INTERACTIONS BETWEEN CARDIAC RESYNCHRONISATION THERAPY AND AMELIORATION OF PERIPHERAL VASCULAR DYSFUNCTION: IMPACT UPON OUTCOMES.

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Dedication

This thesis is dedicated to my beautiful and loving wife, Ngozi and to my sweet and phenomenal children: Chidiuso, Chukwudiebube Jnr, Chidiomimi, Chimsinaodigomma and Kaosidichukwunobi.

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ABSTRACT

Cardiac resynchronisation therapy (CRT) has been well established as a treatment option for patients with chronic systolic heart failure (CHF) for over 20 years. While it is clear that benefit depends substantially on the presence of disordered intra-ventricular conduction, leading to potential for 'resynchronisation', it remains unclear precisely how this process induces symptomatic improvement, improved haemodynamic status and reduced mortality rates.

The primary objective of this thesis was to evaluate, pathophysiologically, the factors that could impact outcomes in CRT; specifically, to see, if CRT achieves its beneficial effects via a combination of improvement in cardiac contractility as well as improvement in peripheral vascular endothelial function. The secondary objectives of the thesis were to evaluate whether the extent of improvement in functional status is associated with improvement in parameters of dyssynchrony, and whether change in electrical remodeling is related to change in dyssynchrony and which is achieved through change in neurohumoral activation and change in redox stress.

We evaluated 33 consecutive patients who were scheduled for routine insertion of CRT, according to current guidelines and irrespective of ischemic or non-ischemic basis of heart failure.

Peripheral vascular function was assessed with radial artery applanation tonometry that evaluated changes in augmentation indices to sublingual nitroglycerin and inhaled salbutamol for endothelium-independent and endothelium-dependent NO effects respectively. Inhibition of ADP- induced platelet aggregation was assessed, as well as

changes in plasma concentrations of asymmetric dimethyl arginine (ADMA), symmetric dimethyl arginine (SDMA), and matrix metalloproteinases -2 and-9, NT-proBNP, and catecholamine metabolites. Endothelial shedding of glycocalyx layer was assessed by measurement of plasma levels of syndecan-1. Platelet content of thioredoxin interacting protein (TXNIP) was used to assess the potential for changes in redox stress. Standard echocardiographic measurements were used to evaluate changes in cardiac functions while cardiopulmonary exercise testing and 6-minute walk distance were utilised to assess functional changes. Electrical changes were measured with changes in intrinsic QRS duration, inter-ventricular conduction times using intra-cardiac electrocardiograms, and right ventricular effective refractory periods. All of these parameters were measured at baseline and at 6 months post CRT insertion.

Our results showed that at baseline, there was an inverse relationship between vascular NOS function, (as assessed by fall in AIx to salbutamol) and intrinsic QRS duration, (r=-0.40, p =0.01). CRT did not result in improvement in peripheral vascular endothelial function in spite of improvement in cardiac contractility, (LVESV: 136.6 [57.5] to 98.9 [52.1] ml, p <0.001) and in most measures of functional status such as New York Heart Association (NYHA) functional class (2.7 [0.8] to 1.9 [0.7], p <0.001); 6MWD (314.5 [112.8 to 357.0 [117.0] meters, p = 0.005); and quality of life score (QOL) (40.7 [25.4] to 22.9 [22.3], p = 0.001). Apart from a significant reduction in plasma concentrations of SDMA (0.83 [0.28] to 0.74 [0.20], p =0.013), which was independent of changes in renal function, there were no other significant changes in other measures of inflammatory activation. CRT also did not affect parameters of glycocalyx shedding or redox stress although it resulted in significant reduction in plasma concentrations of NT-pro BNP (1862 [1091-3185] to 1469 [774-2841] ng/L, p = 0.008) Although there was no change in right ventricular refractoriness, there was significant improvement in inter- ventricular electrical

conduction assessed by onset left ventricular pacing to the onset of right ventricular intra-cardiac electrocardiogram, (LVp-RVegm): (117 [44.5] to 97.0 [45.0] ms, p =0.019.

In summary, the studies in this thesis showed that CRT exerted its salutary effects independent of changes in peripheral vascular endothelial function and without significant changes in most parameters of inflammatory activation apart from reduction in SDMA levels independent of changes in renal function. There was also improvement in measures of inter-ventricular electrical conduction following CRT.

Certification

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

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Chukwudiebube Nnanna Ajaero

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Publications, presentations and awards related to work performed towards this thesis.

Publications.

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3. **C. Ajaero,** S. Chua ,B. Assadi-Khansari 1, J. Horowitz , A. Sverdlov , D. Ngo
Galectin 3 is Markedly Elevated in Severe Heart Failure and Predicts Improvement in LV Volumes Post Cardiac Resynchronisation Therapy. Heart, Lung and Circulation. August 2016,Vol 25, S 110-111 (ABSTRACT)

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LIST OF ABBREVIATIONS

6MWD	6-minute walk distance
ACE	Angiotensin converting enzyme
ADMA	Asymmetric dimethyl arginine
ADP	Adenosine diphosphate
AIx	Augmentation index
ANP	Atrial natriuretic peptide
ARB	Angiotensin receptor blocker
ATP	Adenosine triphosphate
BNP	Brain natriuretic peptide
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosyl monophosphate
CHF	Chronic heart failure
CPET	Cardiopulmonary exercise testing
CRT	Cardiac resynchronisation therapy
ECG	Surface electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rate
EPS	Electrophysiological study
FMD	Flow mediated dilatation
GSHPx	Glutathione peroxidase
GTN	Glyceryl trinitrate
GTP	Guanosine triphosphate
HED	Hydroxy ephedrine
HHF	Hospitalized heart failure

hsCRP	High sensitive C-reactive protein
ICD	Internal cardioverter defibrillator
iQRSD	Intrinsic QRS duration
ISDN	Isosorbide dinitrate
IVMD	Inter-ventricular mechanical delay
LBBB	Left bundle branch block
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVegm	Left ventricular intra-cardiac electrogram
LVESV	Left ventricular end-systolic volume
LVP	Left ventricular pacing
MLHFQ	Minnesota Living with Heart Failure Questionnaire
MMP	Matrix metalloproteinase
MSNA	Muscle sympathetic nerve activity
NADP	Nicotinamide adenine dinucleotidephosphate
NO	Nitric oxide
NOS	Nitric oxide synthase
NT-proBNP	N-terminal pro brain natriuretic peptide
NYHA	New York Heart association
OH-	Hydroxyl
ONOO-	Peroxynitrite
PARP	Poly ADP-ribose polymerase cleavage
PDGF	Platelet derived growth factor
PET	Positron emission tomography
PRMT	Protein arginine methyltransferase

QOL	Quality of life score
RAAS	Renin-Angiotensin-Aldosterone system
RBBB	Right bundle branch block
RER	Respiratory exchange ratio
ROS	Reactive oxygen species
RVegm	Right ventricular intra-cardiac electrogram
RVERP	Right ventricular effective refractory period
SALB	Salbutamol
SDMA	Symmetric dimethyl arginine
SNP	Sodium nitropruside
SOD	Superoxide dismutase
SPWD	Septal-to-posterior wall delay
TSP-1	Thrombospondin 1
TXNIP	Thioredoxin interacting protein
VCO2	Carbon dioxide output
VE	Ventilatory equivalent
VO2max	Peak oxygen consumption
vWF	von Willebrand factor

CHAPTER 1

INTRODUCTION

Systolic Heart failure, the final common pathway of many forms of heart disease, is the syndromic aggregation of signs and symptoms arising from the inability of the heart to maintain adequate cardiac output in the context of normal filling pressures or when it can do so only with elevated filling pressures. Despite ground-breaking advances in the management of heart failure over the last few decades, the health and economic burden of heart failure remain substantial (Krum and Abraham 2009).

1.1 EPIDEMIOLOGY OF HEART FAILURE

Different studies have found different results for both the prevalence and incidence of heart failure. This is in part driven by the different criteria for the diagnosis of heart failure used in those studies, and method of data collection. Both population based studies and hospital/registry data have been used in reporting epidemiology of heart failure.

The earliest and perhaps the most famous population based study is the Framingham Heart Study, which started running in 1948 and has recruited the next two generations of the original study cohort population. It based its diagnosis of heart failure solely on major and minor clinical criteria with major criteria including orthopnoea, paroxysmal nocturnal dyspnoea, raised jugular venous pulsation, presence of S3, cardiomegaly on chest x-ray, and pulmonary oedema. Some of the minor criteria include hepatomegaly, exertional dyspnoea and peripheral oedema (and not attributed to other causes). Definite heart failure was diagnosed in the context of two major criteria or one major plus two minor criteria concurrently. (McKee, Castelli et al. 1971) Apart from the Framingham criteria, other validated criteria commonly used are the Boston criteria (Carlson, Lee et al. 1985) and the European Society of Cardiology criteria. (Remme, Swedberg et al. 2001)

Several other population-based studies have also yielded valuable information in the epidemiology of systolic heart failure. For example, The Hillingdon, West London Study,

(Cowie, Wood et al. 1999) used combinations of clinical parameters, electrocardiography, chest roentgenogram and 2D echocardiogram to make diagnosis of heart failure. In this study, new cases of heart failure were identified from a population of 151,000 utilizing 82 general practitioners and rapid access clinic for new cases of suspected heart failure. The study lasted for 20 months and as such generated data on incidence and aetiology of heart failure.

Another major population-based prospective study was the Rotterdam Study. (Bleumink, Knetsch et al. 2004) In this study, definite heart failure diagnosis required more stringent criteria: the presence of validated clinical signs, objective evidence of heart failure with chest roentgenogram and echocardiogram (M-mode) in accordance with the definition of heart failure by the European Society of Cardiology. (Remme, Swedberg et al. 2001) In addition, diagnosis by a medical specialist was required. 7983 individuals aged 55 years and above were followed up for up to five years

Prior to the Rotterdam study, the Glasgow Study (McDonagh, Morrison et al. 1997) was the first to evaluate the prevalence of heart failure using echocardiogram but by using the biplane left ventricular ejection fraction of 30% or less as definition for heart failure, thus very likely under-estimating the true prevalence.

The Olmsted County Minnesota Study (Redfield, Jacobsen et al. 2003) is a very elegant and perhaps the most comprehensive population-based study of the epidemiology of heart failure. 2042 participants aged 45 years and over were evaluated clinically using the Framingham Heart Failure criteria (McKee, Castelli et al. 1971) together with 2 dimensional echocardiogram and Doppler flow patterns to check both systolic and diastolic functions. Ejection fraction less than 50% was used to define left ventricular systolic dysfunction.

In Australia, the Canberra Heart Study (Abhayaratna, Smith et al. 2006) was the first population-based study in this regard. It evaluated the prevalence of heart failure in 1275 randomly selected participants who are aged 60-86 years of age from February 2002 to June 2003. Clinical parameters as well as echocardiographic assessment of both systolic and diastolic functions were used.

In most developing countries, population-based epidemiological studies of heart failure are virtually non-existent, unsurprisingly due to limited resources in these countries to carry out such research.(Mendez and Cowie 2001)

1.1.1 Incidence of heart failure

A common agreement exists among the major population-based epidemiological study of heart failure: The incidence of heart failure increases exponentially with advancing age. In the Framingham study, the incidence of heart failure per person-year was 0.6-0.8 cases/1000 years in individual aged 29-39 years of age but in those aged 70-74 years of age, there was a more than 10-fold increase in incidence to 8.7 cases/1000 years. (Mahmood and Wang 2013) Similar trends were observed in the Hillingdon study (Cowie, Wood et al. 1999) with incidence of 0.2 cases/1000 person years in the 45-55 years age group compared to 12.4 cases/1000 person years in those aged more than 84 years. Higher values were obtained in the Rotterdam study (Bleumink, Knetsch et al. 2004) with incidence rate of 2.5 cases/ 1000 person years in individuals 55-64 years and 44 cases/1000 person years in those over 85 years of age. These higher values are possibly due to difference in methodology.

No Australian population based study of the incidence of heart failure exists.

1.1.1.1 Trends in incidence of heart failure

There has been demonstrated reduction in the incidence of systolic heart failure secondary to myocardial infarction and this is considered to due to decreased severity and extent of myocardial damage during episodes of myocardial infarction with the availability of reperfusion therapy. (Hellermann, Jacobsen et al. 2003), (Goldberg, Gore et al. 1986) In spite of this, in the Framingham study, the overall incidence of heart failure in men over a 50-year period of 1950-1999 did not change although there was a decrease in women. Similarly, in the Olmsted population based study of 4537 heart failure patients, the incidence of heart failure remained unchanged between 1979 and 2000.(Roger, Weston et al. 2004)
Therefore, although there has been substantial decrease in incidence of heart failure post myocardial infarction, overall incidence of heart failure remains unchanged probably due to other causal factors including hypertension, diabetes, viral causes and ageing population. (Mosterd and Hoes 2007)

Overall, the incidence of heart failure is about 5-10 per 1000 persons per year.(Lloyd-Jones, Adams et al. 2010)

1.1.2 The Prevalence of heart failure

More than 5.8 million people in the United States have heart failure. (McMurray, Petrie et al. 1998) The worldwide estimate of heart failure prevalence is about 23 million. (Gottdiener, Arnold et al. 2000)

The Olmsted County study (Redfield, Jacobsen et al. 2003) provides robust information on the prevalence of heart failure, and found this to be 2.2% for validated heart failure. As

is the case with incidence, the prevalence of heart failure also increases with advancing age. Lower prevalence of 0.7% was found in individuals 45-54 years of age, 1.3% in those aged 55 through 64; 1.5% for those aged 65 through 74; but up to 8.4% in those aged 75 years or older. The prevalence of any systolic dysfunction using EF less than 50% was 6.5% while moderate to severe systolic dysfunction was present in 1.8%. In addition, when adjustment was made for age, prevalence was statistically significantly higher in men than in women.

In the Rotterdam study, (Bleumink, Knetsch et al. 2004) prevalence of heart failure at three different time points ranged between 6-7% with higher prevalence in men. An exponential rise in prevalence was also noted with advancing age: 0.9% in subjects aged 55–64 years, 4.0% in subjects aged 65–74 years, 9.7% in those aged 75– 84 years, to 17.4% in those aged 85 years or over.

The Australian experience is no different. The Canberra Heart Study, (Abhayaratna, Smith et al. 2006) found prevalence of heart failure to be 6.3% and prevalence of any or moderate to severe systolic dysfunction to be 5.9% and 2.1% respectively.
Prevalence also rose sharply with advancing age with 3.1% in participants aged 60-64 years to 13.6% in individuals aged 80-86 years. Men also had higher prevalence than women.

Overall, the prevalence of heart failure is expected to continue to rise even though the incidence may seem to have plateaued. This may be due to the ageing population, improved survival from myocardial infarction and increasing pool of survivors of acute heart failure.

1.1.3 Etiology of Heart Failure in Western Society

1.1.3.1 Ischemic heart disease

Ischemic heart disease is currently the greatest precursor of heart failure in the Western world. (Loehr, Rosamond et al. 2008) In the Atherosclerosis Risk in Communities (ARIC) Study, (Loehr, Rosamond et al. 2008) carried out in a cohort of over 15000 mixed racial groups in the USA, coronary artery disease was present in 53% of cases of incident heart failure. Likewise, the result of the First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (NAHNES 1) showed that the population attributable risk of heart failure was 61.6% for coronary artery disease. (He, Ogden et al. 2001) Undoubtedly, in recent times, many more patients survive acute myocardial infarctions due to advancement in reperfusion strategies and this inevitably increases the proportion of individuals that would go on to develop chronic heart failure.

1.1.3.2 Hypertension

Analysis from the Framingham heart study (Ho, Pinsky et al. 1993) shows that hypertension and ischemic heart disease account for majority of cases of heart failure. Although hypertension is present in 70% of men and 78% of women prior to onset of heart failure, it should also be mentioned that hypertension co-exists with coronary artery disease in 40% of both men and women. Hypertension was present in 42% of heart failure patients in the Studies of Left Ventricular Dysfunction.(Yusuf, Pitt et al. 1991) Similar values were found in the MERIT-HF, (Hjalmarson, Goldstein et al. 2000) and the ATLAS studies.(Ryden, Armstrong et al. 2000)

Concentric left ventricular hypertrophy is usually the first adaptive response to normalize the increased wall stress that follows sustained increase in peripheral resistance. This hypertrophy would subsequently impair diastolic filling and also reduce coronary flow

reserve and consequently trigger cascade of events that would lead to both diastolic and systolic heart failure. Apart from increasing age, left ventricular hypertrophy is the most important determinant of adverse cardiovascular outcomes such as heart failure, strokes and arrhythmia in hypertensive patients. (Gradman and Alfayoumi 2006) Indeed a striking and strong linear relationship exists between left ventricular mass and adverse cardiovascular outcomes.(Schillaci, Verdecchia et al. 2000)

1.1.3.3 Valvular heart disease

Most studies would give valvular heart disease a third place in the etiology of heart failure. In a hierarchical order the Framingham heart study attributed the etiology of heart failure to ischemic heart disease in 54% of cases, hypertension in 24% and valvular heart disease in 16% of cases. (Ho, Anderson et al. 1993) In an Eastern Finland population based study of the incidence of heart failure in individuals aged 45-74 years of age, coronary artery disease was present in 60% of cases, hypertension in 55% and valvular heart disease in 21% of cases. (Remes, Reunanen et al. 1992) The largest pooled population based study of 11,911 adults in the USA as well as 16,501 individuals from Olmsted community study found overall prevalence of moderate to severe valvular heart disease to be 2.5% with a sharp rise after the age of 64 years. (Nkomo, Gardin et al.)

1.1.3.4. Others

Other important contributors to the occurrence of heart failure in Western countries include diabetes mellitus and atrial fibrillation/flutter.

Using 5% of the national sample of United States Medicare claims from 1994 to 1999 to perform a population-based, non-concurrent cohort study in 151,738 beneficiaries with diabetes who were age ≥65 years, Bertoni et al found the prevalence of heart failure to be 22.3% and a cumulative incidence of 12.6% per 100 person years. Furthermore, over 60

months, a high mortality of 32.7% per 100 person years occurred in patients with incident heart failure compared to 3.7% per 100 person years in diabetic patients who did not develop heart failure. (Bertoni, Hundley et al. 2004).

In the United Kingdom, an analysis of a cohort of 1.9 million patients from registry data was evaluated. Individuals 30 years of age or over who were free of cardiovascular disease at baseline were followed up for a median period of 5.5 years. The primary endpoint was the first record of one of 12 cardiovascular presentations. Heart failure and peripheral arterial disease were found to be the most common initial manifestations of cardiovascular disease in the patients with type 2 diabetes. Specifically, of people with type 2 diabetes, 6137 (17·9%) had a first cardiovascular presentation, with the most common form being peripheral arterial disease; reported in 992 (16·2%) of 6137 patients, and then, heart failure in 866 (14·1%) of 6137 patients).(Shah, Langenberg et al. 2015)

Atrial fibrillation is the most common sustained arrhythmia with a prevalence of 0.95% in individuals 45 years of age and a 10-fold increase in those 80 years old and beyond. (Go, Hylek et al. 2001) The relationship between atrial fibrillation and systolic heart failure is bi-directional: thus whilst atrial fibrillation with rapid ventricular rates can cause heart failure,(Brill , Quiniou, Chevalier et al. 2000) with significant reversal of left ventricular systolic dysfunction occurring following reversion to sinus rhythm, (Van Gelder, Crijns et al. , Luchsinger and Steinberg 1998) heart failure itself is known among other things, to cause interstitial fibrosis of the atria thereby creating a substrate for the development of atrial fibrillation.(Li, Fareh et al. 1999) In addition to these, several studies have also found that the development of new-onset atrial fibrillation in patients with heart failure confers significantly higher mortality risk than in heart failure patients with no atrial

fibrillation.(Wang, Larson et al. 2003, Ahmed and Perry 2005, Swedberg, Olsson et al. 2005)

1.1.4 Diagnostic considerations

1.1.4.1 Diagnostic criteria

Because heart failure is a syndromic diagnosis, the goal of diagnosis entails establishing the presence of heart failure, its severity and also its aetiology. Numerous criteria exist to address these. The Framingham criteria (McKee, Castelli et al. 1971) and the Boston criteria (Carlson, Lee et al. 1985) were established prior to the use of non invasive investigations. Notwithstanding, when compared to left ventricular ejection fraction of less than 45% on 2D echocardiogram, the Framingham criteria have sensitivity of 97%, specificity of 79%; excellent negative likelihood ratio of 0.1 though with low positive likelihood ratio of 4.3 for diagnosing systolic heart failure (Maestre, Gil et al. 2009) The Boston criteria also compare favourably with the Framingham criteria in terms of sensitivity and were noted to have a better construct validity than the Framingham criteria even with the use of a higher ejection fraction cut off of 50%. (Di Bari, Pozzi et al. 2004) Interestingly, even the European Society of Cardiology criteria(Remme, Swedberg et al. 2001) were not more sensitive than the Framingham and Boston criteria in diagnosing systolic heart failure.

1.1.4.2 Non-invasive imaging modalities.

Chest roentgenogram has limited utility in heart failure diagnosis. While it can be helpful in diagnosis of decompensated heart failure, it offers limited information to the aetiopathogenesis.

On the other hand, transthoracic echocardiogram yields valuable information associated with heart failure diagnosis. It enables stratification into heart failure with reduced ejection fraction, (systolic heart failure) and heart failure with preserved ejection fraction (diastolic heart failure). It also enables the quantitation of the severity of systolic left ventricular dysfunction. These findings have enormous implications in management as will be discussed later. Echocardiogram can also help in establishing the aetiology of heart failure, for example by identifying previous myocardial infarct and/or valvular dysfunction. Finally, objective response to treatment is usually best assessed by this tool.

Recently, cardiac iodine-123 metaiodobenzylguanidine (MIBG) has been used by some investigators to risk stratify patients with congestive heart failure. The rationale is that chronic sympathetic stimulation in heart failure leads to reflex reduction in beta-adrenergic receptor activity and subsequent fall in catecholamine sensitivity. These would therefore lead to in increased presynaptic accumulation of norepinephrine; the catechol-O-methyl transferase and the monoamine oxidase enzymes subsequently degrade the norepinephrine. Iodine-123 MIBG is an analogue of guanethidine that utilizes the same beta-adrenergic receptors for its uptake but is not degraded by the catechol-O-methyl transferase and the monoamine oxidase enzymes. The pre-synaptic accumulation of Iodine 123 MIBG is thus more measurable. By measuring its Heart-to-Mediastinal ratio, (HMR), values above 1.6 were associated with significantly less mortality over a 2 year period compared to values less than 1.2 in patients with heart failure and ejection fraction of 35% or less. (Jacobson, Senior et al. 2010)

Cardiac Magnetic Resonance (CMR) Imaging with late gadolinium enhancement (LGE) has also been found useful in assessment of heart failure. In a retrospective review of 83 patients, Won et al found that in patients with LVEF less than or equal to 40%, the

presence of ischemic pattern on both LGE and cine sequences has 87% specificity for ischemic aetiology compared to gold standard coronary angiography whereas the absence of ischemic pattern on both LGE and cine sequences had a 94% specificity for non-ischemic aetiology of heart failure.(Won, Donnino et al. 2015) CMR, though not as readily available as echocardiogram, has been considered the gold standard for measuring cardiac volumes and functions with high spatial and temporal resolution of 3D images.(Kassi and Nabi 2013) It is also particularly useful in evaluation of the less common forms of cardiomyopathy such as infiltration with amyloid, sarcoid and/or hemochromatosis.

1.1.4.3 Invasive evaluation

The extensive array of available non-invasive tools for diagnostic evaluations of heart failure has limited the scope of invasive measures. Coronary angiography with or without right heart catheterisation however, remains the gold standard for evaluating ischemic aetiology of heart failure.

1.1.4.4 Biochemical evaluation: Natriuretic Peptides

As a compensatory mechanism to increased myocardial wall stress, cardiomyocytes release pro- brain natriuretic peptide, which is further cleaved to the inactive N terminal pro-brain natriuretic peptide (NT-proBNP) with a half -life of 120 minutes and to the biologically active brain natriuretic peptide (BNP) with a faster half-life of 20 minutes. BNP is natriuretic, vasodilatory, anti-fibrotic and also opposes sympathetic overactivity of heart failure.(Kim and Januzzi 2011) Plasma concentrations of BNP and NT-proBNP, are increased in heart failure, and also have been found to be significantly and negatively correlated with left ventricular systolic function, and positively correlated with

symptoms.(Clerico, Iervasi et al. 1998, Seino, Ogawa et al. 2004) In the Breathing Not Properly (BNP) trial, plasma BNP level was regarded as the single most accurate predictor of the presence or absence of congestive heart failure. Using receiver operating characteristic curve, levels of up to 100pg per milliliter had a sensitivity of 90%, specificity of 76% and accuracy of 83% in differentiating heart failure from other causes of dyspnea.(Maisel, Krishnaswamy et al. 2002) Likewise, the PRIDE study, which used NT-proBNP to rule out acute heart failure in the emergency department, suggested that NT-proBNP testing alone was superior to clinical judgment alone in diagnosing acute heart failure. Whereas levels above 450pg/ml in individuals younger than 50 years of age and more than 900pg/ml in those up to 50 years of age or above were highly sensitive and specific for the diagnosis of heart failure, levels below 300pg/ml were reported to have a negative predictive value of up to 99% for heart failure.(Januzzi, Camargo et al. 2005) This should not be construed to imply that measurement of the natriuretic peptides should replace clinical assessments. On the contrary, combined clinical assessment with the measurements improves diagnostic accuracy and has great utility for cases where clinical assessments are indeterminate.(Steinhart, Thorpe et al. 2009) The direction of change with serial NT-proBNP measurement also adds incremental prognostic information. Bettencourt et al (Bettencourt, Azevedo et al. 2004) followed up 182 patients with heart failure for 6 months and observed that >30% increase in initial NT-proBNP levels was associated with increased primary outcome of death or heart failure hospitalization and vice versa.

1.2 THE BURDEN OF HEART FAILURE

1.2.1 Morbidity with heart failure

1.2.1.1 Common symptoms of heart failure

Heart failure has significant morbidity. The prevalence of heart failure is up to 5.8 million in the United States,(McMurray, Petrie et al. 1998) with a projected world-wide prevalence of 23 million.(Gottdiener, Arnold et al. 2000) In addition, heart failure is associated with significant symptom burden, which negatively impacts quality of life. Barnes et al (Barnes, Gott et al. 2006) in the United Kingdom evaluated the symptom prevalence and burden in 542 symptomatic heart failure patients aged more than 60 years. Kansas City Cardiomyopathy Questionnaire (KCCQ), SF-36, Geriatric Depression Scale (five-item) (GDS-5), NYHA classification, and a service use questionnaire were completed by the patients every 3 months for 2 years. Breathlessness and Fatigue were the two most common symptoms with more than half of the patients experiencing each of these symptoms at least once a day. Nearly a third had to sleep on 2 or 3 pillows at least once a week due to breathlessness. The third commonest symptom was leg swelling which apart from its cosmetic appearance could affect mobility and also result in fragmented sleep due to increased frequency of urination coming from the increased use of diuretics.

