence of these data makes interpretation of arrhythmias data difficult.

Utility of ^{123}I-MIBG imaging for prognostic assessment and risk stratification in patients with CHF

Various clinical innervation studies (summarised in Table 1.3.4) have shown that low "late" ^{123}I-MIBG HMR is an independent correlate of poor outcome in patients with CHF. In a study of 112 patients with dilated cardiomyopathy, Merlet et al. (1999) demonstrated that a decreased late ^{123}I-MIBG HMR was associated with an increase in mortality when compared to other parameters of LV function. A meta-analysis of 18 studies with a total of 1755 patients showed a strong independent relationship between a low "late" HMR or an increased myocardial MIBG washout rate and cardiac events, including cardiac death, MI, cardiac transplantation and CHF hospitalisation (Verberne et al. 2008a). Nakata et al. (1998) investigated 414 patients with or without CHF and found that low late ^{123}I-MIBG HMR, together with low LVEF, NYHA class III or IV, age >60 years, and a history of MI were strong predictors of cardiac death. Furthermore, serial cardiac ^{123}I-MIBG imaging has been shown to be a useful test for the prediction of sudden cardiac death in CHF patients (Kasama et al. 2008, Kasama et al. 2010). In a prospective study of 106 patients with LVEF < 40% over a 65-month follow-up period, using multivariate analysis, Tamaki et al. (2009) reported that increased ^{123}I-MIBG washout rate and reduced LVEF independently predicted the risk of SCD, while HRV, SAECG, and QT dispersion did not. In combination with SPECT perfusion imaging using ^{99m}Tc-Sestamibi, it has been proposed that the extent of mismatch between perfusion and sympathetic innervation may be predictive of VT (Simoes et al. 2004). In a multicentre prospective study of 961 patients, who presented with NYHA class II-III and LVEF < 35%, the ADMIRE-HF trial, a late ^{123}I-MIBG MHR < 1.6 were found to have independent predictive value for identifying patients at high risk of heart failure progression, arrhythmic events, and cardiac death (Jacobson et al. 2010).

There are limited data about the use of ^{123}I-MIBG imaging as a predictor of AICD

Ivestigatiors	n	LVEF	MIBG parameters	Follow-up
Jacobson et al 2010	961	27.1±6.1	late HMR < 1.6	17 months
Boogers et al 2010	116	28±8	late SPECT score > 25	15 months
Imamura et al 2001	171	27±10	WR > 53%	27±11 months
Merlet et al 1999	112	21±9	late HMR <1.2	27±20 months
Nakata et al 1998	414	22±8	late HMR < 1.74	17 months

Table 1.3.4: Utility of ^{123}I-MIBG imaging for prognostic assessment and risk stratification in patients with CHF from previous studies

discharge in patients with CHF. In a study of 54 patients referred for AICD implantation, a strong relationship was found between a low "late" HMR and a significant increase in AICD discharges (Nagahara et al. 2008). A combination of a low late HMR (< 1.9) and high ^{99m}Tc-tetrofosmin rest score, but not late HMR alone, was found to be associated with a significant higher AICD discharge (Nishisato et al. 2010). In a recent study of 116 CHF patients undergoing AICD insertion, Boogers et al. (2010) found a strong correlation between a high late ^{123}I-MIBG SPECT score of greater than 26 (using a visual segmental scoring system on a 17 segmental model of the LV) and a higher incidence of appropriate AICD discharge, compared to those with the score less than 26. However, the use of a visual scoring system which was reported to be non-reproducible in the ADMIRE-HF study by Jacobson et al. (2010), and therefore challenges the results of the prior study.

Utility of ^{123}I-MIBG imaging for treatment assessment

Gerson et al. (2002) reported an improvement in cardiac sympathetic nervous system function in response to carvedilol therapy. Similar results were found with spironolactone (Kasama et al. 2003) and the combination of amiodarone and carvedilol compared to carvedilol alone (Toyama et al. 2008). Moreover, reinnervation after a heart transplantation can be evaluated by serial ^{123}I-MIBG scans (De Marco et al. 1995). In this study of 16 heart transplantation patients, a significant improvement in cardiac sympathetic nervous system function has been observed. However, in patients with decompensated

heart failure receiving a LV support device, the use of ^{123}I-MIBG imaging for assessment of sympathetic nervous activity after LV support device is still controversial. While no improvement in sympathetic nervous function was found in a study by Miyagawa et al. (2001), ^{123}I-MIBG uptake was shown to be significantly improved after LV unloading by LV support device insertion by Drakos et al. (2010).

The majority of studies of ^{123}I-MIBG imaging as a predictor of cardiac events in CHF have used the ^{123}I-MIBG HMR, a global index of sympathetic nervous activity, to indicate to what extent this reflects de novo arrhythmogenesis, deterioration of LV function, or their combination. This approach has several limitations. Firstly, the global uptake of ^{123}I-MIBG does not represent regional sympathetic nervous system, which distributes predominantly in the anterior wall in the normal heart (Janes et al. 1986, Matsuo et al. 2009). Therefore, normal global ^{123}I-MIBG uptake may not reflect a regional abnormality. In diabetic patients with cardiac autonomic neuropathy, early cardiac denervation has been seen in the inferior region, then in the lateral and septal wall, and finally seen in the anterior wall (Scott & Kench 2004). On the other hand, regional cardiac denervation related to ischaemia or MI would be far more variable. Furthermore, most ^{123}I-MIBG-based investigations utilise a categorical evaluation method: a fixed cut-off for low HMR. SPECT ^{123}I-MIBG images can be analysed using a visual 17 segmental scoring system, and total defect scores can be calculated. However, this approach was not reproducible in the ADMIRE-HF study (Jacobson et al. 2010). Moreover, in a large number of these studies, patients with very low ^{123}I-MIBG uptake were excluded from SPECT analysis due to the difficulty in SPECT image reconstruction in those patients. Nevertheless, there has been no evaluation of the implications of regional heterogeneity of MIBG uptake in proximity to potential ischaemic zones, nor of sites (e.g., anterior vs. inferior) of denervation. Moreover, there is very little available evidence about evolution of the denervation process.

Implications for clinical decision making

If the results of ^{123}I-MIBG scanning were to predict arrhythmogenesis with any degree of precision, this technique could be used for decision making regarding AICD insertion. Tellingly, no investigators have reported such a study.

Furthermore, if ^{123}I-MIBG results predict haemodynamic deterioration in CHF, this might be of value in patients with associated cardiac dyssynchrony, who might otherwise have to wait for initiation of CRT. This possibility remains valid even after CRT as a global strategy has been shown to be beneficial in mild CHF (Stevenson et al. 2012, Holzmeister & Leclercq 2011). However, once again, no trials of this type have been undertaken to date.

1.3.9   Treatment of CHF

Management of CHF, including non-pharmacological and pharmacological strategies, and device-based treatment, is aimed at minimising symptoms, preventing remodelling, and prolonging survival. In this section, interactions between CHF therapies and cardiac synpathetic nervous activity will be discussed.

1.3.9.1   Physical exercise

Physical exercise is highly recommended for patients with CHF. Improvement in LV function and patients' symptoms has been reported in stable CHF patients undergoing aerobic and resistant exercise in several controlled studies (Coats 2011, Piepoli et al. 2011). Physical exercise has also been shown to improve sympathetic nervous function in patients with CHF (Roveda et al. 2003).

1.3.9.2   Medications

Neurohormonal systems play a crucial role in CHF progression and its adverse events. Therefore, neurohormonal antagonists, including inhibitors of the renin angiotensin aldosterone system (ACEI, ARB, and aldosterone antagonists), along with sympathetic drive (β-receptor blockers) are vitally important in the management of CHF. A combination of an ACEI or ARB, a β-adrenoceptor antagonist, and an aldosterone antagonist have been strongly recommended in CHF patients because there is persuasive evidence establishing the value of these medications for routine management of CHF (Hunt et al. 2001). An ACEI and/or a β-adrenoceptor antagonist is conventionally utilised initially in most patients.

a. Inhibitors of the renin-angiotensin-aldosterone system (RAAS) Activation of the RAAS is associated with several cardiovascular diseases. Inhibitors of the renin-angiotensin-aldosterone system can act at several sites along the RAAS pathway. For example, ACEIs inhibit angiotensin-converting enzyme, which converts angiotensin I to II and which inactivates bradykinin, while angiotensin receptor blockers (ARBs) have effects on the angiotensin II receptor type 1 (AT1). Finally, agents such as spironolactone and eplerenone inhibit the effects of aldosterone.

b. Angiotensin converting enzyme inhibitors (ACEI) There have been numerous studies establishing the beneficial effects of ACEI in CHF patients. Two large placebo-controlled trials, the CONSENSUS I and SOLVD studies, showed that enalapril was associated with a reduction in mortality by up to 40% (The CONSENSUS trial study group 1987, The SOLVD investigators 1991). Enalapril also reduced the rate of CHF hospitalisation and cardiovascular deaths in asymptomatic patients (The SOLVD investigators 1992). In the V-HeFT II study, enalapril was more effective in improving survival than a combination of hydralazine and isosorbide dinitrate (Cohn et al. 1991). A meta-analysis has confirmed that ACEI therapy improves CHF symptoms and survival, as well as reducing CHF related hospital admissions (Flather et al. 2000). More recently, a number of trials have suggested that ACEI therapy has other specific benefits: (1) limiting LV remodelling post infarction (Pfeffer et al. 2003), and (2) reducing the risk of myocardial infarction during chronic therapy (St John Sutton et al. 1997). However, a limitation of ACEI therapy is that ACE is only partially inhibited by ACEI due to the existence of other alternative enzymes capable of forming angiotensin II, including cathepsin G, and chymostatin sensitive angiotensin generating enzyme. A phenomenon, which is termed the "angiotensin escape", usually occurs in long term ACEI therapy (Balcells et al. 1997). Therefore, targeting multiple pathways to provide complete RAAS blockade may be another option for un-controlled CHF patients. On the other hand, the effect of ACEI therapy in potentiating bradykinin effect is unique: if this is important in cardioprotection, as it may be (Messadi-Laribi et al. 2008), ACEI therapy would have an advantage over AT1 inhibition.

c. Angiotensin receptor blockers (ARB)   There are a number of trials investigating the role of ARBs in CHF as an alternative option for patients with ACEI intolerance or as an add-on to ACEI therapy. In the ELITE I trial, although there was only a weak trend towards a reduction in death and/or CHF hospitalisation (p = 0.075), a significantly lower rate of all-cause mortality in patients treated with losartan compared to those treated with captopril was observed. Moreover, no difference in the rate of side effects between the two treatments was found (Pitt et al. 1997). However, these findings were not reproducible in a larger study, the ELITE II of 3152 CHF patients (Pitt et al. 2000). In the HEAAL study, a higher dose of losartan (150 mg daily) was shown to be more effective than a lower dose of 50 mg in patients with NYHA class II to IV, LVEF < 40%, and intolerant of ACEI, suggesting the importance of dose optimisation in order to obtain the maximum efficacy (Konstam et al. 2009). The complex CHARM trial programme (Yusuf et al. 2003, Granger et al. 2003, McMurray et al. 2003, Young et al. 2004, Granger et al. 2005, Weir et al. 2008) evaluated a number of permutations of the use of candesartan in CHF: alone, in combination with ACEI's and in treatment of heart failure with preserved systolic function. Reasonable evidence for the utility of candesartan was obtained except in the latter condition. The conclusions of CHARM, therefore, are of utility, but not interchangeability with ACEIs.

The use of combinations of ACEIs and ARBs to constitute a more "aggressive" blockade of the RAAS is controversial. The efficacy of valsartan at a dose of 160mg twice daily in addition to standard therapy was investigated in a study of 5010 CHF patients in which 93% being treated with an ACEI (Cohn & Tognoni 2001). A significant improvement in NYHA class, LV function, and quality of life was found. There was also a marked reduction in the combined mortality and morbidity endpoint, although no difference in overall mortality was seen (Cohn & Tognoni 2001). In the CHARM-added trial which was performed in 2548 patients with NYHA class II-IV who were already on ACEI, candesartan significantly reduced cardiovascular deaths and CHF hospital admissions (McMurray et al. 2003). Therefore, combined therapy may provide more useful effects that reduce neurohormonal activation, limit cardiac remodelling, reduce symptom severity, and improve exercise capacity and LV function (Baruch et al. 1999, Murdoch

et al. 2001, Kum et al. 2008). However, a recent meta-analysis has reported that combined therapy showed no benefit on mortality (Phillips et al. 2007). Moreover, this approach results in more adverse effects such as hypotension, hyperkalaemia and renal impairment (Phillips et al. 2007). Addition of an ARB to an ACEI is recommended only in patients with persistent CHF symptoms despite being on optimal medical therapy, and requires careful monitoring of serum potassium levels and renal function.

d. β-adrenoceptor antagonists Long-term activation of the sympathetic nervous system leads to volume over-load, and an increase in ventricular pressure due to increasing peripheral vasoconstriction and decreasing sodium excretion via the kidneys (Smith et al. 1997, Elhawary & Pang 1994). An increase in noradenaline levels can also induce myocardial remodelling and restrict blood flow to the thickened myocardium, which results in ischaemia (Knowlton et al. 1993). Moreover, sympathetic nervous system activation can trigger cardiomyocyte automaticity leading to an increase in the risk of arrhythmias and hypokalaemia (Kaumann & Sanders 1993). Finally, noradrenaline can activate oxidative stress in myocardial cells, thus provoking apoptosis (Communal et al. 1998). These harmful effects can be inhibited by β-adrenoceptor antagonist therapy.

β-adrenoceptor antagonists differ in their clinical effectiveness. There are three β-adrenoceptor antagonists which have been shown to reduce mortality rate in CHF patients. These are: (1) carvedilol, which is a combination of $\alpha 1$, $\beta 1$, and $\beta 2$-adrenoceptor antagonists (Packer et al. 2002); (2) bisoprolol and (3) metoprolol, both selective $\beta 1$-receptor blockade (Leizorovicz et al. 2002, Hjalmarson & Fagerberg 2000).

A significant decrease in mortality rate in a number of randomised placebo-controlled phase III studies confirms the useful effects of β-adrenoceptor antagonists in patients with CHF. In the CIBIS study, bisoprolol treatment was associated with 29.3% reduction in all cause mortality, and 18.4% reduction in hospital readmission in NYHA class III and IV patients with LVEF < 35% (Leizorovicz et al. 2002). In two large trials, the MERIT-HF and COPERNICUS studies, metoprolol and carvedilol have been shown to reduce mortality in CHF patients (Tepper 1999, Packer et al. 2002). Furthermore, the

use of β-adrenoceptor antagonists in patients with CHF is associated with a reduction in SCD. In patients with ischaemic dilated cardiomyopathy, a significant reduction of SCD was reported in the group taking β-blockers in a prospective investigation of 700 post-infarct patients (Huikuri et al. 2003).

However, not all trials of β-adrenoceptor antagonists in CHF have been positive. For example, the BEST trial showed no significant benefit with bucindolol (Desai et al. 2011). Therefore, it is uncertain whether there is truly a "class effect" in CHF.

A combination of optimal doses of an ACEI (or ARB) and a β-adrenoceptor antagonist is the recommended treatment for patients with CHF. Although early treatment with β-adrenoceptor antagonists has been shown to be theoretically superior to ACEI, the question of which agent should be initiated first is still controversial. In the early stage of CHF, the sympathetic nervous system is activated earlier than the RAAS (Packer 1992). β-adrenoceptor antagonists inhibit the activation of both systems, while ACEI predominantly block the RAAS (Campbell et al. 2001).

e. Aldosterone antagonists   A number of studies have reported that a high circulating level of aldosterone independently exacerbates heart dysfunction, in addition to the harmful effects of angiotensin II (Duprez et al. 1998). Therefore, a low dose of aldosterone antagonist has been suggested as an addition to ACEI and β-adrenoceptor antagonists in patients with moderate to severe CHF dysfunction.

The beneficial effects of aldosterone blockers in CHF might be explained by several mechanisms. For example, aldosterone blockers might enhance the process of myocardial remodelling, fibrosis, and hypertrophy as well as decrease myocardial apoptosis (Nagata et al. 2006). Administration of aldosterone blockers also increases nitric oxide levels and antioxidant reserves, inhibits the formation of oxygen free radicals, and improves endothelial function (Bauersachs & Schafer 2004).

These agents have also been shown to reduce the length of CHF hospitalisation in patients with a low LVEF and signs of CHF early after an AMI (Gheorghiade et al. 2009). The use of spironolactone, a non-selective aldosterone antagonist, was associated with a reduction in mortality and morbidity from 46% to 35% in a large, long-term trial (Pitt

et al. 1999). In a meta-analysis of 10807 CHF or CHF post MI patients, a reduction in all cause mortality of 20% in the aldosterole blocker receiving group compared to the placebo arm has been shown (Ezekowitz & McAlister 2009). Moreover, in patients with severe systolic CHF, aldosterone antagonists have also been shown to be beneficial (Pitt et al. 1999).

A newer drug, a selective aldosterone receptor blocker, eplerenone, has been demonstrated in the EPHESUS trial to reduce the mortality from 13.6% to 11.8% in patients with a LVEF of less than 40% following an AMI (Pitt et al. 2001). Furthermore, the recent EMPHASIS-HF study of 2737 NYHA class I to II CHF patients has shown a substantial benefit of eplerenone in reducing cardiac deaths and CHF hospitalisation (Zannad et al. 2011). This study was terminated early due to the substantial reduction of 37% in the relative risk.

f. Renin inhibitors Aliskiren, a selective renin inhibitor which has been used clinically for the treatment of hypertension since 2007, has been under investigation as a possible therapy for CHF. Because angiotensin II reactivation and aldosterone escape can occur during long-term ACEI and ARB treatment, leading to a reduction in efficacy of the treatment, a number of studies have reported that co-administration of a renin inhibitor is associated with a reduction in circulating renin, AngI, Ang II and aldosterone concentrations (Nussberger et al. 2002). Moreover, aliskiren is safe and well tolerated because it does not accumulate bradykinin and substance P like ACEIs. Therefore, side-effects such as angioedema and cough are rare (Stanton et al. 2003, Pitt et al. 2011).

1.3.9.3 AICDs

a. History Ventricular tachyarrhythmias (a recognised long-term complication of CHF), can be sensed and terminated with the use of automated implantable cardio-defibrillators (AICDs). First utilised in animals at Sinai Hospital of Baltimore in 1969, AICDs were approved for clinical use in the USA by the Food and Drug Administration (FDA) in 1985 (Mower & Hauser 1993). The number of AICD implantations has

increased dramatically over the last decade due to the results of several randomised controlled studies and the improvement in implantation techniques. Many large randomised studies have demonstrated a significantly improved survival in patients with large myocardial infarcts who had been treated with AICDs. In MADIT II, a randomised controlled trial of 1232 patients with previous MI and LVEF<30%, the mortality rate was decreased by 30% in patients who received a device, compared to the anti-arrhythmic treatment arm (Moss et al. 2005). In another study of 2521 patients with ischaemic and non- ischaemic CHF and LVEF<35%, AICD implantation was associated with a 23% reduction in mortality (Poole et al. 2008). A relative risk reduction of 39% at 1 year, 27% at 2 years and 31% at 3 years was reported in a study of 1016 CHF patients (Kim et al. 1997). More importantly, in a meta-analysis of AVID, CASH, and CIDS studies, a 50% reduction for arrhythmic death and 25% reduction for all-cause mortality in the AICD arm was reported (Connolly et al. 2000). Only two studies failed to show any benefit from the use of AICDs. These were CABG-Patch (Bigger et al. 1999) and DINAMIT (Hohnloser et al. 2004). However, these studies were performed very soon after coronary artery bypass graft surgery (CABG-Patch) or AMI (DINAMIT), and therefore, risk indexation may have been suboptimal.

b. Indications of AICDs in patients with CHF   As a result of the above studies, the indications for AICD insertion have been expanded to include their use not only for secondary prevention of SCD (in patients who survived a previous sudden cardiac arrest or sustained VT) but also for primary prevention. Current ACC/AHA guidelines recommend (Class I) an AICD in post-MI patients with a LVEF of < 30%, or in ischaemic or non-ischaemic DCM patients with NYHA Class II or III symptoms and LVEF < 35% (Epstein et al. 2008).

c. Who will benefit from an AICD?   In patients meeting the criteria for AICD implantation, less than 25% have appropriate AICD discharge on follow-up and approximately 50% of patients treated with AICDs never receive defibrillator shocks (i.e. no benefit from the therapy) (Daubert et al. 2008). In a randomised 45.5 month follow-up study (MADIT II), only 31.2% of 829 CHF patients who received an AICD experi-

enced at least one shock (both appropriate and inappropriate shocks). Among those, 11.5% were inappropriate shocks, which are defined as supraventricular rhythm sensed as ventricular arrhythmia by the device (Daubert et al. 2008). The most common inappropriate shocks were atrial fibrillation or flutter, which occurred in 44% of inappropriate shocks in the MADIT II patients. Supraventricular tachycardia accounted for 36%, followed by abnormal sensing in 19% (Daubert et al. 2008). Similar results were reported in the SCD-HeFT study, with total and inappropriate shock occurrences of 33.2% and 17.4%, respectively (Poole et al. 2008). AICD shocks for any arrhythmia are associated with a significant increase in cardiac death in these patients (hazard ratio = 5.68, p < 0.001) (Poole et al. 2008).

Moreover, the cost of AICD devices remains high, and can be a major strain on public health budgets. Therefore, further risk stratification of these patients into those at very high risk and those at low risk (unlikely to benefit from AICDs) would be extremely useful to improve the cost-effectiveness of the therapy, decrease the number needed to treat (NNT) to save one life, and prevent patients from unnecessary implantation and its side effects.

d. The role of AICDs in CHF progression   Although AICDs prevent sudden cardiac death, it has been argued that AICD implantation may increase mortality because AICD shocks (either appropriate or inappropriate) directly lead to localised myocardial injury such as contraction band necrosis, fibrosis, and persistent inflammation (Singer et al. 1987). The issue is that the inappropriate shocks, which occur in one third of patients undergoing AICD implantation, may further worsen LV function in patients who initially have a low LVEF. Furthermore, a higher mortality rate had been reported in patients who experience inappropriate shocks, compared to patients without inappropriate shocks (Daubert et al. 2008).

1.3.9.4   Cardiac resynchronisation therapy (CRT)

Conduction system delay is a common factor in patients with CHF. As a result, asynchronous ventricular contractions can occur. In order to resynchronise ventricular con-

traction, which requires retiming of atrioventricular, interventricular, and intraventricular activation, cardiac resynchronisation therapy was introduced in 1990 (Hochleitner et al. 1990). US Food and Drug Administration (FDA) approval for clinical use was obtained in 2001. Improvement in mechanical synchrony results in an increase in LV filling time, and a reduction in mitral regurgitation and septal dyskinesis, leading to an improvement in LV function and reversal of LV remodelling. Several studies have documented an improvement in acute haemodynamic responses, for instance, an increase in pulse pressure and left ventricular contractility, and a decrease in LV filling pressure, in CHF patients undergoing CRT (Kass et al. 1999, Blanc et al. 1997). Furthermore, it has been demonstrated in a number of randomised controlled clinical investigations in patients with moderate to severe CHF that end-points such as quality of life, 6-min exercise test, peak VO2, and LV function significantly improved after CRT (Cazeau et al. 2001, DeMarco et al. 2008, Cleland et al. 2009). A reduction by 26% in mortality rate had also been reported in a meta-analysis of 1426 CHF patients undergoing CRT compared to 1133 patients without CRT (Salukhe et al. 2004). Moreover, CRT improves the rate of hospitalisation due to decompensation of CHF and LV reverse remodelling (Ghio et al. 2009, Cleland et al. 2006, Anand et al. 2009). In a systematic review, CHF hospitalisation rates decreased by 37% in patients with New York Heart Association class III or IV heart failure undergoing CRT (Mc Alister et al. 2007). Finally, CRT has been reported to be a cost effective therapy for such patients (Calvert et al. 2005). However, adverse effects include lead problems in 6.6 % of patients and device malfunction in 5 % of patients. The overall success rate was 93% and the mortality rate during implantation was 0.3 % (Mc Alister et al. 2007).

CRT not only slows CHF progression in patients with severe cardiac dysfunction, but also might be useful in patients with mild or moderate CHF. In a randomised, controlled study, the REVERSE trial, CRT has been reported to improve LV function, and reduce both LV remodelling and time to the first CHF hospitalisation in patients with NYHA class I or II, compared to the AICD alone arm (Daubert et al. 2009). In the most recent trial of 1820 CHF patients, the MADIT-CRT trial, similar results were found despite the fact that MADIT-CRT patients had lower LVEF, wider QRS complex

at entry, and longer follow-up (2.4 years compared to 1 year in the REVERSE study) (Moss et al. 2009). However, no mortality benefits have been shown in either trial.

The traditional criteria for selecting patients to undergo CRT are NYHA class III or IV symptoms, depressed LVEF ($< 35\%$), and prolonged QRS duration (> 150 ms), which is evidence of electrical delay in the ventricle. Recently, the European Society of Cardiology has extended the indications for CRT implantations to include patients with mild CHF (Dickstein et al. 2010), based on evidence for beneficial effects of CRT in such patients (Lubitz et al. 2010). However, at least 30% of the patients who undergo CRT implantation show no response, perhaps because of inappropriate selection (van Bommel et al. 2009). Furthermore, many patients showed clinical improvement after CRT implantation, without improvement in systolic LV function. Conversely, for some patients, although there was evidence of an increase in LVEF on echocardiography, there was no improvement in functional tolerance.