A retrospective study of over 4000 patients admitted at Worcester Massachusetts with heart failure between 1995-2000 also had somewhat similar results. The mean age of patients was 76 years. The five commonest symptoms included dyspnoea (93%), peripheral oedema (70%), cough (51%), orthopnoea (37%) and chest pain (30%). Elderly patients were also more likely to delay seeking help as they may attribute their symptoms to advancing age.(Goldberg, Spencer et al. 2010)

1.2.1.2 Symptom clusters in heart failure

Heart failure patients experience a myriad of symptoms. It can be quite difficult for patients to accurately recognise these symptoms as cardiac in origin, be able to

differentiate them from other disease conditions or they may even attribute these symptoms as part of normal ageing.(Jurgens 2006) Thus, Symptom Clusters, used in other medical conditions, has been proposed and applied to patients with heart failure. The idea is based on Lenz and Pugh's theory of Unpleasant Symptoms(Lenz, Suppe et al. 1995, Lenz, Pugh et al. 1997) and implies that patient's perception and response to a particular symptom are influenced by prior physiological, psychological and situational factors; symptoms therefore are multidimensional.

With this theoretical background, Jurgens et al (Jurgens, Moser et al. 2009) used relevant part of the Minnesota Living with Heart Failure Questionnaire to identify three cluster categories of symptoms in a secondary analysis from a Heart Failure registry in the United States (N= 687). The identified three clusters are: I) acute volume overload symptoms, which include shortness of breath, fatigue and sleep problems. The acute volume overload cluster accounted for 45.7% variance in symptom impact. II) The emotional cluster of depression, worry and memory problems; with symptom impact variance of 13.1% and III) the chronic volume overload cluster comprising swelling, the need to rest, and dyspnoea on exertion with a variance of 9.3%. Interestingly, comorbid conditions did not influence these clusters except for diabetes that affected the emotional cluster. And although the clusters occurred more frequently in elderly patients 75 years of age and above, their impact appeared more limited.

1.2.1.3 Impact on quality of life

Zambroski and her group(Zambroski, Moser et al. 2005) in a cross-sectional study of 53 heart failure patients, evaluated the impact of symptom prevalence and burden on quality of life in heart failure patients. Symptoms were considered multidimensional with each having frequency, severity and distress. Symptom prevalence was the total sum of the

symptom's frequency, severity and distress whereas symptom burden was the mean of these. Physical and emotional symptoms were assessed using the Memorial Symptom Assessment Scale—Heart Failure (MSAS-HF) which is a heart failure modification of the Memorial Symptom Assessment Scale(Portenoy, Thaler et al. 1994) and is able to assess physical, psychological and heart failure symptoms. Quality of life was assessed with Minnesota Living with Heart Failure Questionnaire. The result showed a wide range of symptoms, with mean number of 15.1+/-8.0 symptoms. However, major symptoms were shortness of breath (85.2%), lack of energy (84.9%), dry mouth (74.9%), drowsiness (67.9%) and difficulty with sleeping (64.2%). Importantly, psychological symptoms such as worry, nervousness were present in more than half of the patients.

As shown in **Figure 1.1**, in terms of symptom severity, sleeping difficulty, lack of energy, worrying and shortness of breath tend to have maximal impact on patients.

Using a multiple regression analysis, the study found that lower age and higher NYHA classification, greater symptom burden and greater symptom prevalence predicted worse quality of life. Symptom burden and symptom prevalence provided the greatest impact on Heart Related Quality Of Life.

1.2.1.4 Co-morbid conditions

As the prevalence of heart failure increases with age, it is understandable that many patients with heart failure would have other co-existing medical conditions.

Cardiac co-morbid conditions refer to those that could lead to heart failure such as ischemic heart disease, hypertension, diabetes, arrhythmias and dyslipidaemia. The rest can be regarded as non-cardiac comorbidities and include conditions like chronic obstructive airway disease (COAD), renal failure, cerebrovascular events, depression, dementia, osteoarthritis, anaemia and so on. These non- cardiac co-morbidities do affect the progression of heart failure(Lang and Mancini 2007) as well as the patient's

perception and discrimination of heart failure symptoms(Jurgens, Moser et al. 2009). In the European Heart Failure Pilot Survey of 3226 patients with heart failure, 74% have at least one co-morbidity with the highest found to be chronic kidney disease (41%), then anaemia (29%), and diabetes (29%). Co-morbidities were independently associated with higher age ($P < 0.001$), higher NYHA functional class ($P < 0.001$), ischaemic aetiology of heart failure ($P < 0.001$), higher heart rate ($P = 0.011$), history of hypertension ($P < 0.001$), and atrial fibrillation ($P < 0.001$)(van Deursen, Urso et al. 2014) Other studies have found higher prevalence of COAD, osteoarthritis, dementia and depression and in some cases, patients have more than five non-cardiac co-morbidities(Havranek, Masoudi et al. 2002, Braunstein, Anderson et al. 2003, Lang and Mancini 2007)

1.2.1.5 Muscle wasting and cachexia in chronic heart failure.

Cachexia is common in advanced heart failure. Various definitions have been proposed for cachexia in heart failure patients some of which include the use of body fat content < 29% in women or < 27% in men, utilizing skin fold thickness measured with calipers; (McMurray, Abdullah et al. 1991) use of ideal body weight less than 85%, (Levine, Kalman et al. 1990) or a documented loss of at least 10% of lean tissue.(Freeman and Roubenoff 1994) It is apparent that definitions based on either body fat content or lean tissue, exclusive of one another are clearly inadequate whereas definition based on ideal body weight will be biased against patients with naturally lower body weight prior to the onset of heart failure.

To overcome these limitations, Anker et al,(Anker and Coats 1999) have proposed a simpler and more comprehensive definition of what they called 'clinical cardiac cachexia' as weight loss of > 7.5% of the previous normal weight observed in patients with CHF of at least 6 months' duration without signs of other primary cachectic states (e.g., cancer,

thyroid disease, or severe liver disease). This weight loss should usually be observed over a period of > 6 months.

The clear observation that cachectic heart failure patients suffer fat, muscle and bone loss with plasma albumin and liver enzymes being normal(Anker, Chua et al. 1997) would argue against a major contribution to this condition by anorexia with poor food intake, deconditioning, impaired nutrient absorption or hepatic hypo perfusion. Immune mediated tumour necrosis factor-alpha (TNF-α) activity has been consistently demonstrated in many studies to be significantly higher in cachectic heart failure patients than in those with no cachexia.(Levine, Kalman et al. 1990, McMurray, Abdullah et al. 1991, Anker, Chua et al. 1997) TNF-α is produced by the macrophages and monocytes in response to inflammation, by the myocardium in response to chronic repetitive stress (Mann 1996)and by endothelial cells where it is known to decrease constitutive nitric oxide synthase (cNOS) mRNA levels by increasing the rate of mRNA degradation, effectively reducing the half-life of cNOS mRNA from 48 hours to 3 hours. (Yoshizumi, Perrella et al. 1993)

TNF-α has been considered a major driving force behind cardiac cachexia. Anker et al (Anker, Chua et al. 1997) also found that plasma levels of norepinephrine, epinephrine and cortisol were markedly elevated in heart failure patients with cachexia than in those without cachexia, with a reduction of the anabolic steroid dehydroepiandrosterone in cachectic patients, thus suggesting an imbalance of cellular metabolism in favour of catabolism.

Peak oxygen consumption (VO_2 max) is known to be significantly reduced in cachectic than in non-cachectic heart failure patients.(Toth, Gottlieb et al. 1997) Furthermore,

cachexia is associated with higher mortality outcome in heart failure.(Anker, Negassa et al. 2003, Kenchaiah, Pocock et al. 2007) Conversely and paradoxically, heart failure patients with high body mass index or obesity seem to have lower mortality outcomes.(Horwich, Fonarow et al. 2001, Fonarow, Srikanthan et al. 2007)

The overall implication here is that exercise intolerance in patients with heart failure is not purely engendered by cardiac dysfunction, and if possible, limitation and reversal of cardiac cachexia represents a component of the therapeutic objective.

1.2.2 Mortality with heart failure

HF has traditionally been associated with high mortality. Analysis of data from the FHS over four decades from 1948 to 1988 did not show any temporal decline in mortality with overall 1-year and 5-year survival rates of 57% and 25% in men and 64% and 38% in women, respectively (Ho, Anderson et al. 1993) During this period however, the pharmacological treatment of heart failure was mainly with digoxin, and later hydralazine/isosorbide dinitrate, whereas neuro-humoral antagonism had not come on board.

In Scotland, between 1986-1995, which corresponds to the era of the introduction of ACE inhibitors, 66,547 patients had a principal diagnosis of heart failure. Crude Case Fatality Rates at 30 days and at 1, 5, and 10 years were 19.9%, 44.5%, 76.5%, and 87.6%, respectively. Median survival was 1.47 years in men and 1.39 years in women (2.47 and 2. 36 years, respectively, in those surviving 30 days). After adjustment, 30-day crude case fatality rates between 1986 and 1995, was reduced by 26% (95% CI 15 to 35, P<0.0001) in men and 17% (95% CI 6 to 26, P<0.0001) in women. Longer-term crude case fatality rates fell by 18% (95% CI 13 to 24, P<0.0001) in men and 15% (95% CI 10 to 20, P<0.0001) in women. Median survival increased from 1.23 to 1. 64 years.(MacIntyre, Capewell et al. 2000) Likewise, further analysis of data from the Framingham Heart study

between 1990-1999 showed an overall improvement in the survival rate after the onset of heart failure of 12 percent per decade (P=0.01 for men and P=0.02 for women).(Levy, Kenchaiah et al. 2002) This again corresponds to the era of commencement of ACE inhibitor use in HF.

With subsequent use of beta-blockers and aldosterone antagonists in the late 1990's(Packer, Bristow et al. 1996, Pitt, Zannad et al. 1999), device- based treatments (Bristow, Saxon et al. 2004) and recently the combined use of ARB and neprilysin inhibitor, 2 year mortality of heart failure, though having been reduced, remains at a substantial 20%(Sacks, Jarcho et al. 2014)

1.2.3 Hospitalized Heart Failure (HHF)

Systolic heart failure drains the health-care system. In Northern America and Europe, HHF is the leading cause of hospitalizations and accounts for 1-2% of all hospitalizations, amounting to about 1 million hospital admissions per year.(Ambrosy, Fonarow et al. 2014) HHF may be on the increase: using data from the National Hospital Discharge Survey, Fang et al(Fang, Mensah et al. 2008) noted that the number of hospitalizations with any mention of heart failure tripled from 1,274,000 in 1979 to 3,860,000 in 2004 with heart failure listed as the first diagnosis in 30-35% of these admissions. A similar trend was also observed in Scotland. Data from the Scottish Hospital In-Patients Statistics (SHIPS) showed that between 1980-1990, discharges for heart failure as the primary diagnosis increased by almost 60%, from 1.30 to 2.12/1000 population in this period; and when considered as either primary or secondary diagnosis, the rate increased from 2.51 to 4.24/1000.(McMurray, McDonagh et al. 1993) However, data from later years covering 2001 to 2009 in the US, showed a slight decrease in primary heart failure hospitalizations

(1,137,944 in 2001 to 1,086,685 in 2009) but a slight increase in secondary heart failure hospitalizations (2,753,793 to 3,158,179 over the same period)(Blecker, Paul et al. 2013)

Although the median length of stay for HHF was found to have declined, from 8 days in 1980–1984 to 5 days in 2000–2004, there was however, an increase in the rate of transfers to both short-term and long-term facilities (12.3% to 26.0%) over the same time period, rather than discharge to home.(Fang, Mensah et al. 2008)

Re-admission is a major problem in HHF. Heart failure was found to be the most common cause of hospital re-admissions within 30 days of discharge; with 26.9% of HHF patients re-admitted at this time. (Jencks, Williams et al. 2009) Re-hospitalization is driven by the presence of cardiac decompensating factors like arrhythmias, ischemia, anemia or infections as well the presence of non-cardiac co-morbidities, and also by suboptimal therapeutic compliance. Apart from being economically very expensive, re-hospitalization also increases mortality. Secondary analysis of data from the 3 arms of the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) trials involving 7572 patients showed that irrespective of whether LVEF was less than or more than 40%, mortality post discharge increases with increased frequency of re-hospitalizations. Longer duration of hospitalization (beyond 21 days) was also associated with higher mortality compared to shorter duration of hospital stay (1-7days). In addition, there was 6-fold excess mortality risk within the initial 30 days post hospital discharge, which fell to 2-fold excess risk by 2 years. And also, after adjustments for baseline predictors of mortality, the hazard ratio for all- cause mortality following first hospitalization relative to patients that were not hospitalized was 3.15; 95% CI, 2.83 to 3.50.(Solomon, Dobson et al. 2007)

1.2.4 The economic burden of heart failure:

It is hardly surprising from the foregoing, that heart failure takes a huge financial toll on society. The cost of heart failure management is mainly driven directly by diagnostic modalities required, long term pharmacological and non- pharmacological treatments (including device-based options) and its requirement for multi-disciplinary care approach. Indirect costs such as loss of income, carer and welfare support also contribute. In most developed economies, heart failure costs about 1-2% of the yearly total expenditure and this is higher than any other medical condition.(Berry, Murdoch et al. 2001) It also correlates directly with the severity and symptomatic class of patients.(Davey, Clarkson et al. 1999) Putting it in perspective, in the United States for instance, the direct medical costs for treating heart failure in the year 2000 was 20 billion US dollars in addition to a further indirect costs of 2 billion US dollars.(Berry, Murdoch et al. 2001)

The greater proportion of heart failure cost is spent on hospitalization with repeat hospitalization consuming up to 60-70% of the total medical costs.(McMurray and Davie 1996) It was thought that the implementation of evidence- based treatment options for heart failure; incorporating pharmacological and device-based measures would reduce heart failure hospitalizations and consequently drive cost downwards. Subsequent analysis of this trend utilizing data from the US Medicare registry has shown that heart failure hospitalization rate adjusted for age, sex, and race declined from 2845 per 100,000 person-years in 1998 to 2007 per 100,000 person-years in 2008 ($P < .001$), a relative decline of 29.5%.(Chen, Normand et al. 2011)

1.2.5 The influence of Race

Several studies, predominantly from American investigators have reported substantially higher prevalence and incidence of heart failure in blacks than other racial groups(Loehr,

Rosamond et al. , Bahrami, Kronmal et al. 2008, Bibbins-Domingo, Pletcher et al. 2009, Eaton, Abdulbaki et al. 2012) This finding has been largely attributed to higher and more severe occurrence of traditional risk factors of hypertension, type 2 diabetes and obesity in blacks.(Loehr, Rosamond et al.) Although the Multi-Ethnic Study of Atherosclerosis (MESA) (Bahrami, Kronmal et al. 2008) found similar higher occurrence of the aforementioned risk factors in blacks, yet blacks were less likely to have incident heart failure attributable to ischemic etiology. On the contrary, 75% of incident heart failure in blacks was not preceded by clinical myocardial infarction. Several other landmark studies have also similarly found that among patients with heart failure, blacks were less likely to have ischemic etiology but more likely to have hypertension- based heart failure than other races.(Cohn, Archibald et al. 1986, Cohn, Johnson et al. 1991, Yusuf, Pitt et al. 1991) Heart failure also occurs earlier in blacks and with greater severity,(Yancy) more hospitalization and worse survival than in whites.(Dries, Exner et al. 1999) The explanation for this variance is at the present postulatory but has included the role of single nucleotide polymorphism in African- Americans involving transforming growth factor (TGF)–β_1, endothelin, β_1-adrenergic receptors, aldosterone synthase, and nitric oxide (NO) synthase. Highest level of (TGF)–β_1 have been found in blacks with hypertension and (TGF)–β_1 itself is thought to induce mesangial and left ventricular hypertrophy and also stimulate the release of endothelin. (Suthanthiran, Li et al. 2000) From a practical perspective, aggressive risk factor control especially with regards to hypertension, diabetes, obesity and left ventricular hypertrophy should reduce the occurrence of heart failure in black subjects. However, this remains an objective that has not yet come close to achievement in practice.

1.3 THE PATHOPHYSIOLOGY OF SYSTOLIC HEART FAILURE

Cardiac output is a function of stroke volume and heart rate. Stroke volume relies on the Frank-Starling mechanism.

1.3.1 Reduced contractile reserve: Failure of Frank-Starling mechanism and blunted treppe phenomenon.

According to the Frank-Starling mechanism, within physiological limits, the normal heart responds to increased stretch by increased sarcomere shortening. Therefore, increase in preload is matched with an increase in the force of contraction. Apart from stretch, it is also known that heart rate affects cardiac contractility such that as the heart rate increases, the force of contraction normally increases (positive staircase or Treppe/Bowditch effect) and vice versa. The Frank-Starling (**Figure 1.2**) and the Bowditch **(Figure 1.3)** phenomena are the two major intrinsic contractile reserves the heart uses to meet up with varying physiologic demands on it, with the Frank-Starling mechanism dominating at low levels of exertion while the frequency treppe/Bowditch effect dominates at high levels of exertion. (Higginbotham, Morris et al. 1986)

It is thought that Starling relationship is due to length-dependent increase in Ca^{2+} transients and/or increased myofibrillar sensitivity to calcium whereas in force-frequency/ 'treppe' effect, the frequency of stimulation is what increases the cytosolic Ca^{2+} content and /or myofibrillar sensitivity to Ca^{2+} .(Allen and Kurihara 1982) In an elegant study, Schwinger et al(Schwinger, Böhm et al. 1994) clearly showed that Frank-Starling mechanism is severely impaired in failing human myocardium. Papillary muscle tissues from brain-dead donors who died of traumatic causes and with no echocardiographic evidence of heart failure were compared with tissues from heart transplant patients: keeping the resting tension, muscle length and muscle cross sectional areas constant between the two groups, they were able to show that increase in preload resulted in higher

force of contraction only in the non-failing myocardium. Also, increasing the muscle length by stretching resulted in significantly higher myofibrillar sensitivity to calcium in non-failing hearts compared to failing myocardium. Furthermore, pre-treatment with the cardiac glycoside ouabain significantly improved stretch-force response in failing myocardium compared to non-failing myocardium. Other studies have found similar results with replacement of a positive by a negative force-frequency relationship in failing myocardium. (Allen, Jewell et al. 1974, Allen and Kentish 1985, Gwathmey, Slawsky et al. 1990) It is pertinent to note that although there are decreased calcium transients and sensitivity to stretch and stimulation frequency in failing myocardium, baseline calcium content and baseline myofibrillar sensitivity are elevated in failing myocardium compared to non-failing myocardium(Gwathmey, Slawsky et al. 1990, Schwinger, Böhm et al. 1994) This means therefore that the sub-cellular mechanisms responsible for both the Frank-Starling mechanism and the Bowditch effect are already extensively activated at baseline indicating depletion of reserves for these.

1.3.2 Adaptive changes in heart failure

With ensuing heart failure, extensive neuro-hormonal stimulations occur which initially are adaptive but with time, become maladaptive. Target organs involved are predominantly the heart, the kidneys and the vasculature. Inflammatory modulation also occurs.

1.3.2.1 Autonomic nervous system modulation.

The major initial neuro-hormonal modification that occurs in heart failure is the stimulation of the sympathetic nervous system and subsequent down-regulation of the parasympathetic system.

1.3.2.1.1 Acute Sympathetic stimulation effects

Sympathetic stimulation leads to increased pre-synaptic norepinephrine release as well as its decreased post-synaptic re-uptake.(Jacobson, Senior et al. 2010) Acutely on the heart, both chronotropy and inotropy are enhanced via β-adrenergic receptor stimulation, resulting in increased cardiac output. In both the peripheral vessels and the renal vasculature, norepinephrine acts primarily via the α_1-adrenoceptors to promote vasoconstriction and increased renal tubular re-absorption of salt and water as well as non-osmotic release of vasopressin.(Schrier and Abraham 1999) The major net effect is to increase cardiac output through alterations to stroke volume and/or heart rate.

1.3.2.1.2 Chronic Sympathetic stimulation effects

Chronic elevation of norepinephrine has been shown to be associated with increased mortality in patients with heart failure. Prior to the advent of ACE inhibitors and beta blockers in heart failure, Cohn et al (Cohn, Levine et al. 1984)from Minnesota measured norepinephrine in 106 patients with heart failure and followed them up for 1-62 months. They recorded a mortality of 57%. On multivariate analysis, only elevated plasma norepinephrine level (700pg/ml) was associated with increased mortality (P= 0.002) and interestingly, higher levels were found in patients who died of progressive pump failure than in those who died suddenly.

1.3.2.1.2.1 Chronic Sympathetic stimulation on the heart

Many studies point to the deleterious effects of chronic sympathetic stimulation on the heart. Excitation-contraction coupling is known to be impaired in failing human myocytes, with reduced peak systolic Ca^{2+} and slow decay of the Ca^{2+} transients, and reduced sarcoplasmic reticulum Ca^{2+} content and rate of SR Ca^{2+} uptake in failing myocytes compared to non-failing hearts. (Piacentino, Weber et al. 2003) In mice

models, chronic sympathetic stimulation induced cardiac hypertrophy at four months.(Hein, Altman et al. 1999) Several studies have also demonstrated both necrosis and apoptosis occurring in both animal and human heart failure models, with chronic adrenergic stimulation also implicated.(Liu, Cigola et al. 1995, Narula, Haider et al. 1996, Olivetti, Quaini et al. 1996) Mammalian cardiomyoctes' viability decreases inversely with tissue norepinephrine concentrations, an effect thought to be mediated through norepinephrine c-AMP induced cytosolic calcium overload; this was ameliorated by β-adrenoceptor blockade. (Mann, Kent et al. 1992) Cardiac chamber dilatation is also known to occur with chronic adrenergic stimulation.(Osadchii, Norton et al. 2007)

Significant changes occur in the beta-adrenergic receptors with chronic norepinephrine release in an effort to limit the damaging effects of chronic adrenergic stimulation. The β_1 –adrenoceptors are not only quantitatively down-regulated, but are also uncoupled from the stimulatory G_s protein with concomitant increase in the inhibitory G_i protein.(Bristow, Ginsburg et al. 1982) These changes therefore lead to reduced sensitivity to norepinephrine in chronic heart failure. No clear alterations in the concentration of β_2 –adrenoceptors have been found in heart failure. Indeed, very extremely high concentration (up to 350 fold) would be needed to induce pathological changes in heart failure albeit in mice model. (Liggett, Tepe et al. 2000) Because β_2 –adrenoceptors contribute to the levels of inhibitory G_i protein, it is probably a good thing that the levels are not changed in heart failure. However in states of high adrenergic surge, as in Takotsubo cardiomyopathy, a switch from G_s to G_i can occur via β_2 –adrenoceptors leading to myocardial stunning. The effect is more profound in the apical region with higher density of β - adrenergic receptors.(Lyon, Rees et al. 2008)

Excess β 3–adrenoceptor activity can occur in heart failure and can exert negative inotropic effect via nitric oxide and inhibition of calcium transients.(Morimoto, Hasegawa et al. 2004)

1.3.2.1.2.2. Sympathetic stimulation on the kidneys and peripheral vasculature.

On the kidneys, sympathetic stimulation increases renal tubular re-absorption of sodium and water, enhances efferent arteriolar tone, decreases tubular response to natriuretic peptides and increases renin production, which plays an important role on the renin-angiotensin-aldosterone system, described below. On the peripheral vasculature, sympathetic stimulation, acting via the alpha-adrenoceptors increases peripheral vascular resistance. These effects although initially beneficial, subsequently become mal-adaptive.

1.3.3 The Renin-Angiotensin- Aldosterone System (RAAS)

The renin-angiotensin-aldosterone system has a profound effect on the progression and outcome of heart failure. Consequently, pharmacological agents targeting this system have significantly ameliorated morbidity and mortality associated with heart failure.(Swedberg and Kjekshus 1988, Pitt, Zannad et al. 1999) Activation of RAAS starts with increased production of renin from the juxtaglomerular apparatus of the kidneys in response to a decrease in effective arterial blood volume and or increased sympathetic drive. Renin, being an aspartic proteinase, cleaves angiotensin I from angiotensinogen. Angiotensin I is converted to angiotensin II, a very potent vasoconstrictor, mainly by angiotensin converting enzyme (ACE) and also by alternative local tissue chymases. The role of the chymases was indirectly targeted in the CHARM-Added trial(McMurray, Östergren et al. 2003) in which candesartan was added to ACE-inhibitors in patients with heart failure: the incremental benefit of treatment with an angiotensin receptor blocker over that of an ACE inhibitor alone is consistent with multiple pathways of angiotensin activation.

Angiotensin II is further converted by local tissue amionopeptidases to angiotensin III and angiotensin IV. This is elaborated further in **Figure 1.4**

Angiotensin III stimulates aldosterone production to a similar extent as angiotensin II but exerts lesser vaso-constrictory effects. Angiotensin IV causes renal vasoconstriction, but unlike angiotensin II and III, it has no vaso-relaxant effects on adrenal arteries.(Kopf and Campbell 2013) This notwithstanding, it has been implicated in regulating cell growth in cardiac fibroblasts, endothelial cells, and vascular smooth muscle cells.(Ruiz-Ortega, Esteban et al. 2007)

A second 'converting' enzyme, the angiotensin converting enzyme 2 (ACE 2) is a carboxypeptidase that is predominantly expressed in the heart and the kidneys (Soler, Ye et al. 2009) and converts angiotensin I and II to angiotensin-(1-9) and angiotensin- (1-7) respectively. Angiotensin- (1-7) binds to mas receptors and is known to interact with prostaglandin-bradykinin-NO systems to oppose vaso-constrictive and proliferative effects of angiotensin II(Ferrario 2006). Angiotensin II and angiotensin III exert their vascular effects via two receptors: angiotensin type 1 and type 2 (AT_1R and AT_2R) receptors.

Angiotensin II signalling occurs via AT_1R through various pathways including

1. G-protein coupled cascade (which activates phospholipase- C, A_2 and D),
2. Mitogen-activated protein kinases,
3. NAD(P)H and ROS signalling,
4. Non-receptor tyrosine kinases (including JAK/STAT pathway and FAK and Pyk2 pathway) and
5. Receptor tyrosine kinases (including PDGF receptor pathway, Epidermal growth factor receptor pathway (EGFR) and the Insulin Receptor Signaling Pathway).

These cascades of events would affect vascular smooth muscles, endothelial cells, cardiac myocytes and extracellular matrix leading to a myriad of effects including proliferation, hypertrophy, fibrosis, endothelial dysfunction, and matrix synthesis and degradation. (Mehta and Griendling 2007) These effects are accentuated in the development and progression of the failing myocardium.