Several factors can influence the success rate of CRT, including LV lead position, the presence of myocardial scar, and inappropriate device programming. Although LV dyssynchrony associated with wide QRS duration is a fundamental determinant of CRT, LV pacing at the area of maximal delay results in more improvement in myocardial efficiency. As a result, more optimal geometric remodelling can be achieved, leading to a further reduction in LV end-systolic volume and an improvement in EF. Accordingly, several methods to identify the area of maximal dyssynchrony have been utilised, such as pulse wave Doppler imaging, tissue synchronisation imaging, real time 3D echocardiography, and speckle tracking (Ansalone et al. 2002, Murphy et al. 2006, Becker et al. 2007, Becker et al. 2007a). However, echographic parameters were shown to have low accuracy for predicting response to CRT in a prospective, multicenter study (Chung et al. 2008).

Given that most candidates for CRT have severely impaired LV systolic function, the majority receive concurrent AICD insertion. However, a prognostic benefit of CRT is seen even without concomitant defibrillator implantation (Moss et al. 2009, Tang et al. 2010).

1.4   Stress-induced ("Tako-tsubo") cardiomyopathy (TTC)

The term "stress cardiomyopathy" was first introduced by Cebelin & Hirsch (1980) who found evidence of myocardial contraction-band necrosis in autopsies of physical assault victims. In 1986, the first acute heart failure case due to emotional stress was reported (records of the Massachusetts General Hospital 1986). Two years later, a case report of acute LV dysfunction associated with subarachnoid haemorrhage was published (Pollick et al. 1988). Reversible cardiac dysfunction was also observed in patients with pheochromocytoma, which led to the theory that a surge in catecholamine release can result in myocardial damage (Iga et al. 1989). However, stress-induced cardiomyopathy may also exhibit regional variability within the LV. For example, the term "tako-tsubo" cardiomyopathy was first adopted in 1990 by Japanese investigators who named the condition on the basis of the similarity in the shape of the left ventricle with a round bottom and a narrow neck to a Japanese octopus pot or "tako-tsubo" (Sato et al. 1990). From 1998, alternatively named as "broken heart syndrome", TTC attracted worldwide attention. Although hundreds of papers have been published, TTC is still a mysterious disease due to its difficult diagnosis, obscure pathophysiology, and uncertain prognosis. This condition manifests as reversible regional left ventricular dyskinesis usually involving the apical or mid ventricle (Sato et al. 1990, Angelini 2009). It typically affects post-menopausal women, and is often triggered by acute emotional or physical stress (Gianni et al. 2006, Sharkey et al. 2010). Clinical presentation of TTC typically mimics an acute myocardial infarction (MI), with acute onset of severe chest pain, dyspnoea, and ECG changes typical of MI (Bybee et al. 2004). However, coronary arteries are often normal, and there is no supporting evidence of acute infective myocarditis. In this section, the incidence, diagnosis, prognosis and pathophysiology of TTC are reviewed.

1.4.1 Incidence of TTC

TTC has often been misdiagnosed because of its novel nature and variable presentation. Therefore, the incidence of TTC may be higher than the reported incidence, which is about 1% to 2% of patients presenting with an acute coronary syndrome or myocardial infarction symptoms (Bybee et al. 2004, Pilliere et al. 2006, Gianni et al. 2006). The majority of patients diagnosed with TTC are post-menopausal women, who account for approximately 90% of all reported cases (Gianni et al. 2006). In a cohort study of 136 TTC patients, 96% were female and about 90% were over 50 years of age (Sharkey et al. 2010). Among these patients, 98% presented with at least one recent severe stress event (Sharkey et al. 2010). Depression has been found to be a very common antecedent factor in TTC with a prevalence of 40% in a study involving 110 TTC patients by Mudd et al (Mudd et al. 2007). In another study of 34 TTC patients, 21% of patients had anxiety or depression (Vidi et al. 2009). Another feature of TTC, recently described in a multicenter study of 90 patients, is that the onset of TTC is characterised by a peak in the summer and morning occurrence (Citro et al. 2009).

1.4.2 Prognosis of TTC

In general, the prognosis of TTC is favourable. Although several investigations have shown that the LV function in TTC patients may return to normal within a few weeks and symptoms may subside within a few days (Akashi 2005), severe complications such as LV free wall rupture, pulmonary oedema, heart failure, arrhythmias, dynamic LV outflow tract obstruction, hypotension and even death may occur (Akashi et al. 2004b). Moreover, several studies have suggested that recovery from TTC may be incomplete. Firstly, persistent abnormality of LV histology 3 months after presentation has been documented (Yoshida et al. 2007, Nef et al. 2007). Secondly, myocardial metabolism appears to remain abnormal for a long period of time (Nef et al. 2008b). Finally, it is also widely recognised that TTC tends to be recurrent : two Mayo Clinic series documented distinct recurrences in 11% of patients over a mean follow-up period of 4 years (Dib et al. 2009, Elesber et al. 2007). Other investigators have detected a lower

rate of recurrence of TTC (about 5%) (Sharkey et al. 2010).

1.4.3   Atypical contractile pattern of TTC

There are various forms of LV contractile abnormalities, including classical TTC with apical ballooning, or atypical forms of midventricular ballooning, and "inverted" TTC, i.e. dysfunction of basal segments with sparing of the mid-ventricle and apex (Cacciotti et al. 2007, Kurowski et al. 2007, Botto et al. 2008). Right ventricular dysfunction and/or diastolic dysfunction have been widely observed in TTC and are associated with a higher rate of complications, a prolonged recovery, and worse prognosis (Elesber et al. 2006a, Nef et al. 2007).

1.4.4   Diagnosis of TTC

There are several issues regarding the diagnosis of TTC. Firstly, although criteria to identify TTC have been proposed by a number of groups, no consensus diagnostic criteria have been proposed. The most commonly used Mayo clinic criteria, which were last modified in 2008, requires the presence of all four of the following criteria: (1) regional wall motion abnormalities (mid-LV and/or apical transient hypokinesis, akinesis, or dyskinesis) (2) the absence of obstructive coronary artery disease or acute plaque rupture on angiography; (3) new ST-segment elevation and/or T-wave inversion; or modest serum cardiac troponin elevation; (4) the absence of pheochromocytoma or myocarditis (Prasad et al. 2008). However, this definition has been criticised because no convincing rationale for excluding patients with a history of coronary artery disease, pheochromocytoma, or myocarditis is provided (Omerovic 2011). Japanese and Italian investigators also have their own criteria. Therefore, it is essential to establish consistent diagnostic criteria for TTC.

Secondly, the process of diagnosis of TTC is inherently complicated by selection bias. The condition is usually recognised, particularly in the early series of cases, as a result of cardiac catheterisation of patients presenting with acute chest pain in which the initial diagnosis was an acute coronary syndrome (including a substantial proportion of

patients initially diagnosed with S-T segment elevation myocardial infarction [STEMI]). The most commonly utilised definition of TTC reflects the historical practice of making the diagnosis via cardiac catheterisation. While initial reports of TTC largely related to patients presenting with initial S-T segment elevation on ECG, and therefore triaged to emergency cardiac catheterisation, more recent studies indicate that this is not the usual mode of presentation.

In recent publications, typically only about 40% of presentations include initial S-T segment elevation (Dib et al. 2009, Madhavan et al. 2011). Very elderly, frail patients presenting with prolonged chest pain, especially without S-T elevation, may not undergo cardiac catheterisation, and therefore the diagnosis of TTC may easily be missed. All of these issues, as well as the possible presence of incidental coronary disease, represent recognised problems with the Mayo clinic criteria (Madhavan & Prasad 2010).

Finally, there is an additional theoretical difficulty in the exclusion of single or multi-vessel coronary spasm as an underlying disorder: in practice the total exclusion of spasm is usually impracticable. However, the distribution of hypokinesis in TTC rarely accords with the course of a single coronary artery.

In this section, the clinical presentation of TTC and the tools available for the diagnosis of TTC are discussed.

1.4.4.1 Clinical presentation of TTC

A common feature of TTC is that it is often triggered by sudden emotional or physical stress brought about by individually or communally stressful circumstances. Lists of TTC precipitants have been compiled (Tsuchihashi et al. 2001, Abe et al. 2003, Bybee et al. 2004, Sharkey et al. 2005, Watanabe et al. 2005, Berman et al. 2007, Tiong 2009, Christoph et al. 2010).

While such lists are extensive, TTC precipitants fall into several categories (see Table 1.4.5). The critical issue is that of the probability of precipitation of TTC by the stimuli concerned, which is unknown. One unusual relationship is between acute cerebral infarction or haemorrhage and TTC: while TTC is a well-recognised complication of both cerebral infarction and haemorrhage (Rahimi et al. 2008), embolic cerebral infarction is

Category of precipitants	Examples
Severe personal emotional stress	Unexpected death of relatives
	Domestic abuse
	Arguments/conflict
	Situational anxiety/panic
	Catastrophic medical diagnoses
	Devastating financial or gambling losses
	Work place-related stress
Communal disasters	Earthquakes
Chemical precipitants	Adrenaline/Dobutamine/Isoprenaline
	Serotonin-noradrenaline reuptake inhibitor
	Envenomation: eg. Irukandji jellyfish
Physical illness precipitants	Acute medical illness
	Infection
	Malignancy
	Mechanical fall
	Post operation
Centre nervous system conditions	Subarachnoid haemorrhage
	Stroke
	Epilepsy
Endocrinopathy	Phaeochromocytoma
	Addisonian crisis
	Thyrotoxicosis

Table 1.4.5: Precipitants of TTC

also a potential complication of TTC (Kato et al. 2009).

Although no stressors can be identified in some patients. 98% of patients presenting with TTC in a prospective study at the Mayo clinic reported having at least one recent severe stress event and over 90% of the patients were female (Wittstein 2008).

The clinical presentation of TTC usually mimics the symptoms of an acute coronary syndrome with variable degrees of chest pain at rest in 50% to 60% of patients, and/or dyspnoea, but rarely with cardiogenic shock or syncope (Bybee et al. 2004).

1.4.4.2 Available diagnostic modalities

a. Nonspecific modalities

(i) ECG changes in TTC Changes seen in the electrocardiograms of TTC patients vary depending on the individuals, but may be similar to changes in MI patients with ST elevation, T-wave inversion, and pathological Q-waves (Gianni et al. 2006). In a systemic review by Pilgrim & Wyss (2008), ST segmental elevation was documented in more than 70% of patients, particularly in the precordial leads. However, in another study of 105 TTC patients, only 34.2% had ST segmental elevation on ECG (Dib et al. 2009). Subsequently, T-wave inversion has been found to be very common. Furthermore, a prolonged QT interval has often been recognised in TTC (Mahfoud et al. 2009) and torsades de pointes may occur as a result (Denney et al. 2005). Several attempts have been made to distinguish the ECG changes between TTC patients and AMI patients, who present with anterior ST-segment elevation due to left anterior descending (LAD) occlusion. However, ST-segment elevation and progressive T-wave inversion on ECG are not reliable criteria to differentiate these conditions (Sharkey et al. 2008a).

(ii) Laboratory findings in TTC Laboratory findings are also similar to patients who present with acute coronary syndrome. A significant elevation of catecholamine levels on admission has been reported by Wittstein et al compared to that of MI patients (Wittstein et al. 2005). However, no significant difference in catecholamine levels between MI and TTC patients has been documented in other studies (Madhavan

et al. 2009). This may be explained by the differences in time-course from time of the first symptom presentation, to the time that the actual blood sample is taken. Cardiac biomarkers such as Troponin-T, CK, and CK MB are slightly elevated in most TTC cases (Pilgrim & Wyss 2008). Interestingly, substantial increases in BNP and NT-pro BNP have been found in several studies (Nef et al. 2007, Pilgrim & Wyss 2008).

b. Specific modalities

(i) Echocardiography   Echocardiography has been demonstrated to be a useful method for identifying TTC. It often shows apical and/or midventricular akinesis/hypokinesis and basal hyperkinesis, distorting the shape of the LV, "LV apical ballooning". The contractile dysfunction of the LV results in a lowered LVEF (Wittstein et al. 2005). Moderate to severe LV systolic/diastolic dysfunction is common but not ubiquitous. Although the wall thickness in the akinetic segments may be reduced in the acute phase of TTC, the characteristics of these thinned regions have been reported to be different from the hyperechogenic fibrosis signals of a transmural MI. More importantly, the segmental rather than global wall motion abnormalities, which are not associated with any single coronary artery territory, could be used to distinguish TTC from AMI (Citro et al. 2011).

Using a speckle tracking echocardiographic methods to quantitate regional LV systolic function, Mansencal et al. (2009) reported that there were significant differences in strain, strain rate and systolic peak velocities between acute and chronic phases in patients with TTC. Furthermore, in the acute phase, systolic dysfunction in TTC was circular with similar velocity among basal, mid ventricle, and apical segments and was different from that of patients with LAD coronary artery obstruction.

(ii) Coronary angiography   The diagnosis of TTC is most commonly made by invasive coronary angiography, and it is rare for TTC to be the initial diagnosis at hospital admission. In most cases, no significant coronary artery stenoses are found. Provocation tests can be utilised to exclude myocardial stunning due to coronary vasospasm but these are rarely performed in practice. Left ventriculography is also a useful

method to diagnose and classify forms of TTC, typically demonstrating the distinctive LV shape (similar to a Japanese octopus pot with a round bottom and a narrow neck). Due to the need for cardiac catheterisation (using the Mayo clinic criteria), the diagnostic process is subject to potential selection bias, as this investigation is more likely to be performed in patients with severe symptoms.

(iii) Cardiac MRI Cardiac MRI allows assessment of cardiac function and LV regional wall motion abnormalities with high resolution. Using T2-weighted MRI, periapical oedema is observed in most patients (Rolf et al. 2009). Although delayed enhancement is not usually seen in patients with TTC on contrast-enhanced MRI, it has been reported in some cases (due to increase in extracellular matrix content, confirmed with collagen-1 staining) (Rolf et al. 2009).

(iv) Nuclear medicine imaging

- SPECT perfusion imaging

  The interpretation of results from nuclear medicine perfusion imaging of patients with TTC is controversial. ^{99m}Tc-MIBI SPECT shows an early reduction of tracer uptake in affected LV myocardium despite the presence of normal coronary arteries, which recovers within days or weeks (Bybee et al. 2006, Ito et al. 2005, Kurisu et al. 2003). However, no significant abnormality in myocardial perfusion defects on ^{99m}Tc SPECT myocardial perfusion imaging has been found in other studies, possibly related to the timing of SPECT imaging (Burgdorf et al. 2008, Cimarelli et al. 2009).

- ^{123}I-MIBG

  The significant decrease in the wash-out rate and the uptake of ^{123}I-MIBG in planar and SPECT MIBG scans, reported in several studies, suggests that the sympathetic nervous system is overactive in TTC (Akashi et al. 2004a, Burgdorf et al. 2008, Cimarelli et al. 2009). Significantly reduced MIBG uptake in TTC suggests cardiac autonomic damage and/or increased activation of cardiac sympathetic nervous function (Akashi et al. 2004a, Morel et al. 2009).

- Positron emission tomography (PET)

  Utilising fluorine 18-fluorodeoxyglucose PET, a number of investigations have reported decreased myocardial glucose uptake in the context of a relatively reserved coronary flow (assessed via either ^{13}N-ammonia PET or ^{99m}Tc-MIBI SPECT, suggesting abnormal myocardial metabolism (Bybee et al. 2006, Yoshida et al. 2007). Reduced coronary flow reserve in the acute phase of TTC has also been demonstrated using nitrogen-13 ammonia PET (Feola et al. 2008). Furthermore, flow-metabolism mismatch between fatty acid uptake and myocardial perfusion has been shown in the mid ventricular and apical regions (Ito et al. 2005, Kurisu et al. 2003).

1.4.5   Pathophysiology of TTC

Despite the increasing recognition of this condition, the precise underlying pathophysiology associated with the distinctive contractile pattern in TTC remains unknown. Acute elevation of plasma catecholamine levels are usually noted, suggesting some form of catecholamine-mediated myocardial toxicity or myocardial stunning (Wittstein et al. 2005, Madhavan et al. 2009). Proposed mechanisms for the transient cardiac dysfunction include epicardial coronary artery vasospasm, impaired multi-vessel coronary microcirculation, direct myocyte damage due to cyclic-AMP mediated calcium overload, transient mid-ventricular cavity obstruction related to elevated catecholamine levels and dehydration in patients with sigmoidal bulging of the interventricular septum, and diffuse ischaemia related to superoxide release from platelets as a result of acute oxidative stress (Ueyama et al. 2009, Akashi et al. 2010).

1.4.5.1   The role of oestrogen in TTC pathophysiology

The occurrence of TTC in post-menopausal women raises the issue of its possible association with the effects of oestrogen. The level of oestrogen may indeed play a fundamental role in TTC pathophysiology. There is established evidence from animal models that oestrogen diminishes adrenaline effects in the pre-synaptic cardiac sympathetic

nerve fibres through three metabolic cascades involving calcium metabolism: (1) the sarco-endoreticular Ca^{2+} phosphatase (SERCA) 2-phospholamban cascade (Bupha-Intr & Wattanapermpool 2006); (2) the L-type Ca^{2+} channel modulated by cyclic AMP (Li et al. 2000, Ma et al. 2009); and (3) the ATP-sensitive K^+ channels (Ranki et al. 2001). These effects protect the epicardium from "adrenergic surge" by preventing calcium entering into the myocardial cells. As a result, myocardial contraction is reduced leading to a reduction in oxygen consumption.

Furthermore, administration of oestrogen in a TTC model (immobilisation stress induced rats) markedly improved LV dysfunction (Ueyama 2004). In postmenopausal females, a significant depletion in oestrogen level may in theory leave the epicardium unprotected against the strong adrenergic stress, leading to TTC. This idea is supported in a study by Nef et al in which there was a reduction in gene expression of Ca^{2+}-handling proteins in TTC patients in comparison with control patients (Nef et al. 2009a). However, there is no current evidence that post-menopausal oestrogen therapy protects against occurrence of TTC, nor does TTC occur predominantly in the immediate post-menopausal period.

1.4.5.2 Is TTC caused by multivessel epicardial coronary artery spasm?

It has been suggested that reversible myocardial dysfunction may be caused by epicardial coronary artery spasm and insufficient coronary blood flow can lead to myocardial ischaemia/stunning in TTC (Sato et al. 1990). However, there are several studies which do not support this idea. Firstly, spontaneous multivessel spasm was observed in only 1.4% patients with TTC in a systematic review (Gianni et al. 2006). Whether using ergonovine or acetylcholine during provocative tests, multivessel spasm occurred in only 28% of TTC patients (Gianni et al. 2006). Secondly, there is histopathological evidence from several TTC cases that myocardial contraction-band necrosis and focal myocardial fibrosis can occur (Yoshida et al. 2007, Prasad et al. 2008). These changes are not observed in ischaemic myocardial stunning. Furthermore, the predominant peri-apical hypokinesis/akinesis in the majority of TTC cases does not correspond to any myocardial territory supplied by a single coronary artery. Accordingly, epicardial coronary

artery spasm is unlikely to be the mechanism underlying TTC. However, the idea that myocardial ischaemia may occur during the acute phase of TTC does not require that spasm is present: an intense inflammatory response may compromise regional blood flow by compressing intramyocardial small coronary arteries.

1.4.5.3   Is TTC a "catecholamine cardiomypathy"?

Reversible cardiomyopathy has been well documented in patients with phaeochromocytoma, in whom catacholamine levels are usually elevated. This observation leads to the hypothesis that excessive catecholamine production associated with high stress levels might be a major mechanism in TTC. In an animal model of TTC, ECG changes and LV apical ballooning have been seen in rats stressed by immobilisation (Ueyama 2004). Additionally, treatment of rats with isoproterenol may induce predominantly apical LV hypokinesis, prompting the suggestion that this may be regarded as a model of TTC (Grosjean et al. 1999). Activation of several enzymes and genes, including mitogen-activated protein kinase and natriuretic peptide genes, was observed in these rats and attenuated by α-1 and β blockers. Wittstein and workers reported that in patients with TTC, catecholamine levels were 2 to 3 times higher than those of patients with Killip class III myocardial infarction (Wittstein et al. 2005). The fact that catecholamine levels were still elevated after one week, despite their very short half-life of 3 minutes, suggests a continuous activation and/or impaired clearance (Wittstein et al. 2005). Furthermore, a recent study by Kume et al. (2008) has shown that TTC is associated with an increase in catecholamine spill-over from the coronary sinus to the aortic root. Finally, a few case reports have documented that intravenous administration of adrenaline or dobutamine provokes TTC, providing further evidence that catecholamines play a role in the pathophysiology of TTC (Abraham et al. 2009, Manivannan et al. 2009, Volz et al. 2009, Litvinov et al. 2009, Zubrinich et al. 2008, Tomcsanyi et al. 2008, Saeki et al. 2006). However, elevated catecholamine levels have not always been found in TTC. In one study of 19 TTC patients, no significant differences in adrenaline and noradrenaline levels between the TTC group and the control group were seen (Madhavan et al. 2009).

It has been suggested that direct myocyte injury due to acute catecholamine surge may be a predominant mechanism underlying the pathophysiology of TTC. Elevated catecholamine levels may have a negative impact on myocyte viability by inducing cAMP-mediated calcium overload. High catecholamine levels also increase production of oxygen-derived free radicals leading to myocyte injury (Adameova et al. 2009). Moreover, free radicals can interfere with sodium and calcium transporters, leading to cellular calcium overload and trans-sarcolemmal calcium influx, which result in myocyte dysfunction. An excesses of catecholamines also results in the activation of an inflammatory process. For example, elevated levels of pro-inflammatory cytokines have been shown in a rat model after catecholamine infusion (Murray et al. 2000). Moreover, myocardial necrosis, leukocyte infiltration, and myofibrillar degeneration have been observed as early as 1 hour after administration of isoproterenol (Mikaelian et al. 2008).

Another possible mechanism which might explain the vulnerability of the LV apex to a surge of catecholamine release is that the apex is highly trabeculated, leading to a larger exposure to circulating catecholamines (Bielecka-Dabrowa et al. 2010). Furthermore, β-adrenoreceptors are more concentrated in the apex compared to the base (Handy et al. 2009). Therefore, the apex may be more sensitive to circulating catecholamines. Moreover, in isolated rat LV myocardium exposed to high adrenaline levels, the $\beta2$ adrenoreceptor Gs coupling may be switched to Gi coupling, leading to a negative inotropic response (Heubach et al. 2004). This pathway is also known to have protective effects against apoptosis, which may be an explanation for the essentially reversible impairment of LV contractility in TTC (Zhu et al. 2001).

1.4.5.4   Is TTC a active inflammatory disease?

Although the original Mayo clinic criteria suggested that myocarditis should be excluded in patients with suspected TTC (Bybee et al. 2004), several investigations have reported that TTC may be essentially an inflammatory process. In a study in which endomyocardial biopsy was performed, evidence of regional inflammatory responses was found, in the form of areas of interstitial infiltrates and myocardial fibrosis; and contraction bands with or without necrosis (Nef et al. 2007, Nef et al. 2008). These distinctive his-

tological features are different from coagulation necrosis seen in myocardial infarction. Recently, in studies using T2-weighted cardiac MRI, regional myocardial oedema has been reported (Otsuka et al. 2008, Abdel-Aty et al. 2009, Eitel et al. 2010). The oedema region improved significantly in the chronic phase, compared to the acute phase (Eitel et al. 2010).

1.4.5.5   Is TTC a microcirculatory abnormality?

The extensive reversible myocardial dysfunction in TTC suggests that this disturbance could be related to microcirculatory impairment. The severe myocardial metabolic abnormalities seen in ^{18}F-fluorodeoxyglucose myocardial PET studies (Yoshida et al. 2007), and the alterations to the coronary flow velocity spectrum and the coronary flow velocity reserve (CFVR) seen in Doppler guidewire studies, suggests a microcirculatory disturbance underlying the mechanism of TTC (Kume et al. 2005). Furthermore, Elesber et al. (2006) reported in a study of 42 TTC patients that 69 % of the patients had evidence of abnormal microvascular perfusion at presentation, using the TIMI myocardial perfusion grade on coronary angiography. Similar results were reported in another multicentre study of 24 patients (Fazio et al. 2010). However, no significant abnormalities in myocardial perfusion defects have been detected in TTC patients using ^{99m}Tc SPECT myocardial perfusion imaging (Burgdorf et al. 2008, Cimarelli et al. 2009). Furthermore, the possibility of inflammatory change within myocardium and/or microvessels could account for microcirculatory abnormalities, as discussed in Section 1.2.2.

1.4.6   Management of TTC

Currently, there is no therapeutic consensus for the management of TTC, either in the acute phase or in the longer term. There have been no randomised studies in TTC to test the benefit of any medications. The most important issue is that diagnosis of TTC should be considered early, particularly in post-menopausal woman presenting with chest pain after physical or emotional stress, to avoid inappropriate therapies such as thrombolysis.