Angiotensin III is thought to have similar effects on blood vessels and aldosterone release as angiotensin II, presumably through AT_1R. In addition to these, angiotensin III is also generally considered to have cardio-protective and counteracting effects to angiotensin II when it binds to AT_2R. For example, Park et al (Park, Oh et al. 2013) recently showed in rat model that angiotensin III stimulated stretch-induced atrial natriuretic peptide (ANP) secretion in a dose-dependent manner without change in atrial contractility. This release of ANP was blocked by AT_2R antagonist but not by AT_1R or mas receptor antagonists. Furthermore, pre-treatment with inhibitors of phosphoinositide 3-kinase (PI3K), Akt, nitric oxide synthase, soluble guanylyl cyclase, or protein kinase G (PKG) attenuated angiotensin III-stimulated ANP secretion, suggesting a critical role of NO/soluble guanylate cyclase as the effector pathway involved.

In summary, angiotensin II is the most potent vasoconstrictor relative to angiotensin III and IV, while angiotensin IV is the least potent vasoconstrictor and angiotensin III acting via AT_2R, exerts largely vasodilatory effects. The renin-angiotensin system is considered to be composed of two arms: the excitatory arm consisting of angiotensin II, AT_1R and ACE, and the protective arm consisting of AT_2R, Ang- (1-7), mas receptor and ACE 2. In the failing myocardium, the balance is shifted in favour of the excitatory arm.

Aldosterone is the terminal effector of the RAAS. Its release in heart failure is mediated through stimulation of AT_1R by angiotensin II and angiotensin III which causes paradoxical relaxation of adrenal arteries, and thus enhances blood flow to the zona glomerulosa. The initial compensatory effect of aldosterone is to enhance renal tubular re-absorption of Na^+ and water with concomitant loss of K^+. Its effects in chronic stimulation are clearly deleterious: aldosterone induces the multifunctional Ca(2+)/calmodulin-dependent protein kinase II (CaMKII) oxidation by recruiting NADPH oxidase, and in a mouse model, this oxidized and activated CaMKII promotes matrix metalloproteinase 9 (MMP-9) expression in cardiomyocytes.(He, Joiner et al. 2011) Aldosterone also up-regulates plasminogen activator inhibitor-1 (PAI-1). Through these, it regulates matrix deposition, promotes vascular dysfunction and promotes fibrosis.(Brown, Kim et al. 2000)

Consequently, aldosterone antagonists have been shown in major landmark trials to significantly improve morbidity and mortality in systolic heart failure with mild and severe symptoms as well as following acute myocardial infarction.(Pitt, Zannad et al. 1999, Pitt, Remme et al. 2003, Zannad, McMurray et al. 2011)

1.3.4 The natriuretic peptides

Three types of natriuretic peptides have been discovered since the last three decades and include the atrial natriuretic peptide (ANP), the brain natriuretic peptide (BNP) with its related N-terminal BNP, and the C-type natriuretic peptide (CNP). Their effects are mediated through three trans-membrane receptors. Two of these (the natriuretic peptide receptors [NPR] -A and B) are are guanylyl cyclases, which catalyse the conversion of intracellular guanosine triphosphate to cyclic guanosine monophosphate (cGMP) whereas

the third, NPR-C acts via the inhibitory G protein to inhibit adenylyl cyclase and activate phospholipase C.(Zois, Bartels et al. 2014) In the failing myocardium, atrial or ventricular stretch lead to release of the natriuretic peptides. BNP enhances natriuresis and diuresis, antagonizes both sympathetic and RAAS activities and also exerts anti-proliferative and anti-fibrotic changes in the heart and the vasculature(Fujisaki, Ito et al. 1995)

In spite of well-known elevation in values of BNP in systolic heart failure, a randomised controlled trial of the recombinant BNP analogue, nesiritide in the ASCEND-HF trial failed to show any clinical benefit.(O'Connor, Starling et al. 2011) Tsutamoto et al(Tsutamoto, Wada et al. 1997) had shown that in systolic heart failure patients, plasma concentrations of ANP, BNP, c-GMP and norepinephrine increased with the severity of heart failure. Yet in spite of the finding of plasma levels of ANP and BNP that were three to five-fold higher in non-survivors compared to survivors, there was no difference in plasma levels of c-GMP between the two groups. This finding suggests the possibility of 'BNP resistance' in heart failure. In a similar result, Liu et al (Liu, Ngo et al. 2014)had initially shown that one of the physiologic mechanisms for the beneficial effect of BNP in heart failure is through its suppression of the generation of superoxide by neutrophils. They were able to show subsequently that this mechanistic effect of BNP on neutrophils was attenuated significantly in patients with acute heart failure compared to normal controls. Furthermore, there was partial recovery of this BNP function with conventional treatment of heart failure for five weeks which was not due to any significant alterations in the level of BNP.(Liu, Ngo et al. 2015)

1.3.5 Oxidative stress

1.3.5.1 Generation of reactive oxygen species (ROS)

In health, a fine balance between reactive oxygen species and antioxidants maintains 'redox neutrality'. ROS include the primary superoxide free radical ($O_2^{\cdot -}$), hydroxyl radical (OH^-), peroxynitrite ($ONOO^-$) and the non radicals such as hydrogen peroxide (H_2O_2). Their oxidising effects are counteracted by scavenging enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase, and also by NO. Whereas SOD neutralises superoxide, GSHPx reduces H_2O_2 and other hydroperoxidases, prevents the formation of the potent secondary free radical, OH^-. Therefore, GSHPx itself is considered to play a very prominent role in redox protection in the heart. Transgenic mice model with gain of function mutation in the gene for GSHPx had amelioration of left ventricular remodeling in comparison with wild type mice model. (Matsushima, Kinugawa et al. 2006) Catalase reduces H_2O_2 but to a lesser extent as GSHPx.

1.3.5.2 Sources of ROS in the failing heart.

Direct evidence for elevated ROS levels in failing myocardium has been demonstrated in adult mongrel dogs with the use of electron spin resonance spectroscopy combined with the nitroxide radical 4-hydroxy-2,2,6,6-tetramethyl-piperidine-*N*-oxyl.(Ide, Tsutsui et al. 2000) This also showed that OH^- could be derived from both $O_2^{\cdot -}$ and H_2O_2. When this increase in ROS cannot be matched with a commensurate increase in the amount of scavengers, the 'redox neutrality' shifts to 'redox stress'. Neutrophils, endothelial cells and cardiac myocytes are cells that generate ROS in heart failure whereas at the sub-cellular level, mitochondria, NAD(P)H oxidase, xanthine oxidase, uncoupled NO synthase, cytochrome c oxidase and archidonic acid metabolism are implicated.

Mitochondria in failing myocardium are known to produce more $O_2^{\cdot -}$ than normal mitochondria in the presence of NADH.(Tsutsui, Kinugawa et al. 2011) NAD(P)H

oxidase and/or xanthine oxidase are important in ROS generation by vascular endothelial cells whereas NAD(P)H oxidase is relevant in activated leucocytes.

The role of uncoupled NOS is also very important in the failing myocardium. Under physiological conditions, NOS 3 (eNOS) in the presence of its co-factor, tetrahydrobiopterin (BH4) consumes NADPH and generates NO from L-arginine and oxygen. Oxidative stress or deficiency of BH4 uncouples NOS leading to formation of more ROS including $ONOO^-$.

1.3.5.3 Effects of ROS in heart failure.

A multitude of deleterious effects are seen with ROS increase in heart failure including protein and lipid peroxidation, DNA damage, apoptosis, impairment of oxidation-contraction coupling, fibroblast activation and activation of matrix metalloproteinase. ROS also stimulate cardiac hypertrophy via the tyrosine kinase Src, GTP-binding protein Ras, protein kinase C, mitogen-activated protein kinases (MAPK), and Jun-nuclear kinase (JNK) (Tsutsui, Kinugawa et al. 2011)and also cause DNA breaks and mitochondria damage. ROS generation also affects skeletal muscle function and health, which can impair $VO_{2\ max}$ as well as exercise tolerance in heart failure.

1.4 PHARMACOLOGICAL TREATMENT OF SYSTOLIC HEART FAILURE.

1.4.1 ACE inhibitors and angiotensin receptor blockers

ACE-inhibitors have firmly established benefits in systolic heart failure in terms of mortality and HHF. They were also the first class of pharmacotherapy to demonstrate such

benefits. In the CONSENSUS trial(Swedberg and Kjekshus 1988), which was the first of these trials, enalapril use resulted in 31% reduction in mortality at 12 months in patients with severe heart failure relative to placebo, largely due to attenuation of progressive pump failure. In patients with left ventricular systolic dysfunction following acute myocardial infarction, captopril use was associated with statistically significant 19% reduction in all-cause mortality, 22% risk reduction for HHF and 37% risk reduction for development of severe heart failure.(Pfeffer, Braunwald et al. 1992) On this basis it is recommended not only to start treatment with a low dose of an ACE inhibitor but also to titrate upwards to the maximum tolerated dose(Hunt, Abraham et al. 2009). In the ATLAS trial, although high-dose lisinopril, was associated with an insignificant trend towards improved mortality compared to low-dose regimen, nonetheless, high-dose regimens significantly lowered the combined end point of mortality and hospitalization for any cause by 12 percent and hospitalizations for HF by 24 percent(Packer, Poole-Wilson et al. 1999).

Rationales exist for consideration of ARBs in systolic heart failure and include the non-ACE pathway for generation of angiotensin II such as local tissue chymases and intolerability to ACE especially with the accumulation of bradykinin. The ELITE II trial(Pitt, Poole-Wilson et al. 2000) assessed whether losartan 50mg daily was superior to captopril in improving survival outcomes in elderly heart failure patients. There were no significant differences in all-cause mortality (11.7 vs 10.4% average annual mortality rate) or sudden death or resuscitated arrests between the two treatment groups. In the VALIANT study(Pfeffer, McMurray et al. 2003), the effect of valsartan on mortality and morbidity in heart failure following myocardial infarction was compared to captopril alone and also in combination with captopril. In terms of mortality, with a hazard ratio in the valsartan group as compared with the captopril group of 1.00; 97.5 percent confidence

interval 0.90 to 1.11; P=0.98, it is clear that no major mortality difference exists between the two groups. In addition, no mortality benefit was found by combining the two medications. In the CHARM-added trial(McMurray, Östergren et al. 2003), candesartan in addition to an ACE inhibitor was associated with a significant reduction in the composite of all-cause mortality and HHF, driven mainly by reduction in HHF. The different outcomes in VALIANT and CHARM-Added trials may be explained by the different population characteristics: while candesartan was added to patients who remained symptomatic on an ACE inhibitor, valsartan was used in patients post acute myocardial infarction. Therefore, the candesartan group possibly had a more extensive stimulation of RAS than the valsartan group.

ARBs therefore are acceptable alternatives in heart failure patients who could not tolerate ACE inhibitors, but are not preferable as initial treatment nor is there a strong indication for combined therapy.

1.4.2. Mineralocorticoid-receptor antagonists

After successful inhibition of the effect of angiotensin II in systolic heart failure, attention shifted to inhibition of aldosterone activity. The RALES trial(Pitt, Zannad et al. 1999) studied the effect of spironolactone on mortality in patients with severe heart failure and moderate to severe left ventricular systolic dysfunction (NYHA III and IV; EF ≤ 35%) when added to the then standard heart failure regimen that included an ACE inhibitor, a loop diuretic and very likely, digoxin. Compared to placebo, spironolactone resulted in 30% reduction in risk of death, (HR 0.70; 95 percent confidence interval, 0.60 to 0.82; P<0.001) with positive effects on progressive pump failure as well as sudden cardiac death. In patients with moderate LV systolic dysfunction post acute myocardial infarction, eplerenone, a selective aldosterone blocker reduced all-cause mortality by 15% (P = 0.008) and cardiovascular deaths or hospitalizations by 13% (P = 0.002). Gynecomastia

did not occur, but hyperkalemia was more common than in the placebo group(Pitt, Remme et al. 2003). The mortality and HHF benefits of aldosterone antagonism in patients with mild heart failure symptoms (NYHA II and EF ≤ 35%) were also demonstrated in the EMPHASIS-HF trial(Zannad, McMurray et al. 2011) with a 37% reduction in the primary outcome of a composite of death from cardiovascular causes or hospitalization for heart failure. It is important to monitor potassium levels in patients on aldosterone antagonists.

1.4.3 Beta-adrenoceptor antagonists.

Four β -adrenoceptor antagonists have proven efficacy in systolic heart failure: metoprolol is a selective inhibitor of beta$_1$-adrenergic receptors. When metoprolol succinate was added to optimal medical treatment, the MERIT-HF(Hjalmarson, Goldstein et al. 2000) showed that it resulted in 34% relative risk reduction for all cause mortality in patients with moderate to severe heart failure. Deaths from progressive pump failure and from sudden cardiac arrest were both significantly reduced. In a similar result, bisoprolol, a highly selective β $_1$-adrenergic receptor antagonist, improved left ventricular dimensions and also reduced the risk of all-cause mortality in patients with moderate to severe systolic heart failure by 34% relative to placebo.(1994) Carvedilol and Nebivolol are classified as 'third-generation' β -adrenoreceptor antagonists due to their ability to cause endothelial-dependent vasodilatation through NO release, a property absent in the other β -adrenoceptor blockers used in heart failure treatment.(Kalinowski, Dobrucki et al. 2003) Carvedilol is a racemic lipophilic agent with selective α_1-adrenoreceptor–antagonist and nonselective β-adrenoreceptor-antagonistic activities in a 1:10 ratio.(Ruffolo, Sauermelch et al. 1990) Carvedilol was also shown in a rat model to possess antioxidant properties; it was able to rapidly inhibit ferrous initiated lipid peroxidation via scavenging of free radicals.(Yue, Cheng et al. 1992) Results from the COPERNICUS trial(Packer, Fowler et

al. 2002) showed that carvedilol reduced the combined risk of death or hospitalization for a cardiovascular reason by 27%, P=0.00002 compared to placebo in patients with severe left ventricular systolic dysfunction.

The SENIORS trial(Flather, Shibata et al. 2005) demonstrated a 14% reduction in the composite outcome of all cause mortality or cardiovascular hospital admission in patients with moderate heart failure, who were 70 years of age or above, and were treated with Nebivolol, when compared to placebo. This less than impressive effect has not impeded subsequent use of this drug.

The effect of β –adrenoceptor blockers in systolic heart failure may not be a class effect. For example, bucindolol, in spite of possessing a weak α_1-adrenoreceptor–antagonist effect and nonselective β-adrenoreceptor-antagonistic activities like carvedilol, failed to show mortality benefit in heart failure.(Eichhorn, Domanski et al. 2001) Bucindolol however has intrinsic sympathomimetic properties unlike carvedilol. It is not known whether this difference is particularly important in the observed lack of benefit with bucindolol.

1.4.4 Angiotensin-Neprilysin inhibition

Recently, inhibition of neprilysin, a neutral peptidase that normally degrades bradykinin, natriuretic peptides and other vasoactive peptides has been targeted therapeutically. In combination with valsartan, neprilysin inhibition was found to be superior to enalapril alone in the reduction of the combined primary end point of death from cardiovascular cause or heart failure hospitalisation with a statistaically significant relative risk reduction of 20% and 21% respectively at 27 months in patients with moderate to severe systolic heart failure.(McMurray, Packer et al. 2014)

1.4.5 The role of ‘ancient therapies’

1.4.5.1 Frusemide/Loop diuretics

Frusemide is the most commonly used loop diuretic in acute decompensated heart failure and its use has a class A recommendation but with a C level of evidence and this is a reflection of the ethical difficulty of organising a randomised placebo-controlled trial with frusemide in such a setting. Loop diuretics compete for the chloride site in the Na-K-2Cl carrier in the luminal membrane of the thick ascending loop of Henle and the macula densa in the distal tubule. This interaction prevents tubular re-absorption of sodium and potassium. Passive re-absorption of calcium is also impaired. Other loop diuretics include bumetanide, torsemide and ethacrynic acid. Frusemide is the most widely used clinically. The utility of frusemide is essentially to improve dyspnea and feeling of well being irrespective of whether it is given as a bolus or as an intravenous infusion.(Felker, Lee et al. 2011) This is achieved not only by diuresis but also by venodilatation, an effect noted to occur well before the commencement of diuresis in patients with acute pulmonary oedema.(Dikshit, Vyden et al. 1973) There is no convincing evidence that frusemide improves mortality in heart failure. On the other hand, loop diuretics may increase the risk of arrhythmic deaths through electrolytes imbalance such as hypomagnesemia, hyponatremia, hypocalcemia and especially hypokalemia.(Cooper, Dries et al. 1999) Furthermore, acute vasoconstriction has been observed with the intravenous administration of frusemide in chronic heart failure and is thought to be due to a reflex increase in sympathetic discharge and RAS stimulation.(Francis, Siegel et al. 1985) Patients with decompensated heart failure that also have chronic kidney failure present particular challenge to the use of intravenous loop diuretics. Diuresis in this subgroup of patients tend to worsen renal function and a meta analysis has found worsening renal function to be associated with a higher risk for mortality (odds ratio = 1.62; 95%

confidence interval 1.45-1.82, P < .001) and hospitalization (odds ratio = 1.30, 95% confidence interval 1.04-1.62, P = .022) (Damman, Navis et al. 2007)

In summary, the use of frusemide in decompensated heart failure remains ubiquitous but the potential side effects should be considered.

1.4.5.2 Digoxin.

Although digitalis-containing compounds have been in use for centuries, it was William Withering who in 1785 published his very popular monograph: 'An account of the foxglove and some of its medical uses', in which he scrupulously described the successful use of digitalis to treat congestive states, its effects on the heart and its toxic effects in overdose. Wade has published review on this.(Wade 1986) Digoxin, whilst able to potentiate inotropy, also exerts a negative chronotropic effect on the heart. By inhibiting the Na^+ -K^+ ATPase, digoxin causes increased intracellular Na^+, consequently activating the Na^+-Ca^{2+} exchanger to subsequently enhance influx of calcium and contractility. It also modulates the autonomic nervous system by inhibiting sympathetic outflow in heart failure and increasing parasympathetic tone.

The clinical use of the foxglove extract has been associated with seemingly unending controversies. Two trials: PROVED(Uretsky, Young et al. 1993) and RADIANCE(Packer, Gheorghiade et al. 1993) in the pre- beta-blocker and mineralocorticoid antagonism era, examined the effects of withdrawing digoxin from stable heart failure patients. In the PROVED study, patients were on digoxin and diuretics and 3 months withdrawal of digoxin led to increased incidence of treatment failures, lower ejection fractions, increased body weight, and higher heart rate and a fall in exercise

capacity even in patients with mild heart failure. Similar observations were noted in the RADIANCE study in which patients were on digoxin, diuretics and ACE inhibitor.

To examine the effect of digoxin on mortality, the largest digoxin trial, DIG(Group 1997) was designed as a randomized, double-blind clinical trial. Most patients were in NYHA II-III with LVEF of 45% or less and were treated with an ACE inhibitor. After 3 years, there was no difference in mortality between digoxin and placebo although there was a trend towards reduced death from worsening heart failure, a benefit that was found to be counter-balanced by an upward trend in arrhythmic deaths in the digoxin group. Digoxin use however, was associated with 6 percent fewer hospitalizations overall than in the placebo group, and fewer patients were hospitalized for worsening heart failure (26.8 percent vs. 34.7 percent; risk ratio, 0.72; 95 percent confidence interval, 0.66 to 0.79; P<0.001)

Post hoc analysis of the DIG trial has shown that in men, higher serum digoxin levels were associated with worsened crude all cause mortality (0.5-0.8 ng/mL, 29.9%; 0.9-1.1 ng/mL, 38.8%; and>or =1.2 ng/mL, 48.0%; P = 0.006 for trend) There was reduced mortality in the low range patients, no effect on mortality in the middle group and increased mortality in the high end group. These findings persisted after multivariable adjustments.(Rathore, Curtis et al. 2003) Similar associations were found in women as well.(Adams, Patterson et al. 2005)

In the light of all these, digoxin use in heart failure is now seems to be for symptomatic relief in patients in sinus rhythm who continue to be symptomatic on current optimal heart failure treatment. It provides additional rate control in patients with heart failure and atrial fibrillation- and even then, attention to serum digoxin level is important.

1.4.6 Specific niche treatment

1.4.6.1 Ivabradine

Ivabradine inhibits the "funny current" or I_f channel in the sinus node. The I_f channel is an inward depolarizing current that is activated in early diastole by hyperpolarization and is considered a key component of the 'membrane clock'. Diastolic depolarization of the channel occurs more rapidly with sympathetic discharge and more slowly with enhanced vagal tone, an effect mediated via cyclic AMP. The SHIFT study randomized 6558 patients with symptomatic heart failure and a left-ventricular ejection fraction of 35% or lower, in sinus rhythm and with heart rate 70 beats per min or higher whilst on optimal medical treatment including maximum tolerated doses of beta-blockers, to either ivabradine or placebo. The primary endpoint was the composite of cardiovascular death or hospital admission for worsening heart failure and was significantly lower in the ivabradine group (HR 0.82, 95% CI 0.75-0.90, p<0.0001). This was mainly driven by reduction in HHF.(Swedberg, Komajda et al. 2010) The European Society of Cardiology guideline of 2012 recommends ivabradine as add-on therapy to patients with strict criteria of sinus rhythm with LVEF ≤35 percent, a heart rate remaining at ≥70 bpm, and persisting symptoms (New York Heart Association class II to IV) despite treatment with an evidence-based dose (or maximum tolerated dose) of beta blocker, ACE inhibitor (or ARB), and an mineralocorticoid antagonist; and this indication is essentially to reduce HHF(McMurray, Adamopoulos et al. 2012)

1.4.6.2 Hydralazine and isosorbide dinitrate.

In 2005, BiDil, a combination of hydralazine and isosorbide dinitrate became the first drug ever to be approved for use on a specific racial group. This followed overwhelming evidence from the African-American heart failure trial (A-HeFT). The road map to A-

HeFT started from the Vasodilator- Heart failure trials (V-HeFT) I and II. In the V-HeFT I of 1986(Cohn, Archibald et al. 1986), men with impaired cardiac function and reduced exercise tolerance were double-blind randomised to conventional heart failure treatment (which included diuretics and digoxin at that time) plus either prazosin, or a combination of hydralazine and isosorbide dinitrate or placebo. Mean follow-up was 2.3 years by which time the mortality risk reduction among patients treated with both hydralazine and isosorbide dinitrate was 34 percent ($P<0.028$) compared to placebo. There was no statistical difference between placebo and prazosin.

Subsequent to this finding and the commencement of ACE inhibitors in heart failure (following the results of CONSENSUS trial),(Swedberg and Kjekshus 1988) the effect of hydralazine (300mg/day) and isosorbide dinitrate, H-ISDN (160mg/day) on mortality in heart failure was compared to enalapril in the V-HEFT II(Cohn, Johnson et al. 1991). At 2 years, enalapril was associated with statistically significant reduction in mortality over isosorbide dinitrate. Retrospective analysis of V-HeFT I and II suggested an association between race and response, thus in V-HeFT I, blacks had a significant survival response to H-ISDN (hazard ratio, 0.53; 95% confidence interval, 0.29 to 0.98), with no such benefits in whites. (Hazard ratio, 0.88; 95% confidence interval, 0.63 to 1.24)(Carson, Ziesche et al. 1999) In V-HeFT II, whites demonstrated higher survival with enalapril over H-ISDN whereas there was no difference in blacks with either therapy.

With this background in mind, as well as the fact that the balance between NO/superoxide/peroxynitrite is known to be tilted towards higher superoxide/peroxynitrite in blacks than in whites, (Kalinowski, Dobrucki et al. 2004) led to the design of the A-HeFT trial. Isosorbide dinitrate itself is a nitric oxide donor and a potent venodilator. The use of nitrates in clinical practice has long been known to be associated with nitrate tolerance, pseudo-tolerance and nitrate resistance (Elkayam, Kulick et al. 1987) Nitrate

resistance (or de novo hypo responsiveness to NO) can be due to excessive generation of ROS, reduced scavenging of ROS or a combination of both- features which are not lacking in heart failure.(Ide, Tsutsui et al. 2000, Matsushima, Kinugawa et al. 2006, Tsutsui, Kinugawa et al. 2011) Hydralazine on the other hand is not only a potent direct arterial dilator but also has been shown to be a highly potent radical scavenger against peroxynitrite as well as against superoxide generation by the xanthine oxidase and NADPH oxidase systems(Daiber, Oelze et al. 2005). As mentioned before, blacks tend to generate more superoxide and peroxynitrite and less NO than whites.(Kalinowski, Dobrucki et al. 2004) The A-HeFT(Taylor, Ziesche et al. 2004) enrolled 1050 black Americans with severe heart failure and randomized them to either H-ISDN or placebo. The study was terminated earlier due to significantly higher mortality in the placebo group, and with 43% reduction in the risk of death from any cause in the H-ISDN arm, 33% reduction in HHF and significantly improved quality of life in the H-ISDN arm. These benefits were obtained with the addition of H-ISDN to RAS inhibitors, aldosterone antagonists and beta-blockers.

1.5 NON-PHARMACOLOGICAL TREATMENT OF SYSTOLIC HEART FAILURE

Non-pharmacological treatment options in systolic heart failure are clearly complementary to optimal medical therapy and are never stand-alone therapies. These options would include:

1.5.1 Implantable cardiac defibrillators (ICD)

There is considerable evidence regarding the efficacy of ICD in primary prevention of sudden cardiac deaths in patients with systolic heart failure of both ischemic and non-ischemic aetiology.

The landmark MADIT I trial(Moss, Hall et al. 1996) evaluated the mortality benefits of ICD when compared with anti-arrhythmic drugs in 196 patients with prior myocardial infarction (MI) who also had non-sustained VT (NSVT) on monitoring, reduced LVEF (≤35%), and inducible sustained monomorphic VT during electrophysiology study (EPS) that was also inducible after administration of intravenous procainamide. In the ICD group, there was a statistically significant reduction in overall mortality, cardiac mortality, and arrhythmic deaths compared to the anti-arrhythmic arm.

The results from MADIT II(Moss, Zareba et al. 2002) trial obviated the need for EPS prior to implantation of ICD in patients with prior MI who have LVEF of 30% or less. With 1232 patients randomised to either ICD or conventional medical treatment, there was 31% relative risk reduction in death from any cause at 20 months in the ICD arm. It is noteworthy that no mortality benefit has been shown when ICD is implanted early post myocardial infarction, for instance, less than forty days as in DINAMIT trial(Hohnloser, Kuck et al. 2004) or at the time of surgical revascularisation as in the CABG-Patch trial.(Bigger 1997) It is possible that significant functional recovery may have occurred in these patients thereby reducing the chances of lethal arrhythmias in the first instance.

The DEFINITE trial(Kadish, Dyer et al. 2004) provided evidence for the use of ICD in patients with severe systolic heart failure of non-ischemic aetiology. Compared with medical therapy alone, ICD in addition to medical therapy resulted in a non-significant reduction in death from any cause, (P=0.08) and a significant reduction in the risk of death

from arrhythmia (P=0.006) Further evidence for the use of ICD in non-ischemic heart failure also comes from the SCD-HeFT trial which enrolled patients with either ischemic (52%) or non-ischemic aetiology (48%) and compared ICD with amiodarone and with placebo. Whilst amiodarone showed no mortality benefit over placebo, ICD significantly reduced mortality in both the ischemic and the non-ischemic groups.

1.5.2 Cardiac resynchronisation therapy (CRT)

The major landmark trials that paved the way for the widespread use of cardiac resynchronisation therapy in systolic heart failure date from 2001. The initial trials used soft clinical endpoints while the later trials studied hard clinical end-points of mortality and heart failure hospitalisation. The first of these trials was the Multisite Stimulations in Cardiomyopathies (MUSTIC)(Cazeau, Leclercq et al. 2001), which was a single-blind, randomised crossover study that evaluated the hemodynamic changes associated with biventricular pacing. The primary end-point of distance walked in six minutes increased significantly by 22 per cent with active pacing while the secondary endpoints of peak oxygen consumption improved significantly by 8 per cent (P< 0.03) together with the quality of life score that improved by 32 per cent (P <0.001). In likewise manner, the Pacing Therapies in Congestive Heart Failure (PATH-CHF) Study Group also evaluated the primary endpoint of exercise capacity with biventricular pacing. Oxygen uptake during bicycle exercise increased from 10.0 to 10.7 ml/kg/min at the anaerobic threshold (p = 0.2) and from 13.4 to 15.2 ml/kg/min at peak exercise (p = 0.002). The 6-min walk distance increased from 342 m at baseline to 386 m (p<0.001)(Auricchio, Stellbrink et al. 2002)

The Multicentre Insync Randomized Clinical Evaluation (MIRACLE)(Abraham, Fisher et al. 2002) of 2002 was the first double-blind randomized CRT trial and evaluated 453 patients. The primary end points of distance walked in six minutes improved in the treatment group relative to the control arm (+39 vs. +10 m, P=0.005), functional class (P<0.001), and quality of life (-18.0 vs. -9.0 points, P= 0.001). The peak oxygen consumption was part of the secondary endpoints and was significantly improved in the treatment group by 1.1ml/kg/min (P= 0.009). The MIRACLE-ICD(Young, Abraham et al. 2003) went a step further with the addition of ICD to CRT but with similar endpoints as the MIRACLE study. Although quality of life score and functional class improved significantly, there was no difference in 6-min walk distance, nonetheless, peak oxygen consumption improved by 1.1ml/kg/min (P=0.04). Surprisingly, no significant differences were observed in left ventricular size or function.

Hard clinical endpoints were evaluated in studies such as the COMPANION(Bristow, Saxon et al. 2004), CARE-HF(Cleland , Daubert et al. 2005) and RAFT(Tang , Wells et al. 2010) and the results have helped to firmly entrench CRT as a conventional mode of treatment in systolic heart failure.

Briefly, the Comparison of Medical Therapy, Pacing, and Defibrillator in Heart Failure, COMPANION (Bristow, Saxon et al. 2004) studied 1520 patients with advanced heart failure and evidence of left ventricular dyssynchrony for the combined primary endpoint of death from or hospitalisation for heart failure, and after twelve months, found a 34% reduction in the patients with biventricular pacemaker only, CRT-P (P<0.002) and a 40% reduction in the group with biventricular pacemaker plus ICD, CRT-D (P<0.001) Furthermore, CRT-P reduced the risk of the secondary end point of death from any cause by 24 percent (P=0.059), and a CRT-D reduced the risk by 36 percent (P=0.003). The lack of benefit in the secondary endpoint of death from any cause in the CRT-P arm of the

COMPANION partly led to the design of the Cardiac Resynchronisation- Heart Failure (CARE-HF) study(Cleland , Daubert et al. 2005) This study also recruited patients with advanced heart failure but used only CRT-P. Eight hundred and thirteen patients were followed up for up to 29 months. The primary end point was the time to death from any cause or an unplanned hospitalization for a major cardiovascular event and this was reduced by 34% relative to 'optimal' medical therapy alone (P<0.002) Thus the COMPANION and the CARE-HF studies provided overwhelming evidence for the use of CRT with or without defibrillator in patients with advanced heart failure.

Patients with mild to moderate systolic heart failure and evidence of ventricular dyssynchrony can also potentially benefit from cardiac resynchronisation. The RAFT trial demonstrated a clear mortality benefit in patients in NHYA II and III (with up to 80% of them in NYHA class II) who received CRT in addition to ICD and optimal medical treatment when compared to patients ICD and optimal medical treatment alone. After follow-up of 1798 patients for about 40 months, there was 25% relative reduction in the primary end-point of death from any cause or heart failure hospitalisation in the CRT-D group compared to ICD-Medical treatment group (P <0.001).

Even in milder forms of heart failure, CRT has shown promise. The Multicentre Automatic Defibrillation Intervention trial-CRT (MADIT-CRT)(Moss, Hall et al. 2009) recruited 1089 patients with NYHA functional class I and II and LVEF of 30% or less and randomized them to either ICD or CRT-ICD. Although there was 34% reduction in the primary end-point of death from any cause of heart failure hospitalisation, which ever occurred first, this reduction in the primary end-point was driven mainly by 41% in heart failure hospitalisation. Patients with left bundle branch and wider QRS duration responded better. Further support for the use of CRT in milder forms of heart failure comes from the

Resynchronisation reverses remodelling in systolic left ventricular dysfunction (REVERSE) trial(Linde, Abraham et al. 2008) which showed a significant reduction in heart failure hospitalisation as well as in indices of left ventricular reverse remodelling in patients with LVEF of 40% or less, NHYA class I and II, wide QRS complex, on optimal medical treatment and randomised to active CRT ± ICD programmed 'on' compared to the patients whose devices were programmed 'off'.

1.6 FOCUS ON CARDIAC RESYNCHRONISATION THERAPY.

1.6.1 How to measure response

Response to CRT has been measured in different ways and there seems to be no generally accepted single measure of response to CRT This in part could reflect the complexity of the effect of cardiac resynchronisation on the whole individual as well as underscoring a limited understanding of how CRT fully works in heart failure. **Table 1.1** summarises the different outcome measures in some of the major outcome trials.

Therefore, response to CRT could be categorised as clinical (with soft endpoints of functional status, quality of life perception and exercise capacity and also with hard endpoints of hospitalised heart failure or mortality), echocardiographic measures of reverse remodelling as well neurohormonal alterations. It is clear that the outcomes evaluated were not uniform among the different studies. Furthermore, even when similar endpoints were evaluated by different studies, the results were not always similar. For example, echocardiographic measures were not significantly improved in the MIRACLE-ICD.(Young, Abraham et al. 2003) These underscore the concept of considerable

heterogeneity of response to CRT, and result in a dilemma regarding choice of appropriate marker of recovery for clinical trials and for clinical practice.

1.6.2 Concept of considerable heterogeneity of response to CRT

A major issue with CRT is the lack of symptomatic improvement of some patients. Fornwalt et al(Fornwalt, Sprague et al. 2010) extensively evaluated the different response criteria used in twenty-six most cited publications of CRT response. Seventeen different response criteria were identified and the agreement among fifteen of these criteria were assessed in 426 patients in the PROSPECT study(Chung, Leon et al. 2008) using the Cohen's κ-coefficient. (Two out of the seventeen criteria could not be calculated from the PROSPECT study). The PROSPECT study itself was the largest, multicentre, prospective study that sought to identify which echocardiographic parameters best predict response to CRT.

Table 1.2 shows the different criteria identified.

The findings from Fornwalt et al(Fornwalt, Sprague et al. 2010) speak volumes. Using Cohen's κ-coefficient of ≤0.4 to indicate poor agreement, <0.4 $_{K}$ < 0.75 as moderate and ≥0.75 as high agreement, up to seventy-five percent of the comparisons showed poor agreement, 21% showed moderate agreement, while only 4% showed strong agreement. Specifically, the 15 response criteria as a group showed a poor response agreement, (κ=0.22±0.24 median=0.14, range=−0.2 to 0.97), the seven echocardiographic response criteria also showed poor agreement among each other, (κ=0.35±0.28, median=0.29, range=−0.2 to 0.88), the seven clinical parameters showed a moderate agreement among each other, (mean κ=0.44±0.23, median=0.43, range=0.14 to 0.97) whereas the worst agreement was the comparison between echocardiographic and clinical parameters (mean κ=0.05±0.05, median=0.04, range=−0.03 to 0.17). Another reason that

may affect the heterogeneity of reported response rates is the duration of follow-up. While most studies followed up patients for 3-6 months, the CARE HF trial extension phase(Cleland, Daubert et al. 2006) has shown that CRT has consistent, progressive benefits for up to 37.4 months of follow-up.

1.6.3 How does CRT work?

The basic rationale for the use of CRT in heart failure is to 'correct' an abnormal left ventricular activation delay relative to the right ventricle. Patients are identified traditionally, (and currently) by the presence of prolonged QRS duration on the electrocardiograph and more specifically, left bundle branch block (LBBB) morphology. Different intra-cardiac perturbations can occur with LBBB and include abnormal ventricular septal motion that results in abnormal inter-ventricular pressure gradients. There can be delays with aortic and mitral valve opening and closing times as well as delay in the onset of left ventricular systole leading to ventricular-atrial gradient and hence, diastolic mitral regurgitation.(Nishimura, Hayes et al. 1995) Delayed left ventricular activation is the basis for inter-ventricular mechanical dyssynchrony and therefore left ventricular mechanical inefficiency: all of these can be improved acutely with biventricular pacing. For example, Auricchio et al(Auricchio, Stellbrink et al. 1999) demonstrated an acute increase in maximum LV pressure derivative (LV+dP/dt) and aortic pulse pressure (PP) at the onset of biventricular or left ventricular pacing in patients with wide QRS duration; this effect was greater than with right ventricular pacing alone. However, simply correcting mechanical contractile dyssynchrony does not fully explain how CRT confers symptomatic benefits to patients. Indeed, results from the landmark trial, PROSPECT(Chung, Leon et al. 2008) showed that no single echocardiographic parameter of inter-ventricular or intraventricular mechanical dyssynchrony is able to predict response to CRT.

Furthermore, the EchoCRT (Ruschitzka , Abraham et al. 2013), a randomised double blind prospective multicentre trial, subsequently showed that in patients with moderate to severe heart failure and with echocardiographic evidence of mechanical dyssnchrony but no electrical dyssynchrony on the ECG, CRT failed to improve mortality or heart failure hospitalisation; instead, increased mortality was observed in the group randomised to CRT 'on' compared to the CRT 'off' group (P=0.02). Nonetheless, it is thought that the acute mechanical changes induced by CRT could in the long run induce chronic adaptive changes that add to the beneficial effects of the procedure. These chronic adaptive changes may be intra-cardiac or extra-cardiac.

1.6.3.1 The Neurohumoral effect of CRT

1.6.3.1.1. The natriuretic peptide:

Further analysis from the CARE-HF(Cleland , Daubert et al. 2005) with regards to NT-proBNP has shown two things. First, the level of NT-proBNP prior to CRT intervention predicts mortality in both the CRT and non-CRT study groups. This is true for all deaths, sudden cardiac deaths and deaths from progressive pump failure. Second, the NT-proBNP levels three months post CRT implant was a stronger predictor of mortality than the baseline levels. In CRT patients with <median baseline NT-proBNP levels, the proportion of sudden death was 41% (absolute rate 5%), and the proportion of death from pump failure was 27% (absolute rate 3%). Amongst CRT patients with ≥median baseline NT-proBNP levels, the proportion of sudden death was lower (29%), but the absolute rate was higher (15%) (Berger, Shankar et al. 2009).

In the Netherlands, Hoogslag et al(Hoogslag, Hoke et al. 2013) found similar results in a retrospective analysis of 170 patients that received CRT and were followed up for six months: although of these patients, 66% improved on the basis of clinical parameters, 58% on the basis of echocardiographic measures and 54% based on NT-proBNP, yet NT-proBNP response showed better agreement with echocardiographic response than with clinical response and patients with neurohormonal response demonstrated a superior long-term outcome compared to patients without neurohormonal response (log-rank P = 0.02). It may therefore be construed that by reversing left ventricular remodelling and volumes, CRT also ameliorates some of the maladaptive systemic neurohumoral response of heart failure.

1.6.3.1.2. Catecholamine spill and signalling

Several studies have shown an improvement in adrenergic signalling following CRT. Indirect evidence of this includes reduction in mean heart rate as well as increase in heart rate variability (Auricchio, Stellbrink et al. 2002). More direct evidence was provided by Cha et al(Cha, Chareonthaitawee et al. 2011) who evaluated presynaptic cardiac sympathetic activity as determined by ^{123}I-MIBG scintigraphy in 24 patients at baseline and then 6 months post CRT implantation. Response to CRT was defined as a reduction in LVESV index of at least 15%. The delayed heart/mediastinal ratio of ^{123}I-MIBG increased (from 2.04 to 2.30; P=0.04) and the heart/mediastinal washout rate decreased (from 39% to 21%; P=0.004) in responders only. Furthermore, the changes in the LVESV index after CRT were significantly correlated with delayed heart/mediastinal ratio (r=−0.42; P=0.048;) and heart/mediastinal washout rate (r=0.45; P=0.03)

Positron emission tomography (PET) offers better sensitivity and resolution than scintigram and also overcomes the limitation of relying on an index ratio between tracer

uptake in the heart and the upper chest. Capitanio et al (Capitanio, Nanni et al. 2015) therefore used ^{11}C-hydroxyephedrine (HED) PET/CT to evaluate cardiac sympathetic activity in ten patients with idiopathic heart failure who received CRT. HED uptake was measured at baseline, CRT was implanted and turned 'off', then 1 week after, HED uptake was measured again and CRT turned 'on'. Subsequent HED uptake assay was performed one week and three months later. The results showed a heterogeneous distribution of HED in the myocardium at baseline, but at three months, although there was no significant change in the total uptake of HED, there was significantly improved homogeneity, reflecting increased uptake in areas with greater neuronal damage. This improved uptake was also associated with a fall in arterial HED level and was considered to be a reflection of late systemic response. Because these changes were absent at one week of CRT 'on', the authors argued that the results were not due to a different activation pattern induced by CRT pacing but rather a late systemic response to CRT.

Further evidence for extra-cardiac sympathetic modulation of CRT was provided in the study of muscle sympathetic nerve activity (MSNA) using the peroneal nerve in eleven patients who had CRT and were compared to heart failure patients with no CRT and also with normal controls. The CRT group showed a significant fall in MSNA bursts/minute at rest (48.9 ± 11.1 bursts/min vs 33.7 ± 15.3 bursts/min, $P < 0.05$) and with handgrip (62.3 ± 13.1 bursts/min vs 46.9 ± 14.3 bursts/min, $P < 0.05$) at three months of follow-up while the heart failure group with no CRT conversely demonstrated a significant increase (Kuniyoshi, Martinelli et al. 2014).

1.6.3.2. Molecular adaptations

Spragg and Kass (Spragg and Kass 2006) have found striking regional molecular changes in dogs with pacing induced heart failure with superimposed LBBB, which were absent in dogs with pacing induced heart failure but with no LBBB. In the LBBB group,

the lateral wall of the left ventricle (the region of delayed activation), showed increased phosphorylation of the mitogen-activated protein kinase ERK42/44, and reduced expression of SERCA 2a, phospholamban and connexion 43. In yet another subsequent study of twenty-two dogs with pacing-induced dyssynchronous heart failure, in which half received CRT, Kass et al (Chakir, Daya et al. 2008)showed that CRT ameliorated the abnormal regional stretch kinases. Global up-regulation of apoptotic proteins in dyssynchronous hearts as measured by terminal deoxynucleotide transferase–mediated dUTP nick-end labeling (TUNEL), caspase-3 proteolytic activity and nuclear poly ADP-ribose polymerase cleavage (PARP) were also down-regulated with CRT through up-regulation of anti-apoptotic proteins.

Furthermore, CRT has also been shown to modulate mitochondrial metabolic pathways and ATP production in dog models. Specifically, there was an increase in the expression of pyruvate carboxylase (necessary for replenishment of the Krebs cycle) as well as an improvement in oxidative stress achieved via an increase in the expression of thioredoxin peroxidase reductase, and also through post-translational modification of mitochondrial ATP synthase by a reduction in cysteine residue. (Agnetti, Kaludercic et al. 2010, Wang, Foster et al. 2011) These results strongly suggest that CRT does far more than correction of intra-cardiac mechanical dyssynchrony.

1.6.3.3. Vascular endothelial function.

Few studies have evaluated the effect of CRT on vascular endothelial function. In a study of thirty-three CRT patients in whom flow mediated dilatation (FMD) was used to assess endothelial function, baseline endothelial dysfunction predicted response to CRT at 3 months: responders had significantly worse baseline FMD than non responders. There was an improvement in endothelial function in responders, which however did not attain

statistical significance (P=0.12). Nonetheless, change in FMD correlated with change in 6MWD at 3 months (r = 0.34, P = .05)(Akar, Al-Chekakie et al. 2008). A study by Santini et al(Santini, Capria et al. 2013) of 2013 showed a significant improvement in FMD twelve months post CRT (8.8 ± 4.8% vs 4.1 ± 3.8%; P < .05).

The effect of CRT on the microvasculature has also been evaluated. This study involved twenty-two patients with severe dilated cardiomyopathy. Ten were randomised to optimal medical therapy and twelve to CRT. Endothelial function was measured by reactive hyperemia peripheral arterial tonometry (RH-PAT), follow-up was for 3 months. NYHA class, LVEF, end-diastolic left ventricular dimension and plasma levels of brain natriuretic peptide (BNP) were significantly and equally improved in both arms but only CRT significantly increased RH-PAT index (medical therapy group: 1.5 ± 0.2 to 1.5 ± 0.3, p = 0.824; CRT group: 1.4 ± 0.2 to 1.7 ± 0.4, p = 0.003). This increase in RH-PAT also correlated with an increase in cardiac output that occurred with CRT only but not with medical treatment (r = 0.600, p = 0.003).(Enomoto, Yamabe et al. 2011)

1.6.3.4 Any effects on platelet functions?

Human platelets are small, anucleate discoid corpuscles fragmented from megakaryocytes. Measuring 3-5µm, platelets generally have a life span of about 7 days unless activated earlier. The role of platelets in health and disease is increasingly understood to go beyond the traditional function of haemostasis to include mediation of inflammation, immunopathogenesis and maintenance of vascular integrity.

Platelets have membrane receptors of the *integrin family* (which includes glycoprotein IIb-IIIa complex for fibrinogen binding, glycoprotein Ia-IIa receptor, fibronectin receptors) and *Leucine-rich repeat* receptors (which include Gp Ib-IX-V that interacts

with collagen, von Willebrand Factor (vWF), thrombospondin, P-selectin, and leukocyte integrin Mac-1; and many toll-like receptors necessary for inflammation) There are also numerous transmembrane receptors like thrombin receptors, ADP receptors (P2Y1, P2Y12), prostaglandin receptors (thromboxane A2, PGE2, PGI2), lipid receptors, chemokine receptors and cathecolamine receptors.

Beside these receptors, platelets are also rich in granules: α-granules, dense granules and lysosomes. Alpha granules contain adhesion molecules, growth factors, coagulation and fibrinolytic factors, chemokines and immunologic factors. Dense granules on the other hand contain ADP, GTP, ATP, ionic calcium, magnesium, phosphates and serotonin. Lysosomal granules contain similar enzymes like other lysosomes elsewhere which include hydrolases and cathepsins. (Review by Matthew Linden 2013) (Linden 2013)

1.6.3.4.1 Role of thrombospondin-1 (TSP-1) release from alpha granules:

Vascular haemostatic thrombosis usually starts with adhesion of platelets to the injured surface. In the setting of shear force, this adhesion is facilitated by large multimers of von Willebrand factor (vWF) stored in the endothelium and platelets. ADAMTS13 cleaves the large multimers of VWF to prevent excessive and unchecked platelet aggregation. TSP1 is also a mild reductase of vWF and a competitive inhibitor of ADAMTS13 and in one report, up to 70% of the activity of ADAMTS13 was inhibited by TSP1(Wang, Liu et al. 2010).

1.6.3.4.2 Impaired platelet nitric oxide signalling

Shah et al(Shah, Passacquale et al. 2011) demonstrated that although patients with heart failure had increased basal platelet NOS activity relative to controls, there was no increase in platelet NOS activity with albuterol in contrast with the control group. Furthermore, there were increased basal and stimulated superoxide levels in the heart failure group as

well as decreased scavenging capacity for superoxide in heart failure group compared with the control group. The authors therefore reasonably concluded that in the setting of heart failure, platelet produce less bioactive NO.

1.7. SCOPE OF THE PRESENT STUDY

The benefits of CRT can be categorised as:

1. Improved exercise tolerance
2. Improved left ventricular systolic function
3. +/- Reduced left ventricular excitability.

The precise bases underlying these changes remain controversial. For example, other potential benefits of CRT within the heart might include

a. Improvement of diastolic ventricular interactions
b. Amelioration of septal ischemia.

Furthermore, restoration of coordinated left ventricular contraction might theoretically affect the neurohumoral milieu of heart failure, reducing homeostatic function to platelets, and thus reducing afterload.

The planned PhD studies will evaluate the central-peripheral interactions in CRT to determine the changes induced by the process and their various roles in modulating overall clinical response.

1.7.1 Principal Hypothesis:

That optimal outcome in CRT is engendered according to degree of correction of dyssynchrony and is achieved via a combination of:

1. Amelioration of Peripheral Circulatory Dysfunction
2. Reversal of left ventricular contractile dysfunction.

1.7.2 Secondary Hypotheses

1. That change in function is proportional to change in dyssynchrony (and via change in peripheral circulatory dysfunction.)
2. That change in cardiac excitability is proportional to change in dyssynchrony via:
    a. Change in neurohumoral activation
    b. Change in redox stress

1.8 TABLES AND FIGURES FOR CHAPTER 1

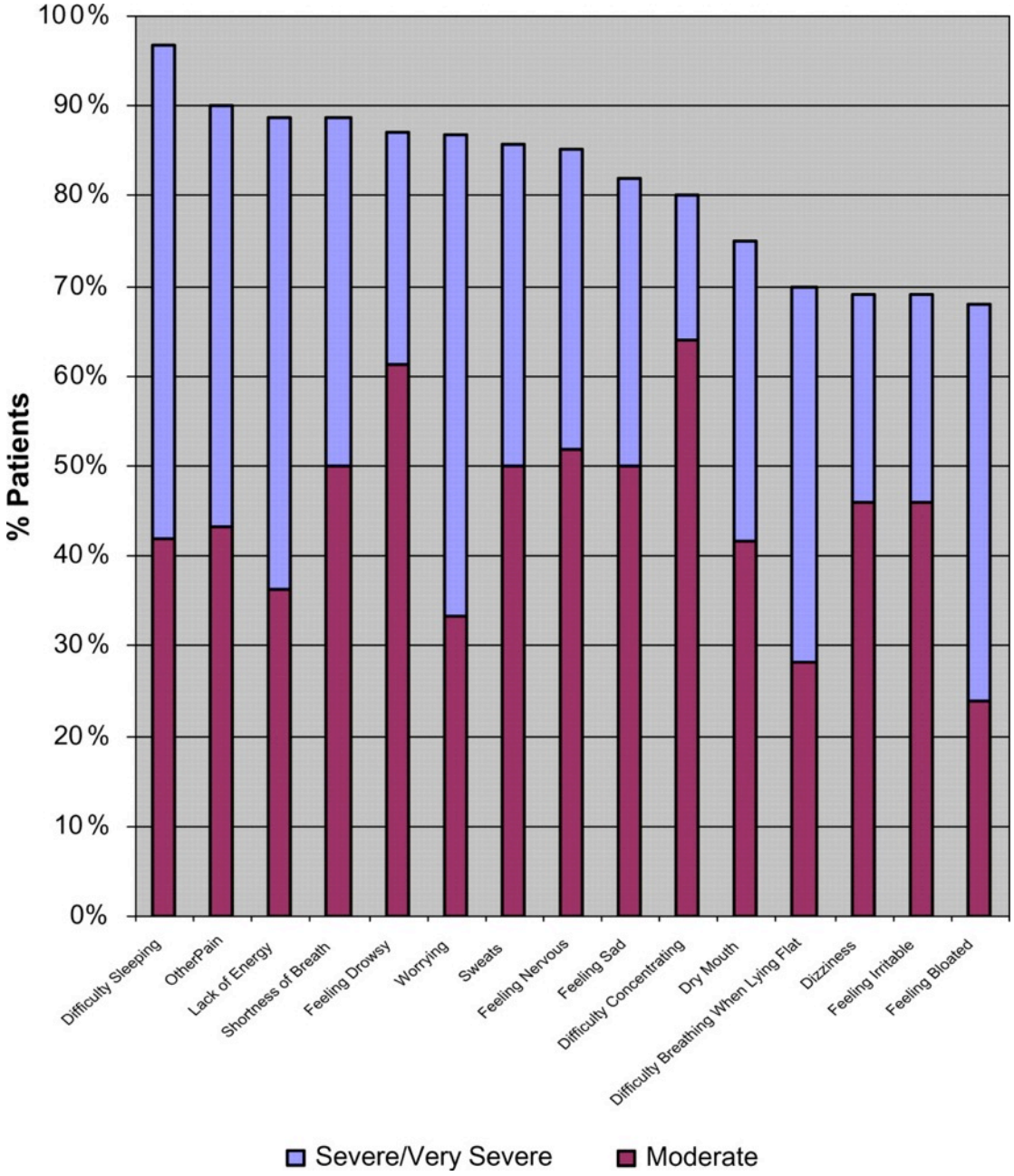


Figure 1.1 *Select symptom severity among a cohort of patients with heart failure described as moderate to severe/very severe.(Zambroski, Moser et al. 2005)*