β-adrenoceptor antagonists may be theoretically useful, given that there is substan-

tial evidence of sympathetic nervous activation and elevation of catecholamines in TTC (Prasad 2007). Moreover, in the immobilisation animal model of TTC, the combination of α and β-receptor blockade successfully reversed LV motion abnormalities and improved ST segment elevation on ECG (Ueyama 2004, Akashi et al. 2010). In a human study, intravenous administration of propranolol has been reported to be beneficial in TTC patients with midventricular obstruction variant (Yoshioka et al. 2008). However, this benefit was confined to the relief of obstruction, which should be regarded only as a surrogate end-point. Indeed, there is evidence from human studies that propranolol may aggravate microvascular constriction in the coronary arteries under circumstances associated with sympathetic discharge, via unopposed α-adrenoceptor stimulation (Gunther et al. 1981, Kern et al. 1983). Finally, it has been suggested that recurrence of TTC may be prevented by the use of combined α and β blockers (Uchida et al. 2009).

In the acute phase of TTC, several complications such as LV free wall rupture, pulmonary oedema, heart failure, arrhythmias, and severe hypotension may occur. Pulmonary oedema has also been reported occasionally (Gianni et al. 2006). There has been several reports of the occurrence of thromboembolic stroke post TTC, presumably secondary to LV mural thrombosis (Azzarelli et al. 2008). This is an intriguing complication, given the relatively transient nature of hypokinesis in most cases (Silva et al. 2009), and raises the issue of dysfunction of the LV endocardial endothelium. However, anticoagulation with heparin is not universally recommended, because of the occasional occurrence of cardiac rupture. Identifying these complications and their causes is essential for appropriate treatments. For example, hypotension may be due to acute CHF or due to LV outflow tract obstruction. Inotropes should be avoided because they may worsen the existing high level of catecholamines and LV outflow tract obstruction (Hurst et al. 2010). In cases of shock, intraaortic balloon pumping may be required. No treatment regimens have been established for TTC patients with QT prolongation. However, cardiotonic drugs should be avoided.

1.5   Scope of the present studies

Given that catecholamines were postulated to contribute to cellular damage in both CHF and acute cardiomyopathies such as TTC, the studies described in this thesis examined a number of aspects of this putative toxicity.

Specifically:-

(1) In chapter 2, results of a study evaluating the prognostic impact of cardiac denervation (a "high catecholamine" state) on outcomes of CHF are presented.

(2) In chapter 3:

(a) the relationship between severity of the acute phase of TTC, catecholamine release, and natriuretic peptide secretion is evaluated

and (b) the potential of the NO system to act as a further modulator of all survival or damage in this high catecholamine environment is assessed.

Chapter 2

Cardiac denervation in chronic heart failure: relationship to changes in LV function and to arrhythmogenesis

2.1 Introduction

The pathogenesis, economic impact and conventional therapeutics of CHF have been discussed in Section 1.3.4. Furthermore, Section 1.3.9.3 has highlighted both the potential prognostic advantages of AICD insertion in such patients, and the fact that in approximately 50% of selected patients, AICD discharge never occurs (Daubert et al. 2008), while SCD occurs up to 33% of patients who are conventionally "ineligible" for an AICD implantation (LVEF > 35%). These data raise the issue of potential refinement of the criteria for defibrillator insertion in combination with or without concurrent CRT.

Cardiac denervation, both regional and general, is an important basis for cardiac hypersensitivity to catecholamines (see Section 1.3.8) and hence may modulate both the negative impact of catecholamines on myocardial function as well as their pro-arrhythmic effects (see Section 1.3.8.1). In practice, imaging of cardiac sympathetic nervous activity, utilising ^{123}I-MIBG has been recommended as a possible basis for risk

indexation in potential candidates for defibrillator insertion (see Section 1.3.8.2).

On the other hand, the evidence base for this recommendation is uncertain: there are few large studies (see Table 1.3.4), these do not evaluate the relationship between changes in contractile function and potential arrhythmogenesis, and in the current literature, the decision-making utility of ^{123}I-MIBG imaging for AICD implantation is still uncertain.

Therefore, we have designed a study to evaluate the utility of ^{123}I-MIBG imaging to predict both haemodynamic deterioration and arrhythmogenesis in patients with CHF.

The objectives of this study were to:

1. Examine the determinants of the extent and localisation of cardiac denervation in the setting of chronic systolic heart failure (CHF)

2. Evaluate potential relationships between extent of denervation and

(i) **Evolution of LV systolic dysfunction** (principal objective)

(ii) Propensity towards development of ventricular tachyarrhythmias

As regards this latter objective, the issue to be evaluated was the likelihood that cardiac denervation could be utilised to identify populations with > 4-fold heterogeneity of defibrillator discharge risk, rather than the question of a statistically significant association alone.

3. Identify determinants of progression of cardiac denervation in such patients

2.2   Methodology

2.2.1   Patient population

The study population consisted of patients with a known history of moderate to severe CHF (LVEF $< 40\%$) who were prospectively enrolled to undergo cardiac defibrillator implantation for primary or secondary prevention (prior VT or VF) by cardiologists at both the Queen Elizabeth and Lyell McEwin Hospitals between October 2008 and March 2011.

Indications for AICDs were: (a) CHF of ischaemic or non-ischaemic origin with a LVEF $< 40\%$, or (b) NYHA class II or III and EF $< 35\%$, or (c) documented ven-

tricular tachyarrhythmias with LVEF < 40%. In many cases, these conditions were also associated with evidence of LV dyssynchrony, providing an indication for simultaneous CRT insertion. Patients with clinically significant valvular heart disease, and/or contra-indication to MRI, e.g. previous insertion of permanent pacemaker or valvular prosthesis, metallic implant, claustrophobia or severe renal impairment (creatinine > 200mol/L) were excluded from CMR examination.

Tricyclic antidepressants were ceased prior to the ^{123}I-MIBG scanning (Solanki et al. 1992). Other standard CHF medications were continued. Because ^{123}I is a radionuclide with a short half-life of 13 hours which emits only γ rays, no thyroid blocker was required (Yamashina & Yamazaki 2007). Patients' history and clinical profiles were evaluated by interviewing all patients and reviewing hospital and physician records at the time of the first MIBG study and at follow-up. This information included pre-admission likelihood of coronary artery disease (CAD), past history, cardiac risk factors, clinical symptoms, and NYHA class. Because chronic renal failure results in an acceleration in washout rate of MIBG, creatinine levels was also checked. The study was approved by the institutional Ethics of Human Research Committee and written informed consent was obtained before study entry.

2.2.2 Study protocol

Enrolled subjects underwent the following tests:

2.2.2.1 Baseline investigations

A. Imaging techniques

a. Transthoracic echocardiogram Transthoracic echocardiographic data using a Vivid 7 scanner (Vingmed, Norway) were utilised to document changes in left ventricular function with time (given that repeat MRI is contra-indicated once conventional defibrillator insertion takes place). Standard 2 and 4 chamber views were used to measure LV diastolic and sytolic dimensions [LVDd and LVDs (mm)], and LVEF (Simpson's method).

b. ECG An ECG was performed at admission for AICD implantation and follow-up. Corrected QT and QRS intervals were recorded.

c. CMR with gadolinium enhancement In order to detect and size previous myocardial infarctions (if present), cardiac MRI was performed using a Philips (Netherlands) 1.5-Tesla tomograph scanner and five-element cardiac phased-array surface coil. Functional assessment of the left ventricle was performed pre-contrast using cine-CMR with balanced fast field. Delayed enhancement imaging (DE-MRI) was performed at 10-15 min post intravenous injection of 0.2 mmol/kg Gadolinium-diethylennetriamine pentaacetic acid (Magnevist; Schering AG, Germany). Imaging was performed using an inversion recovery, T-weighted 3-D fast field echo sequence. Volumetric coverage of the entire left ventricle was obtained in the short-axis orientation using 6 slices (thickness of 6-8 mm). For reference, 2 and 4-chamber long-axis DE-MRI images were also acquired but not used for infarct quantitation.

MRI quantitative analysis was performed on a Philips View-forum workstation using the MR cardiac enhancement analysis program. In patients with previous MI, quantification of scar volume and peri-infarct viable myocardium was performed using the short-axis delayed enhancement images, and reported as percent of LV mass.

d. ^{123}I-MIBG imaging At the of commencing the project, imaging parameters were based on a number of previous studies using a low dose of ^{123}I-MIBG and a low-energy collimator. Given the need for follow-up studies in each patient, the imaging parameters were not changed during the current project despite a later recommendation that medium energy collimators be utilised in future studies. Early and late planar and SPECT ^{123}I-MIBG images were obtained 30 min and 4 h post IV administration of 110 MBq ^{123}I-MIBG over one minute using a triple-headed Irix gamma camera (Philips; Cleveland, Ohio) with a low-energy, high-resolution collimator. In order to assist in optimal reconstruction of SPECT ^{123}I-MIBG images, additional cardiac SPECT-CT-based images were obtained utilising Symbia SPECT-CT scanner (Siemens Medical Solution USA, Inc. Molecular Imaging).

(i) Planar [123]**I-MIBG image processing** In order to calculate ventricular ^{123}I-MIBG activity, a region of interest (ROI) was manually drawn over the LV myocardium and the mean heart counts per pixel was recorded. A 5x5 pixel ROI of the mediastinum was positioned in the mid-line 1/3 upper chest where the lowest activity was visualised, and the mean counts were recorded. These ROIs were then saved and duplicated for the delayed image and follow-up ^{123}I-MIBG scans. The early and late heart to mediastinum ratios (HMR) and the wash-out rate (WR) were calculated as:

$$\text{HMR} = \frac{\text{Heart counts per pixel}}{\text{Mediastinum counts per pixel}} \qquad (2.2.1)$$

$$\text{WR} = \frac{\text{Early MIBG activity} - \text{Late MIBG activity}}{\text{Early activity}} \times 100\%. \qquad (2.2.2)$$

Figure 2.2.1 is an example of planar ^{123}I-MIBG scans at baseline and after 1 year of a patient with dilated cardiomyopathy.

(ii) SPECT [123]**I-MIBG image processing** Because no normal data base for ^{123}I-MIBG SPECT quantitation is commercially available, it was impossible to use commonly used myocardial perfusion soft-ware packages such as 4D-MSPECT (the University of Michigan, USA) and QPS (Cedars Quantitative Perfusion SPECT) (Cedars-Sinai Medical Centre). Therefore, an in-house soft-ware program was developed for automated ^{123}I-MIBG SPECT image processing. After acquisition by the SPECT camera, a series of tomographic parallel and transaxial sections were transferred to an Odyssey workstation. The raw myocardial tomographic images were reconstructed to produce short-axis sections. An ellipsoidal mask was created for the image. The operator defined ellipses on a triple image display of short axis, ventricle long axis and horizontal long axis sections. Image locations outside this mask were set to zero. An optional excision from inside the ellipsoidal volume to exclude myocardium locations affected by scatter from hot extra-cardiac organs was then performed if it is necessary. Locations within the excision ellipsoid were excluded from the mask. Utilising a 17 segmental model, the polar
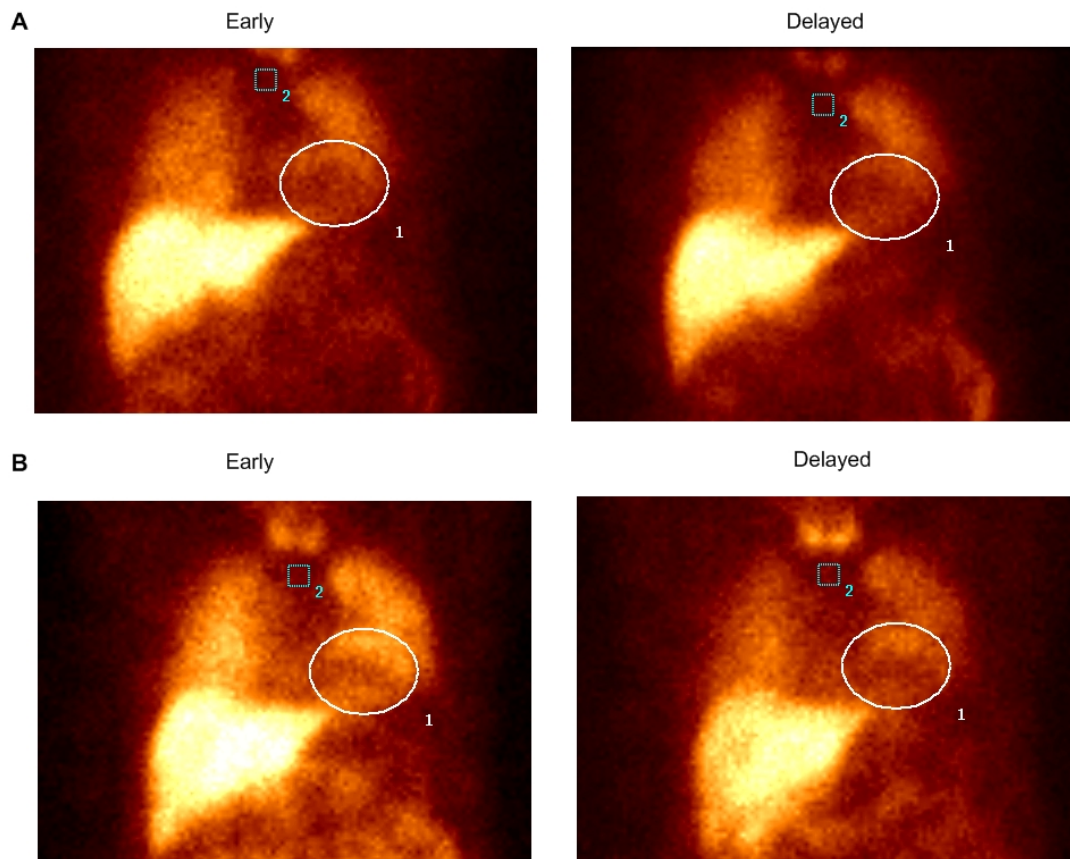
Figure 2.2.1: Early and delayed planar ^{123}I-MIBG scans at the time of AICD implantation (A) and after 1 year (B) in a 64 year old man with dilated cardiomyopathy. The early, delayed HMR, and WR were 1.67, 1.61, 35% vs. 1.76, 1.53, and 46%, respectively.

maps of ventricular ^{123}I-MIBG uptake which allow comparisons of serial studies were generated and normalised to maximum counts. Regional tracer uptake was expressed as percentage of total left ventricle in the 3 main coronary artery territories (left anterior descending artery LAD = 7 segments, right coronary artery RCA and left circumflex artery = 5 segments).

Cardiac ^{123}I-MIBG scanning results were categorised as follows: in general, "late" MIBG activity was utilised for end-point analysis. Mismatch in size between MIBG activity and myocardial perfusion defects, corresponding to the extent of viable but denervated myocardial tissue (SD-PD) was also calculated, again expressed as a percentage of total left ventricle.

e. ^{99m}Tc Sestamibi SPECT myocardial perfusion imaging (MPI)   As it has been reported that "mismatch" between extent of MIBG activity and that of impaired myocardial perfusion might provide additional prognostic information, ^{99m}Tc Sestamibi SPECT MPI was also undertaken. Patients who have had ^{99m}Tc Sestamibi MPI within 3 months (if clinically stable) were not required to undergo repeat Sestamibi scans. A same-day stress-rest ^{99m}Tc Sestamibi SPECT MPI protocol (either exercise or dipyridamole) was utilised. The stress SPECT imaging was performed 30 min after 400MBq ^{99m}Tc Sestamibi administered intravenously at peak stress. A rest study also was performed 3 h after the stress injection. 900MBq Sestamibi was administered intravenously at rest and followed by the performance of SPECT imaging 30 min later.

SPECT resting perfusion defect size was measured (as percentage of left ventricular myocardial volume) using commercially available quantitative perfusion packages [the Cedars Emory quantitative analysis (CEqual) program, Cedars Sinai Medical Center]. The infarct size by the percentage of the total myocardial mass was estimated. The 2 sets of stress and rest myocardial SPECT images were used to measure size and location of ischaemic defects. The extension of perfusion defects and reversibility ($>$ 10% improvement) of each region perfused by the 3 main coronary arteries was calculated from polarmaps and expressed as a percentage of that territory. All perfusion values $<$ 50% were considered non-viable.

Stress Sestamibi scans were used to quantitate size and site(s) of areas of both inducible myocardial ischaemia and myocardial infarction, as well as degree of mismatch between ^{123}I-MIBG denervation and myocardial perfusion defects.

B. Laboratory assessments

a. Determinations of N-terminal proBNP (NT-proBNP) concentrations   Venous blood specimens were collected into tubes containing potassium ethylene diamine tetra acetic acid (EDTA) at the time of ^{123}I-MIBG imaging. Determination of plasma NT-proBNP was performed within 2 hours post-venesection by immunoassay (Elecsys E 170, Roche Diagnostics, Mannheim, Germany).

b. Determinations of plasma concentrations of catecholamine metabolites

Venous blood was drawn into tubes containing EDTA and subsequent assayed for the determination of metanephrine and normetanephrine concentrations utilising liquid chromatography/tandem mass spectrometry (LC-MS/MS) (Lagerstedt et al. 2004).

2.2.2.2   Patients' follow-up

Patients underwent assessment of defibrillator discharges on a 6 monthly basis for a mean follow-up period of 27 ± 10 months, and all discharges were categorised according to precipitants and nature of discharge (i.e. overdrive pacing vs. appropriate AICD therapy). Events, including details of non-planned hospital admissions, deaths (cardiac and otherwise), and appropriate AICD therapy were recorded. Inappropriate AICD discharges and overdrive pacing of nonsustained ventricular tachycardia were excluded from analysis.

At 12 months' follow-up, ECG, ^{123}I-MIBG scanning, and echocardiography were repeated. Repeat ^{123}I-MIBG imaging was performed in only 22 of the 45 patients due to various reasons: 3 patients died within 12 months, 2 had cancer, 2 had stroke, and 15 refused to have the scan repeated.

2.2.3   Statistical methods

2.2.3.1   Sample size calculation

Power calculations were performed to determine the ability of multiple logistic regressions to predict (a) reductions in LVEF of $> 5\%$ and (b) AICD discharge in a period of 27 ± 10 months follow-up. A study of this type would report significant correlation between changes in delayed HMR and reductions in LVEF of $> 5\%$ with power of 85% at $p < 0.05$, if the sample size was 29. To predict AICD discharge, for n = 45, the discharge rate = 24%, and $p < 0.05$, the power of the study was 0.7.

2.2.3.2   Statistical analysis

The data were analysed by the SPSS version 15 software (SPSS, Chicago, Illinois, USA), and presented as mean $\pm$ 1SD. The late HMR was utilised for the primary analysis due to its "proven" prognostic value (see Section 1.3.8.2.

The primary hypothesis concerned the interaction between delayed HMR and change in LVEF. This was evaluated via:

- (a) Linear regression of the delayed HMR and δ LVEF relationship.

- (b) Comparisons between groups using nonpaired t tests for normally distributed data, the Mann-Whitney test for nonparametric data, and Fisher's exact test for categorical parameters after partitioning delayed HMR into lower and upper halves were performed.

- (c) Univariate followed by multivariate analysis of δ LVEF, using stepwise multiple linear regression analysis as potentially modifying parameters.

Furthermore:

(1) Data were analysed separately for patients with and without CRT, to avoid confounding effects of resynchronisation.

(2) Changes in LVDs and NT-proBNP were analysed analogously, given their likelihood of changing similarly to LVEF.

The secondary hypothesis related to rate of appropriate defibrillator discharge. These were analysed according to baseline delayed HMR, utilising Kaplan-Meier analyses and a univariate Cox proportional hazards model. 95% CI values for rates of defibrillator discharge were derived.

Additional analyses concerned other major cardiac events (deaths/HF readmission) evaluated via Kaplan-Meier analyses and a univariate Cox proportional hazards model. A value of P <0.05 was considered significant.

2.3   Results

2.3.1   Study population

Clinical characteristics of the study population are described in Table 2.3.1. 45 consecutive patients, predominantly males, enrolled in the study. The majority of cases were CHF of ischaemic origin. Although most patients were in NYHA class II status, simultaneous CRT insertion was performed in almost half of the population. Critically important to the probability of tachyarrhythmia recurrence was the fact that 28% of patients had already experience at least 1 episode of ventricular tachyarrhythmias. 5 patients were in AF at enrolment, while 3 patients developed AF during follow-up.

In general, the study group was extensively treated medically: overall 58% were receiving combinations of ACEIs/ARBs plus β-adrenoreceptor antagonists plus aldosterone antagonists. Almost half of the population were receiving low dose digoxin.

Table 2.3.2 summarised the critical imaging and laboratory parameters at baseline. The mean value for delayed ^{123}I-MIBG HMR, the principal focus of this study, was 1.57 $\pm$ 0.29, somewhat lower than in most previous reported studies (see Table 1.3.3). Most patients had substantially reduced LVEF values and dilated hearts, while NT-proBNP concentrations were substantially elevated beyond population norms, suggesting poorly controlled CHF despite the relatively good reported symptomatic status. The mean prolongation of QRS duration was consistent with the selection of a group in many of whom CRT was contemplated.

CMR imaging was performed in 30 patients, while 15 patients was excluded from CMR because of several reasons, including previous insertion of permanent pacemaker in 6 patients, no available MRI bookings in 5 patients, claustrophobia in 2 patients, recent stenting (less than 8 weeks) in 1 patient, creatinine $> 200 \mu$mol/L in 1 patient. 15 patients (13 with dilated cardiomyopathy) did not exhibited delayed enhancement on CMR. Therefore, scar size was only quantified in 15 patients [mean infarct size: 27 $\pm$ 12 (% LV mass)].

Complications of AICD implantation occurred in 12 patients with lead dislodgement requiring lead relocation in 9 patients. Among those, 1 patient had an AICD lead perfo-

rating to the right ventricle, causing pericardial haemorrhage and surgical management
was required to extract the AICD and leads via sternotomy.

2.3.2   Methodological problems with SPECT ^{123}I-MIBG

In 14 patients, ^{123}I-MIBG SPECT values were below the limits for reconstruction and
quantitation, precluding analysis of ^{123}I-MIBG SPECT data, and of the relationship
between denervation and perfusion defects. There was a significantly lower delayed
HMR in these patients, compared to those in whom quantitation of SPECT ^{123}I-MIBG
was possible (HMR: 1.4 ± 0.3 vs. 1.6 ± 0.26, p = 0.03). This problem has been
reported by several investigators (Agostini et al. 2009, Ji & Travin 2010) and patients
with marked low ^{123}I-MIBG uptake which resulted in impossible quantitation of SPECT
imaging, were excluded from the analysis in some studies.

2.3.3   Clinical and biochemical correlates of cardiac denervation

Table 2.3.3 compares the characteristics of patients with delayed HMR values below and
above the mean ratio of 1.57.

Although there was a trend for patients with low delayed HMR values to have more
severe CHF symptoms and more dilated hearts with lower LVEF values, this did not
reach statistical significance. On the other hand, NT-proBNP concentrations were sub-
stantially and significantly higher in patients with low HMR values. Baseline NT-
proBNP levels inversely correlated with delayed HMR (r = −0.41, p = 0.004; Figure
2.3.2) and directly correlated with WR (r = 0.36, p = 0.01; Figure 2.3.3).

Data of patients were also stratified according to intention or otherwise to perform
simultaneous CRT, demonstrated in Table 2.3.4. Patients who underwent the combina-

Clinical Characteristics	n = 45
Mean age (range)	62 ± 13 (26 to 79)
Male (%)	93
NYHA class II/III/IV (%)	69/29/2
AICD plus CRT (%)	47
A. Coronary risk factors	
BMI (kg/m²) (mean ± SD)	27 ± 6
Previous/current smoking (%)	60
Systemic hypertension (%)	67
Diabetes mellitus (%)	44
Dyslipidaemia (%)	69
ETOH use (%)	16
B. Aetiology	
Ischaemic/Non-ischaemic origin (%)	71/29
C. Reasons for AICD implantation (%)	
Primary prevention	72
Secondary prevention	28
D. AICD implantation complications	n = 12
Infection	2
Adherent thrombus	1
Lead dislodgement	9
E. Medication use (%)	
ACEIs/ARBs	100
β-adrenoreceptor antagonists	100
Loop diuretics	64
Spironolactone	58
Statins	89
Digoxin	47

Table 2.3.1: Patient baseline characteristics

(BMI = body mass index, NYHA = New York Heart Association, AICD = automated implantable cardio-defibrillator, CRT = cardiac resyncronisation therapy, ETOH = ethanol, ACEIs = angiotensin converting enzyme inhibitors, ARBs = angiotensin receptor blockers)

Baseline parameters	
A. Laboratory assessments	
Normetanephrine (pmol/L)	876 ± 250
Metanephrine (pmol/L)	326 ± 141
NT-proBNP (pg/ml)	$2300 \ (850 - 3743)$
Creatinine (μmol/L)	98 ± 37
Haemoglobin (g/l)	134 ± 14
B. ECG parameters	
QRS duration	128 ± 26
QTc	448 ± 37
C. Echocardiography parameters	
LVDd (cm)	6.5 ± 0.74
LVDs (cm)	5.5 ± 0.89
LVEF (%)	30 ± 7
D. ^{123}I-MIBG parameters	
Early HMR	1.77 ± 0.32
Delayed HMR	1.57 ± 0.29
WR (%)	49.5 ± 18.4

Table 2.3.2: Baseline laboratory and imaging parameters

(HMR = heart to mediastinum ratio , LVDd = left ventricular diastolic dimensions , LVDs = left ventricular systolic dimensions , LVEF = left ventricular ejection fraction, NT-proBNP = N-terminal proBNP, WR = washout rate)
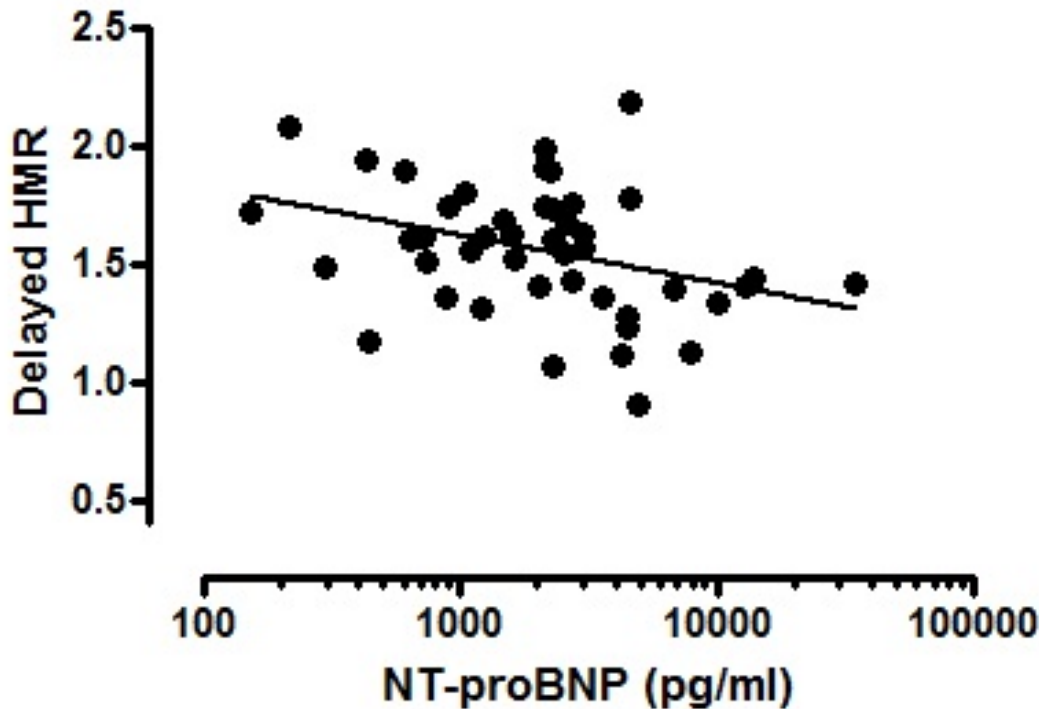
Figure 2.3.2: Correlation between baseline NT-proBNP levels and baseline delayed HMR ($r = -0.41$, $p = 0.004$, Spearman's correlation)

tion of AICD and CRT insertion had significantly prolonged QRS intervals and tended to have higher concentrations of NT-proBNP.