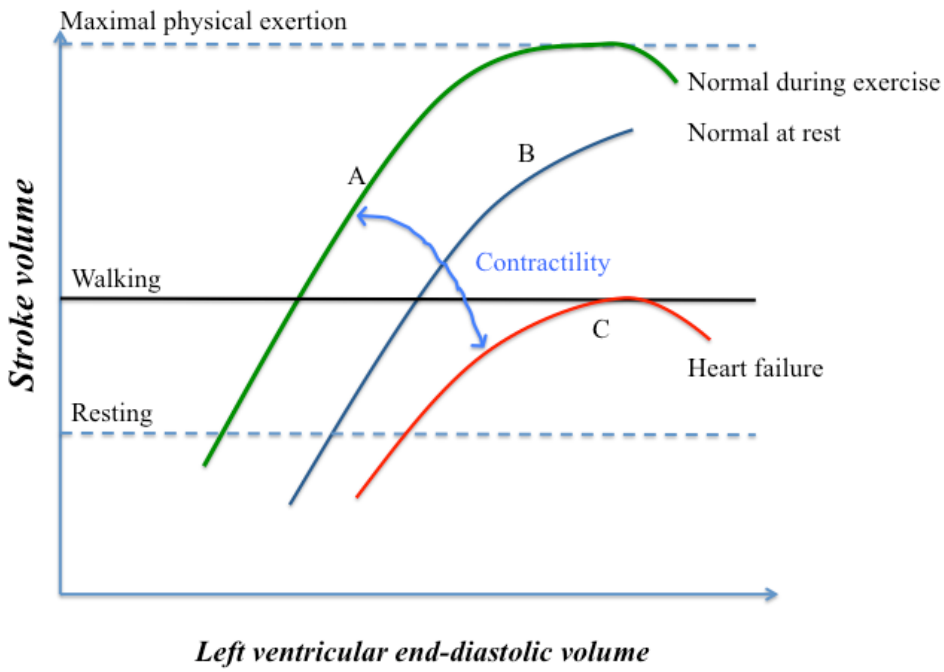


Figure 1.2: *Frank-Starling Relationship.*

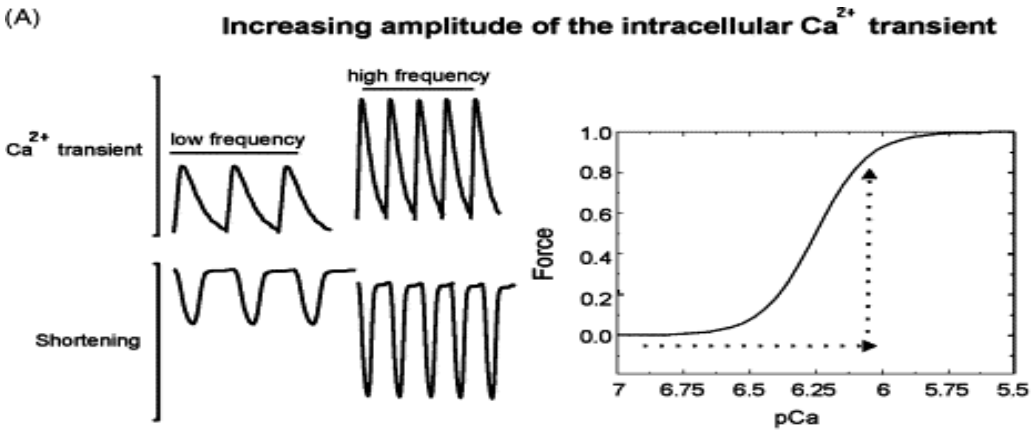


Figure 1.3: *Normal Bowditch effect from Palomeque et al*([Palomeque, Vila Petroff et al. 2004])

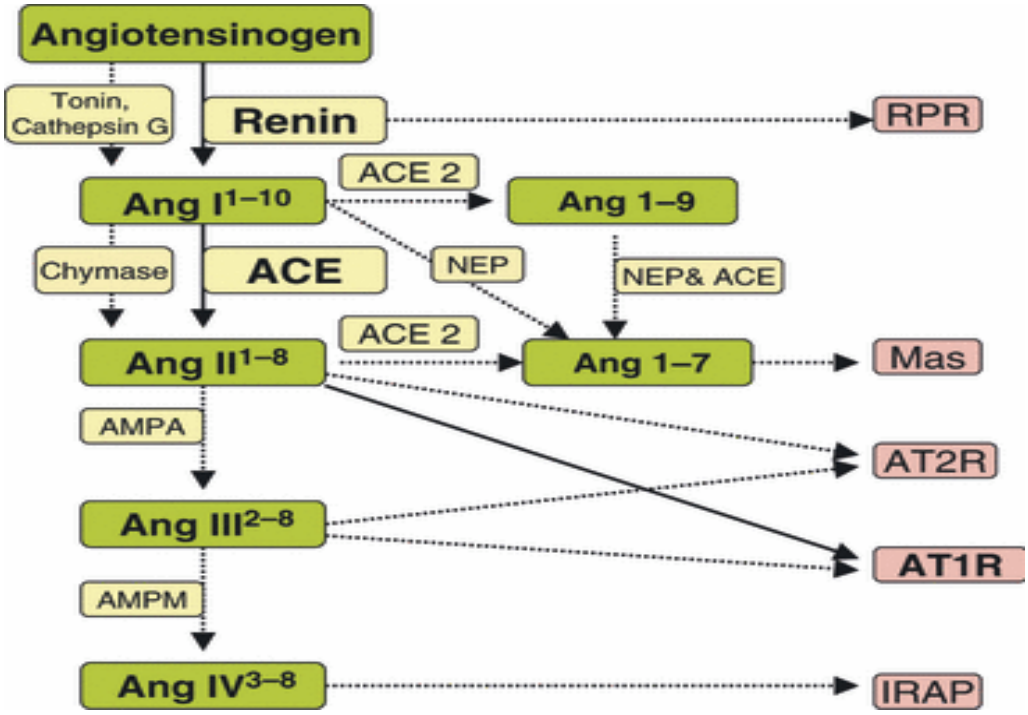


Figure 1.4: *Current view of the expanded renin - angiotensin system. RPR, renin/prorenin receptor; Mas, mas oncogene, receptor for Ang 1–7; AT2R, angiotensin type 2 receptor; AT1R, angiotensin type 1 receptor, IRAP, insulin - regulated aminopeptidase; Ang IV receptor AMPA, aminopeptidase A; AMPM, aminopeptidase M; ACE, angiotensin - converting enzyme; ACE2, angiotensin - converting enzyme 2; NEP, neutral endopeptidase.(Fyhrquist and Saijonmaa 2008)*

Table 1.1 Outcome measures in major CRT trials

Study	Description	Duration	Outcome measures
MUSTIC(Cazeau, Leclercq et al. 2001)	Single blind randomized crossover study: N=67 Severe heart failure NYHA class III QRS duration ≥ 150ms	3 months	***Primary outcome:*** 6 MWD. ***Secondary outcomes:*** QOL score, VO2 max, hospitalizations related to heart failure, the patients' treatment preference (active vs. inactive pacing), and the mortality rate
PATH-CCF (Auricchio, Stellbrink et al. 2002)	Single blind randomised trial. N= 41 Severe heart failure NYHA class III or IV QRS duration ≥ 120ms Any aetiology.	12 months	***Primary outcomes:*** VO2max, 6MWD ***Secondary outcomes:*** NHYA, QOL score
MIRACLE (Abraham, Fisher et al. 2002)	First double blind randomised trial. N= 453 Severe heart failure NYHA III or IV LVEF ≤ 35% QRS ≥ 130ms Both IC and NIC	6 months	***Primary outcomes:*** QOL score, NYHA, 6MWD ***Secondary outcomes:*** VO2 max, exercise duration, neurohormones, LV volumes and EF, hospitalisation rates
COMPANION (Bristow, Saxon et al. 2004)	Randomised controlled trial. N=1520 Severe heart failure. NYHA III or IV LVEF ≤ 35% QRS ≥ 120ms Both IC and NIC	16months	***Primary outcomes:*** Composite of death or hospitalisation from any cause. ***Secondary endpoint:*** Death from any cause

	aetiologies		
CARE-HF (Cleland , Daubert et al. 2005)	Randomised controlled trial. N: 404 Severe heart failure. NYHA class III or IV LVEF ≤ 35% LVEDV ≥ 30mm QRS duration ≥ 120ms No ICD	18months	***Primary outcome:*** Composite of death from any cause or hospitalisation for a major cardiovascular event. ***Secondary outcomes:*** NHYA, QOL score, NT-proBNP, LV volumes and EF
RAFT (Tang , Wells et al. 2010)	Double blind randomised trial. N= 1798 Mild to moderate heart failure: NYHA class III or III LVEF ≤ 30% QRS duration ≥ 120ms or paced QRS ≥ 200ms	18months -3.3 years	***Primary outcome:*** Composite of death from any cause or heart failure hospitalisation. ***Secondary outcomes:*** death from any cause, death from CVS cause, heart failure hospitalisation, NYHA class
MADIT CRT (Moss, Hall et al. 2009)	Open label. N= 1820 Mild heart failure: NYHA class I or II IC and NIC aetiologies LVEF ≤ 30% QRS duration ≥ 130ms	2.4 years	***Primary outcome:*** Death from any cause or heart failure hospitalisation
REVERSE (Linde, Abraham	Double blind randomised. N= 684	12 months	***Primary outcome:*** Heart failure clinical composite

et al. 2008)	Mild heart failure Primary ICD indication NYHA class I/II LVEF ≤ 40% LVEDD ≥ 55mm QRS duration ≥ 120ms		response. ***Secondary outcome:*** LVESVI

6MWD: Six-minute walk distance; QOL: Quality of life; LVESVI: Left ventricular end systolic volume index. ICD: implantable cardioverter defibrillator; LVEDD: left ventricular end-diastolic dimension; LVEF: left ventricular ejection fraction; IC: ischemic; NIC: non-ischemic; NYHA: New York Heart Association.

Table 1.2 Different response criteria evaluated in major CRT trials

Response criteria
Echocardiographic
1. ↑LVEF ≥5% (absolute)
2. ↑LVEF ≥15%
3. ↓LVESV ≥10% and did not die of progressive HF within 6 months
4. ↓LVESV >15%
5. LVESV <115% of baseline
6. ↓LVESVI >15%
7. ↓LVEDV >15%
8. ↑Stroke volume ≥15%
Clinical
9. ↓NYHA ≥1
10. ↓NYHA ≥1 and did not die of progressive HF within 6 months
11. ↓NYHA ≥1 and ↑6MWD ≥25%
12. ↓NYHA ≥1 and ↑6MWD ≥25% and did not die of progressive HF within 6 months
13. ↑6MWD >10%, no heart transplant, did not die of progressive HF within 6 months
14. (↓NYHA ≥1 or ↑$\dot{V}O_2$max >10% or ↑6MWD >10%) and alive, no hospitalization for decompensated HF
15. Two of 3:
↓NYHA ≥1
↑6MWD ≥50 m
↓QOL ≥15

16. Clinical composite score improved
Combined
17. (↑LVEF ≥5% {absolute} or ↑6MWD ≥30 m) and (↓NYHA ≥1 or ↓QOL ≥10)

↑ indicates increase; LVEF, left ventricular ejection fraction; ↓, decrease; HF, heart failure; LVESV, left ventricular end-systolic volume; LVESVI, LVESV indexed by body surface area; LVEDV, left ventricular end-diastolic volume; NYHA, New York Heart Association functional class; 6MWD, 6-minute walk distance; $\dot{V}O_2$max, oxygen consumption at peak exercise; and QOL, quality-of-life score. Adapted from Fornwalt et al Circulation.2010; 121: 1985-1991(Fornwalt, Sprague et al. 2010)

CHAPTER 2:

METHODS AND RESULTS

METHODS

2.1 Recruitment of Participants

Patients who were scheduled for routine CRT insertion with or without AICD implantation at The Queen Elizabeth hospital (TQEH), the Lyell McEwin Hospital (LMH) and the Flinders Medical Centre (FMC), irrespective of the underlying aetiology of heart failure, were invited to participate in the study. Prospective participants were initially contacted by telephone call and consenting participants were consecutively enrolled.

Exclusion criteria include anticipated inability to perform cardiopulmonary exercise testing due to non-cardiac causes like severe osteoarthritis or severe chronic obstructive airway disease; severe renal impairment (creatinine > 265.2μmol/L or on dialysis) because of the use gadolinium; life expectancy less than 1 year due to non-cardiac causes, concurrent treatment with inhibitors of platelet ADP receptor activation e.g clopidogrel, and inability to give consent.

2.2 Power calculation

The primary end point of the study will be change in augmentation index response to salbutamol post CRT.

With a total of 33 patients, and paired analysis, the study was powered to detect approximately a 1.25 SD difference in augmentation index in response to salbutamol at α =0.05, β =0.8 level.

Approval for the study was granted by the Ethics and Human Research Committee of The Queen Elizabeth Hospital.

2.3 Study Protocol:

Consenting participants were required to present to TQEH for baseline evaluations within two weeks prior to the planned CRT implant. The baseline evaluations included clinical evaluations, a self-administered questionnaire, physiological assessments, echocardiographic evaluations, and biochemical analyses. Official interpreters were utilized for patients who were not fluent in English language. Immediately post-CRT implantation, electrical parameters were measured through the device. All the above baseline evaluations were repeated 6 months after CRT implant.

2.3.1 Clinical evaluations:

Baseline patients' characteristics were delineated. The underlying etiology of heart failure was classified as either ischemic or non-ischemic. The presence of other cardiovascular risk factors was sought. Hypertension was defined as blood pressure of up to or more than 140/90 mmHg (or less if on antihypertensive). Likewise, dyslipidemia and diabetes were defined according to current guidelines or if patient was on treatment for either or both of them. Smoking status was also documented. Functional status was determined, based on the New York Heart Association functional class, and quality of life was evaluated using the standard (and well-validated) Minnesota Living with Heart Failure Questionnaire (MLHFQ)(Rector, Carson et al. 2012)

2.3.2 Physiological evaluations:

2.3.2.1 Radial artery applanation tonometry.

Peripheral vascular endothelial function was assessed by changes in augmentation index (AIx) as defined by Kelly et al(Kelly, Hayward et al. 1989), using radial artery applanation tonometry as previously described (Cameron, McGrath et al. 1998) (Ngo, Sverdlov et al. 2009). To summarise, patients were first rested in a supine position for 30minutes. Using a commercially available pulse waveform analyser, the SphygmoCor system (AtCor Medical, Sydney, Australia, model CvMS V9) baseline AIx was computed as the average of three readings. 300mcg of sublingual nitro-glycerine (GTN) was administered and augmentation index measured every 5 minutes for twenty minutes. The difference between the lowest value of AIx with GTN and the baseline AIx (that is, the maximum change in AIx with GTN) is a measure of endothelium- independent nitric oxide signalling. (Donald, Charakida et al. 2006) Subsequently, 400mcg of inhaled salbutamol was administered and measurements were repeated every 5 minutes for twenty minutes. The difference between the lowest Aix with salbutamol and the baseline (the maximum change in AIx with salbutamol) is a measure of endothelium-dependent nitric oxide signalling (Donald, Charakida et al. 2006). Using the acquired radial artery waveform, a validated, generalized transfer function was used to generate the corresponding central aortic pressure waveform from which the augmentation indices were calculated. This is depicted in **Figures 2.1(A-C).** Because AIx inversely correlates with heart rate, all measurements were indexed to a heart rate of 75 beats per minute and only high fidelity tracings were used.

2.3.2.2 Six- minute walk distance. (6MWD)

The protocol for the 6MWD has been described.(2002) This test was performed indoors in a dedicated and marked hallway that is 30 metres long and has a hard floor. Cones were used to delineate the turn-around points. The procedure was duly explained and patients were asked to walk as far as possible and as fast as they could for a timed period of 6 minutes. They were allowed to slow down, to rest or to lean against the wall if necessary, at their own discretion and to resume walking again at their possible fastest pace. Running or jogging was not allowed. The test was terminated if patient developed chest pain, became unduly dyspnoeic, or looked ashen with significant diaphoresis. If patient ordinarily mobilised with a walking aid, he or she was allowed to use the same during the test. The maximum distance walked at 6 minutes was recorded for each patient.

2.3.2.3 Cardiopulmonary exercise testing (CPET)

CPET was performed according to established guidelines. (Society and Physicians 2003, Balady, Arena et al. 2010) Briefly, a bicycle ergometer (Model: ergoline /100/200 GmbH, Germany) and linked to ExpAir Medisoft S.A Belgique 1.31.02 software were utilised in all cases. Before performing each test, the equipment was calibrated both for airflow or volume, including low and high flows (with calculated volumes within +/-3%) using a 3Litre syringe and also for gases with carbon dioxide set at 4% and oxygen at 20%. A semi- automated progressive incremental (ramp) protocol in which the pre test was set at 0 watts while the workload started from 10watts and increased by 10 watts every minute, was used. Patients pedalled for a minute on the pre test setting of 0 watts before the loading commenced. They were encouraged to exercise as long as possible, ideally for up to 8 to 12 minutes, and especially aiming to achieve a respiratory exchange ratio (RER) of 1 and above, with cycling rate kept at 60-70 revolutions per minute. Volitional exhaustion was the usual endpoint although exercise was terminated if patient developed chest pain, acute ischemic changes on the ECG or hypotension. After

unloading, the patient pedalled for a further 1 minute. Measurements of oxygen consumption, (VO_2) carbon dioxide output (VCO_2) ventilator equivalent, (VE) and RER were automatically acquired and finally averaged and displayed at 10 seconds interval. **Figure 2.2 A-B** shows the instruments used. Measured gas volumes are graphically displayed in **Figure 2.3**. The highest reading of the three last averages of VO_2 max at peak exercise was chosen as the VO_2 max as shown in **Table 2.1**

2.3.2.4 Platelet aggregometry test.

Platelet response to nitric oxide was assessed in vitro by whole blood aggregometry test according to previously described protocol by Chirkov et al(Chirkov, Holmes et al. 1999). Briefly, 9mL of whole blood was collected into plastic tubes containing 1mL of acid citrate anticoagulant, which helps to preserve platelet function during the test. The blood was allowed to stand at room temperature for 20 minutes. 500µL of normal saline was pre-warmed to 37^{o}c in a 4-channel impedance aggregometer (Chrono-Log corporation, model 700, shown in **Figure 2.4**), after which 450µL of whole blood was added to the saline and both pre warmed for 5 minutes. All tests were run at 37^{o}c and a stirring speed of 900 rpm. Platelet aggregation was tested by the addition of 5µL of ADP 2.5µM and response was measured as the amount of electrical impedance (in Ohms) at 7 minutes. A repeat run was subsequently performed with the addition of 10µl of sodium nitroprusside (SNP) 10µM and then ADP and the results were used to calculate the percentage inhibition ADP-induced platelet aggregation by nitric oxide. Only results with ADP-induced electrical impedance response greater than 4 ohms were used. Patients who were on platelet ADP inhibitors like clopidogrel were required to stop the medication 2weeks prior to testing. This is illustrated in **Figure 2.5**.

2.3.3 Echocardiographic measurements

All echocardiographic measurements were performed according to the American Society of Echocardiography guidelines. Phillip echocardiographic machine model iE33, 2009, Bothell WA, 98041 USA was used for image acquisition and analyses were performed using EchoPac Software Only BT 11 Version 113, 2013 General Electric Co. M-modes, Doppler, 2D and 3D images were acquired.

The M-mode was primarily used to assess left ventricular intra-ventricular dyssynchrony. Septal to posterior wall delay (SPWD), was calculated as the time difference between the onset of the QRS to the peak of deformation of the inter-ventricular septum and the left ventricular posterior. SPWD of 130ms or more was considered diagnostic of intraventricular dyssynchrony (Anderson, Miyazaki et al. 2008).

Inter-ventricular mechanical delay (IVMD) was assessed by Doppler and was the difference between the onset of QRS complex to the opening of the aortic valve and the onset of QRS complex to the opening of the pulmonic valve. IVMD of 40ms or more was diagnostic of inter-ventricular mechanical dyssynchrony.

Left ventricular volumes including end-diastolic, end-systolic and stroke volumes were measured in two dimensions and ejection fraction calculated by the modified Simpson's method in biplane(Otterstad, Froeland et al. 1997).

2.3.4 Biochemical analyses

2.3.4.1.Platelet Thioredoxin interacting protein (TXNIP) content.

TXNIP, also known as Vitamin D3 upregulated protein 1 VDUP1, is a negative regulator of thioredoxin (TRX) and exerts a number of thioredoxin-independent pro-inflammatory effects as shown in **Figure 2.6.** TRX itself plays a pivotal role in regulation of redox stress, cellular proliferation, apoptosis and gene expression.(Nishiyama, Matsui et al. 1999)

Platelet expression of thioredoxin-interacting protein (TXNIP) was quantitated by immunofluorescence as previously described.(Procter, Goh et al. 2016) Briefly, blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and spun at 250g for 10 minutes at room temperature. Slides were smeared, air-dried and fixed with 4% paraformaldehyde and stored in -70^{0}C freezer, and immunofluorescence staining performed within 6 months.

For immunofluorescence staining, the slides were allowed to warm to room temperature and then washed 3 times in phosphate buffer saline (PBS) for 5 minutes per wash. 100µl of diluted goat's serum 1:5 with PBS (as a blocking solution) was added and incubated at room temperature for 30 minutes. Primary antibody was prepared by diluting rabbit anti-VDUP, (Invitrogen Corporation, Carlsbad, CA, USA) with 1% (w/v) bovine serum albumin in PBS in 1:50 ratio. The blocking solution was discarded without washing the slides and then 100µL of the primary antibody added and incubated overnight at 2-4^{0}C. The primary antibody was discarded the next day and the slides were washed 3 times in PBS for 5 minutes per wash. Two conjugated 2^0 antibodies: dilute phycoerythrin (PE)-labelled CD41 antibody (Becton, Dickinson and Company, USA), in 1:50 with PBS, serving as a platelet marker, and dilute fluorescein isothiocyanate (FITC)-labelled anti-rabbit polyclonal antibody (Becton, Dickinson and Company, USA), in 1: 100 with PBS were prepared whilst shielded from light. 100µL of each of the 2^0 antibodies was added to the slide and incubated at room temperature for 60 minutes. The slides were again washed

3 times in PBS for 5 minutes per wash, dried with lens tissue, and 1 drop of Dako fluorescent mounting medium was added, covered with a slip and allowed to sit at room temperature for 10 minutes. Image acquisition was performed with Carl Zeiss Microscope, Germany, using 400x magnification, multichannel for rhodamine and green fluorescent protein (GFP) and exposure time set at 5000ms. The intensities of TXNIP staining for each platelet were obtained utilizing image analysis software (AxioVision 40 version 4.8.2, Carl Zeiss Microscopy, Germany). Subsequent analysis or counting was performed by randomly identifying 100 platelets per slide and average TXNIP obtained. **Figure 2.7 (A-C)** illustrates this

2.3.4.2 Asymmetric dimethyl arginine (ADMA) and symmetric dimethyl arginine (SDMA)

ADMA and (to a lesser extent) SDMA are considered markers of endothelial dysfunction. Whereas ADMA inhibits endothelial nitric oxide synthase (eNOS), SDMA does not inhibit eNOS but rather competes for cellular uptake with arginine. The resultant effect of either of these is reduction of nitric oxide production. (Leone, Moncada et al. 1992)

For ADMA and SDMA estimation, 10ml of blood was collected in heparinised tubes and immediately put in ice. Spinning was performed at 4^0C at 1800g for 15 minutes. Plasma was collected in Eppendorf tubes and stored at -70^0C until analysed. Subsequent analysis was as described by Heresztyn et al(Heresztyn, Worthley et al. 2004). Briefly, this involves extraction, derivatisation with fluorescent derivatising reagent (AccQ-Fluor™) and chromatography.

2.3.4.3 Matrix Metalloproteinases (MMP)

MMPs are zinc–dependent enzymes that are initially secreted in the inactive forms but become activated to modulate extracellular matrix degradation. MMP-2 and MMP-9 specifically belong to the subclass of gelatinases that are known to be up regulated in heart failure of both ischemic and non-ischemic etiologies. (Spinale, Coker et al. 2000)

MMP-2 was estimated from blood samples collected in EDTA bottles while MMP-9 was estimated from blood samples collected in heparinised tubes. For both, collected blood was immediately put in ice and was centrifuged for 15 minutes at 1800g at 4^{o}c within 30 minutes of collection. Platelet-poor plasma was collected and stored at -70^{o}c until analysed. Assays were performed with RnD Quantakile quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits (Minneapolis USA) according to the Manufacturer's instructions.

Briefly, samples were allowed to thaw at room temperature. All reagents, standards and samples were prepared accordingly.

For MMP-2:

50 µL of Assay Diluent was added to each well. Then, 50 µL of Standard, control, or sample was added to each well, covered with a plate sealer, and incubated at room temperature for 2 hours on a horizontal orbital micro plate shaker. The wells were aspirated and washed 4 times. Subsequently, 200 µL of Conjugate was added to each well, covered with a new plate sealer, and incubated at room temperature for 2 hours on the shaker. Aspiration and washing were performed again, 4 times. Then 200 µL of Substrate Solution was added to each well and incubated at room temperature on the benchtop for 30 minutes whilst being protected from light. 50 µL of Stop Solution was then added to

each well and reading done within 30 minutes at 450 nm and wavelength correction of 570 nm.

For MMP-9:

100µL of diluent was added to each of the 96 wells. Then, 100µL of standard, sample or control was added. The wells were covered with a plate sealer and then incubated at room temperature for 2 hours on a horizontal orbital microplate shaker. Aspiration and washing of each well was done 4 times. 200 µL of conjugate was added thereafter, covered with a new plate sealer and then incubated at room temperature on the shaker for 1 hour. Aspiration and washing were done 4 times. Then 200µL of substrate was added to each well, protected from light, and incubated at room temperature for 1 hour at the benchtop. 50µL of stop solution was then added to each well and reading was done within 30 minutes at 450 nm with wavelength correction set at 570 nm.

2.3.4.4 Plasma metanephrine and normetanephrine

Analyses were performed on fresh samples collected in K3EDTA bottles by the Queen Elizabeth hospital biochemistry department according to standard procedure and essentially involved the use of liquid chromatography-tandem spectrometry. The normal range was <500 pmol/L for free metanephrine and < 900 pmol/L for free normetanephrine.

2.3.4.5 NT-ProBNP measurement

Samples were collected in heparinised tubes and analysed with the Elecsys proBNP system (Roche diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim) by the Queen Elizabeth hospital biochemical laboratory. The Sandwich principle was used and this involves a first incubation with a biotinylated monoclonal NT-proBNP-specific

antibody, and a monoclonal NT-proBNP-specific antibody labelled with a ruthenium complex. A second incubation was performed with the addition of streptavidin-coated microparticles, and the microparticles were subsequently captured on the surface of the electrode magnetically. A photomultiplier finally measured the chemiluminiscent emission induced by application of voltage to the electrode.

2.3.4.6 Plasma syndecan-1 level estimation.

For syndecan-1 assay, blood was collected in EDTA tubes and immediately put on ice and centrifuged for 15 minutes at 1800g at 4ºC within 30 minutes of collection. Plasma was collected and stored at -70ºC until analysed. Analysis was performed utilizing Human sCD138 ELISA kit (Diaclone SAS, France version 7, 2015) according to manufacturer's instructions. .

2.3.4.7 High sensitive C-reactive protein:

Quantitative measurements of hs-CRP was performed in the hospital biochemistry laboratory on stored plasma using the immuno-turbidimetric test method on Beckman Coulter AU analysers and this has an application range of 0.08-80mg/L.

2.3.5 Electrical measurements through the device

Baseline intrinsic intervals were measured at sweep speed of 50mm/s as described previously.(Zucchelli, Soldati et al. 2010) All measurements were in ms.

The P-R interval was measured from the onset of the P wave to the onset of the QRSD complex.

The Q-RVegm was measured from the onset of the QRS complex to the onset of right ventricular intracardiac electrogram.

The RVegm-LVegm was measured from the onset of the right ventricular intracardiac electrogram to the onset of the left ventricular intracardiac electrogram. An example is shown in **Figure 2.8**

Measurement of basal QRS duration was performed routinely.

The Q-LVegm was calculated as the sum of Q-RVegm and RVegm-LVegm while the LVegm-QRSend was calculated as the difference between the basal QRS duration and Q-LVegm.

Electrophysiological measurements through the device were performed at the speed of 100mm/s and included:

1. During right ventricular-only pacing, in VVI mode and rate of 100bpm

   (RVp)-LVegm: the onset of RVp to the onset of left ventricular intracardiac electrogram.