2.3.4   Primary hypothesis: interaction between delayed HMR and changes in LV systolic function

Figure 2.3.4 depicts changes in LV systolic function parameters and NT-proBNP concentrations in the patients studied, stratified according to CRT status. In neither subgroup, nor in the study population as a whole (Table 2.3.5), did LVEF change significantly over the course of the study.

If patients were stratified according to baseline delayed HMR status, the relationship between this parameter and δ LVEF was evaluated categorically by Chi-square

Parameters	HMR > 1.57	HMR < 1.57	P value
	n = 22	n = 23	
Age	62 ± 14	62 ± 12	0.99
NYHA III/IV status (%)	23	39	0.19
CRT	41	57	0.23
QRS	124 ± 25	131 ± 27	0.39
NT-proBNP levels (pg/ml)	1851 (725 − 2314)	3000 (1320 − 6347)	0.01
Coronary risk factors			
BMI (kg/m^2)	27 ± 5	28 ± 5	0.49
Previous/current smoking (%)	59	57	0.55
Systemic hypertension (%)	64	52	0.32
Diabetes mellitus (%)	32	48	0.22
Dyslipidaemia (%)	64	52	0.32
ETOH use (%)	14	17	0.53
Echocardiography			
LVDd	6.4 ± 0.77	6.7 ± 0.72	0.35
LVDs	5.4 ± 0.82	5.6 ± 1.0	0.55
LVEF	31 ± 7.8	29 ± 6.7	0.42

Table 2.3.3: Comparisons between 2 groups of patients with delayed ^{123}I-MIBG HMR > 1.57 and with delayed ^{123}I-MIBG HMR < 1.57

Parameters	AICD alone n = 24	AICD plus CRT n = 21	P value
Age	60 ± 12	64 ± 13	0.39
NYHA III/IV status (%)	29	35	0.45
QRS	115 ± 20	141 ± 25	0.0009
NT-proBNP levels (pg/ml)	$1590\ (765 - 4479)$	$2534\ (1906 - 3744)$	0.14
Coronary risk factors			
BMI (kg/m^2)	27 ± 8	26 ± 5	0.46
Previous/current smoking (%)	65	52	0.29
Systemic hypertension (%)	65	48	0.19
Diabetes mellitus (%)	39	43	0.52
Dyslipidaemia (%)	52	62	0.37
ETOH use (%)	13	19	0.45
Echocardiography			
LVDd	6.5 ± 0.75	6.7 ± 0.73	0.38
LVDs	5.4 ± 0.98	5.6 ± 0.82	0.46
LVEF	30 ± 6	30 ± 8	0.81

Table 2.3.4: Comparisons between patients who underwent AICD plus CRT implantation and those underwent AICD implantation alone
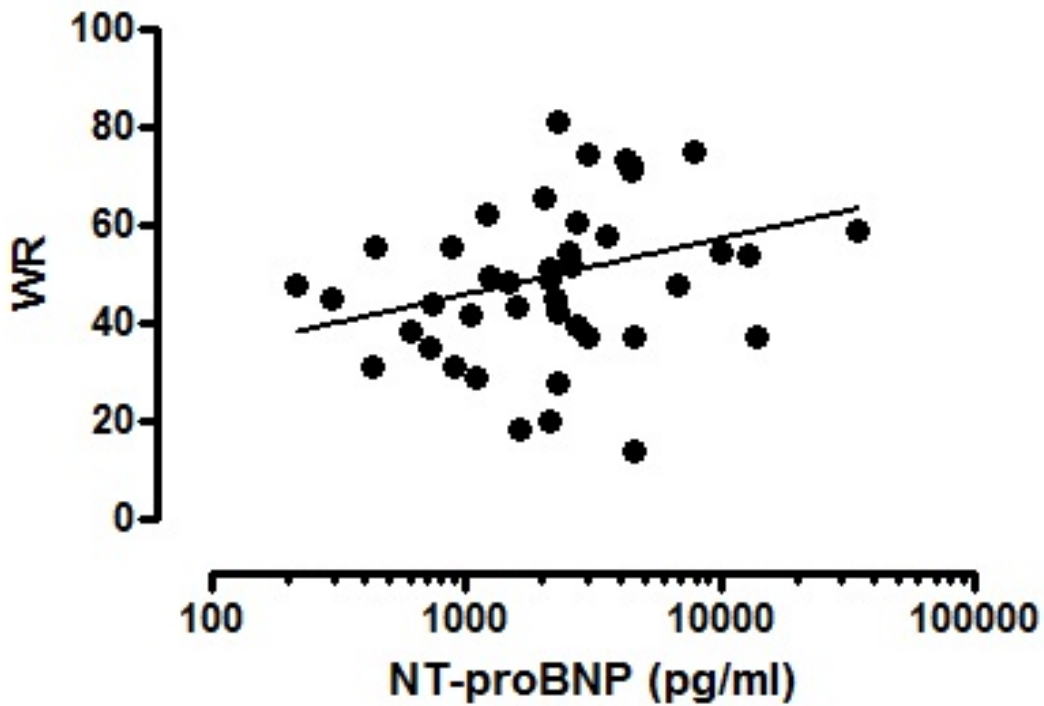
Figure 2.3.3: Correlation between baseline NT-proBNP levels and baseline WR (r = 0.36, p = 0.01, Spearman's correlation)

analysis relative to mean values of delayed HMR. In neither case was there a significant relationship between baseline HMR and parameters of LV function changes.

Furthermore, if this methodology was extended to comparisons between delayed HMR and changes in (a) LVDs or (b) NT-proBNP, again no significant correlation was found.

Finally, because ^{123}I-MIBG WR has been suggested to predict CHF progression (Currie et al. 2011), the relationship between WR and changes in LVEF, LVDs, and LVDs was sought. However, no correlations between WR and LV function deterioration were found.
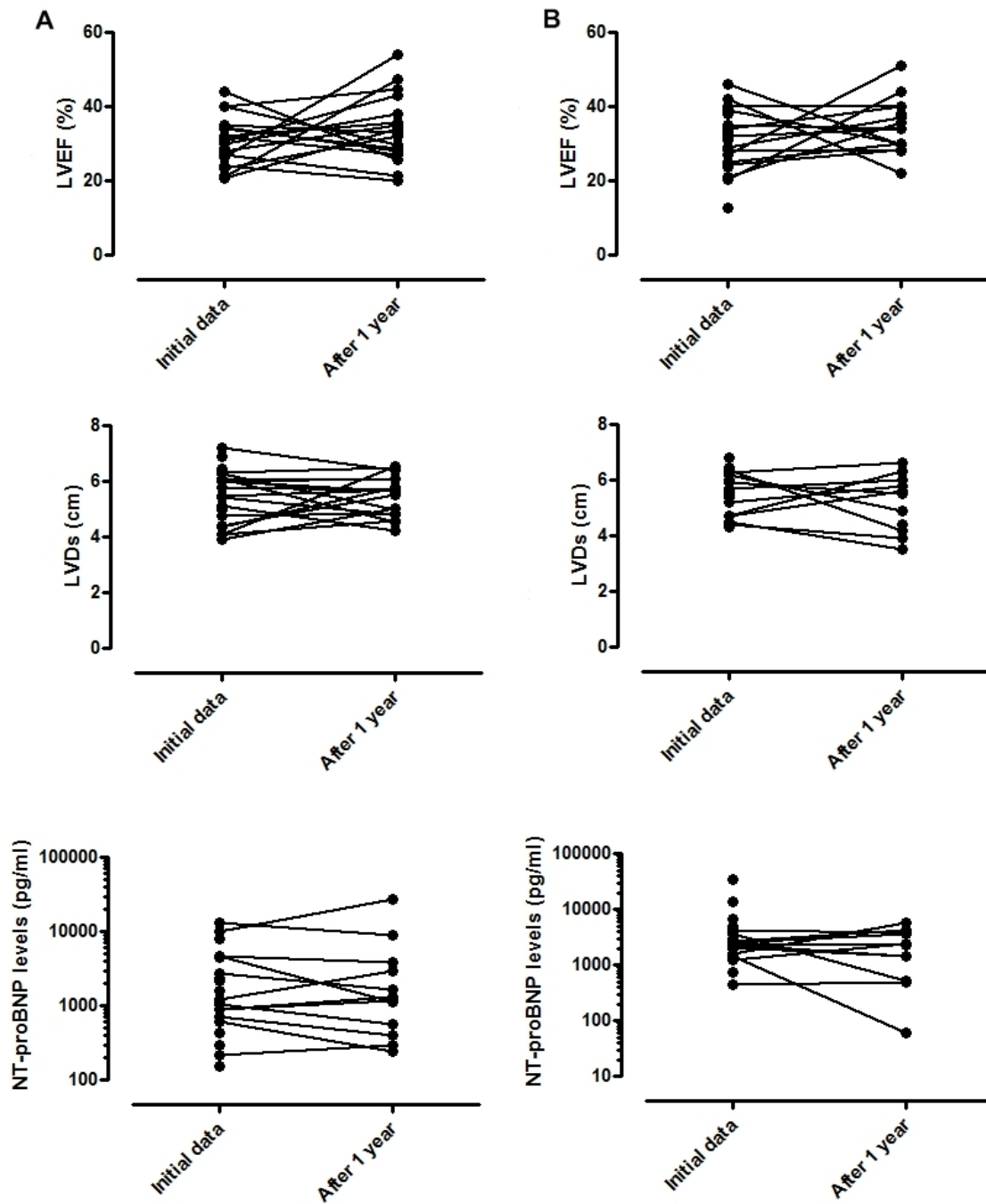
Figure 2.3.4: Changes in LV systolic function parameters and NT-proBNP concentrations in the AICD alone group (A), compared to the AICD plus CRT group (B). NT-proBNP levels were repeated in 22 patients who underwent the second MIBG scanning

Follow-up	Initial results	After 1 year	P value
QRS	128 ± 26	132 ± 41	0.68
NT-proBNP levels (pg/ml)	$1532\ (725 - 2745)$	$1251\ (498 - 3575)$	0.9
Echocardiography			
LVDd	6.5 ± 0.4	6.4 ± 0.8	0.75
LVDs	5.3 ± 0.9	5.4 ± 0.9	0.71
LVEF	31 ± 8	34 ± 9	0.23
MIBG imaging			
Early HMR	1.78 ± 0.32	1.79 ± 0.35	0.76
Delayed HMR	1.60 ± 0.27	1.61 ± 0.33	0.85
WR (%)	45.5 ± 14.2	48.4 ± 17.6	0.42

Table 2.3.5: Comparisons between the initial QRS intervals, NT-proBNP levels, echocardiographic data, ^{123}I-MIBG parameters and after 1 year

2.3.5 Secondary hypothesis: delayed ^{123}I-MIBG HMR and appropriate AICD discharge

Over the course of the study 24% of patients experienced an appropriate AICD discharge. Kaplan-Meier analysis (Figure 2.3.5) revealed no significant difference between the probabilities of discharge according to baseline HMR values (using a cut-off of mean HMR at baseline). Cox proportional hazard analysis revealed a hazard ratio of 1.49 (95% CI = 0.45 to 4.9, p = 0.5) for low: high delayed HMR status.

2.3.6 Multivariate analysis

As planned, correlates of (a) δ LVEF, (b) AICD discharge were sought via multivariate analysis. Results are summarised in Table 2.3.6 and Table 2.3.7. No correlations between changes in LVEF and age, diabetes mellitus status, delayed HMR, WR, and CRT insertion were found. Furthermore, none of the parameters, including NT-proBNP
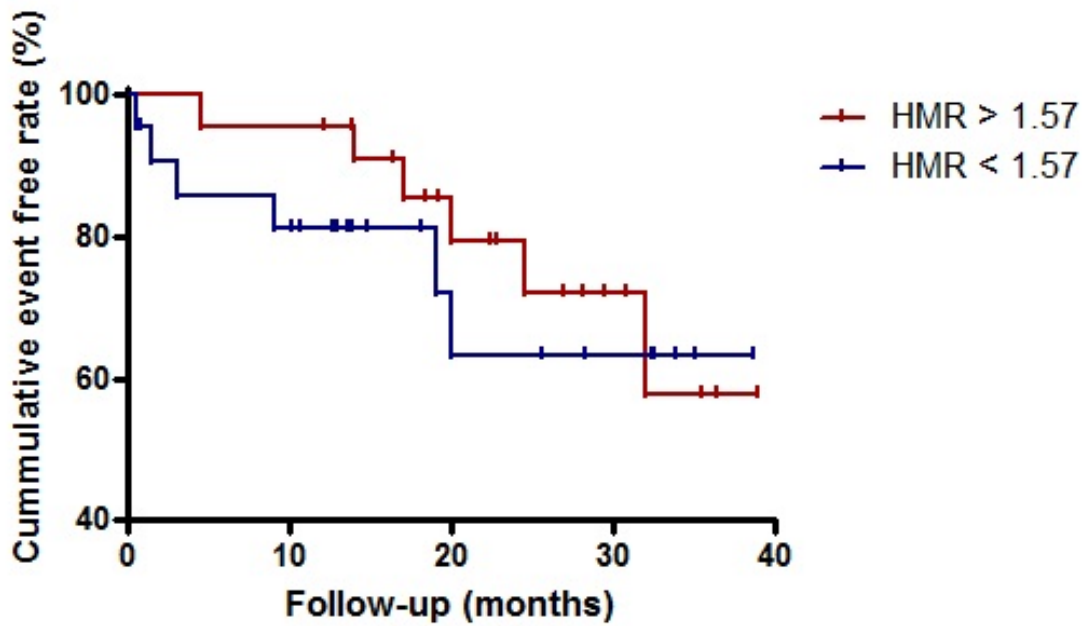
Figure 2.3.5: Kaplan-Meier analysis for patients with delayed ^{123}I-MIBG HMR > 1.57, compared to those with ^{123}I-MIBG HMR > 1.57 for prediction of AICD discharge

levels, LVEF, delayed HMR, WR, and CRT insertion were significantly correlated with AICD discharge.

Parameters	β coefficient	P value
Age	−0.07	0.66
Diabetes mellitus	−0.06	0.71
Delayed HMR	0.2	0.21
WR	−0.09	0.56
CRT insertion	−0.006	0.97

Table 2.3.6: Determinants of changes in LVEF after 1 year of AICD implantation (Stepwise multiple linear regression analysis)

Parameters	β coefficient	P value
EF	0.05	0.77
NT-proBNP levels (pg/ml)	-0.13	0.42
Delayed HMR	0.14	0.39
WR	-0.13	0.43
CRT insertion	0.21	0.18

Table 2.3.7: Determinants of AICD discharge during follow-up (Stepwise multiple linear regression analysis)
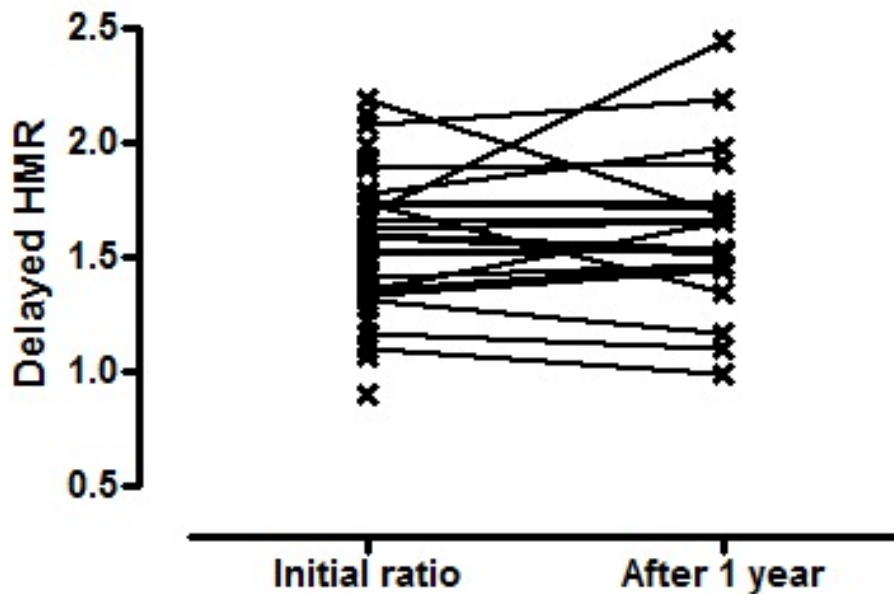
Figure 2.3.6: Lack of progression of myocardial denervation: Changes in delayed HMR after 1 year (n = 22).

2.3.7 Change in delayed HMR

There was no significant difference in delayed HMR between enrolment and 1 year thereafter (Figure 2.3.6).
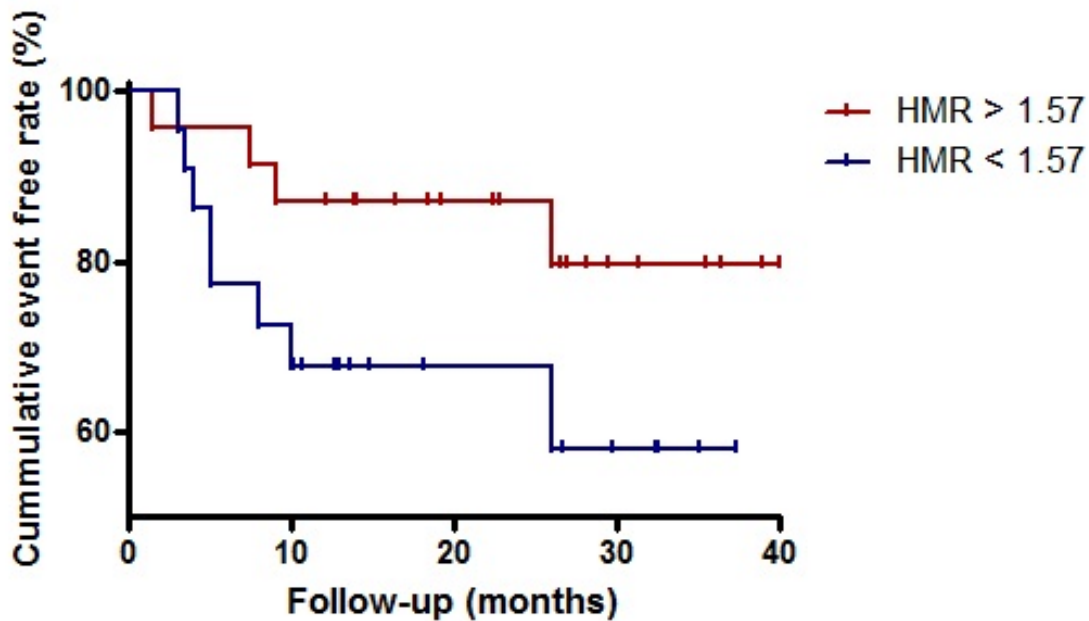
Figure 2.3.7: Kaplan-Meier analysis for patients with delayed ^{123}I-MIBG HMR > 1.57, compared to those with ^{123}I-MIBG HMR > 1.57 for prediction of HF admission or HF deaths (p = 0.1)

2.3.8   Non-arrhythmic cardiac events

13 patients were hospitalised due to decompensated CHF during the follow-up period. 4 patients died of severe heart failure, while 1 patient died of prostate cancer. There was a trend toward a higher rate of HF admission or HF deaths in patients with delayed HMR < 1.57, compared to those with delayed HMR > 1.57 (mean HMR at baseline) (hazard ratio = 2.5, 95% CI = 0.84 to 7.4, p = 0.1), as shown in Figure 2.3.7.

2.4   Discussion

The basis for this investigation was the findings, by a number of Japanese (Nakata et al. 1998, Imamura et al. 2001, Matsuo et al. 2003, Fujimoto et al. 2004) and other (Cohen-Solal et al. 1999, Bengel et al. 1999, Jacobson et al. 2010, Boogers et al. 2010)

groups, that extent of cardiac denervation, as exemplified by parameters of ^{123}I-MIBG scanning results, was predictive of ventricular arrhythmogenesis in patients with both ischaemic (McGhie et al. 1991, Simoes et al. 2004) and non-ischaemic (Merlet et al. 1999, Momose et al. 2011) CHF. While the results of the abovementioned studies were generally consistent with one another, a number of problems remained:

(1) There was no absolute agreement on the optimal ^{123}I-MIBG parameters for measurement. The majority of investigators (Nakata et al. 1998, Merlet et al. 1999, Jacobson et al. 2010) utilised, as we have in this study, delayed ^{123}I-MIBG HMR. However, one notable group (Boogers et al. 2010) found that information from delayed ^{123}I-MIBG HMR was not significantly related to arrhythmogensis, and utilised instead summed defect score on SPECT ^{123}I-MIBG. However, other investigators (Jacobson et al. 2010) have reported that this parameter is not readily reproducible.

(2) Despite the extensive data on ^{123}I-MIBG imaging, there was no consensus on the "potency" of ^{123}I-MIBG-based analysis for therapeutic decision making. For example, as to whether an individual was likely to benefit from AICD implantation. An evaluation of the data from Nishisato et al. (2010) suggests a mean AICD discharge ratio of about 2:1 for patients with and without the combination of low ^{123}I-MIBG HMR and high summed ^{99m}Tc-tetrofosmin rest score, which falls short of the requisite degree of clinical certainty.

(3) There has been no serious previous attempt to test the hypothesis that the reported impact of ^{123}I-MIBG scanning results on arrhythmogenesis might be mediated or at least modulated by deterioration in LV systolic function. This possibility is theoretically attractive because cardiac deterioration may increase free catecholamine concentrations and may promote catecholamine-induced myocardial damage (see Section 1.2.2).

The current study, although somewhat limited by relatively small sample size, reached several important conclusion, namely:

- (1) There is no significant correlation between delayed ^{123}I-MIBG HMR and changes in LVEF over a period of 12 months, either overall or in patients without concomitant CRT insertion.

- (2) There is no marked relationship between delayed HMR and appropriate AICD discharge rate.

- However, (3) It is possible that low delayed ^{123}I-MIBG HMR are predictive of cardiac failure-related events.

A number of reasons may explain the results of the study. Firstly, patients were receiving extensive medical therapy, including 100% of the patients being treated with both ACEIs/ARBs and β-adrenoreceptor antagonists. This may have contributed to the relative stability of LVEF over time. Nevertheless, it has been reported that LVEF correlates weakly with delayed HMR (r = 0.5) (Merlet et al. 1992) and delayed HMR is a marker of SCD risk, while washout rate may be a marker of HF progression (Currie et al. 2011). Secondly, the combination of AICD and CRT (utilised in approximately half of the patients) may have affected outcomes in this study. However, substantial effect of CRT was excluded by stratifying patients according to intention or otherwise to performed simultaneous CRT. Furthermore, the heterogeneity of patients' background may have had an impact on the study results.

A further conclusion of the study, obtained from the 22 patients who underwent repeat ^{123}I-MIBG scanning 12 months after entry, is that cardiac denervation was not demonstrably progressive over this period of time. Although plasma concentrations of baseline NT-proBNP inversely correlated with baseline delayed HMR and directly with baseline WR, there were no significant correlations between changes in NT-proBNP levels and changes in either delayed HMR or WR. Kasama et al. (2008) have previously suggested that serial ^{123}I-MIBG assessment improves prediction of cardiac events including sudden cardiac deaths in patients with CHF. However, in the current data set the concordance between baseline and 12 months data was very strong (Figure 2.3.6), suggesting that there is nothing to be gained from serial assessment.