   QRS (RVp): the QRS duration during RV pacing.

2. During left ventricular-only pacing in VVI mode at the rate of 100bpm.

   (LVp)-RVegm: the onset of left ventricular pacing to the onset of right ventricular intra-cardiac electrogram. This is shown in **Figure 2.9**

   QRS(LVp): the QRS duration during LV pacing.

3. During biventricular pacing in VVI mode at the rate of 100bpm.

   QRS (BVp): QRS duration during biventricular pacing.

4. Right ventricular effective refractory periods (RVERP):

   RVERP were determined using a decremental extra-stimulation protocol. The S1 was the drive train, which was delivered at a burst of 8 cycles followed by an S2 that is less than the S1 cycle length. The S2 cycle length was gradually decreased by 10ms until ventricular capture was lost or until 200ms was reached, (which ever occurred first, so as to avoid the induction of ventricular fibrillation). The longest coupling interval of S1-S2 that failed to capture the ventricle was the RVERP. The S1 drive trains were 600ms, 500ms and 400ms. Tests were conducted twice at 30 seconds interval and averages taken. An example is shown in **Figure 2.10**

2.3.6 Statistical Analyses.

All data are expressed as mean ± SD unless indicated otherwise. The effects of cardiac resynchronisation therapy on clinical, biochemical, endothelial, electrical and echocardiographic parameters were assessed using paired t- test for normally distributed variables, and Wilcoxon matched-pairs signed rank test for non-parametric data. Each patient served as his/her control.

The interactions between changes in left ventricular contractility and endothelial functions as well as the interactions between changes in function and neurohumoral and electrical parameters were correlated using Pearson correlation coefficients for normally distributed data and Spearman correlation for non-parametric data. A two-tailed P value <0.05 was considered statistically significant.

All data were analysed with Prism 6 for Mac OS X version 6.0h October 2015.

RESULTS

2.4 Baseline characteristics of patients.

Baseline characteristics are summarised in **Table 2.2**

A total of 34 patients were recruited. One patient died of sepsis before baseline evaluations could be performed and was therefore not included in the analyses. Of the remaining 33 patients, the mean age was 71.2 ± 9.7 years and 10 (30.3%) were females. The aetiology of heart failure was ischemic in 17 (52%) patients and the cohort was overweight with a mean body mass index of 29.3 ± 6.1 kg/m^2. 23 (70%) patients were in NYHA functional class III and ambulatory class IV. While 3 patients were technically in class I status on the basis of reported symptoms and exercise capacity, all had moderate to severely depressed left ventricular systolic dysfunction and they did not have substantial levels of physical exercise. Treated hypertension was present in 21 (64%) patients with mean systolic blood pressure for the entire cohort being 126.4 ± 17.1 mmHg. 14 (42%) had type 2 diabetes and 5 (15%) had atrial fibrillation. Only 3 (9%) were current smokers although 16 (48%) were ex-smokers.

The mean eGFR was 56.4 ± 22.2 ml/min/1.73 m^2 and no patient was on dialysis.

30 (91%) were either on an angiotensin converting enzyme inhibitor or an angiotensin receptor blocker while 25 (76%) were on heart failure specific beta-adrenoceptor blockers. 18 (55%) were on a statin. Importantly, 18 (55%) were also on aldosterone antagonist, while only 2 (6%) were on amiodarone.

The mean baseline QRS duration was 157.9 ± 27.2ms with 31 (93.9%) having a left bundle branch block morphology and 2 (6.1%) having an intra-ventricular conduction delay. There was no patient with a right bundle branch block.

The mean left ventricular end-systolic volume, left ventricular end-diastolic volume and left ventricular ejection fraction were 137.4 ± 55.3 ml, 192.8 ± 67.4 ml and 29.8 ± 6.1 % respectively.

Mean baseline augmentation index (AIx) was 19.9 ± 7.8 %. This was not very different from the previously reported normal values of 18 ± 16 % for adults of 48 ± 20 years of age reported by McEniery et al.(McEniery, Wallace et al. 2006) The mean AIx in this study was also substantially lower than the values of 27.2 ± 8.3 % reported by Sverdlov et al (Sverdlov, Ngo et al. 2014)among patients who had multiple cardiovascular risk factors of whom only 34-41% of them were on ACEI/ARBs. Thus it can be concluded that these patients were in a state of arteriolar dilatation at baseline. The mean platelet SNP response however was 33.9 ± 26.5% and this was lower than the previously reported normal values of 54 ± 24% (Chirkov, Holmes et al. 2002) thus indicating some measure of platelet resistance to nitric oxide. The mean plasma ADMA concentration was 0.6 ± 0.07 μM (normal 0.5 ± 0.08 μM), consistent with endothelial dysfunction, (Heresztyn, Worthley et al. 2004) and the median plasma SDMA concentration was 0.7 μM (IQR 0.6-1.0).

2.5 The effects of cardiac resynchronization therapy.

Table 2.3 summarizes the effects of cardiac resynchronization therapy on clinical, vascular and platelet, biochemical, echocardiographic and electrical parameters before and 6 months after CRT implant. Out of the 33 enrolled patients, 1 died a month after CRT implant, and three declined the 6 months follow-up visit for personal reasons.

2.5.1 Effects of cardiac resynchronization on clinical parameters

CRT resulted in significant improvement in NYHA functional class. There was a statistically significant decrease in NYHA class score from 2.7 (0.8) pre-CRT, to 1.9 (0.7)

post-CRT, p <0.001. There was also a statistically significant increase in the six minutes walk distance from 314.5 (112.8) m pre- CRT to 357.0 (117.0) m post-CRT, p= 0.005. Likewise, the quality of life score, assessed with the Minnesota Living with Heart failure questionnaire showed statistically significant improvement with values of 40.7 (25.4) pre-CRT decreasing to 22.9 (22.3) post-CRT, p=0.001. Lower scores indicate better quality of life. On the other hand, there was no statistically significant difference in peak oxygen consumption with exercise, VO_2 max13.8 (4.67) ml/min/kg pre-CRT to 14.1(5.3) ml/min/kg post-CRT. The effects of cardiac resynchronization therapy on clinical parameters are summarized in **Figures 2.11 (A-D)**

2.5.2.The effects of cardiac resynchronisation therapy on vascular and platelet functions

Augmentation index (AIx) was not altered with CRT, being 20.3 (8.2)% pre- CRT and 20.3 (8.1)% post- CRT, p=NS. Also, there was no statistically significant difference in AIx response to GTN: -14.1 (10.0)% pre-CRT and -16.6 (8.1)% post-CRT, p=NS and in AIx response to salbutamol: -9.9 (10.5)% pre-CRT and -11.9 (8.3)% post-CRT, p=NS. While there was no statistically significant difference in plasma levels of ADMA: 0.66 (0.08) μM pre-CRT and 0.65 (0.09) μM post- CRT, p= NS; there was a significant decrease in plasma levels of SDMA: 0.83 (0.28) μM pre-CRT to 0.74 (0.20) μM post-CRT, p= 0.013. (**Figure 2.43**)

There was no improvement noted in platelet functions with CRT. Specifically, the inhibition of ADP-induced platelet aggregation by sodium nitroprusside was 30.5 (21.8) % pre-CRT and 25.2 (19.7) % post-CRT, p= NS while platelet TXNIP content was 144.8 (122.6) AU pre-CRT and 188.5 (126.6) AU post CRT, p= NS.

2.5.3 The effects of cardiac resynchronisation therapy on echocardiographic parameters.

Cardiac resynchronisation resulted in statistically significant decrease in left ventricular end systolic volume from 136.6 (57.50) ml pre-CRT to 98.9 (52.1) ml post-CRT, p <0.001. Left ventricular ejection fraction was increased from 31.0 (6) % pre-CRT to 38 (10) % post-CRT, p <0.001.

Septal to posterior wall delay (SPWD), a measure of intra-ventricular mechanical dyssynchrony was significantly improved from 119.1 (201.2) ms pre-CRT to 1.74 (141.4) ms post CRT, p= 0.005. Inter-ventricular mechanical delay (IVMD) was also significantly reduced from 43.6 (44.6) ms pre-CRT to 19.9 (33.9) ms post-CRT, p= 0.012

2.5.4 Effects of cardiac resynchronisation therapy on biochemical parameters

There was a statistically significant reduction in the plasma levels of NT-pro BNP from a median of 1862 ng/L pre-CRT to 1469 ng/L post- CRT, p= 0.008. There was however no significant decrease in plasma levels of metanephrine from 257.8 (167.2) pmol/L pre-CRT to 239.2 (295.6) pmol/L post-CRT, p= NS and in plasma levels of normetanephrine from 918.8 (356.6) pmol/L pre-CRT to 900.7 (295.6) pmol/L post-CRT, p= NS. Likewise, plasma levels of matrix metalloproteinase- 2 and 9 were not significantly altered by cardiac resynchronisation. MMP-2 level was 217.2 (52.5) ng/ml pre-CRT and 219.8 (49.9) post-CRT, p= NS while MMP-9 was 27.5 (9.6) ng/ml pre-CRT and 26.5 (10.0) ng/ml post-CRT, p= NS.

2.5.5 Effects of cardiac resynchronisation therapy on electrical parameters

Although CRT led to a decrease in intrinsic QRS duration from 158.7 (23.7) ms pre-CRT to 147.9 (29.4) ms post-CRT, this did not reach statistical significance (p= 0.08) in the

whole cohort. However, although not pre-specified, there was significant reduction in the intrinsic QRS duration from 165.4 (20.32) ms pre-CRT to 140.5 (28.67) ms post-CRT, p= 0.012 in the subgroup of patients with non-ischemic aetiology, whereas in the subgroup with ischemic aetiology, there was no significant change: 152.6 (25.62) ms pre-CRT and 154.2 (28.44) ms post-CRT, p= NS. The difference between the two groups was statistically significant (unpaired t-test P= 0.017). Also, there was significant reduction in intra-cardiac conduction times with CRT. The LVp-RVegm was reduced from 116.7 (44.5) ms at baseline to 97.0 (45.0) ms at six months of CRT, p= 0.019. In likewise manner, LVegm-QRSend also significantly decreased from 77.5 (53.3) ms at baseline to 53.9 (35.5) ms at six months of CRT, p= 0.024. These denote reversal of adverse electrical remodelling.

CRT did not significantly alter the PR interval and right ventricular effective refractory periods. The PR interval was 206.2 (40.3) ms pre-CRT and 211.0 (44.9) ms post-CRT, p= NS. The RVERP at 600ms drive train was 279.1 (24.7) ms at baseline and 283.0 (22.8) ms at six months of CRT, p= NS. The RVERP at 500ms drive train was 267.4 (23.7) ms at baseline and 270.7 (19.0) at six months, P=NS and at 400ms drive train was 258.7 (26.6) at baseline and 260.9 (19.3) at six months, p=NS.

2.6 Univariate correlations:

2.6.1 Left ventricular contractility and vascular endothelial function

There was no significant correlation between the change in baseline augmentation index (AIx) pre-CRT implantation and 6 months thereafter and the change in left ventricular contractility at 6 months as measured by the change in LVESV (r=0.07, p=NS). Change in

LVESV also did not correlate significantly with change in AIx with GTN (r=0.02, p= NS) nor with change in AIx with salbutamol (r= -0.25, p= NS) See **Figures 2.12-2.14**

2.6.2 Left ventricular contractility and nitric oxide signalling

The change in LVESV showed no significant correlation with change in platelet responsiveness to sodium nitroprusside (r= 0.10, p= NS), or with change in platelet content of TXNIP (r= 0.17, p= NS); neither was there any significant correlation with plasma levels of ADMA (r= 0.24, p= NS) nor SDMA (r= 0.21, p= NS) **Figures 2.15-2.17** illustrate these investigations.

2.6.3 Six-minute walk distance and mechanical dyssynchrony

There was a significant and strong negative correlation between change in 6MWD and change in inter-ventricular mechanical delay (r= -0.54, p= 0.005). On the other hand, there was no significant correlation (nor any obvious trend towards one) between change in 6MWD and change in intra-ventricular septal to posterior wall mechanical delay (r= 0.08, p=NS) as shown in **Figures 2.18** and **2.19** respectively.

2.6.4 Quality of Life score and mechanical dyssynchrony

There was no significant correlation between quality of life score measured with The Minnesota Living with Heart Failure Questionnaire, and changes in inter-ventricular mechanical delay (r=0.04, p=NS) or with changes in intra-ventricular septal to posterior wall mechanical delay, (r= 0.21, p=NS) as shown in **Figures 2.20 and 2.21** respectively.

2.6.5 Six-minute walk distance and electrical dyssynchrony

There was no significant correlation between change in 6MWD and change in conduction time between the onset of left ventricular pacing and the onset of right ventricular intra-cardiac electrocardiogram (r= 0.21, p =NS) and no significant correlation between change in 6MWD and change in intrinsic QRS duration (r= -0.01, p= NS). These are shown in **Figures 2.22 and 2.23** respectively.

2.6.6 Quality of Life score and electrical dyssynchrony.

A significant positive correlation was found between change in quality of life score and change in intrinsic QRS duration (r=0.38, p=0.04). This implies that patients with improvement in quality of life score had significant reduction in intrinsic QRS duration. However, there was no correlation between quality of life score and LVp-RVegm. See **Figures 2.24 and 2.25.**

2.6.7 Intrinsic cardiac intervals and measures of mechanical dyssynchrony

A significant positive correlation existed between change in PR interval, as a measure of atrio-ventricular conductivity and change in septal to posterior wall delay, as a measure of mechanical dyssynchrony (r= 0.56 p= 0.009) as shown in **Figure 2.26**. Considering that the baseline PR interval was prolonged prior to CRT implant, this result implies that a reduction in atrio-ventricular conduction time is associated with an improvement in the measure of intra-ventricular dyssynchrony. There was however no significant correlation between change in septal to posterior wall delay and change in intrinsic QRS duration (r= -0.18, p= NS; **Figure 2.27**)

2.6.8 Cardiac excitability and mechanical dyssynchrony.

There was no significant correlation between change in right ventricular effective refractory period at 600ms, as a measure of cardiac excitability and change in septal to posterior wall delay as a measure of mechanical dyssynchrony (r= -0.26, p= NS; **Figure 2.28**)

2.6.9 Electrical dyssnchrony and mechanical dyssynchrony

There was no significant correlation between LVp-RVegm as a measure of electrical dyssynchrony and septal to posterior wall delay as a measure of mechanical dyssynchrony (r= -0.28, p= NS; **Figure 2.29**)

2.6.10 Intrinsic cardiac intervals and neurohumoral activation

A significant negative correlation was found between changes in plasma normetanephrine and those in P-R interval (r= -0.45, p= 0.04) and also between changes in NT-proBNP and P-R interval (r= -0.44, p= 0.04). See **Figures 2.30 and 2.31** respectively. On the other hand, no significant correlations were found between changes in plasma normetanephrine and intrinsic QRS duration (r= -0.17,p= NS) or between changes in NT-proBNP and intrinsic QRS duration (r= -0.14, p= NS) as shown in **Figures 2.32 and 2.33**

2.6.11 Cardiac excitability and neurohumoral activation

No significant correlations were found between changes in plasma normetanephrine and RVERP at 600ms (r= -0.01, p= NS) or between changes in NT-proBNP and RVERP at 600ms (r= 0.07, p= NS) as in **Figures 2.34-2.35**

2.6.12 Redox stress and electrical dyysynchrony.

Figure 2.36 shows no significant correlation between changes in platelet content of thioredoxin interacting protein and intrinsic QRS duration as a measure of electrical dyssynchrony (r= -0.26, p= NS)

2.6.13 Relationship between cardiac dyssynchrony and nitric oxide signaling

Figure 2.37 shows a significant inverse correlation between the width of intrinsic QRS complex at baseline and endothelium-dependent nitric oxide signaling as measured by the effect of inhaled salbutamol on augmentation index (r=0.40, p=0.02)

2.6.14 Relationship between cardiac refractoriness and nitric oxide signalling

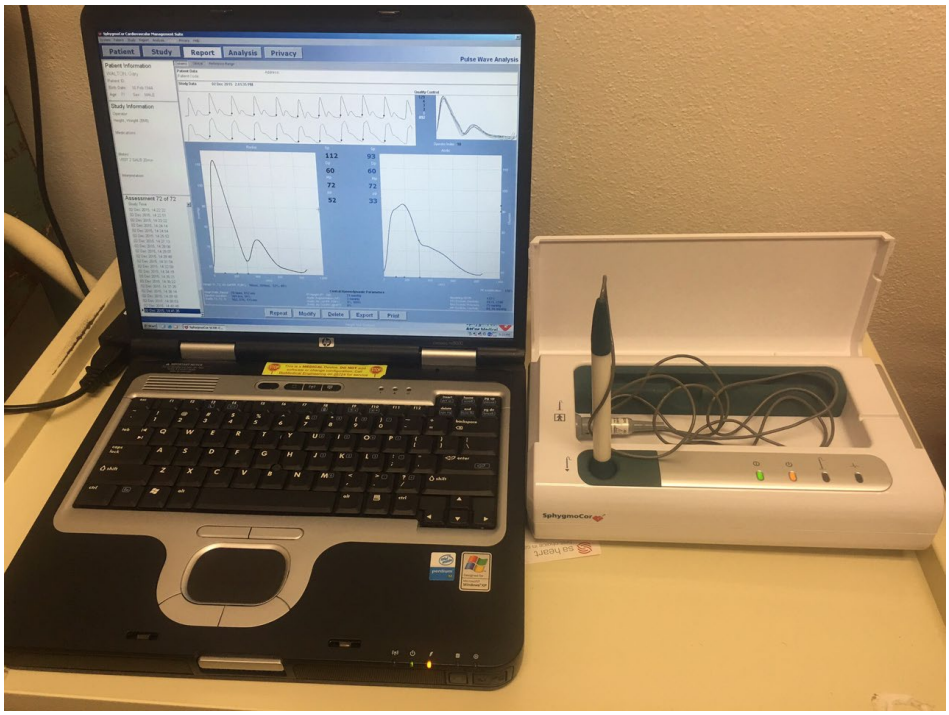
We also observed a significant negative correlation between endothelium-dependent nitric oxide signaling as measured by the effect of inhaled salbutamol on augmentation index and right ventricular effective refractory period at drive trains of 600ms (r= -0.45, p=0.01), 500ms (r= -0.44, p=0.01), and 400ms (r= -044, p=0.03). These are shown in **Figures 2.38-2.40**. Also, intrinsic QRS duration correlated directly with right ventricular effective refractory period at drive trains of 600ms (r= 0.41, p=0.02) and 500ms (r= 0.37, p=0.04) as shown in **Figures 2.41-2.42**.

Summary

In **Table 2.4,** the results of these extensive correlations are now summarised.

2.7 TABLES AND FIGURES FOR CHAPTER 2

A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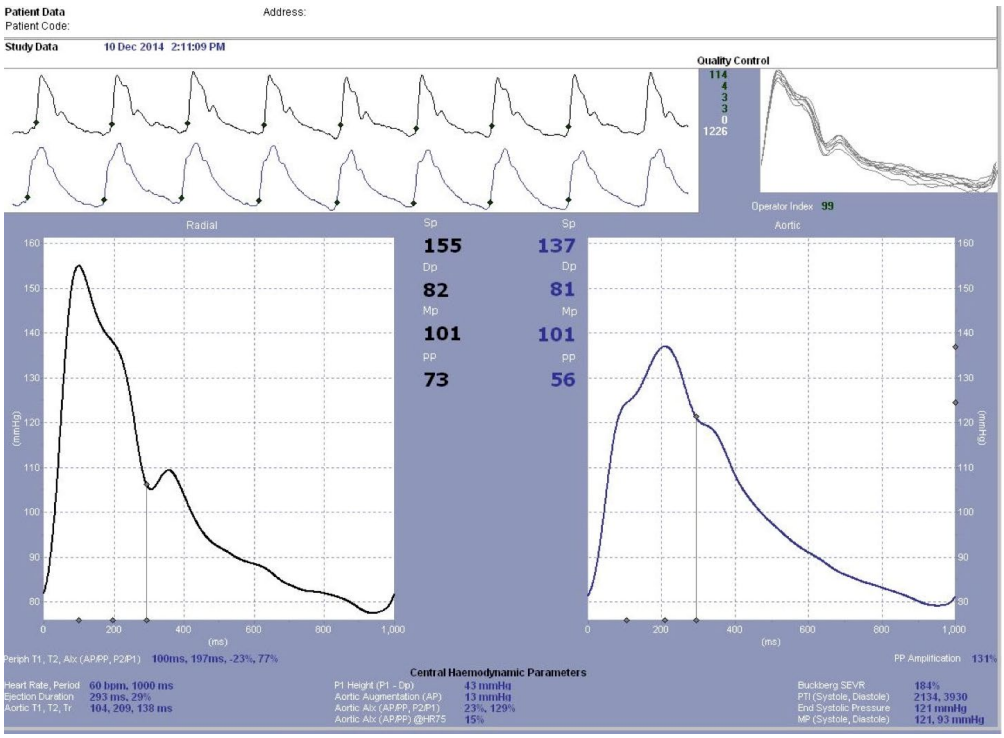
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Aortic
Sp
Dp
Mp
PP
155
82
101
73
137
81
101
56
(mmHg)
(ms)
Periph T1, T2, Alx (AP/PP, P2/P1) 100ms, 197ms, -23%, 77%
PP Amplification 131%
Central Haemodynamic Parameters
Heart Rate, Period 60 bpm, 1000 ms
Ejection Duration 293 ms, 29%
Aortic T1, T2, Tr 104, 209, 138 ms
P1 Height (P1 - Dp) 43 mmHg
Aortic Augmentation (AP) 13 mmHg
Aortic Alx (AP/PP, P2/P1) 23%, 129%
Aortic Alx (AP/PP) @HR75 15%
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End Systolic Pressure 121 mmHg
MP (Systole, Diastole) 121, 93 mmHg

C

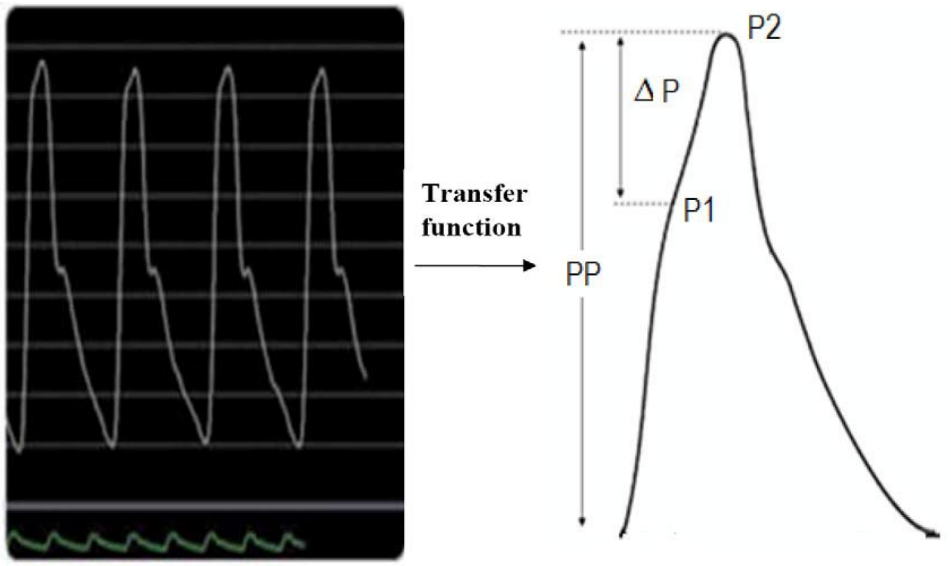


Figure 2.1 (A-C). ***(A)***. *SphygmoCor system (AtCor Medical, Sydney, Australia, model CvMS V9 used for radial artery applanation tonometry.* ***(B).*** *Applanation tonometry tracing from one of the study participants.*

(C). *A graphic representation of pulse wave analysis and AIx derivation. Peripheral pressure waveforms are recorded electronically and converted to a central waveform via a transfer function to derive a rate-corrected augmentation index. Augmentation index is the difference (ΔP) between the first (P1) and second systolic (P2) pressure peaks, divided by pulse pressure (PP), expressed as a percentage of the pulse pressure. (Crilly, Coch et al. 2007)*

A

B

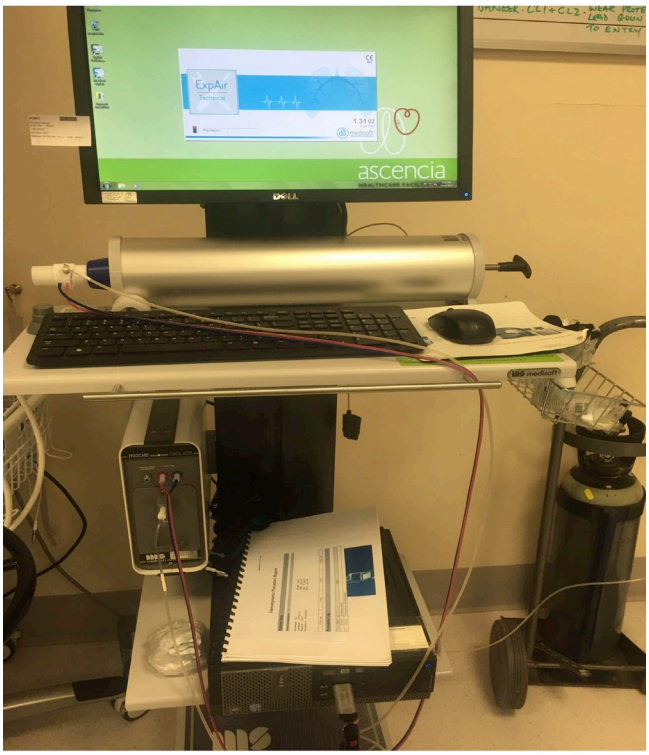


Figure 2.2 *Instruments used for the cardiopulmonary exercise testing.* ***(A)*** *Bicycle ergometer, ergoline /100/200 GmbH, Germany,* ***(B)*** *ExpAir Medisoft kit S.A Belgique 1.31.02*