2.5 Limitations of the study

In a relatively small study of this type, the lack of demonstrated statistical correlation between HMR and LVEF may reflect type II error. Secondly, low energy collimators
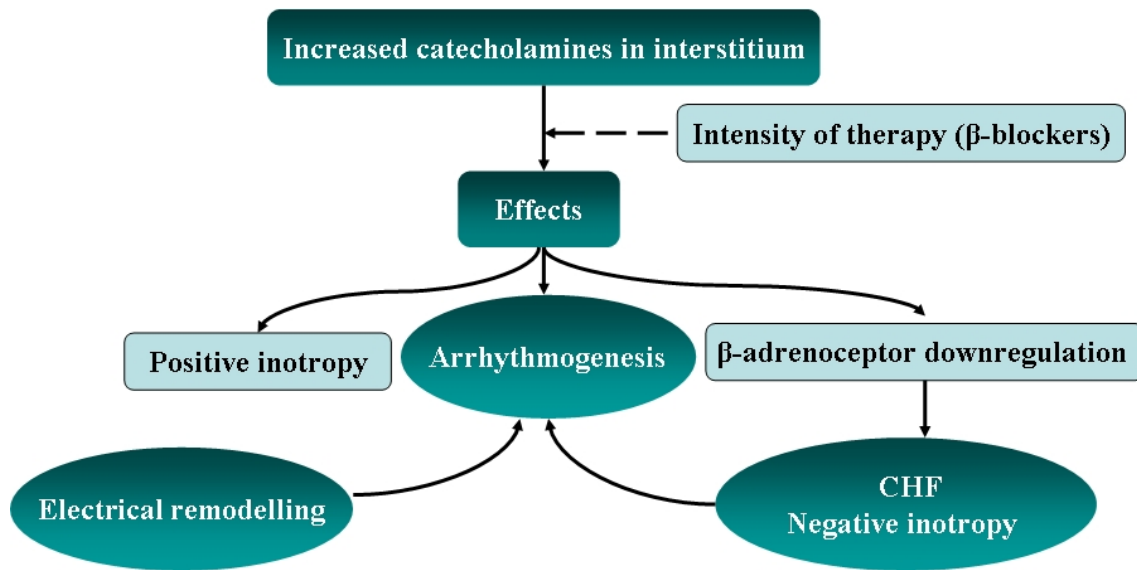
Figure 2.5.8: Relationship between cardiac denervation, LV function deterioration and arrhythmogenesis

were used in this study because the study was commenced before the recent recommendation to use medium energy collimators was published (Bax et al. 2008). This may have had an impact on the quality of the MIBG SPECT images, resulting in the difficulty in reconstructing SPECT data, particularly in patients severe LV dysfunction. Furthermore, if the postulated physiological "cascade" presumed to underlie the relationship (or otherwise) between cardiac denervation, LV function deterioration and potential arrhythmogenesis is schematically depicted (Figure 2.5.8), it is integral to the thinking behind this study that LV function deterioration is considered an essential step towards long-term arrythmogenesis. While this might be correct, the LV dysfunction/arrhythmogenesis nexus is only approximate. It is possible for induction of VT to occur without substantial permanent functional deterioration, for example in the presence of concomitant ischaemia. The non-feasibility of obtaining reliable SPECT MIBG data was regrettable, precluding detailed ischaemia : denervation correlations. In theory, the use of provocative EP testing would have been preferable to AICD discharge rate, but was ethically contra-indicated.

2.6   Conclusions

The results of the current study demonstrated that cardiac denervation detected by ^{123}I-MIBG imaging utilising heart to mediastinum ratio, does not predict changes in LVEF and does not closely predict AICD discharge. However, the small number of subjects included in this study limited statistic power to detect small changes in both LVEF and HMR overtime which makes it less conclusive.

Chapter 3

Implications of catecholamines in the pathophysiology of Tako-tsubo cardiomyopathy

3.1 Introduction

In the last few years, TTC has been the focus of considerable interest, based upon its obscure pathophysiology, difficult diagnosis and uncertain prognosis.

At present, TTC represents a multifaceted clinical and pathophysiological enigma. From a clinical point of view, the majority of patients present with chest pain which is usually initially attributed to myocardial ischaemia: indeed TTC is usually treated initially as a form of thrombotic ACS. TTC is therefore often diagnosed only after institution of initial emergency therapeutic measures for ischaemia. The current diagnosis of TTC depends heavily on the demonstration of periapical hypokinesis/akinesis and the differentiation of TTC from STEMI, to avoid unnecessarily urgent cardiac catheterisation. Furthermore, thrombolysis, which may be required in AMI patients, is dangerous in TTC. It is also reported that routine echocardiography at presentation would potentially improve the diagnostic accuracy (Donohue & Movahed 2005, Fazio et al. 2009). However, this is impractical in clinical settings. Therefore, there is an obvious need to improve the diagnosis of TTC.

It has been widely assumed, on the basis of reasonable associative and clinical chemical evidence, that hypersecretion of catecholamines plays a part in the initiation of TTC (Akashi et al. 2003, Wittstein et al. 2005, Yoshioka et al. 2008) and a marked release of BNP/NT-proBNP has been documented in TTC (Akashi et al. 2004, Wittstein et al. 2005, Grabowski et al. 2008, Madhavan et al. 2009, Morel et al. 2009). Furthermore, a number of current investigations from both biopsy studies and CMR have been suggested that TTC is associated with intramyocardial inflammation activation (Nef et al. 2007, Otsuka et al. 2008, Rolf et al. 2009, Abdel-Aty et al. 2009, Eitel et al. 2010). Both activation of inflammatory process and extensive release of catecholamine could precipitate formation of BNP. More interestingly, a recent study by Chan et al. (2012) has documented that BNP in turn, induces release of catecholamines from the heart and from the synaptic endings, leading to activation of protein kinase A and increase in cAMP formation.

Therefore, the scope of the current study focuses on two main aspects of this novel disease, including improving the diagnostic process of TTC, understanding its pathophysiology. Firstly, we examined the basis for elevation of BNP/NT-proBNP in TTC and tested the hypothesis that NT-proBNP could be utilised as a primary test to differentiate TTC from myocardial infarction in patients with chest pain or acute dyspnoea. In regards to the pathophysiologic aspects of TTC, we sought to determine the evidence for catecholamine effects in patients with TTC by evaluating the generation and modulation of NO signalling in TTC.

3.2   Incremental utility of Brain Natriuretic Peptides in TTC

3.2.1   Perturbations in BNP kinetics in patients with TTC: patterns, mechanisms and potential diagnostic utility

TTC is a disorder characterised by acute development of segmental (usually periapical) left ventricular systolic dysfunction. The differential diagnosis of TTC includes both

myocardial ischaemia and infarction, and traditionally TTC has become, to a large extent a diagnosis of exclusion. Patients with TTC not infrequently have low cardiac output status at initial presentation (Akashi et al. 2003) but definite pulmonary oedema is very rare (Jabara et al. 2009). Nevertheless, a number of investigations have reported that at the time of diagnosis, plasma levels of brain natriuretic peptide (BNP) (Akashi et al. 2004, Wittstein et al. 2005, Grabowski et al. 2008, Madhavan et al. 2009, Morel et al. 2009) or its precursor NT-proBNP (Nef et al. 2008) are markedly elevated in the majority of cases. The reported increases in NT-proBNP/BNP levels in TTC are surprising, given that left ventricular filling pressure is not markedly elevated (Nakagawa et al. 1995, Forfia et al. 2005). Indeed, the extent of elevation of BNP and NT-proBNP levels reported in TTC seems to be somewhat greater than that of patients with acute myocardial infarction, although detailed comparisons are lacking.

The marked and parallel release of BNP and NT-proBNP suggest acute cleavage of pro-BNP. Corin, a transmembrane serine protease present both in myocardium and plasma (as discussed in Section 1.3.5.5), has been shown by some investigators to cleave proBNP (Wu et al. 2009). Furthermore, absence of corin I1555 (P568) allele has been found in African-Americans and is associated with an increase in hypertension and left ventricular hypertrophy (Rame et al. 2009).

Therefore, the objectives of the current study were:

1. To determine the basis for the putative elevation in natriuretic peptide levels in TTC, utilising predominantly NT-proBNP as a marker because of its longer half-life in plasma (Pemberton et al. 2000).

2. To test the hypothesis that the extent of elevation of plasma levels of NT-proBNP may serve as a component of a diagnostic algorithm to facilitate differentiation of TTC from (thrombotic) ACS.

3. To test the hypothesis that corin is the major enzyme responsible for the acute rise in BNP.

3.2.2   Methodology

3.2.2.1   Patient population

The investigations consisted of two linked components: an evaluation of the time-course and extent of NT-proBNP/BNP release in the context of newly diagnosed TTC, and a comparison of NT-proBNP elevation at diagnosis in patients with TTC vs. age-matched female patients with ACS [without acute pulmonary oedema (APO)].

a. TTC group   Consecutive TTC patients were prospectively identified on the basis of the following criteria: acute chest pain and/or dyspnoea of greater than 30 minute duration with ST elevation or T-wave inversion and/or cardiac biomarker elevation; detection of periapical or midventricular akinesis/hypokinesis; and no evidence for a diagnosis of myocardial infarction on coronary angiography or cardiovascular magnetic resonance (CMR).

b.   ACS patients   A control group of age-matched females with obstructive ACS were selected on the following criteria: female patients presenting with chest pain $\pm$ dyspnoea of greater than 30 minute duration, and associated with transient elevation of plasma concentrations of troponin T beyond the normal laboratory range, together with evidence of obstructive coronary artery disease on coronary angiography. Patients with ACS associated with overt acute pulmonary oedema were excluded, given that (a) pulmonary oedema was not encountered among our TTC patients and (b) that pulmonary oedema is known to be associated with marked elevations of BNP and NT-proBNP levels (Ray et al. 2005).

In order to establish the base-line characteristics of the two study groups, patients' history and clinical profile were evaluated by interviewing all patients and reviewing hospital and physician records. This information included pre-admission likelihood of coronary artery disease (CAD), past history, cardiac risk factors, clinical symptoms, and recent stress events. Haemoglobin level and estimated glomerular filtration rate (eGFR) at admission were also reported. The study was approved by the institutional Ethics of Human Research Committee and written informed consent was obtained before study

entry.

3.2.2.2 Assay methodology

a. Determination of plasma BNP and NT-proBNP concentrations Venous blood specimens were collected into tubes containing potassium ethylene diamine tetra acetic acid (EDTA) at the time of diagnosis and daily thereafter for 3 days. Determination of plasma NT-proBNP and BNP was performed by immunoassay (Elecsys E 170, Roche Diagnostics, Mannheim, Germany and AxSYM, Abbott Laboratories, Illinois, USA, respectively). NT-proBNP level was assayed within 2 hours post-venesection, whereas for BNP assay, blood was centrifuged at 1680 relative centrifugal force (RCF) for 10 minutes at 2°C and frozen plasma was stored at -80°C until analysis. In 40 patients, in whom presentation occurred within 12 hours of onset of symptoms, NT-proBNP assay was performed at presentation and 24 hours thereafter to determine the pattern of early NT-proBNP release. NT-proBNP and BNP assays were repeated 10 days and 3 months post diagnosis.

b. Determination of plasma corin concentrations Plasma samples were collected and stored as described above. Plasma concentrations of corin (pg/ml) were measured utilising a quantitative sandwich ELISA immunoassay (Quantikine Human Corin, Research and Diagnostics Systems, Inc., Minneapolis, USA) at 3 time points.

c. Additional routine investigations Additional serial routine biochemical investigations including assays of troponin-T and creatine kinase (CK) (Elecsys, Roche Diagnostics, Mannheim, Germany) were performed at admission and daily thereafter. Serial (daily) determination of plasma C-reactive protein (CRP) concentrations was performed utilising a latex-enhanced immunoturbidometric assay (Elecsys 1010, Roche Diagnostics, Mannheim, Germany). All CRP results of <10 mg/L were then subjected to high sensitivity (hs-CRP; Olympus au5400, Dallas, Texas, USA) determination.

Plasma concentrations of normetanephrine, a non-acidic derivative of norepinephrine reflecting extraneuronal uptake and O-methylation, were measured as an index of cate-

cholamine exposure in TTC. Venous blood at diagnosis was drawn into tubes containing EDTA and centrifuged at 1680 RCF for storage at $-80°$C for subsequent assay of normetanephrine by liquid chromatography/tandem mass spectrometry (LC-MS/MS) (Lagerstedt et al. 2004).

3.2.2.3   Further diagnostic/correlative investigations

- (a) The diagnosis of TTC was established via a combination of transthoracic echocardiography (performed in all patients) and coronary angiography (performed in 73 patients). In 18 patients, coronary angiography was not performed because of extreme age and/or disability; however, both absence of myocardial infarction, by DE-MRI, and resolution of wall motion abnormalities on echocardiography were established in these cases.

- (b) At the time of cardiac catheterisation determination of PCWP via right heart catheterisation was performed in 42 patients for correlation with NT-proBNP release.

- (c) CMR imaging, performed acutely in 36 patients, was utilised to quantitate regional hypokinesis and to calculate regional left ventricular wall stress. CMR was performed utilising a 1.5 Tesla Philips Intera magnet (Netherlands). DE-MRI was performed at 10-15 min following intravenous injection of 0.2 mmol/kg Gadolinium-diethylennetriamine pentaacetic acid (Magnevist; Schering AG, Germany). Short axis images of the left ventricle (8 mm slice thickness), were obtained, employing SENSE. LVEF utilising Simpson's method, was calculated off-line by a single trained operator, by planimetry of systolic and diastolic endocardial borders, in all complete slices.

- (d) Wall motion was analysed, according to a sixteen-segment model (Schiller et al. 1989) by a MR-trained cardiologist. The thickening and excursion of each segment was described and scored (normal: 1, hypokinesis: 2, akinesis: 3, dyskinesis: 4). The wall motion score index (WMSI) was prospectively selected as a measure of extent of regional wall motion impairment (Schiller et al. 1989).

- (e) Regional systolic wall stress (δ) was calculated at basal (δBASE), mid-ventricular (δMID) and apical (δAPEX) levels, utilising the LaPlace-derived formula of Grossman et al, (Grossman et al. 1975) as adapted for use in CMR by Delepine et al (Delepine et al. 2003). Average end-systolic internal radius, with corresponding average radial thickness of myocardial wall, was determined via offline-analysis of CMR SENSE images, with the end-systolic frame being defined by AV closure on a LV outflow tract view. Whereas LV basal (δBASE) and mid-ventricular (δMID) wall stress was determined from short-axis images, apical wall stress (δAPEX) utilised measurements from corresponding long-axis views, given that δAPEX is known to be underestimated when wall thickness is determined from short axis slices, a consequence of tangential slicing due to the increased radius of wall curvature at the LV apex (Delepine et al. 2003).

3.2.2.4 Statistical methods

a. Power calculations

(i) **Aims 1 and 2** <u>Rationale</u> The mean value of NT-proBNP concentrations in post-menopausal women is approximately 60 ± 16 (SD) (pg/ml) (these data were obtained by measurement of NT-proBNP levels in a cohort of 10 age-matched women). However, NT-proBNP is known to be moderately elevated in most ACS cases [644 pmol/L (Galvani et al. 2004)].

We rationalised that for elevations of NT-proBNP to have potential diagnostic utility in TTC, they would have to be at least 5-fold greater than the normal range in 95% of cases.

Therefore, we postulated that the mean NT-proBNP level in TTC would be sufficiently elevated that 2SD below that mean would still be greater than population norms.

In normal age-match female subjects, the SD value is 27% of the mean. Given that TTC is likely to be subject to greater heterogeneity, we postulated a 50% increase in SD : mean.

<u>Differentiation from normals</u>: > 3 SD, > 10 subjects per group serve 90% power at

p = 0.001.

Differentiation from ACS: > 2.5 SD, > 16 subjects per group serve 90% power at p = 0.001.

(ii) Aims 3 Power calculations were performed to determine the ability of linear regression to detect a significant correlation between corin and NT-proBNP concentrations. With n = 19, SD value was 39% of mean, the power of the study was 0.43 at p = 0.05.

b. Data analysis Data were analysed using SPSS version 15 software (SPSS, Chicago, Illinois, USA) and presented as median and interquartile range. Correlations between NT-proBNP levels and PCWP, regional systolic wall stress and WMSI were assessed with Spearmans correlation analysis. Fisher's exact test was used to compare the differences in patients characteristics between two groups. The Wilcoxon signed rank test was used to compare differences between medians of two groups. One-way ANOVA analysis was used to assess the impact of age on NT-proBNP concentrations in both TTC and ACS patients. A value of $p < 0.05$ was considered significant.

In order to test the hypothesis that marked elevation of NT-proBNP constitutes a possible means for differentiating TTC from ACS, our initial approach was to examine this issue in isolation (i.e. in the absence of clinical correlates, other than gender or age). Therefore, receiver operating characteristic (ROC) curves including sensitivity and specificity relationships for a series of putative NT-proBNP levels at the time of diagnosis were analysed.

3.2.3 Study results

3.2.3.1 Patient characteristics

Clinical characteristics of the study population (both TTC and ACS) are described in Table 3.2.1. Ninety one patients (88 females, 3 males) were diagnosed with TTC over a 3.5 year period from March 2008 to December 2011. Systolic hypertension (55%) and dyslipidaemia (43%) were the most common coronary risk factors in this study group.

Potential emotional or physical stressors were identified in 70 patients (77%). Only 6% patients with TTC had been treated with β-adrenoreceptor antagonists prior to the diagnosis.

Of the 35 TTC patients exhibiting anterior ST-elevation, the diagnosis was made on Day 1, during urgent cardiac catheterisation, in all but one case. Of the remaining 56 patients, 27 were also diagnosed on the day of presentation, 13 the day after presentation (Day 1), and 16 on Day 2 or Day 3. 6 patients were initially hypotensive; one died on day seven of multi-organ failure. Median pulmonary capillary wedge pressure (PCWP) was 14mmHg. Whilst none of the patients exhibited clinical manifestations of pulmonary oedema, 20 patients had a PCWP of 15 mmHg or above, the maximum being 36 mmHg. All patients in the TTC group had troponin T elevations beyond the normal range for the laboratory, while 55% had CK elevations.

The ACS group consisted of 40 age-matched female patients presenting with chest pain of < 48 hours duration. There were no differences in coronary risk factors and clinical presentation between 2 groups, except for a higher proportion of diabetes in the ACS group. Peak creatine kinase concentrations were significantly greater in ACS patients ($p = 0.01$), but Troponin T concentrations were similarly (and marginally) elevated in both groups. Furthermore, there was a significantly higher levels of CRP in the TTC group, compared to ACS patients ($p = 0.009$) (Table 3.2.1).

3.2.3.2   Bases for BNP/NT-proBNP in TTC

a. Time course of elevation   NT-proBNP and BNP concentrations were markedly elevated beyond population norms at diagnosis, with progressive resolution over 3 months (Figure 3.2.1). At 10 days, both NT-proBNP and BNP were still elevated beyond age-corrected norms in all patients, while at three months, there was residual, but borderline elevation. Peak NT-proBNP [median 5839 (interquartile range 2646-10881) pg/ml] and BNP [617 (426-1026) pg/ml] were closely correlated ($R = 0.93$, $p < 0.0001$). In patients presenting within 12 hours of onset of symptoms ($n = 40$), median NT-proBNP levels were 1095 (425-4016) pg/ml at presentation, with peak concentrations being attained on day 2 [4860 (2882-8793) pg/ml, $p < 0.0001$]; see Figure 3.2.2.

Clinical Characteristics	TTC (n = 91)	ACS (n = 40)	P value
Mean Age (range)	68 ± 13 (31 to 95)	69 ± 11 (42 to 90)	0.68
A. Coronary risk factors			
BMI (kg/m)	28 ± 7	30 ± 6	0.4
Previous/current smoking	23%	35%	0.2
Systemic hypertension	55%	68%	0.23
Diabetes mellitus	21%	26%	0.49
Hyperlipidemia	43%	66%	0.03
B. Clinical presentation			
Potential acute stress	82%	NA	
Chest pain	98%	97%	0.9
Dyspnoea	5%	9%	0.71
S-T elevation on ECG	41%	43%	0.46
C. Laboratory assessment			
Troponin T (mg/dL) median (interquartile range)	0.41 (0.24-0.57)	0.36(0.06-1.14)	0.82
Creatine Kinase (IU/L) median (interquartile range)	173(110-289)	358(109-738)	0.01
CRP/hs-CRP (mg/dL) median (interquartile range)	18(7-56)	6(3-21)	0.009
Renal dysfunction eGFR < 60 ml/min (%)	25%	35%	0.18
Hemoglobin level (g/l) mean ± SD	130 ± 15	129 ± 13	0.88

Table 3.2.1: Patient characteristics

(BMI = body mass index, CK = Creatine Kinase, hs CRP = high sensitive C-reactive protein, eGFR = estimated glomerular filtration rate)

b. Correlations between peripheral biochemical markers and NT-proBNP levels There was a direct correlation ($r = 0.56$, $p < 0.0001$) between plasma normetanephrine concentrations at diagnosis and peak NT-proBNP concentrations (Figure 3.2.3). Plasma concentrations of hs-CRP were directly correlated with peak NT-proBNP levels ($r = 0.44$, $p < 0.0001$) (Figure 3.2.4).

c. Correlations between NT-proBNP level and haemodynamic and functional indices

- (a) There was no significant correlation between NT-proBNP concentrations at diagnosis and PCWP at diagnostic cardiac catheterisation ($r = 0.22$, $p = 0.16$).

- (b) As it is possible that NT-proBNP release might result from any component of regionally increased systolic wall stress, these correlations were determined at three levels (δBASE, δMID and δAPEX; see methods). There was no significant correlation between peak NT-proBNP concentrations and regional systolic wall stress at these three levels ($p > 0.1$ in all cases).

- (c) Comparisons between extent of impairment of wall motion score index (WMSI) and peak NT-proBNP (Figure 3.2.5) demonstrated a moderately correlation ($r = 0.3$, $p = 0.008$). For example, a WMSI > 2, implying severe periapical hypokinesis or akinesis, corresponded to mean NT-proBNP concentrations > 10000 pg/ml.

3.2.3.3   Comparison of NT-proBNP concentrations between TTC and ACS patients

Initial NT-proBNP levels were 3-fold greater in TTC than ACS patients [median 3000 (interquartile range 930-7085) pg/ml vs. 778 (385-1863) pg/ml, p = 0.0003, Mann-Whitney; see Figure 3.2.6]. Furthermore, the increase in NT-proBNP between days 1 and 2 observed in TTC patients (Figure 3.2.2) was absent in the ACS cohort. Specifically, δ NT-proBNP levels (day 2 - day 1) were significantly higher in TTC patients, compared to ACS [2801 (953 - 6268) vs 629 (139 - 1243) pg/ml, p = 0.002, respectively).

Although NT-proBNP increased significantly with patient age, in both TTC and ACS patients (p=0.008, ANCOVA), TTC was associated with 2-3 fold incremental elevations at any particular age (Figure 3.2.7). ROC curve-derived data for differentiation of TTC from ACS, at various NT-proBNP concentrations at diagnosis, showed that NT-proBNP concentrations > 2500 pg/ml were relatively specific for TTC (55% sensitivity, 83% specificity), but above 6000 pg/ml, where 95% specificity was attained, there was low sensitivity (26%) for the diagnosis (area under the curve AUC = 0.7) (see Figure 3.2.8).

Given that TTC patients exhibited higher NT-proBNP levels, together with lower peak CK concentrations than ACS patients, a ROC analysis for the initial NT-proBNP: CK ratio was also performed. The analysis increased the AUC to 0.75 (see Figure 3.2.9).

3.2.3.4   Potential role of corin in BNP release

At diagnosis, although NT-proBNP levels were markedly elevated above population norms, corin concentration were within the previously described normal range [mean 1168 ± 453 (SD) pg/ml] (see Table 3.2.2). Furthermore, although plasma NT-proBNP

concentrations were significantly associated with extent of catecholamine release, there was no significant correlation between corin and either NT-proBNP or normetanephrine concentrations at any time points. Finally, there was a subsequent marked decline in NT-proBNP levels at 3 months without any significant change in corin concentration. Thus, the NT-proBNP/corin ratio fell markedly over 3 months (see Figure 3.2.10).

Parameters	At diagnosis	10 days	3 months
NT-proBNP levels (pg/ml) median (interquartile range)	6517 (4217 - 16326)	1989 (837 - 4392)	394 (165 - 636)
Corin levels (pg/ml) mean ± (SD)	1168 ± 453	1200 ± 353	988 ± 319

Table 3.2.2: NT-proBNP and corin levels in 19 TTC patients at 3 time points

3.2.4 Discussion

TTC eluded detection until 1990 (Sato et al. 1990), despite subsequent data suggesting that it accounts for at least 1% of presentations with acute coronary syndromes in a Western setting (Pilliere et al. 2006) and presumably more in post-menopausal females. Although it has been suggested that the incidence is increasing (Buchholz & Rudan 2007), it is far more likely that this reflects increased awareness of the phenomenon. Even though there is no agreement to date as to the appropriate acute management of TTC, earlier diagnosis would at least enable clinicians to avoid unnecessary investigations or treatments (e.g. fibrinolytic therapy) and the risks which might attend them. Furthermore, greater diagnostic precision would facilitate prospective study of the disorder, including, ultimately, the evaluation of potential therapeutic interventions.

The current investigations demonstrate that both BNP and NT-proBNP plasma concentrations are appreciably elevated at diagnosis in all TTC patients. This is not in itself novel: six previous studies have documented that BNP elevation occurs commonly

in TTC (Akashi et al. 2004, Wittstein et al. 2005, Grabowski et al. 2008, Madhavan et al. 2009, Morel et al. 2009) while one investigation obtained similar data with NT-proBNP (Nef et al. 2008). Moreover, the extent of such elevation is similar in the current series to the previously described data.

The current data demonstrate additionally that BNP and NT-proBNP plasma concentrations are closely correlated in TTC patients and that, while NT-proBNP concentrations are elevated within 12 hours of symptom onset, they continue to rise in TTC patients during the next 24 hours. This suggests that release of NT-proBNP in TTC patients results from increased expression/synthesis, rather than release of preformed peptide.

Surprisingly, given the reported rapid resolution of wall motion anomalies in such patients, both NT-proBNP and BNP concentrations remained markedly elevated above population norms for at least 10 days and marginally at three months. These findings suggest that the factors precipitating BNP and NT-proBNP release in such patients are not rapidly reversible. Indeed, while left ventricular function appears to normalise rapidly, histochemical, histological and metabolic anomalies within the LV myocardium in TTC patients are more persistent (Nef et al. 2007); it is possible that these may be more relevant to BNP/NT-proBNP expression and release. Analogous data for patients with ACS (Ang et al. 2009) suggested that BNP levels frequently remain elevated for at least 7 weeks, and that this carries adverse prognostic implications. Whether persistent NT-proBNP/BNP elevation in TTC correlates with slow recovery remains to be determined.

Given the suggestion by several investigators that TTC may present, in whole or in part, a response to excessive catecholamine secretion (Akashi et al. 2003, Wittstein et al. 2005, Yoshioka et al. 2008), we sought correlations between admission normetanephrine concentrations and peak NT-proBNP. The actual demonstration of a direct correlation, as shown in Figure 3.2.3, is somewhat surprising, given that some investigators have failed to observe any elevation of plasma catecholamine levels in the acute phase of TTC (Madhavan et al. 2009), while other data suggest that the development of TTC may reflect individual variability in cardiac responsiveness to catechols (Barletta et al.

2009, Previtali et al. 2010). The current data tend to suggest otherwise: essentially, within the spectrum of a TTC population, the extent of NT-proBNP elevation appears to reflect a predictable response to catecholamine stimulus.

None of the TTC patients were in APO and PCWP was only marginally elevated. Thus, the extent of the NT-proBNP and BNP elevation, while comparable to that seen in decompensated heart failure (Nakagawa et al. 1995), reflects different physiologic stimuli. There were no significant correlations between NT-proBNP concentrations and either PCWP or systolic wall stress, again suggesting a different stimulus for release to that applicable in APO.

On the other hand, the extent of the apical hypokinesis, as quantified by WMSI, was moderately well correlated with NT-proBNP concentrations (Figure 3.2.5). This closer correlation raises the possibility that stimuli inducing BNP/NT-proBNP release are also those inducing hypokinesis. It has been reported that myocardial inflammation may trigger BNP release: - in the context of heart transplantation, markers of inflammatory activation were strong correlates of BNP concentrations in the absence of haemodynamic disturbance (Mehra et al. 2006, de Bold 2009). As inflammation has been documented via biopsy in TTC (Nef et al. 2007), this may represent a basis for these findings.

The second objective of the study was to determine to what extent NT-proBNP plasma concentrations might be useful to differentiate patients with TTC from those with ACS. In the past, the vast majority of patients with TTC have generally had ST segment elevation on admission ECG (Gianni et al. 2006): however, this was not the case in the current large series. However, a recent review of 105 patients diagnosed via cardiac catheterization at the Mayo Clinic (Dib et al. 2009) reveals that only 34% of subjects had initial ST-segment elevation, consistent with the findings in the currently reported series. Therefore, it cannot be anticipated that TTC would "automatically" be diagnosed at emergency cardiac catheterization on presentation. Madhavan et al. (2009) have reported that BNP plasma concentrations in TTC are substantially greater that those in ACS patients presenting with ST-elevation myocardial infarction. In the current study, we demonstrated that in post-menopausal females, NT-proBNP levels were about 3-fold greater in TTC than ACS. Hence ROC construction confirmed that NT-proBNP

concentrations of > 2500 pg/mL tended to suggest a diagnosis of TTC, while those $>$ 6000 pg/mL were strongly suggestive of TTC. Thus, it appears that determination of NT-proBNP might assist in the early differentiation of TTC from ACS. Furthermore, evaluation of NT-proBNP/CK ratio might improve the diagnostic utility of this process.

Therefore, a potential applicability of a "TTC score" system is proposed in this current study. The potential utility of NT-proBNP elevation to distinguish TTC from ACS/AMI is taken largely in "isolation". However, a clinically valid algorithm to differentiation between these conditions might also utilise other data such as patients' gender, precipitating stressors, and ECG parameters rather than NT-proBNP levels alone.

Finally, corin does not appear to modulate proBNP cleavage in TTC. Corin expresion has been reported to be up-regulated in hypertrophic and failing cardiomyocytes (Tran et al. 2004, Wang et al. 2008, Chen et al. 2010). An increase in corin myocardial expresion was also shown in rats with acute myocardial infarction (Calderone et al. 2006). However, limited data demonstrated that there was a decrease in corin concentrations, despite a marked increase in BNP and NT-proBNP levels in CHF patients, suggesting defective BNP processing and/or BNP resistance (Chen et al. 2010). In the current study, the findings that TTC is associated with marked and persistent elevation of NT-proBNP/BNP levels which increased rapidly and peaked within 48 hours, may suggest an intense release of BNP and NT-proBNP. Furthermore, a significant correlation was found between elevation of NT-proBNP levels and catecholamine concentrations. However, corin levels did not correlate with the extent of normetanephrine release. The next step would be to evaluate furin levels (given that furin represents the only other well-characterised enzyme which cleaves pro-BNP) and the clearance rates of NT-proBNP and BNP in these patients.

3.2.5   Study limitations

The study has a number of potential limitations. First, it is not always easy to determine precisely the time of onset of symptoms in TTC and the data presented may be distorted because of less than abrupt onset in some patients. Second, 21 patients with TTC did not undergo cardiac catheterisation and it is possible that they might have had "fixed"

coronary disease. In this regard, we did not investigate the issue of extent of regional ischaemia in individual TTC patients: even without "fixed" coronary disease. This might occur, for example, via focal coronary spasm or endothelial swelling (Yamamoto et al. 2002) and may stimulate release of natriuretic peptides (Zhang et al. 2004). Although we could not document any close association between regional wall stress and NT-proBNP release, wall stress is likely to vary widely within the LV in TTC patients and, therefore, such an association cannot be totally excluded.

While the comparison with ACS patients is internally valid, it has two major limitations: in "real life" the number of ACS patients is likely to be substantially greater that that of TTC patients, and so the current comparison is a little artificial. Similarly, the ROC-based diagnostic inferences are taken in isolation, not clinical context. In practice, a more appropriate diagnostic algorithm for TTC would certainly include potential precipitants, evaluation of ECG changes and also a coronary risk.

In this regard, it should be mentioned that we also determined NT-proBNP concentrations in whole blood utilising a point of care (POC) device (COBAS h232, Roche Diagnostics, Mannheim, Germany): data accorded closely with those from the plasma Elecsys E 170 assay, but it appeared from Bland-Altman correlation(Figure 3.2.11) , that the POC device may have somewhat over-estimated NT-proBNP concentrations that were over 3000pg/ml. Nevertheless, this would have had little impact on the conclusions of this study.

3.2.6   Conclusions

In conclusion, TTC is associated with marked and prolonged elevations of NT-proBNP and BNP, which are disproportionate with PCWP, but correlated with each other and with the extent of regional hypokinesis; this suggests a local and persistent "chemica" cause for NT-proBNP/BNP release. As NT-proBNP concentrations are substantially greater than those seen in ACS, this assay is likely to assist in the differentiation of TTC from ACS. Therefore, a clinically based algorithm for early consideration of the

diagnosis of TTC, using a "TTC score" system is proposed. For example, a "TTC score" might assign points values to (1) postmenopausal female patients with chest pain, based on the presence of a well-defined stressor (1 point), (2) Troponin T elevation (1 point), (3) NT-proBNP elevation to > 2500pg/ml (1 point) or > 6000 pg/ml (2 points), and (4) localised (1 point) or multiregional (2 points) T wave changes on ECG. However, this algorithm needs to be validated prospectively against ACS/AMI and it utility, rather than merely statistical validity, demonstrated. Finally, corin plays no role in the modulation of proBNP cleavage in TTC. Thus, further investigation is needed to evaluate whether furin is responsible for the acute rise in BNP in patients with TTC.
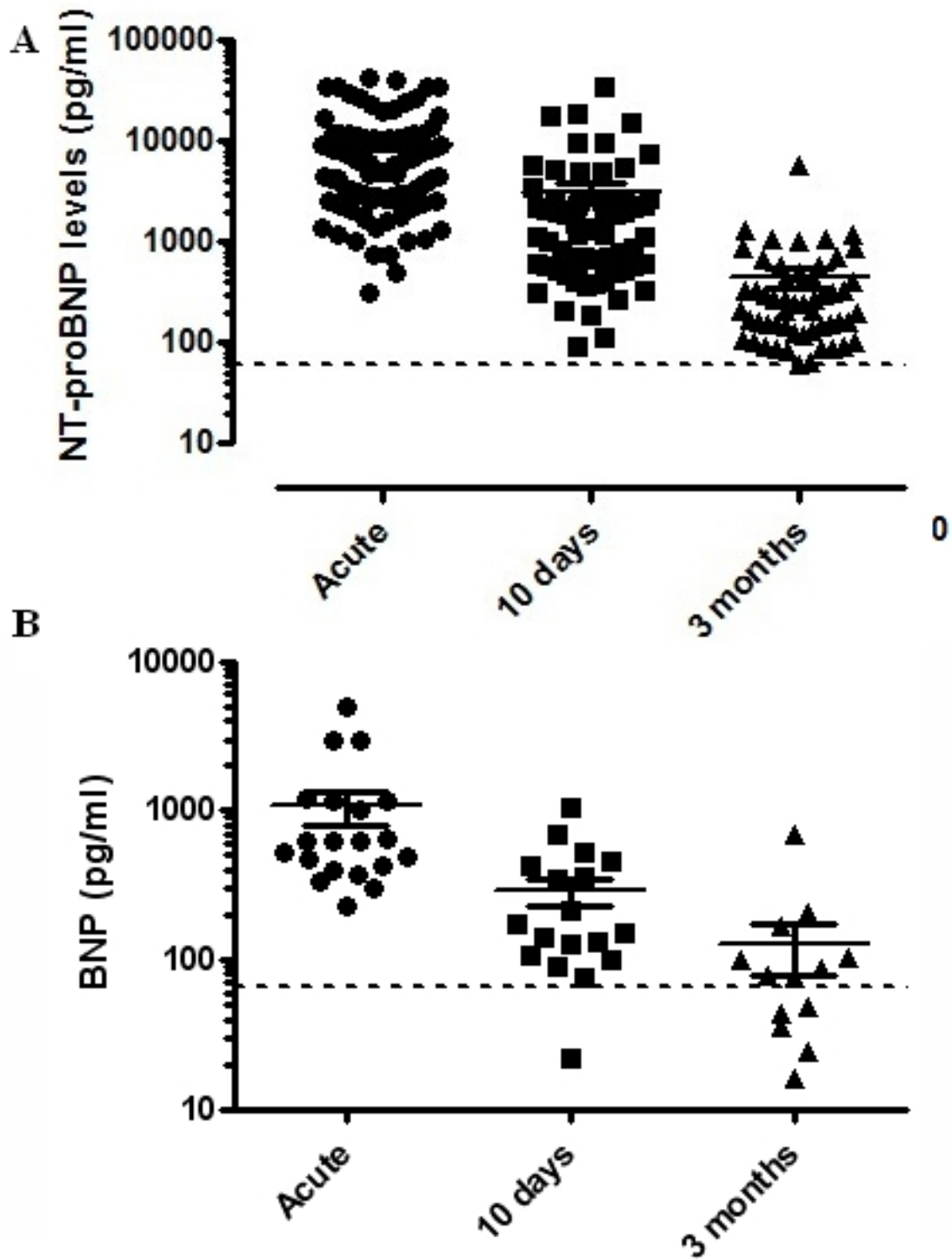
Figure 3.2.1: NT-proBNP (A) and BNP (B) concentrations in TTC at diagnosis, 10 days, and 3 months. Mean values for age-adjusted controls are indicated by the dotted lines.
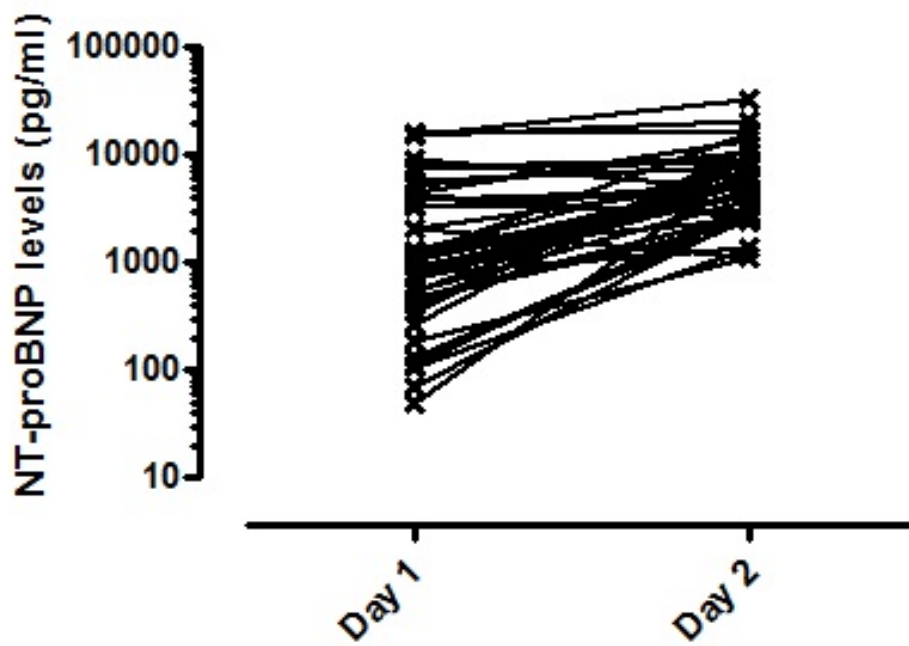
Figure 3.2.2: Changes in NT-pro BNP over first 48 hours in individual patients (p < 0.0001 for day 2 vs. day 1)
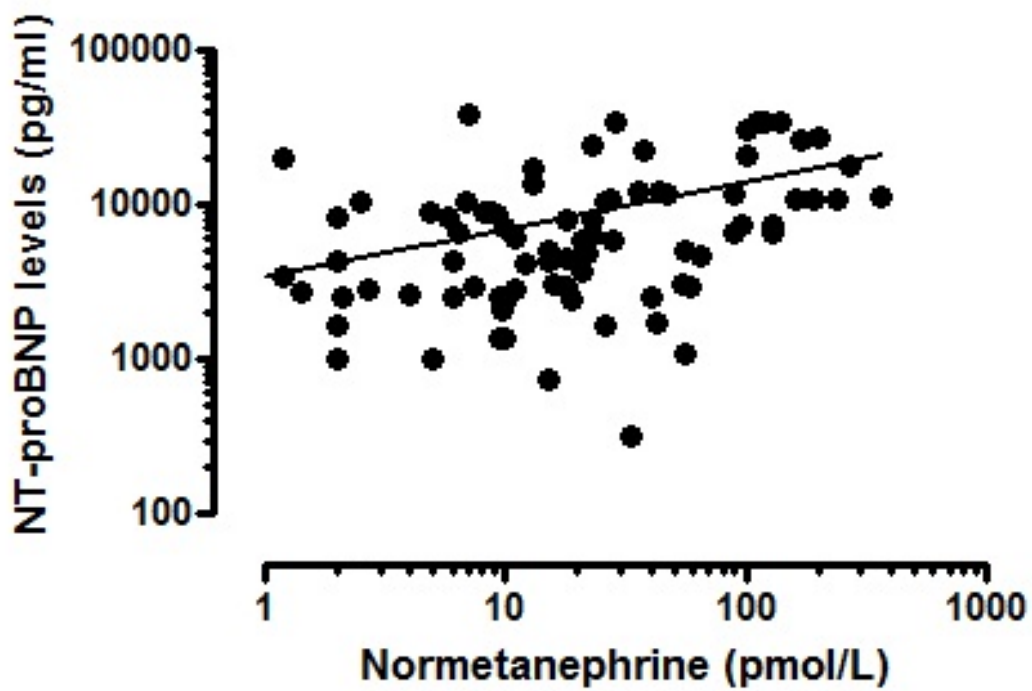
Figure 3.2.3: Correlations between normetanephrine (n = 63) and peak NT-proBNP concentrations (r = 0.56, p < 0.0001)
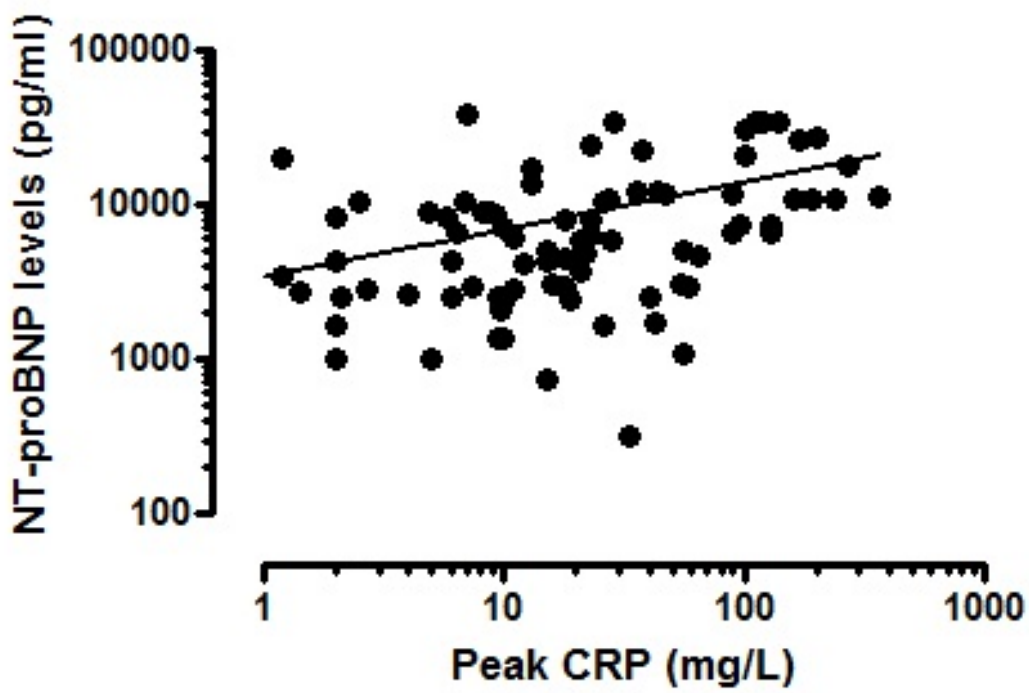
Figure 3.2.4: Correlations between hs-CRP levels (n = 84) and peak NT-proBNP concentrations (r = 0.44, p < 0.0001)
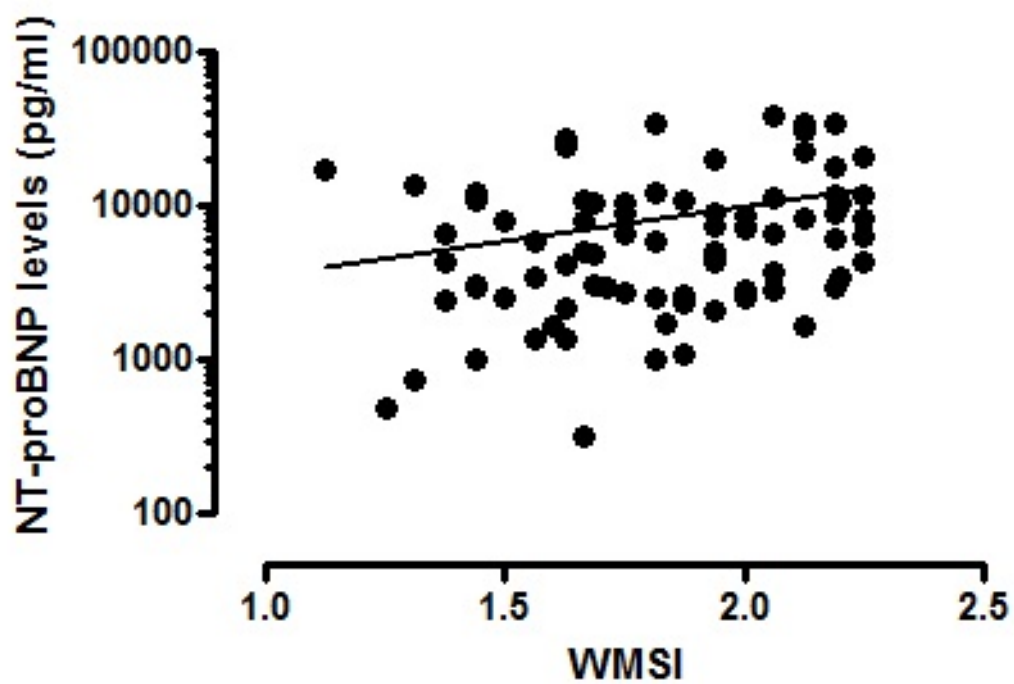
Figure 3.2.5: Correlations between wall motion score index (WMSI) and peak NT-proBNP concentrations (r = 0.3, p = 0.008).
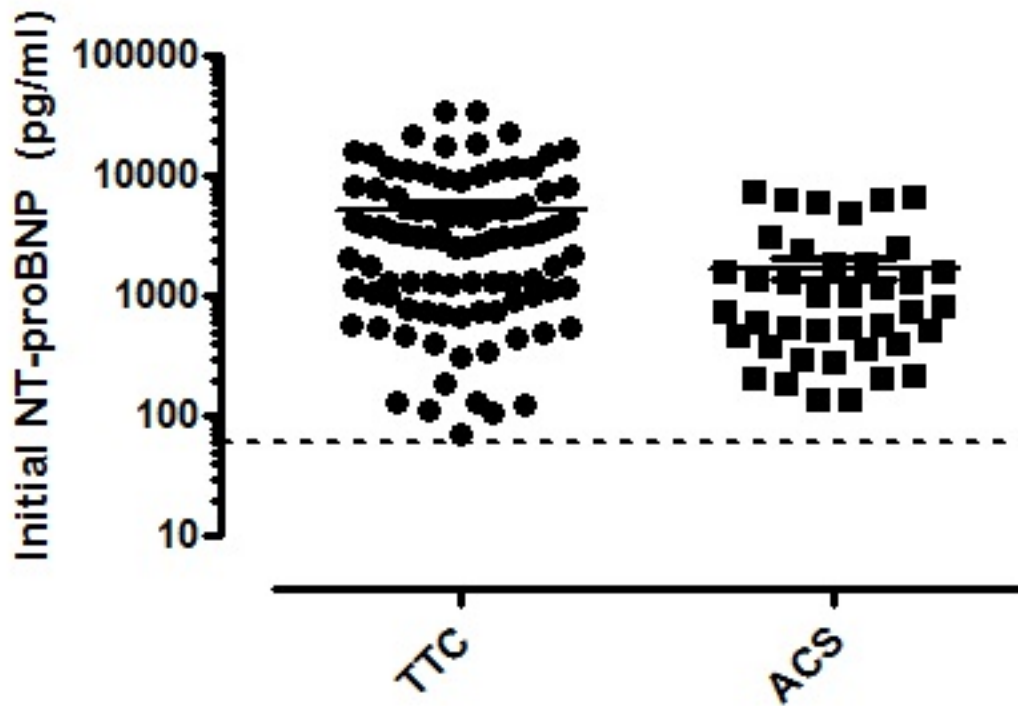
Figure 3.2.6: NT-proBNP concentrations in TTC and ACS patients [median 3000 (interquartile range 930-7085) pg/ml vs. 778 (385-1863) pg/ml, p = 0.0003; Mann-Whitney]
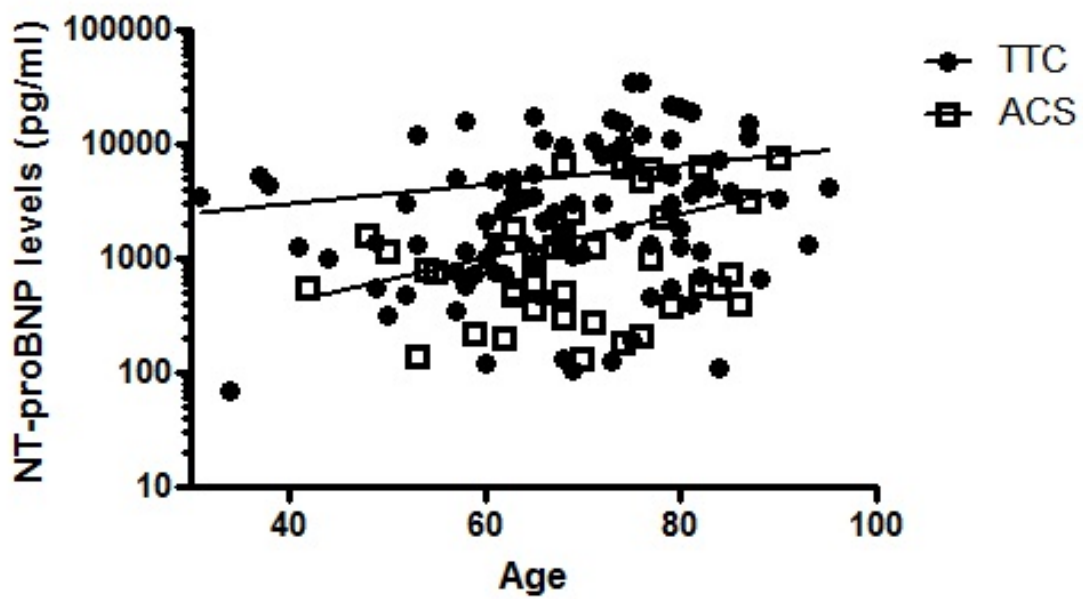
Figure 3.2.7: Impact of age on NT-proBNP concentrations (ANCOVA: F = 5.3, p= 0.02 for TTC versus ACS; F= 7.4, p= 0.01 for impact of increasing age in TTC (closed symbols) and ACS (opened symbols) patients.
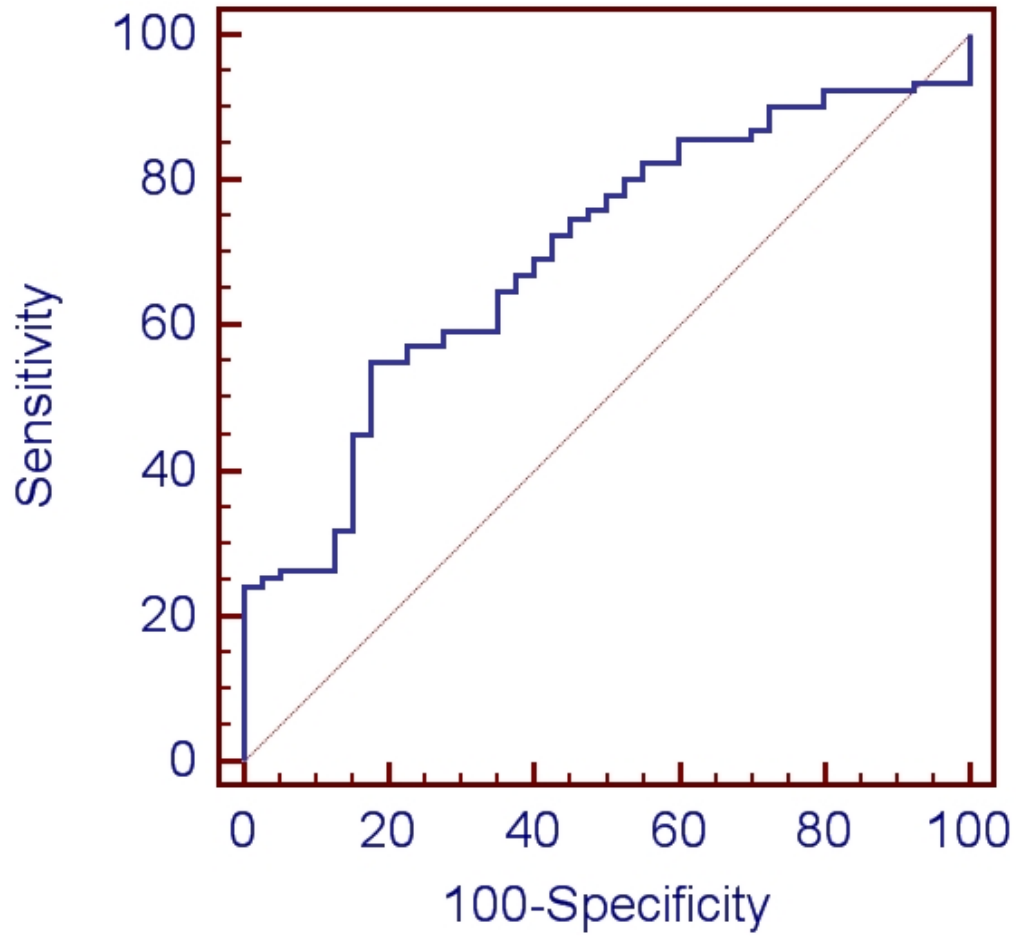
Figure 3.2.8: ROC curve-derived data of NT-proBNP concentrations for differentiation of TTC from ACS
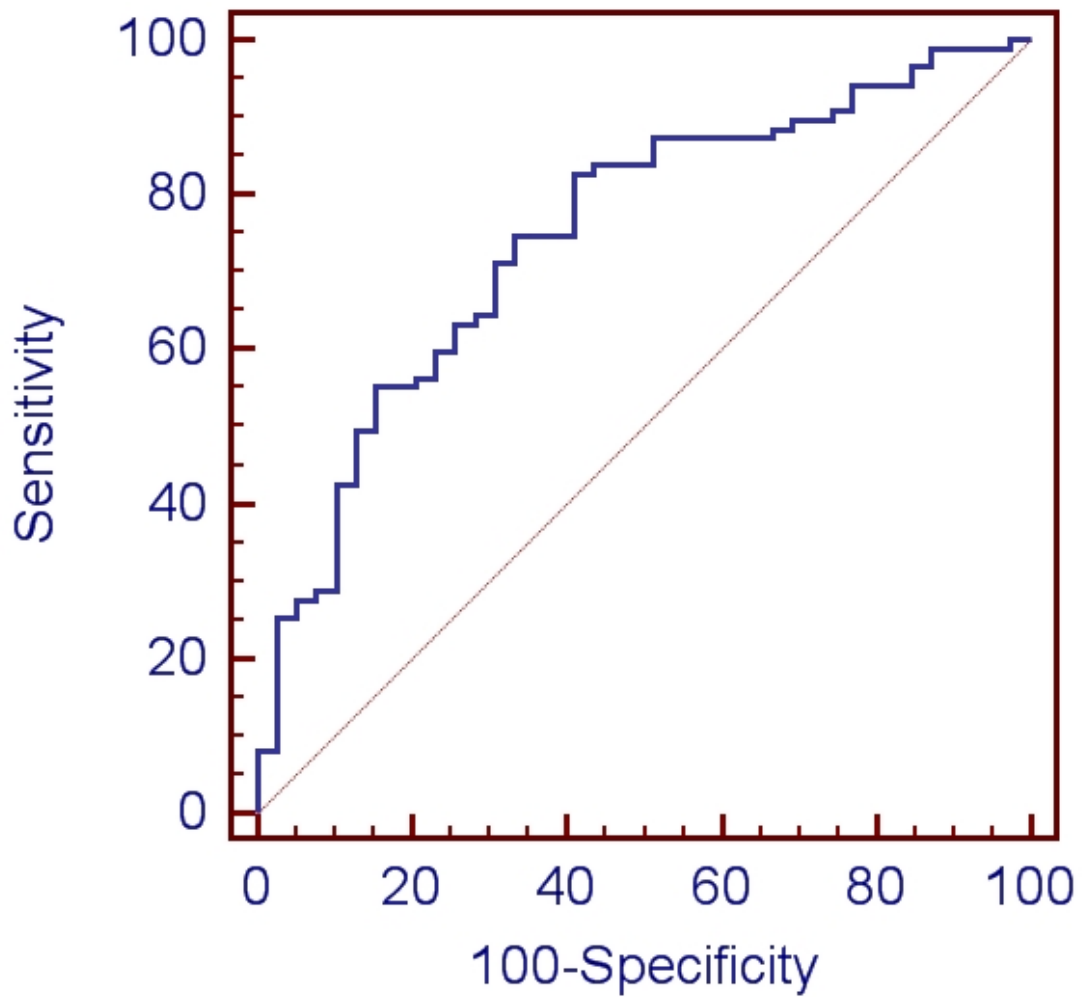
Figure 3.2.9: ROC curve-derived data of the NT-proBNP/CK ratio for differentiation of TTC from ACS
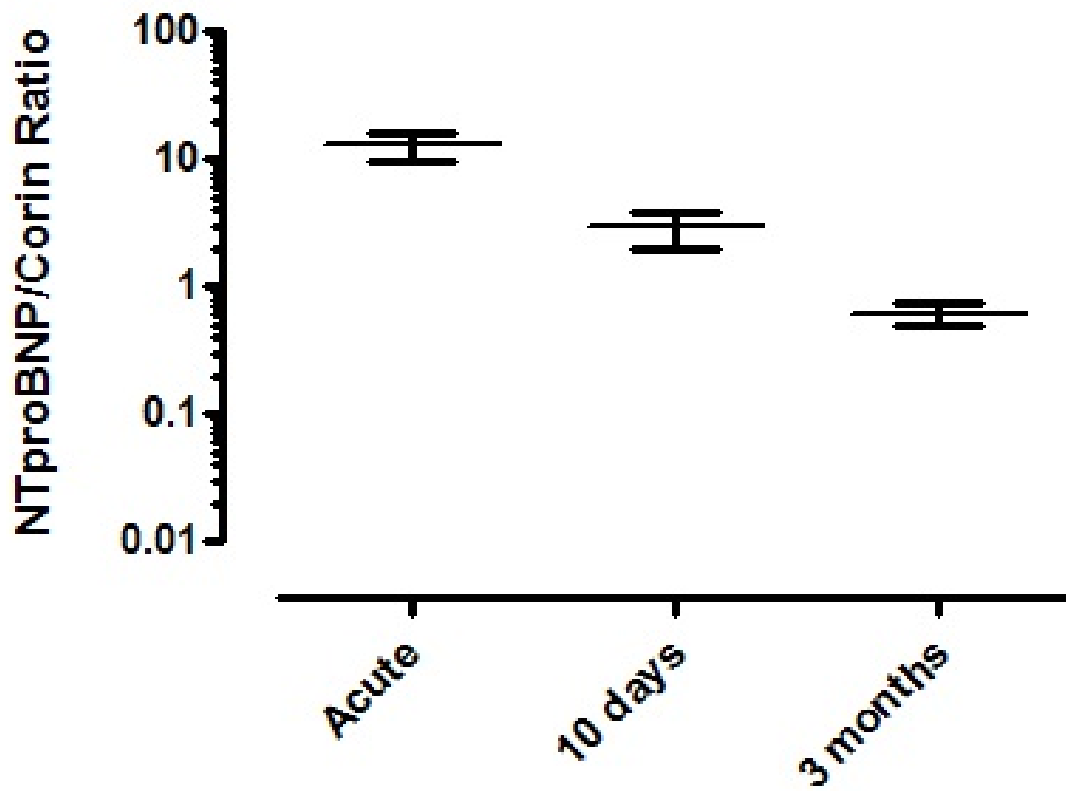
Figure 3.2.10: NT-proBNP/corin ratio in TTC patients at the acute phase, 10 days , and 3 months
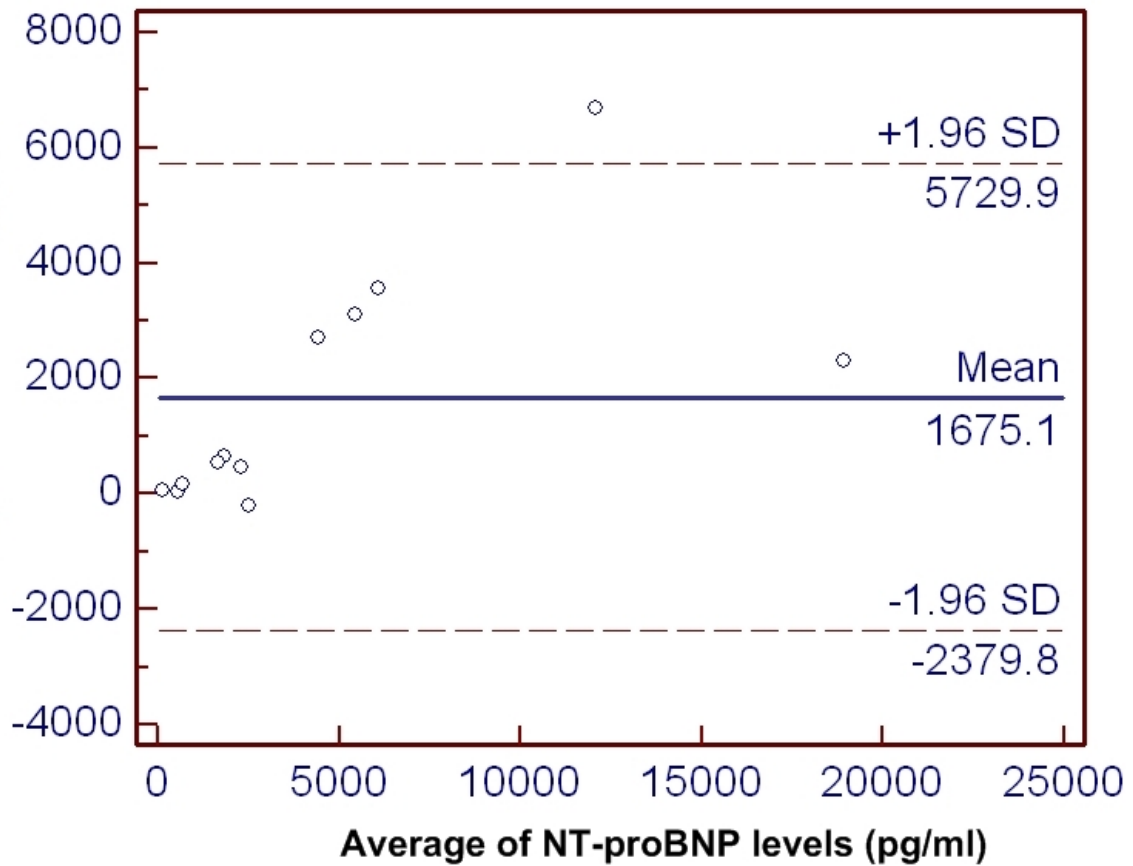
Figure 3.2.11: The differences in NT-proBNP levels measured by COBAS h232 POC device and the Elecsys E 170 assay against their mean using Bland-Altman analysis