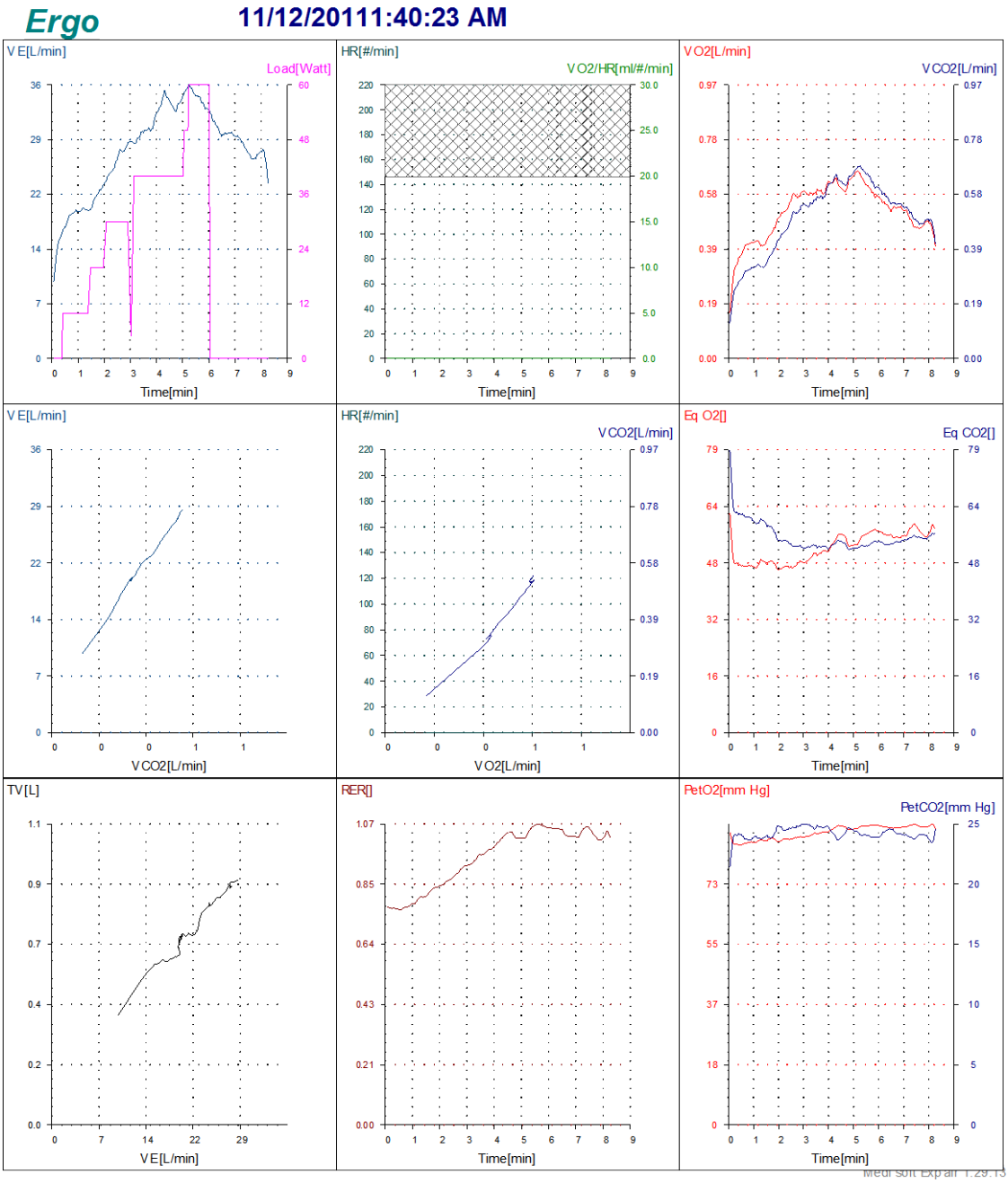


Figure 2.3 *Graphical displays of measured gas volumes during CPET*

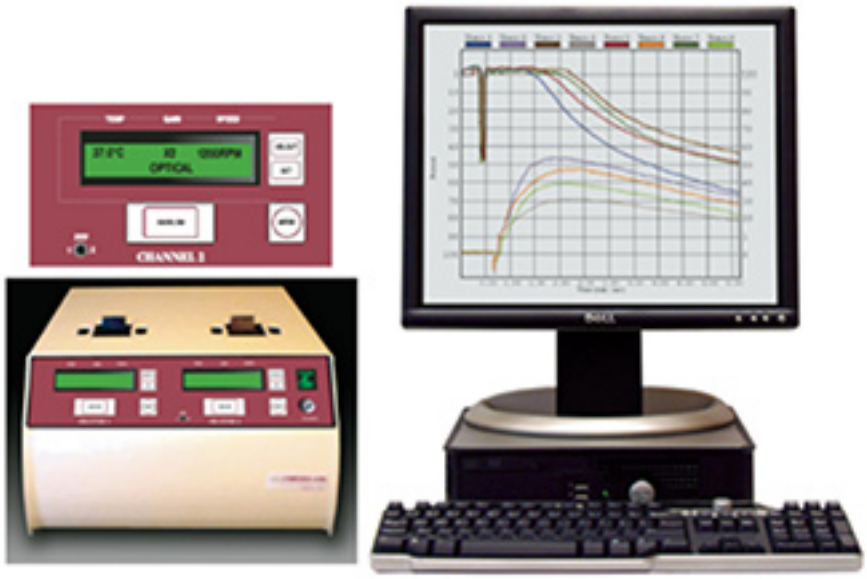
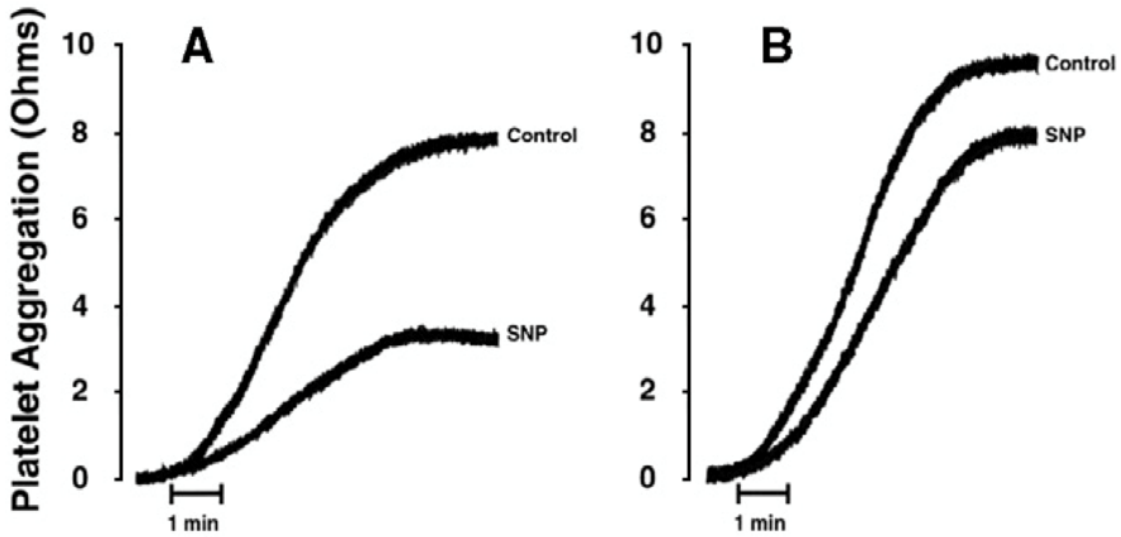


Figure 2.4 *Chrono-Log corporation, model 700, used for the assessment of inhibition of*



ADP-induced platelet aggregation in whole blood by SNP

Figure 2.5 *Graphic representation of inhibition of ADP-induced platelet aggregation in whole blood by SNP: demonstration of NO resistance. (A) Normal subject. (B) Study subject, showing both hyperaggregability and reduced responsiveness to antiaggregatory effect of SNP.(Chirkov and Horowitz 2007)*

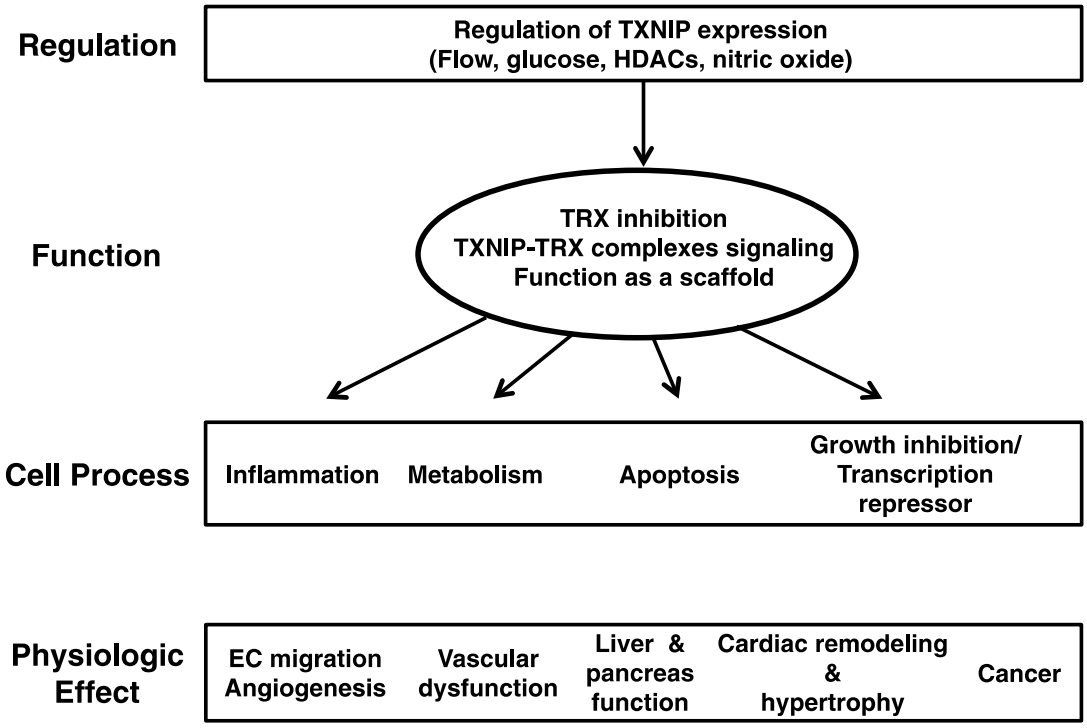


Figure 2.6 *Summary of TXNIP regulation and function.*

Several factors are known to regu- late TXNIP expression, such as glucose, blood flow, HDACs, nitric oxide, and more. As a result of changes in TXNIP ex- pression, three main effects can be noticed: (i) TRX inhibition, (ii) TXNIP-TRX complexes formation and functions, and (iii) TXNIP ability to function as a scaffold. Alterations in those three mechanisms are proposed to be the underlying mechanism involved in TXNIP- regulated cellular processes. (Reproduced from Spindel et al 2012 (CITE)

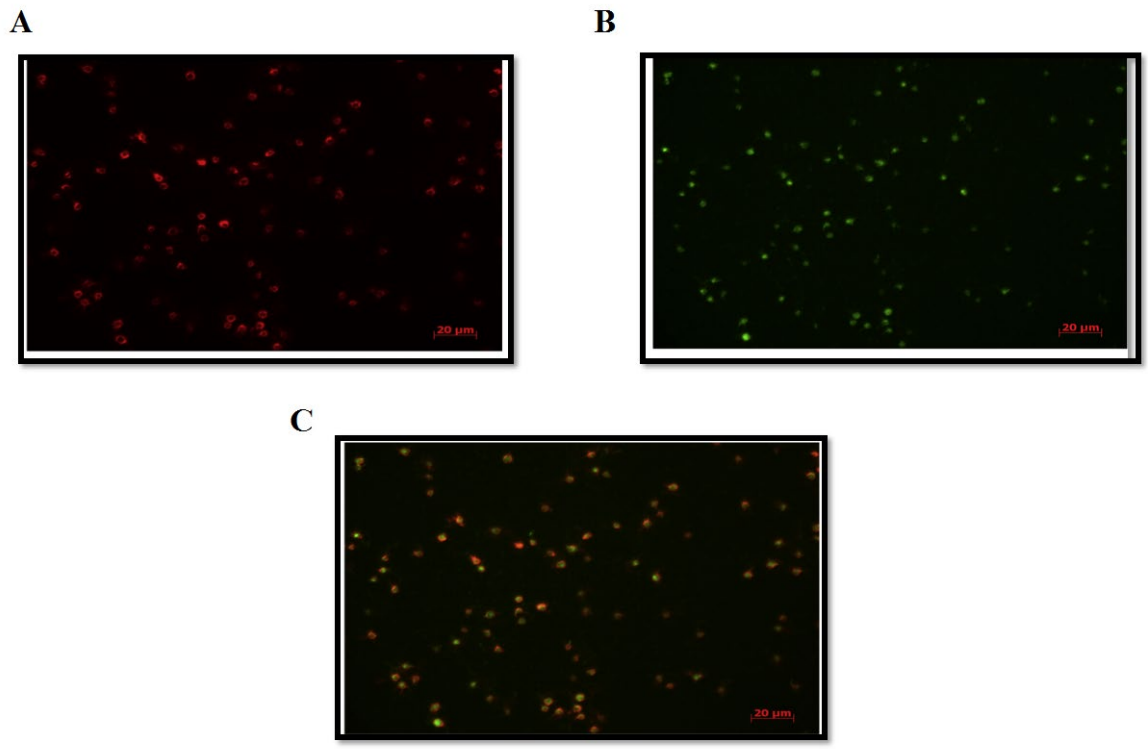


Figure 2.7 A- C *Demonstration of TXNIP on platelets by immunohistochemistry staining. A: Immunohistochemistry for CD41 platelet marker; B: immunohistochemistry for TXNIP; C: simultaneous acquisition of both channels A and B*

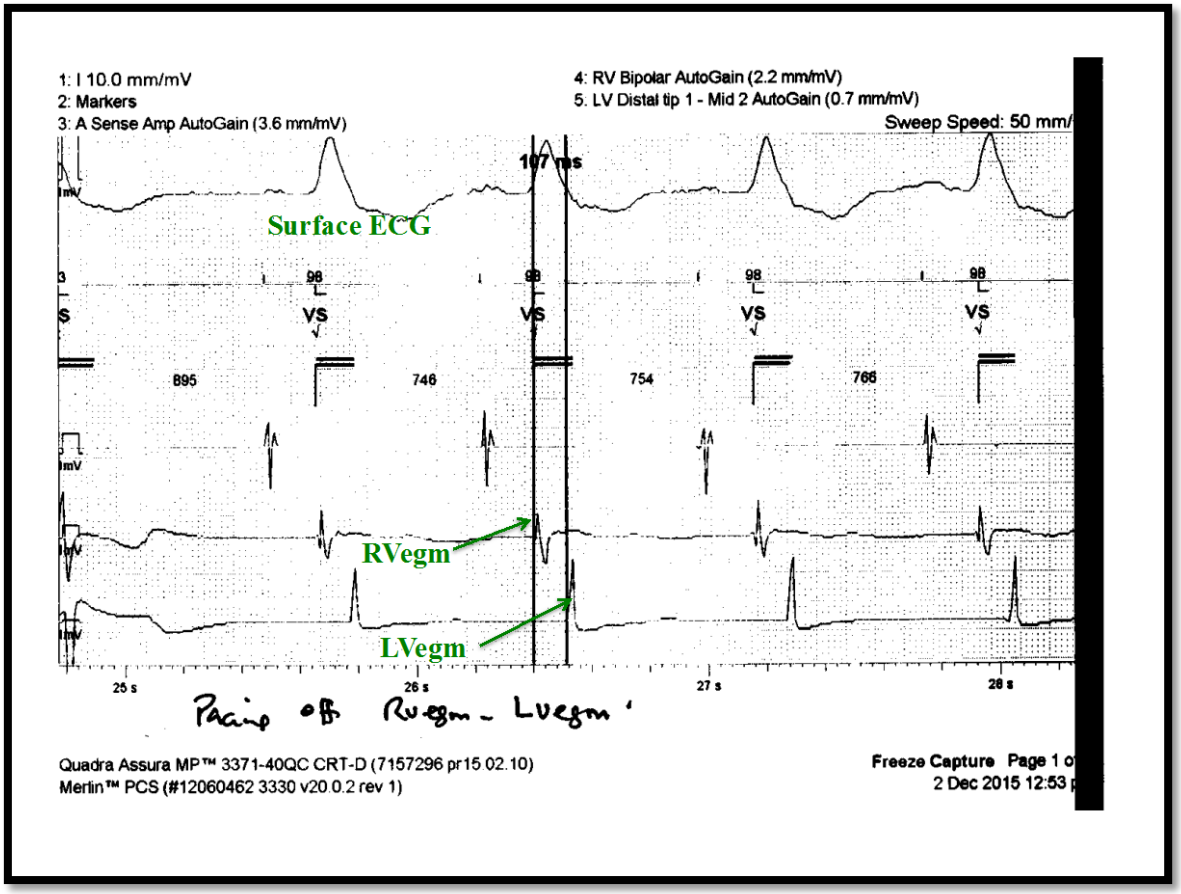


Figure 2.8 *Measurement of intrinsic inter-ventricular electrical conduction time using intra-cardiac electrocardiogram with pacing turned off. RVegm: right ventricular intra-cardiac electrocardiogram, LVegm: left ventricular intra-cardiac electrocardiogram*

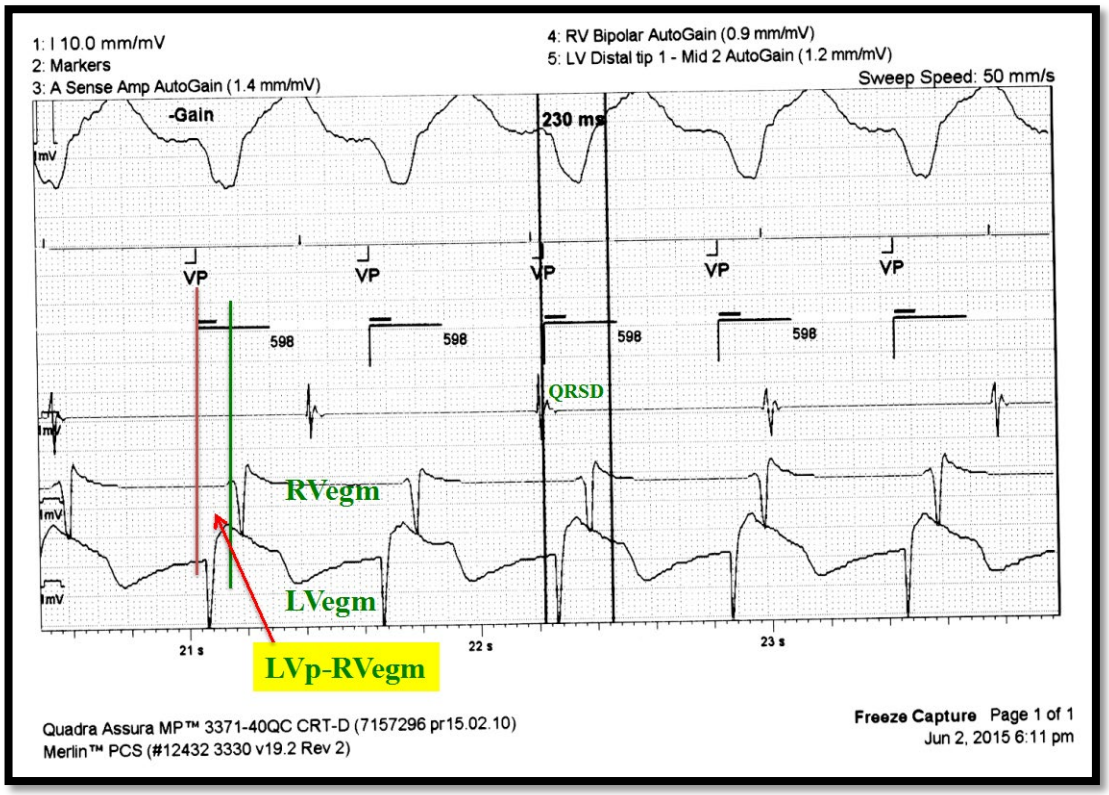


Figure 2.9. *Measurement of LVp-RVegm.*

This is the conduction time from paced left ventricle to sensed right ventricle.

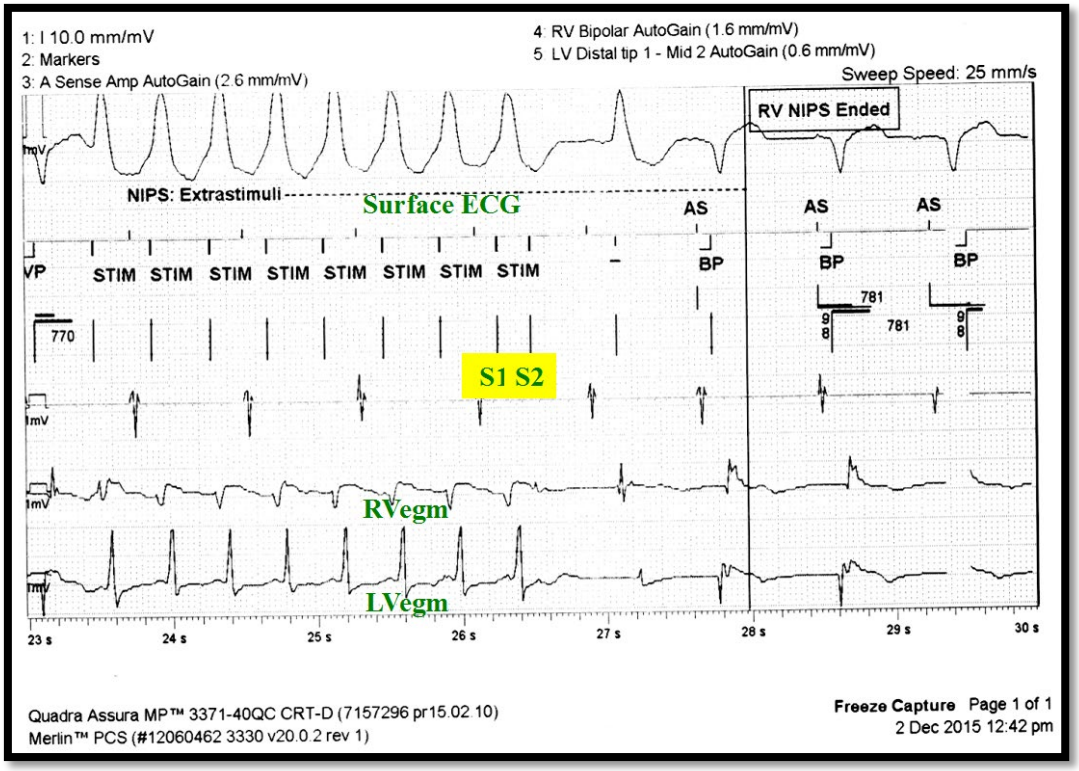


Figure 2.10 *Assessment of right ventricular effective refractory period.*

The S1 is part of the drive train while S2 is the extra-stimulus which in this case fails to capture the ventricle as shown by the absence of a corresponding surface ECG as well as the absence of a corresponding right ventricular intra-cardiac electrocardiogram. RVegm: right ventricular intra-cardiac electrocardiogram, LVegm: left ventricular intra-cardiac electrocardiogram.

Table 2.1. Calculation of VO_2 max

Metabolic Exercise of 10/06/2014 to 4:06: - Protocol : RESTING Page : 1

Time min	Load Watt	Speed Km/h	Grade %	VE L/min	V.I. L/min	VO2 L/min	VO2 / kg ml/kg	Ref VO2 L/min	VCO2 L/min	RER	I. Eff %Watt	Eq O2	Eq CO2	SpO2 %	FeO2 %	FeCO2 %	PetO2 mmHg
Every 10sec																	
00:09	0	0	0	17.5	11	0.65	8	0.30	0.51	0.79	0.0	27	34	0	16.61	3.58	103
00:19	0	0	0	12.8	10	0.42	5	0.30	0.32	0.76	0.0	30	40	0	17.42	2.81	106
00:30	0	0	0	12.0	13	0.39	5	0.30	0.28	0.72	0.0	30	42	0	17.52	2.67	106
00:39	0	0	0	16.8	17	0.62	7	0.30	0.46	0.75	0.0	27	36	0	16.80	3.36	106
00:50	0	0	0	15.1	17	0.42	5	0.30	0.36	0.86	0.0	36	42	0	17.69	2.92	114
01:00	0	0	0	14.2	14	0.43	5	0.30	0.36	0.85	0.0	33	39	0	17.40	3.16	111
01:10	20	0	0	11.0	11	0.32	4	0.50	0.26	0.81	0.0	34	42	0	17.51	2.98	110
01:19	20	0	0	10.6	11	0.32	4	0.50	0.25	0.77	0.0	33	43	0	17.48	2.89	108
01:30	20	0	0	14.9	14	0.58	7	0.50	0.43	0.74	0.0	26	35	0	16.57	3.53	101
01:40	20	0	0	15.7	15	0.59	7	0.50	0.45	0.76	0.0	27	35	0	16.68	3.50	103
01:49	20	0	0	19.0	18	0.67	8	0.50	0.54	0.81	0.0	28	35	0	16.80	3.52	106
02:00	20	0	0	14.6	14	0.46	5	0.50	0.39	0.84	0.0	32	38	0	17.26	3.24	109
02:09	30	0	0	14.8	14	0.47	5	0.60	0.39	0.83	0.0	31	38	0	17.25	3.23	108
02:20	30	0	0	22.4	25	0.70	8	0.60	0.57	0.81	0.0	32	39	0	17.37	3.08	112
02:30	30	0	0	21.0	15	0.70	8	0.60	0.55	0.79	0.0	30	38	0	17.07	3.38	106
02:39	30	0	0	20.1	17	0.70	8	0.60	0.56	0.80	0.0	29	36	0	16.82	3.51	104
02:50	30	0	0	15.6	16	0.58	7	0.60	0.47	0.82	0.0	27	33	0	16.71	3.64	106
03:00	30	0	0	17.3	17	0.64	7	0.60	0.52	0.82	0.0	27	33	0	16.70	3.65	106
03:09	40	0	0	15.8	17	0.50	6	0.70	0.41	0.82	0.0	32	39	0	17.26	3.18	108
03:20	40	0	0	14.9	11	0.57	7	0.70	0.43	0.76	0.0	26	34	0	16.62	3.61	100
03:30	40	0	0	16.2	16	0.65	8	0.70	0.49	0.75	0.0	25	33	0	16.31	3.76	98
03:39	40	0	0	20.3	21	0.76	9	0.70	0.61	0.80	0.0	27	33	0	16.57	3.67	103
03:50	40	0	0	23.6	24	0.71	8	0.70	0.65	0.92	0.0	33	36	0	17.35	3.38	113
04:00	45	0	0	23.3	23	0.67	8	0.75	0.64	0.96	0.0	35	36	0	17.48	3.39	114
04:10	50	0	0	21.5	21	0.62	7	0.80	0.60	0.98	0.0	35	36	0	17.45	3.43	113
04:19	50	0	0	18.1	18	0.57	7	0.80	0.51	0.90	0.0	32	36	0	17.27	3.44	109
04:30	50	0	0	19.0	18	0.64	7	0.80	0.54	0.85	0.0	30	35	0	17.06	3.50	107
04:40	50	0	0	21.0	20	0.72	8	0.80	0.62	0.86	0.0	29	34	0	16.94	3.60	107
04:49	50	0	0	21.2	21	0.72	8	0.80	0.63	0.88	0.0	30	34	0	16.93	3.65	107
05:00	50	0	0	22.7	22	0.78	9	0.80	0.70	0.90	0.0	29	32	0	16.90	3.75	107
05:10	60	0	0	23.4	23	0.77	9	0.90	0.70	0.92	0.0	31	33	0	17.08	3.64	109
05:19	60	0	0	22.1	21	0.72	8	0.90	0.67	0.92	0.0	31	33	0	17.07	3.67	108
05:30	60	0	0	23.6	23	0.77	9	0.90	0.73	0.94	0.0	30	32	0	17.01	3.77	109
05:40	60	0	0	25.4	25	0.80	9	0.90	0.76	0.95	0.0	32	33	0	17.19	3.66	110
05:49	60	0	0	26.6	26	0.82	10	0.90	0.80	0.97	0.0	32	33	0	17.22	3.68	111
06:00	60	0	0	25.0	25	0.74	9	0.90	0.74	1.00	0.0	34	34	0	17.31	3.63	112
06:10	70	0	0	29.1	28	0.87	10	1.00	0.87	0.99	0.0	33	34	0	17.31	3.65	113
06:19	70	0	0	34.4	33	0.97	11	1.00	0.98	1.02	0.0	36	35	0	17.50	3.51	115
06:30	70	0	0	31.5	33	1.03	12	1.00	1.00	0.97	0.0	31	32	0	17.00	3.90	109
06:40	70	0	0	31.8	30	0.96	11	1.00	0.97	1.00	0.0	33	33	0	17.24	3.74	112
06:49	70	0	0	30.2	28	0.93	11	1.00	0.93	1.00	0.0	32	33	0	17.21	3.76	111
07:00	70	0	0	28.6	27	0.85	10	1.00	0.86	1.00	0.0	34	33	0	17.34	3.63	111
07:10	80	0	0	35.6	34	1.01	12	1.10	1.05	1.05	0.0	35	34	0	17.42	3.65	114
07:19	80	0	0	32.9	29	0.96	11	1.10	0.99	1.04	0.0	34	33	0	17.31	3.78	113
07:30	80	0	0	39.8	38	1.08	13	1.10	1.18	1.09	0.0	37	34	0	17.53	3.64	116

Metabolic Exercise of 10/06/2014 to 4:06: - Protocol : RESTING Page : 2

Time min	Load Watt	Speed Km/h	Grade %	VE L/min	V.I. L/min	VO2 L/min	VO2 / kg ml/kg	Ref VO2 L/min	VCO2 L/min	RER	I. Eff %Watt	Eq O2	Eq CO2	SpO2 %	FeO2 %	FeCO2 %	PetO2 mmHg
07:40	80	0	0	41.8	41	1.06	12	1.10	1.20	1.13	0.0	40	35	0	17.71	3.53	118
07:49	80	0	0	35.9	37	0.88	10	1.10	1.02	1.16	0.0	41	35	0	17.79	3.50	119
08:00	85	0	0	46.0	44	1.15	13	1.15	1.33	1.15	0.0	40	35	0	17.72	3.56	118
08:09	90	0	0	44.7	44	1.12	13	1.20	1.29	1.15	0.0	40	35	0	17.73	3.57	118
08:20	90	0	0	43.8	43	1.18	14	1.20	1.30	1.10	0.0	37	34	0	17.51	3.70	116
08:30	90	0	0	46.2	44	1.25	15	1.20	1.38	1.10	0.0	37	34	0	17.48	3.69	116
08:39	90	0	0	45.3	43	1.19	14	1.20	1.34	1.13	0.0	38	34	0	17.56	3.66	117
08:50	90	0	0	49.0	46	1.32	15	1.20	1.48	1.12	0.0	37	33	0	17.44	3.77	116
09:00	90	0	0	43.5	43	1.15	13	1.20	1.30	1.13	0.0	38	34	0	17.54	3.72	117
09:09	0	0	0	34.4	35	0.98	11	0.30	1.04	1.08	0.0	36	33	0	17.38	3.81	114
09:20	0	0	0	39.6	37	1.14	13	0.30	1.28	1.12	0.0	35	31	0	17.28	3.96	114
09:30	0	0	0	46.7	45	1.16	13	0.30	1.37	1.18	0.0	40	34	0	17.69	3.64	118
09:39	0	0	0	37.1	37	1.04	12	0.30	1.12	1.07	0.0	36	33	0	17.32	3.87	113

Computer display of a study participant's CPET averaged at 10 seconds intervals. The highest value of the last three averages at peak exercise (shown in the red box) is the VO_2 max. The blue line indicates end of exercise and beginning of recovery. In this case, the maximum load was 90W.