3.3   Role of nitric oxide in pathogenesis of TTC

3.3.1   Introduction

It has been widely assumed that hyper-secretion of catecholamines plays a part in the initiation of TTC (Akashi et al. 2003, Wittstein et al. 2005, Yoshioka et al. 2008) but the precise pathological process remains uncertain. Thrombotic events such as LV apical thrombus formation and embolic stroke have been reported in a number of studies. While this might in part result from regional stasis, the pathophysiology of these events is poorly understood. Furthermore, there is little available information concerning the impact of TTC on integrity of cardiovascular homeostatic mechanisms. In theory, pronounced catecholamine stimuli may impair nitric oxide (NO)-based signalling, for example by increasing concentrations of the competitive NOS inhibitor ADMA (Mallamaci et al. 2004) and/or via induction of redox-based dysfunction of the NO-soluble guanylate cyclase "cascade" (Lee et al. 2009).

Therefore, the objectives of the current study were:

1. To determine whether NO generation and/or signalling are impaired in TTC.

2. To evaluate putative relationships between severity of impairment of NO signalling and that of acute episodes in individual patients.

The results of the study reveal evidence that TTC, rather than representing a form of marked impairment of NO signalling, is paradoxically associated with preservation of these process. Furthermore, it appears that this preservation may contribute to rapid recovery of cardiovascular homeostasis after TTC.

3.3.2   Methodology

The study was designed as a comparison between TTC cases and normal aging female controls.

3.3.2.1   Patient population

a. TTC group   The current study was performed in a subgroup of 56 TTC patients, who had been recruited since March 2009.

b. Control group   A control group of 110 consecutive females were randomly selected from a population-based study (Grant et al. 2006). Subjects with current or previous symptomatic myocardial ischaemia were excluded.

3.3.2.2   Parameters of NO signalling

Assessment of integrity of the NO/soluble guanylate cyclise (sGC) system was undertaken in 3 ways:-

- (a) Platelet NO signalling was evaluated utilising anti-aggregatory effects of the NO donor sodium nitroprusside (SNP) (Chirkov et al. 2001).

- (b) Plasma concentrations of ADMA were determined as a biochemical index of potential endothelial dysfunction (Boger 2003).

- (c) Circulating endothelial progenitor cell (EPC) counts were determined via flow cytometry: EPC counts are substantially, although not entirely, NO-modulated (Fleissner & Thum 2011).

Venous blood specimens were collected into corresponding tubes for measurements of inhibition of platelet aggregation by SNP, ADMA levels, and EPC counts. In the TTC patients, these evaluations occurred at the time of diagnosis, 10 days, and 3 months thereafter.

For platelet aggregation studies, blood samples were drawn by venesection from an antecubital vein and 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 utilised in order to minimise deterioration of platelet function during experiments. Aggregation in whole blood was examined utilising a dual-channel impedance aggregometer (Model 560, Chrono-Log, Havertown, PA, USA) (Chirkov

et al. 1999). Tests were performed at 37°C and stirring speed of 900 rpm. Samples of blood were diluted twofold with normal saline (final volume 1 ml) and warmed for 5 min at 37°C. Aggregation was induced with ADP (final concentrations of 2.5mol/L) and monitored continuously for 7 min, and responses were recorded for electrical impedance, in Ohms. In order to determine platelet responsiveness to NO, inhibition of aggregation by the NO donor sodium nitroprusside (SNP) was utilized (Anderson et al. 2004, Chirkov et al. 1999). A final concentration of 10mol/L of SNP was added to samples 1 minute before ADP. Duration of the incubation was estimated as the optimal in preliminary experiments. In control tests, physiological saline was added in appropriate volumes. Inhibition of aggregation was evaluated as a percentage of maximal aggregation in the presence and absence of SNP.

Plasma concentrations of ADMA were determined using high-performance liquid chromatography (HPLC) using the derivatisation reagent AccQ-Fluor after solid phase extraction (Heresztyn et al. 2004). Blood was collected into heparinised tubes, centrifuged at 2°C and plasma was stored at −80°C until assay.

For determination of EPC counts, blood samples were collected in tubes containing potassium ethylene diamine tetra acetic acid (EDTA). Flow cytometric analysis (FAC-Scan, Becton Dickinson) of mononuclear cells positive for both cell surface antigens, CD34 fluorescein isothiocyanate and CD133 phycoerythrin (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany) was assessed (Rajendran et al. 2009). Corresponding negative controls with IgG2a and IgG1 were obtained.

3.3.2.3   Relationships between NO signalling and severity of TTC

a. Biochemical correlates   Plasma concentrations of normetanephrine, NT-proBNP, hs-CRP, CK, troponin T were measured as previously described.

b. Physiological correlates   Wall motion score index and cardiac T2-weighted signal intensity were performed as previously described.

3.3.2.4 Statistical methods

a. Power calculation The mean value of platelet responsiveness to NO in post-menopausal women is approximately 21 ± 23 (SD). In order to detect 30% difference in NO response between TTC and controls, > 39 subjects per group serve 90% power at $p = 0.05$.

b. Data analysis In order to test the hypothesis that the severity of TTC attacks and/or the speed of recovery from TTC are correlated with markers of NO signaling, both SNP responses and ADMA concentrations were correlated with (1) markers of TTC severity: wall motion score index, T2-SI, NT-proBNP release, normetanephrine concentrations, peak CRP and troponin T levels (2) markers of recovery at 3 months: plasma NT-proBNP concentrations via linear regression or Spearmans correlation as appropriate.

Determinants of SNP responses and ADMA concentrations in TTC and control populations were compared via backwards stepwise multiple linear regression. Comparison between TTC and control data were corrected for multiple comparisons utilizing Bonferroni and Kruskal-Wallis tests as appropriate. Post-hoc comparisons based categorically on SNP responses and ADMA concentrations were partitioned relative to median/mean values. A value of $p < 0.05$ was considered significant.

3.3.3 Study results

3.3.3.1 Patients characteristics

Clinical characteristics of the study population (both TTC and normal groups) are described in the Table 3.3.3. The study was performed in fifty-six TTC patients (54 females, 2 males) and 110 consecutive female controls. While there was no difference in Framingham 10 year coronary artery disease risk score between the 2 groups, hypertension and diabetes occurred more frequently in TTC than control subjects.

Clinical Characteristics	TTC (n = 56)	Controls (n = 110)	P value
Mean Age	67 ± 14	61 ± 8	0.001
BMI (kg/m)	27 ± 5	27 ± 7	0.29
Previous/current smoking	22%	22%	0.55
Systemic hypertension	56%	36%	0.03
Diabetes mellitus	24%	8%	0.007
Hyperlipidemia	48%	53%	0.35
Framingham 10 year CAD risk score	5.4 (3.8-7.9)	5.9 (3.8-8.9)	0.65
hs-CRP (mg/dL)	16(6.8 38)	1.7 (0.94 3.8)	< 0.0001

Table 3.3.3: TTC patient characteristics compared to controls

3.3.3.2 NO signalling in TTC

a. Comparisons with controls and changes post TTC

(i) SNP responses   Figure 3.3.12 summarises SNP responses at admission, 10 days and 3 months compared with control values. Acute NO effect on platelet aggregation was increased in TTC relative to controls and this difference persisted at 3 months [median 43% (interquartile range: 11 57), 38% (17-55), and 30% (12-64) for TTC vs. 13% (4-34) for controls, p = 0.0001].

(ii) Plasma ADMA   Plasma ADMA levels were lower at the acute phase, 10 days and after 3 months post TTC compared to controls (mean 0.54 0.08 (SD) vs. 0.58 0.1mol/L, p = 0.03) (Figure 3.3.13). Although ADMA concentrations increased significantly with patient age, TTC was associated with incremental elevations at any particular age.

(iii) EPC counts   EPC counts did not differ significantly between TTC and control subjects, nor fluctuate significantly between admission, 10 day, and 3 months in TTC
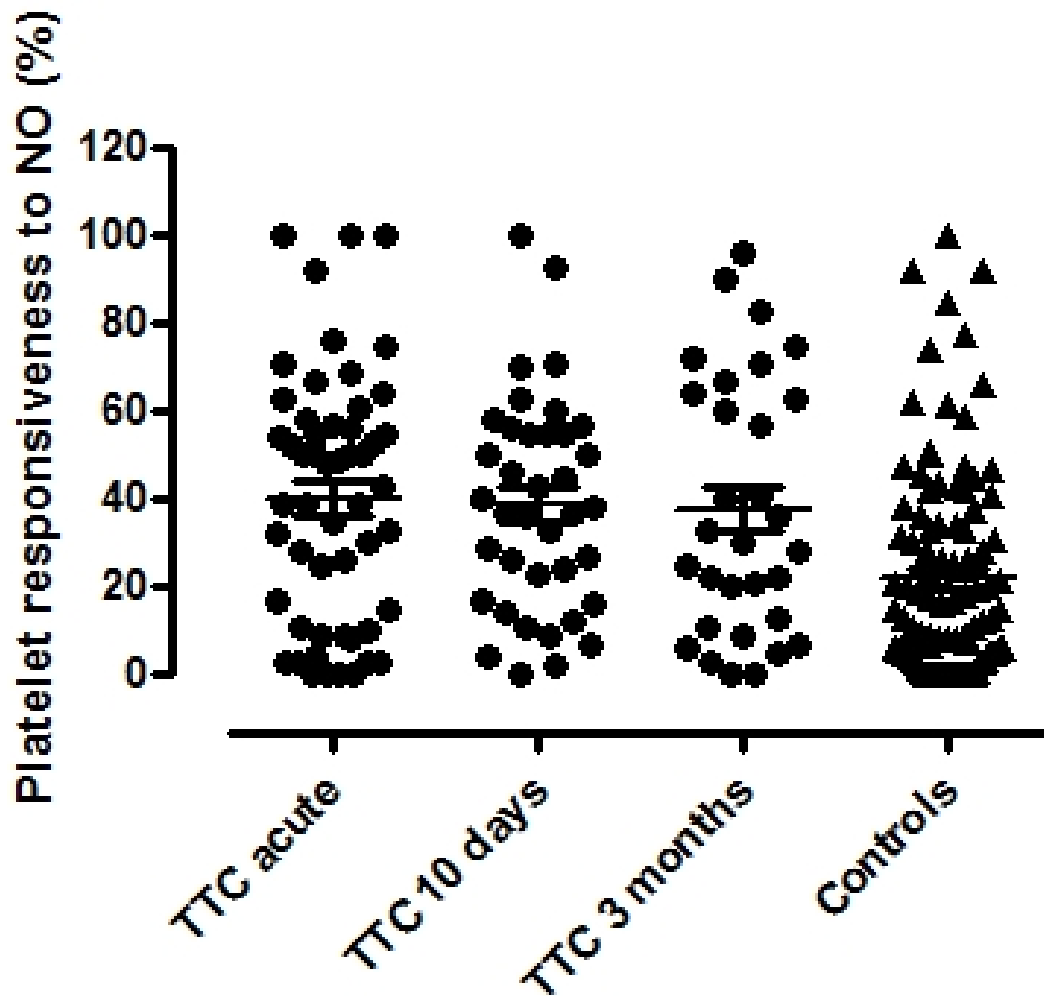
Figure 3.3.12: Plasma concentrations of platelet responsiveness to SNP at the acute phase, 10 days and 3 months in TTC patients, compared to controls

patients (Figure 3.3.14).

b. Correlations with acute markers of severity of TTC There were direct correlations between platelet responsiveness to SNP at the acute phase of TTC and peak normetanephrine (Figure 3.3.15) and peak NT-proBNP (Figure 3.3.16) (r = 0.31, p = 0.03; r = 0.31, p = 0.02, respectively).
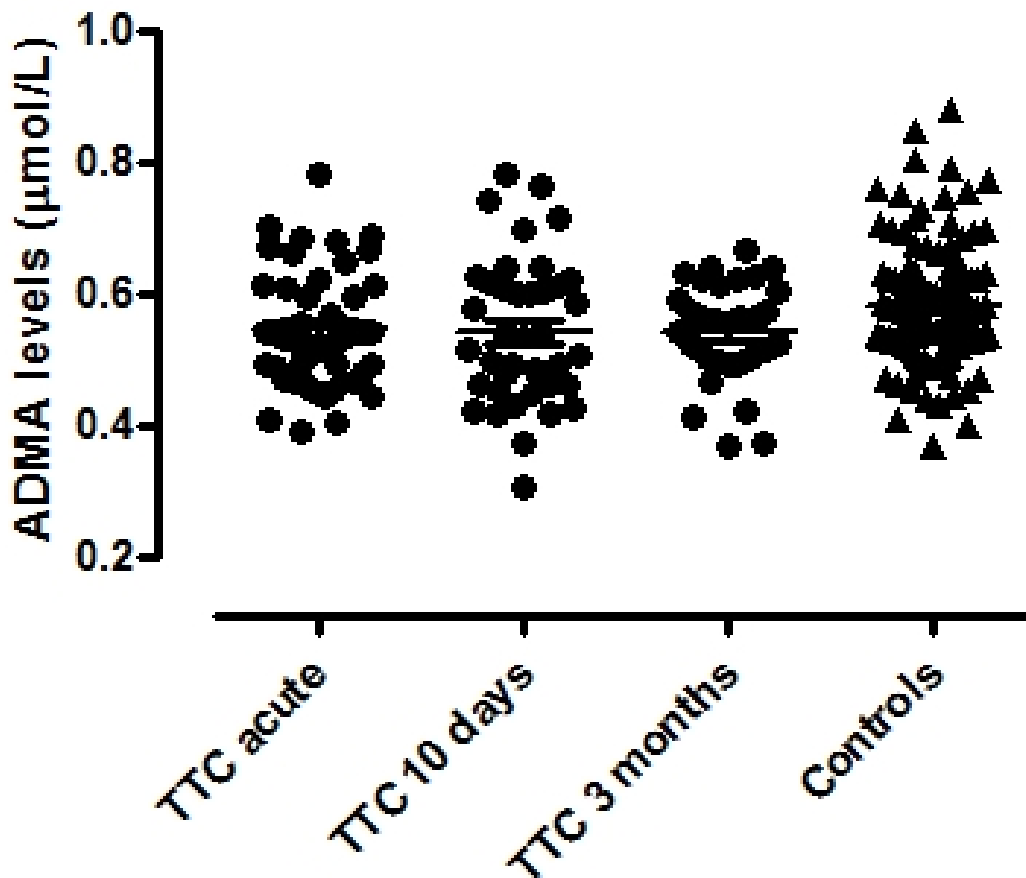
Figure 3.3.13: Plasma concentrations of ADMA at the acute phase, 10 days and 3 months in TTC patients, compared to controls

c. Correlations with extent of recovery at 3 months   At 3 months, ADMA levels were directly correlated with simultaneous NT-pro BNP concentrations ($r = 0.5$, $p = 0.003$; Figure 3.3.17).

At no stage was there any significant correlation between either SNP responses or ADMA concentrations and either: (1) wall motion score index on echocardiography or
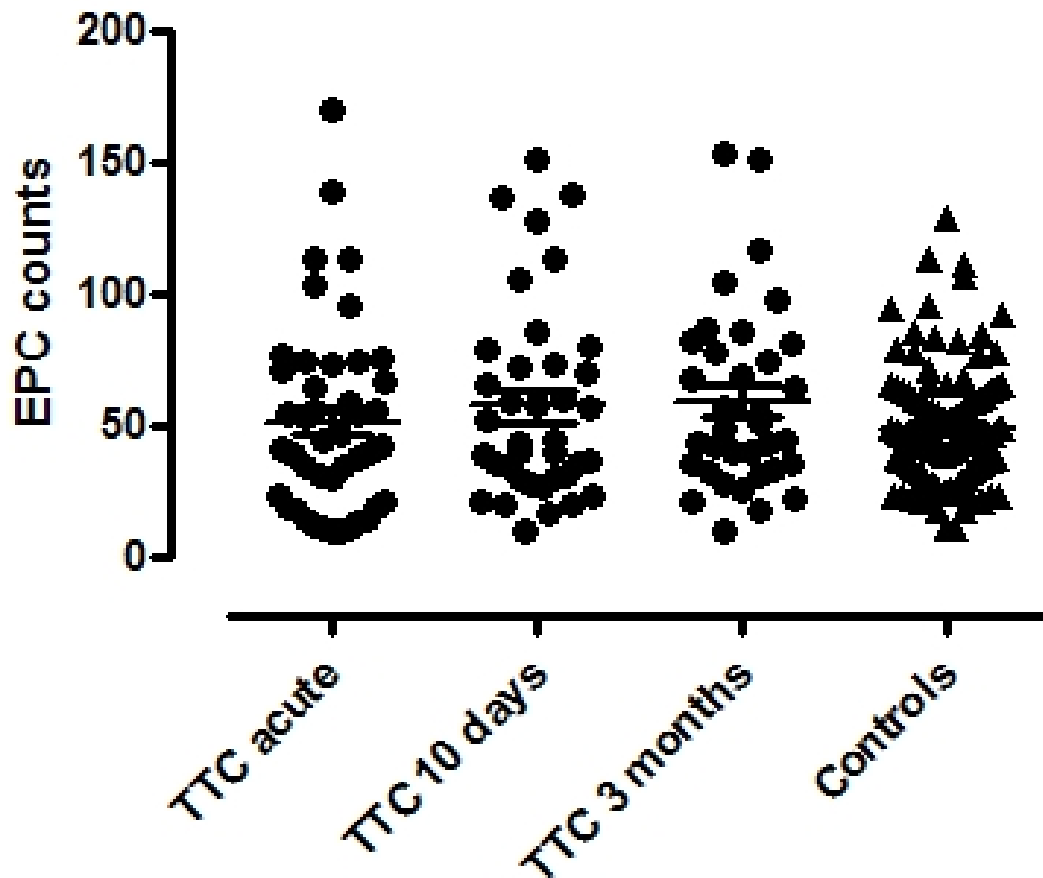
Figure 3.3.14: EPC counts at the acute phase, 10 days and 3 months in TTC patients, compared to controls

(2) extent of oedema via CMR T2 signal intensity.

3.3.4 Discussion

This controlled study establishes that TTC is not associated with the expected impairment in NO signalling, but rather with a "paradoxical" finding: increased platelet response to SNP relative to population norms, together with significantly lowered ADMA levels. Furthermore, circulating EPC counts, which partially reflect NO stimulation (Fleissner & Thum 2011), showed no evidence of reduction in comparison to norms. The observed changes were not restricted to the acute phase of TTC, but persisted for
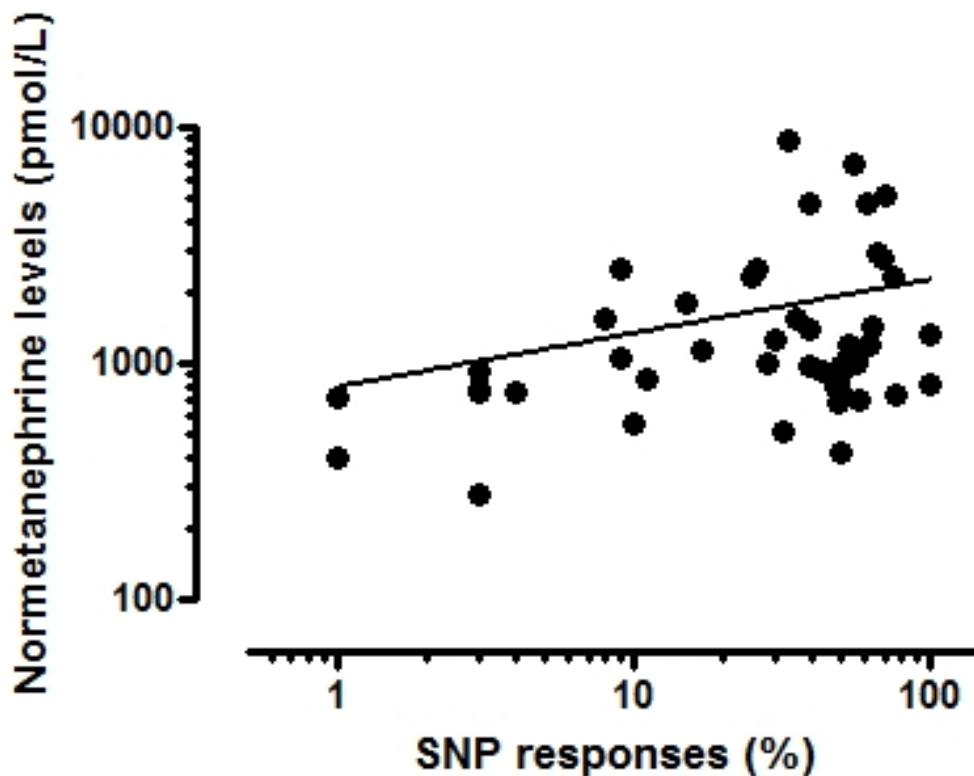
Figure 3.3.15: Correlation between platelet responsiveness to SNP at the acute phase of TTC and peak normetanephrine concentrations

at least 3 months thereafter.

A secondary conclusion was that the extent of SNP response was directly correlated to some, but not all, markers of severity of individual TTC attacks: - while there was no significant correlation with extent of wall motion impairment or oedema, there were significant correlations with normetanephrine and NT-proBNP concentrations. As regards ADMA concentrations, these were directly and strongly correlated with levels of NT-proBNP at 3 months (Figure 3.3.17). Thus, intact NO signalling tended to be associated with more severe episodes of TTC, while ADMA, a marker of impaired NO generation, was correlated with biochemical evidence of impaired recovery. It is therefore appropriate to consider the possible mechanisms and implications of the surprising findings.

In retrospect, there have never been data to suggest that TTC occurs particularly
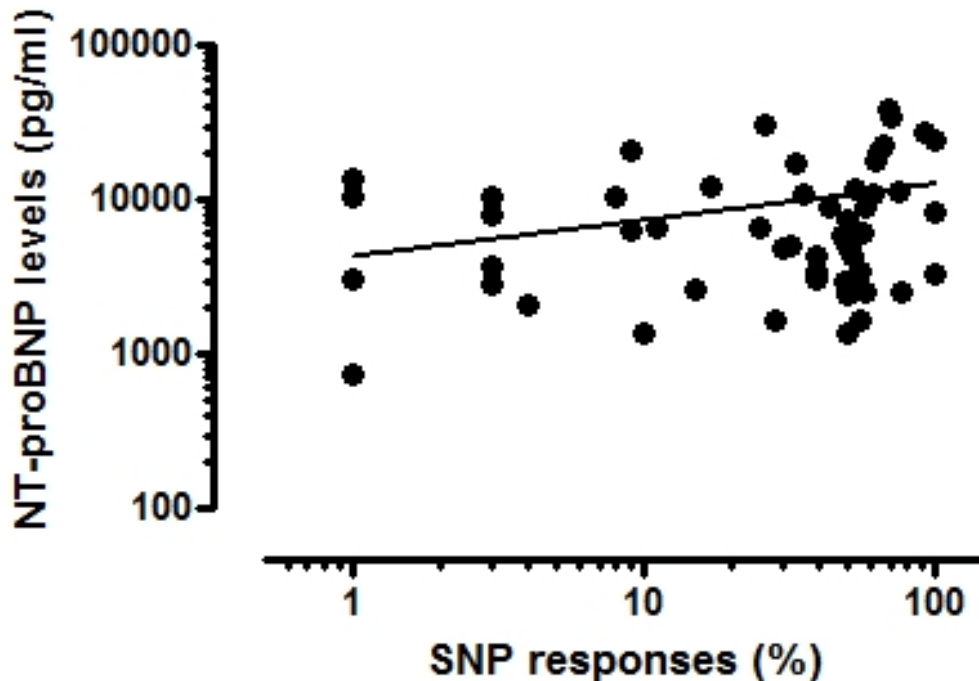
Figure 3.3.16: Correlations between platelet responsiveness to SNP at the acute phase of TTC and peak NT-proBNP concentrations

in individuals with endothelial dysfunction or other disorders of NO signalling. The tendency to diagnose TTC primarily in patients with normal epicardial coronary arteries (Prasad et al. 2008), might have introduced some selection bias in this regard. In the current study, Framingham risk scores were similar for TTC and control populations.

In theory, exposure to high concentrations of catecholamines might have increased NOS activation in platelets (Anfossi et al. 2002), but this would not have increased response to SNP. Furthermore, catecholamines may increase generation of reactive oxygen species, thus "scavenging" NO and/or inactivating sGC, the main biochemical components underlying impaired response to NO (Chirkov & Horowitz 2007). Similarly, catecholamines may increase ADMA concentrations (Mallamaci et al. 2004). The direct correlation between normetanephrine concentrations and SNP response is therefore surprising.

However, we have previously provided evidence that both extent of NT-proBNP
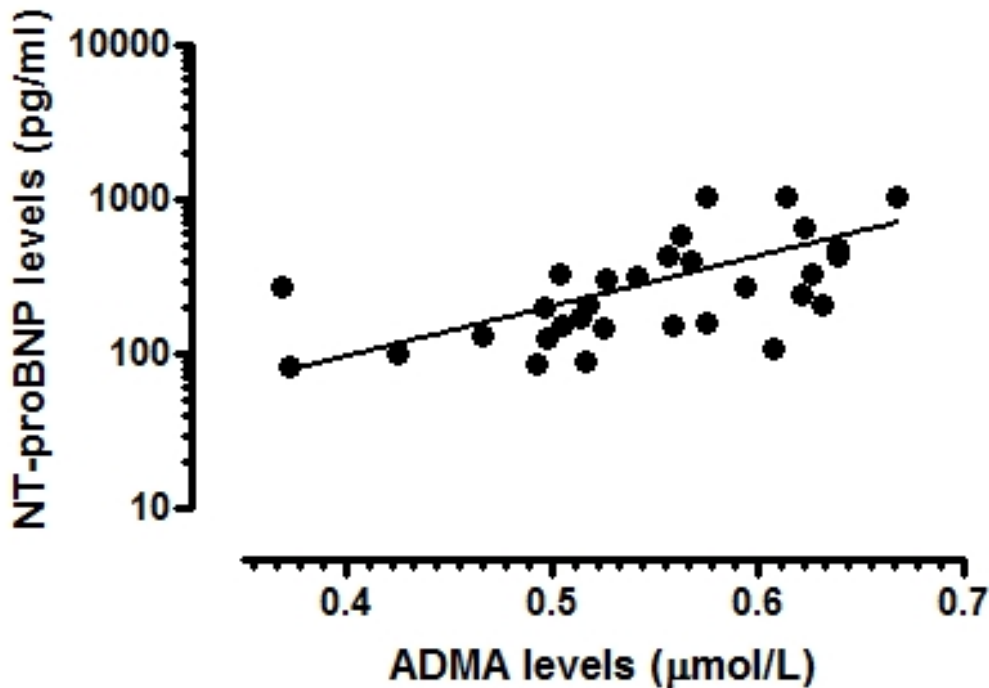
Figure 3.3.17: Correlation between ADMA and NT-proBNP levels at 3 months

elevation and of increase in normetanephrine concentrations are markers of severity of TTC attacks (Nguyen et al. 2011). We have postulated that NT-proBNP release may be predominantly inflammatory in origin. Furthermore, elevation of catecholamine levels after onset of TTC may represent both cause and consequence, in uncertain proportions. Considering the 3 month data, the main conclusion to be drawn is that patients with TTC have paradoxically preserved NO signalling, which may well have antedated as well as extended beyond the acute illness. Whether this supra-normal NOS signalling in some way predisposes towards TTC is an issue raised by the current data.

As regards the correlation between 3-month ADMA concentrations and incomplete recovery from TTC, this is partially consistent with existing data. Nef et al demonstrated, via myocardial biopsy in TTC, that there is early activation of RISK pathway (Nef et al. 2009), which limits extent of cellular necrosis under oxidant stress. Nitric oxide is a major physiological activator of this pathway (Penna et al. 2011), and integrity of NOS would therefore tend to limit long-term impairment post TTC.

3.3.5 Study limitations

The major limitations of this study are that the observed aberrations were diametrically opposite to those hypothesised and that the pathogenic significance of the current findings is uncertain (and cannot easily be ascertained in humans). However, the differences observed between TTC patients and controls, especially as regards SNP responses, are both large and highly statistically significant; this difference was present despite the fact that the TTC patients were somewhat older than the controls, and a higher proportion of TTC patients had diabetes and/or hypertension (Table 3.3.3), all conditions associated with elevation of ADMA concentrations and impairment of NO signalling (Maas et al. 2009, Perticone et al. 2005, Pitocco et al. 2010).

One possible consequence of increased NO signalling in TTC is the precipitation of early hypotension, a common finding in TTC irrespective of the presence or absence of pulmonary congestion (Chong et al. 2011). While this may be primarily due to BNP release, NO may also contribute.

3.3.6 Conclusions

In summary, NO signalling is paradoxically increased in TTC both acutely and for at least 3 months thereafter, and is correlated with some markers of TTC severity. The possible role of NO as a modulator of the pathogenesis of TTC is worthy of further exploration.

Chapter 4

Conclusions and future considerations

Excessive release of catecholamines exerts a number of deleterious effects on the heart both chronically and acutely. It has been suggested that catecholamines induce redox stress via release of oxygen-derived free radicals and promote inflammatory processes, leading to myocyte toxicity, apoptosis, and fibrosis. These effects not only worsen the function of the heart but also induce arrhythmias.

The objectives of the current studies were to address a numbers of issues regarding catecholamine-related pathophysiology in two forms of cardiac muscle dysfunction: chronic heart failure, a state of prolonged exposure of a failing heart to high concentrations of catecholamines, and Tako-tsubo cardiomyopathy, a form of "stress-induced" cardiomyopathy.

The results of the current study raise a number of important issues.

(1) In the case of chronic heart failure, the main aim of this study was to examine prognostic/functional implications of regional denervation in CHF. The current study demonstrated that cardiac denervation detected by ^{123}I-MIBG imaging does not predict changes in LVEF and does not closely predict AICD discharge. Thus, the utility of ^{123}I-MIBG scanning as a means of prognostication and therapeutic decision-making in patients with CHF is questionable.

Chronic exposure to elevated plasma catecholamines desensitises and down-regulates

myocardial α and β-adrenoceptors, leading to decreased contractile performance and aggressive remodelling of the left ventricle and stimulation of myocardial fibrosis. Furthermore, electrical remodelling of the LV myocardium also occurs, leading to development of micro-reentry circuits, which may trigger VT or VF (Fernandes et al. 2007). As a consequence, progression of CHF is accelerated, worsening cardiac dysfunction and increasing risk of sudden cardiac death. The current principal medical management of CHF includes pharmaceutical intervention and potential implantation of devices such as AICD and CRT. Indications for AICD insertion are currently based on patients' symptoms (NYHA class II or III) and a LVEF $< 35\%$. However, due to the subjective nature of symptomatic indication and low sensitivity and specificity of LVEF measurement, only 23% of patients in the MADIT II study had an appropriate AICD discharge on follow-up (Moss et al. 2002). A number of investigations have been suggested that ^{123}I-MIBG imaging anomalies may be a potential indicator of AICD discharge (Boogers et al. 2010, Jacobson et al. 2010) . However, the use of delayed ^{123}I-MIBG HMR alone may not sufficient to predict AICD discharge. Previous studies have suggested a combination of tests, including BNP concentrations, LVEF, and ^{123}I-MIBG imaging as a means of stratification tool in patients with CHF. Moreover, development of a computerised quantitative tool for SPECT ^{123}I-MIBG imaging may be useful in order to overcome the problems in visual SPECT assessment. In the current investigation, it was found that delayed ^{123}I-MIBG data were poorly predictive of changes in LV function or of AICD discharge. Furthermore, there was no suggestion of progression of cardiac denervation over the follow-up period. These findings should be placed in the context of recent development in therapy of CHF, namely the use of "triple" pharmacotherapy (ACE inhibitor, β-adrenoceptor antagonist, and aldosterone antagonist) in the majority of patients and the more widespread use of CRT. While there was no obvious interaction between CRT interaction and the findings of the current study, it is entirely theoretically possible that extensive inhibition of reactive oxygen species generation in response to catecholamines, for example by the components of "triple" pharmacotherapy, might protect against ongoing LV dysfunction, fibrosis and arrhythmogenesis. This is a hypothesis essentially of the "post-MADIT era" which should now be tested, both in animal models and in

humans.

(2) In the case of TTC, the experiments with BNP and its precursor NT-proBNP revealed that TTC is associated with marked and prolonged elevation of plasma concentrations of NT-proBNP and BNP, even in the absence of pulmonary congestion. Furthermore, we present evidence that the extent of NT-proBNP elevation reflects the magnitude of a putative catecholamine stimulus, and correlates directly with the initial degree of left ventricular wall motion anomaly. Finally, the extent of elevation of NT-proBNP levels may assist in the pre-catheterisation differentiation of TTC from other acute coronary syndromes in ageing females.

The current diagnosis of TTC depends heavily on the demonstration of periapical hypokinesis/akinesis and the differentiation of TTC from ST-elevation myocardial infarction, via urgent/emergent coronary angiography. However, cardiac catheterisation may be problematic in the fragile elderly, who usually present with other co-morbidities. Furthermore, this aging population is likely to have some incidental coronary artery disease. In order to avoid unnecessarily urgent cardiac catheterisation and more importantly thrombolysis, which may be required in acute MI patients, a non-invasive diagnostic methodology to distinguish TTC from AMI in patients with chest pain or acute dyspnoea is obviously needed. It is reported that routine echocardiography at presentation would potentially improve the diagnostic accuracy. However, this is impractical in clinical settings. The use of BNP/NT-proBNP testing in isolation may not helpful because peak concentrations of NT-proBNP were attained on day 2. Nevertheless, a combination of patient's history of severe acute stress events, ECG appearances and NT-proBNP elevation may provide the basis for improved early diagnosis of TTC. A further prospective study is required to validate the use of this algorithm in differentiating TTC from AMI patients.

Moreover, the findings that extensive and persistent elevation of BNP and NT-proBNP concentrations in TTC and the extent of BNP/NT-proBNP correlates with severity of TTC, may set a light in understanding its pathophysiology. A number of current investigations from both biopsy studies and CMR have been suggested that TTC is associated with intramyocardial inflammation activation. Both activation of inflam-

matory processes and extensive release of catecholamines could precipitate formation of BNP. More interestingly, a recent study (Chan et al. 2012) has documented that BNP in turn induces release of catecholamines from the heart and from synaptic endings, leading to activation of protein kinase A and increase in cAMP which is also relevant to the second findings of the studies. Therefore, BNP/NT-proBNP release in TTC might be a "generator" of catecholamines, and the basis for a potential vicious cycle of oxidative stress, rather than a general marker of release of "cardioprotective" autacoids. The findings that hypotension at presentation was commonly observed in TTC patients, together with the evidence that BNP/NT-proBNP levels rapidly increased within 24 hours of admission, suggests an acute release predominantly of the active forms of BNP, rather than the less active BNP 4-32 commonly associated with other forms of CHF. This hypothesis is partially supported by our findings that the plasma concentrations of corin, an enzyme which was suggested to be responsible for cleavage of BNP 4-32 (Semenov et al. 2010), did not increase in the acute phase of TTC. However, this hypothesis needs to be confirmed.

The study with NO signalling in TTC reveals that patients with TTC exhibit "paradoxically preserved" NO signalling at the platelet level, with markers of increased NO effect in other tissues, compared to age and gender norms. Furthermore, some markers of TTC were directly correlated with extent of residual NO signalling. Integrity of NO generation/effect in post menopausal females might predispose these individuals to TTC. It also might facilitate the adverse effects of extensive release of catecholamines. Indeed, catecholamine-induced release of superoxide within the myocardium and endothelium, and the formation of peroxynitrite by combination of nitric oxide with superoxide might play a critical role in cardiac injury in TTC. On the other hand, extensive and prolonged elevation of BNP/NT-proBNP concentrations might in turn induce more catecholamine release (Chan et al. 2012). The other possibility is that extensive catecholamine release might result in β3-adrenoceptor stimulation (Varghese et al. 2000, Kohout et al. 2001), leading to a negative inotropic effect on the heart primary due to coupling with NOS.

The studies in this thesis therefore modulate the classical view of catecholamines effects on the heart in line with the progression of the evidence base for the pharma-

cotherapy of CHF. Pronounced, prolonged catecholamine hypersecretion has multiple adverse effects, but there are potentially modulated by other non-catecholamine factors, such as a previously unsuspected "reverberation" with generally protective autacoids such as BNP and NO. The inference that intensive pharmacotherapy may interrupt this vicious cycle in CHF offers analogous hope for the treatment of TTC.

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