Table 2.2 Baseline characteristics of patients.

Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile values

Age (years)	71.2 ±9.7
Female, n (%)	10 (30.3)
Weight (kg)	87.1 ± 18.6
Height (cm)	172.5 ± 8.1
BMI (kg/m^2)	29.3 ± 6.1
Ischaemic aetiology of CHF, n (%)	17 (52)
History of current/past smoking, n (%)	19 (57.6)
NYHA Class: • I, n (%) • II, n (%) • III, n (%) • IV, n (%)	 3 (9) 7 (21) 19 (58) 4 (12)
Comorbidities: • Hypertension, n (%) • Diabetes, n (%) • COPD, n (%) • Atrial fibrillation, n (%)	 21 (64) 14 (42) 4 (12) 5 (15)
Medications: • ACE inhibitor • Angiotensin receptor blocker • β- adrenoceptor blocker • Aldosterone Antagonist • Digoxin • Frusemide • Statin • Aspirin • Clopidogrel • Perhexiline	 22 (67) 8 (24) 25 (76) 18 (55) 9 (27) 24 (73) 18 (55) 17 (52) 7 (21) 5 (15)

CLINICAL ASSESSMENT	
Systolic BP (mmHg)	126.4 ± 17.1
Diastolic BP (mmHg)	71.2 ± 9.2
Heart rate (bpm)	69 ± 13.6
6-MWD (m)	321.74 ± 104.62
VO_2max (ml/min/kg)	13.8 ± 4.7
QOL Score	41.9 ± 25.6
BIOCHEMISTRY	
NT-pro BNP (ng/L)	1814.0 (1091-3073)
eGFR (mL/min/1.73 m^2)	56.4 ± 22.2
Plasma Metanephrine (pmol/L)	252.1 ± 158.3
Plasma Normetanephrine (pmol/L)	996.5 ± 396.2
Plasma MMP-2 (ng/ml)	217.5 ± 51.2
Plasma MMP-9 (ng/ml)	27.7 ± 9.1
hs- CRP (mg/L)	2.4 (1.4-6.1)
ENDOTHELIAL FUNCTION/NO signaling	
Baseline AIx (%)	19.9 ± 7.8
GTN-induced AIx change (%)	-13.9 ± 10.0
Salbutamol-induced AIx change (%)	-11.3 ± 10.2
ADMA (µM)	0.6 ± 0.07
SDMA (µM)	0.7 (IQR 0.6-1.0)
Platelet SNP response (%)	33.9 ± 26.5
Platelet TXNIP (AU)	136.9 ± 111.2
Syndecan-1 (ng/ml)	55.2 (IQR 39.2-79.2)
VENTRICULAR FUNCTION	
LVESV (ml)	137.4±55.3
LVEDV (ml)	192.8±67.4
EF (%)	29.8±6.1
SPWD (ms)	120.0 ± 195.0
IVMD (ms)	43.9 ± 44.0

ELECTRICAL PARAMETERS	
QRSD (ms)	157. 9 ± 27.2
RVegm-LVegm (ms)	57.8 ± 35.2
LVp-RVegm (ms)	118.0 ± 43.3
RVp-LVegm	88.5 ± 38.6
Q-LVegm	88.6 ± 45.7
LVegm-QRSend (ms)	72.0 ± 53.1
PR interval (ms)	222.3 ± 62.4
RVERP 600 (ms)	297.6 ± 23.8
RVERP 500 (ms)	268.3 ± 23.1
RVERP 400 (ms)	258.0 ± 25.6

BMI: Body mass index; 6-MWD: six minute walk distance; VO_2max: peak oxygen consumption during exercise; QOL: Minnesota Living with Heart Failure questionnaire; eGFR: estimated glomerular filtration rate; MMP: matrix metalloproteinase; Aix: augmentation index; SNP: sodium nitroprusside; ADMA: asymmetric dimethyl arginine, SDMA: symmetric dimethyl arginine; SPWD: septal to posterior wall delay; IVMD: intraventricular mechanical delay; RVegm-LVegm: interval between RV and LV intracardiac electrogram; LVp-RVegm: onset of left ventricular pacing to onset of right ventricular intracardiac electrogram; RVp-LVegm: onset of right ventricular pacing to the onset of left ventricular intracardiac electrocardiogram; Q-LVegm: sum of the onset of QRS complex to right ventricular electrocardiogram and RVem-LVegm; LVP-QRSend: onset of left ventricular pacing to the end of QRS complex; RVERP: right ventricular effective refractory period. AU: arbitrary units.

TABLE 2.3 Effects of cardiac resynchronisation therapy on parameters measured at baseline. Comparisons are via paired Student's t-test or Wilcoxon test as appropriate.

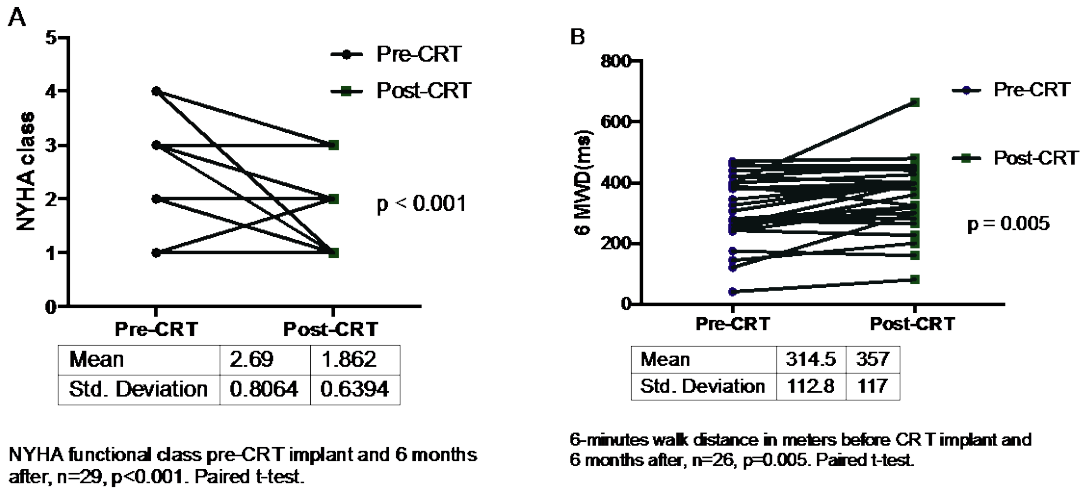
Parameters	Pre CRT	Post-CRT	P-value
CLINICAL			
NYHA	2.7 (0.8)	1.9 (0.7)	< 0.001
VO_2 max (ml/min/kg)	13.8 (4.67)	14.1 (5.3)	NS
6MWD (M)	314.5 (112.8)	357.0 (117.0)	0.005
QOL score	40.7 (25.4)	22.9 (22.3)	0.001
VASCULAR & PLATELET			
Baseline AIx (%)	20.3 (8.2)	20.3 (8.1)	NS
GTN response [AIx change (%)	-14.1 (10.0)	-16.6 (8.1)	NS
Salbutamol response [AIx change (%)]	-9.9 (10.5)	-11.9 (8.3)	NS
SNP response (%)	30.5 (21.8)	25.2 (19.7)	NS
TXNIP (AU)	144.8 (122.6)	188.5 (126.6)	NS
ADMA (μM)	0.66 (0.08)	0.65 (0.09)	NS
SDMA (μM)	0.83 (0.28)	0.74 (0.20)	0.013
Syndecan-1 (ng/ml)	55.2 (39.2-75.3)	59.8 (31.8-79.7)	NS
BIOCHEMISTRY			
Plasma metanephrine (pmol/L)	257.8 (167.2)	239.2 (88.8)	NS
Plasma normetanephrine (pmol/L)	918.8 (356.6)	900.7 (295.6)	NS
NT-pro BNP (ng/L)	1862 (1091-3185)	1469 (774-2841)	0.008
MMP-2 (ng/ml)	217.2 (52.5)	219.8 (49.9)	NS
MMP-9 (ng/ml)	27.5 (9.6)	26.5 (10.0)	NS
hs-CRP (mg/L)	2.4 (1.4-7.1)	3.5 (1.6-11.0)	NS

ECHOCARDIOGRAPHIC			
LV ejection fraction (%)	31.0 (6)	38 (10)	<0.001
LVESV (ml)	136.6 (57.5)	98.9 (52.1)	<0.001
SPWD (ms)	119.1 (201.2)	1.74 (141.4)	0.007
IVMD (ms)	43.6 (44.6)	19.9 (33.9)	0.008
ELECTRICAL MEASUREMENTS			
iQRSD (ms)	159 (23.7)	148 (29.4)	0.08
LVp-RVegm (ms)	117 (44.5)	97.0 (45.0)	0.019
PR Interval (ms)	206 (40.3)	211 (44.9)	NS
LVegm-QRSend (ms)	77.5 (53.3)	53.9 (35.5)	0.024
Q-LVegm (ms)	86.1 (47.1)	91.4 (43.4)	NS
RVegm-LVegm (ms)	59.2 (35.7)	56.2 (34.7)	NS
RVERP 600 (ms)	279 (24.7)	283 (22.8)	NS
RVERP 500 (ms)	267 (23.7)	271 (19.0)	NS
RVERP 400 (ms)	259 (26.6)	261 (19.3)	NS

BMI: Body mass index; 6-MWD: six minute walk distance; VO_2max: peak oxygen consumption during exercise; QOL: Minnesota Living with Heart Failure questionnaire; eGFR: estimated glomerular filtration rate; MMP: matrix metalloproteinase; Aix: augmentation index; SNP: sodium nitroprusside; ADMA: asymmetric dimethyl arginine, SDMA: symmetric dimethyl arginine; SPWD: septal to posterior wall delay; IVMD: intraventricular mechanical delay; RVegm-LVegm: interval between RV and LV intracardiac electrogram; LVp-RVegm: onset of left ventricular pacing to onset of right ventricular intracardiac electrogram; RVp-LVegm: onset of right ventricular pacing to the onset of left ventricular intracardiac electrocardiogram; Q-LVegm: sum of the onset of QRS complex to right ventricular electrocardiogram and RVem-LVegm; LVP-QRSend: onset of left ventricular pacing to the end of QRS complex; RVERP: right ventricular effective refractory period. AU: arbitrary units

Table 2.4 Summary of correlations performed grouped into statistically significant and non-significant correlations

Significant Correlations	Parameter 1 (y-axis)	Parameter 2 (x-axis)	r-value	p-value
	Δ 6-MWD	Δ IVMD	-0.54	0.005
	Δ QOL score	Δ iQRSD	0.24	0.04
	Δ SPWD	Δ P-R interval	0.56	0.009
	Δ normetanephrine	Δ P-R interval	-0.45	0.04
	Δ NT pro-BNP	Δ P-R interval	-0.44	0.04
	AIx to salbutamol	Baseline iQRSD	-0.40	0.02
	AIx to salbutamol	Baseline RVERP at 600ms	-0.45	0.01
	AIx to salbutamol	Baseline RVERP at 500ms	-0.44	0.01
	AIx to salbutamol	Baseline RVERP at 400ms	-0.44	0.03
	Baseline iQRSD	Baseline RVERP at 600ms	0.41	0.02
	Baseline iQRSD	Baseline RVERP at 500ms	0.37	0.04
No significant correlations				
	Δ Baseline AIx	Δ LVESV	0.07	NS
	Δ AIx to GTN	Δ LVESV	0.03	NS
	Δ AIx to salbutamol	Δ LVESV	-0.25	NS
	Δ SNP response	Δ LVESV	-0.10	NS
	Δ ADMA concentrations	Δ LVESV	0.29	NS
	Δ SDMA concentrations	Δ LVESV	-0.21	NS
	Δ TXNIP cconcentrations	Δ LVESV	0.17	NS
	Δ 6-MWD	Δ SPWD	0.08	NS
	Δ 6-MWD	Δ LVp-RVegm	-0.21	NS
	Δ 6-MWD	Δ iQRSD	-0.01	NS
	Δ QOL score	Δ SPWD	-0.21	NS
	Δ QOL score	Δ IVMD	0.04	NS
	Δ QOL score	Δ LVp-RVegm	0.24	NS
	Δ SPWD	Δ iQRSD	-0.18	NS
	Δ SPWD	Δ RVERP at 600ms	-0.26	NS
	Δ SPWD	Δ LVp-RVegm	-0.28	NS
	Δ NT pro-BNP	Δ iQRSD	-0.14	NS
	Δ NT pro-BNP	Δ RVERP at 600ms	-0.01	NS
	Δ Normetanephrine	Δ iQRSD	-0.17	NS
	Δ Normetanephrine	Δ RVERP at 600ms	-0.01	NS
	Δ TXNIP	Δ iQRSD	-0.26	NS



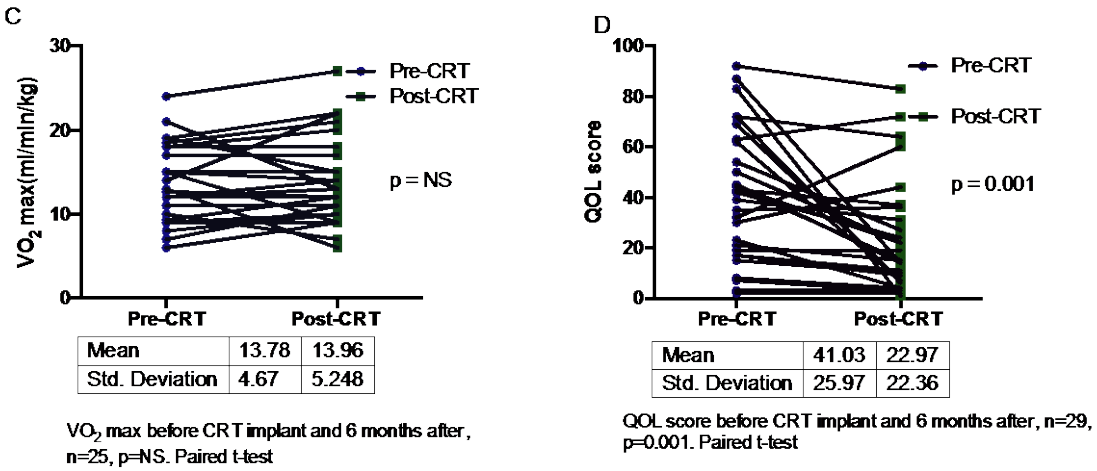


Figure 2.11 *The effects of CRT on clinical parameters*

A: New York Heart Association functional classification

B: 6-minute walk distance

C: Peak exercise oxygen consumption

D: Quality of life score

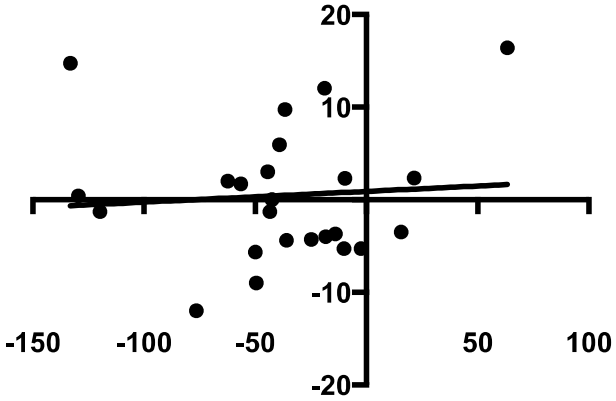


Figure 2.12 *Correlation between change in baseline augmentation index (AIx) and change in left ventricular end-systolic volume (LVESV) r = 0.073, p= NS*

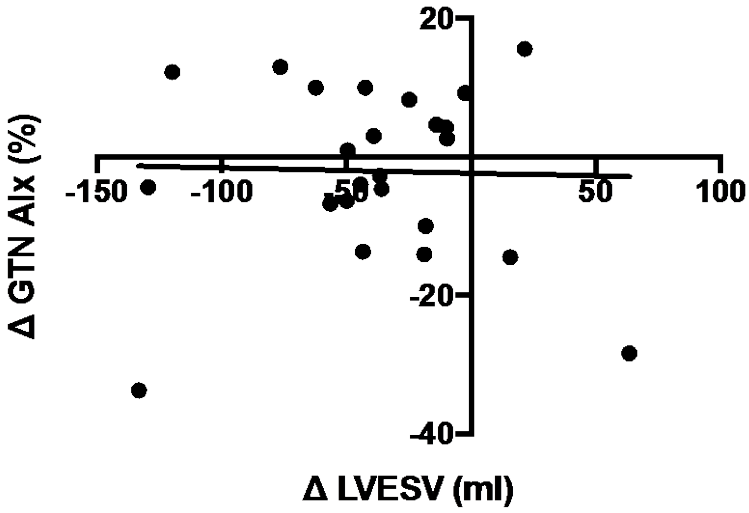


Figure 2.13 *Correlation between change in augmentation index (AIx) with GTN and change in left ventricular end-systolic volume (LVESV) r = 0.03, p= NS*

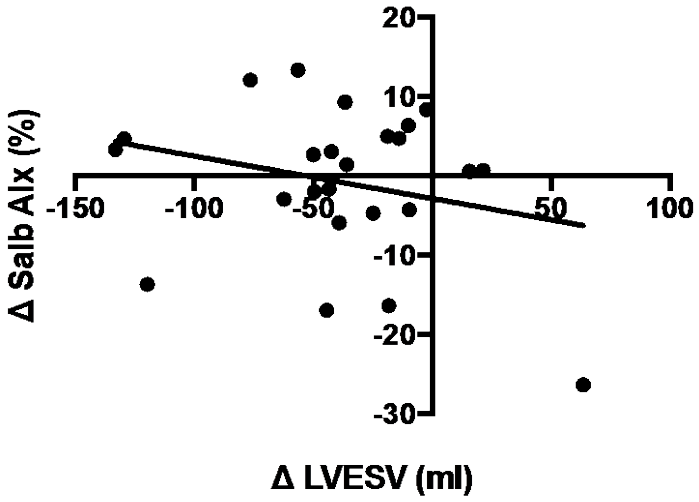


Figure 2.14 *Correlation between change in augmentation index (AIx) with salbutamol and change in left ventricular end-systolic volume (LVESV) r=-0.25, p=NS*

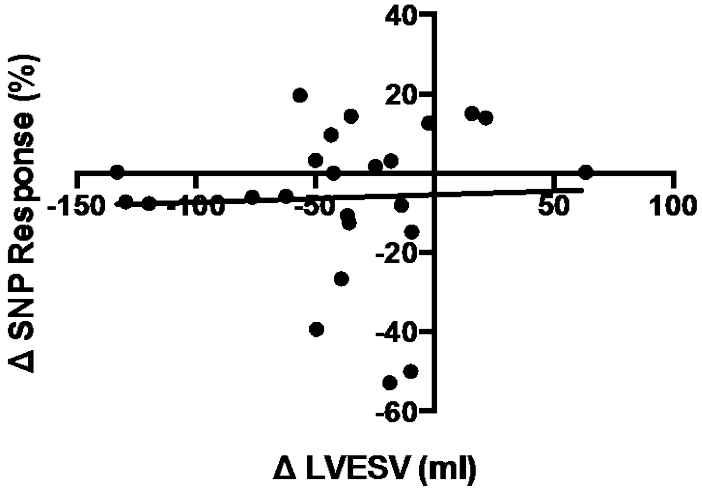


Figure 2.15 *Correlation between change in anti-aggregatory response to sodium nitroprusside (SNP) and change in left ventricular end-systolic volume (LVESV) r=-0.10, p= NS*

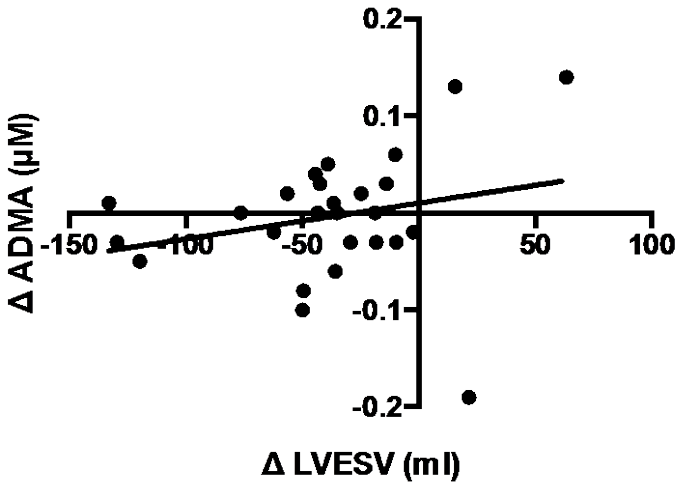


Figure 2.16 *Correlation between change in asymmetric di-methyl arginine (ADMA) concentrations and change in left ventricular end-systolic volume (LVESV) r= 0.24, p= NS*

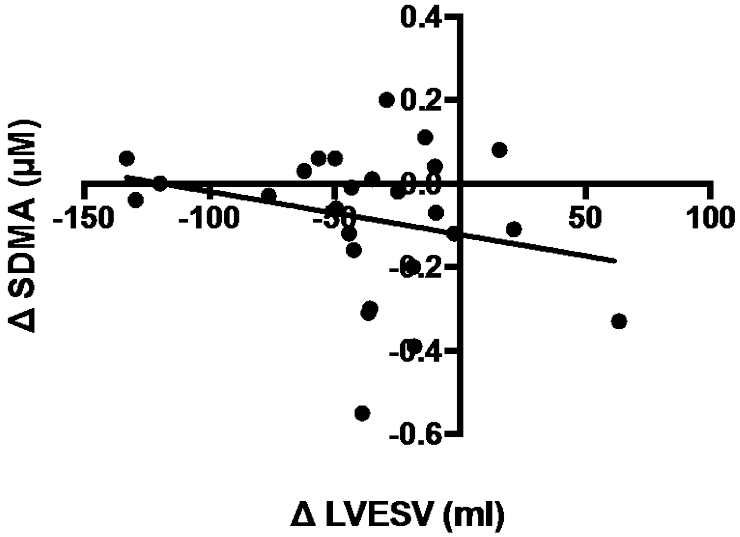


Figure 2.17 *Correlation between change in symmetric di-methyl arginine (SDMA) concentrations and change in left ventricular end-systolic volume (LVESV) r= -0.21 p= NS*

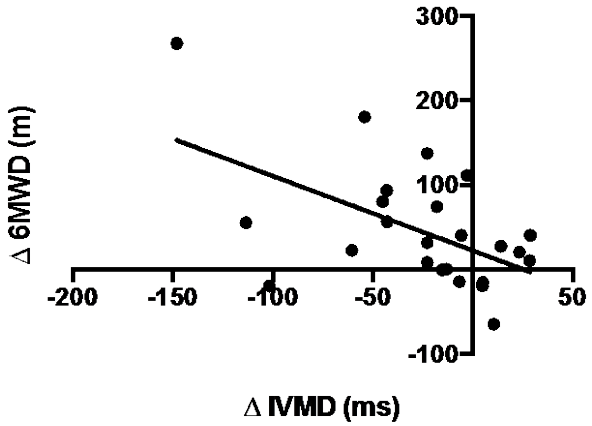


Figure 2.18 *Correlation between change in function measured by six minutes walk distance (6MWD) and change in inter-ventricular mechanical delay (IVMD) as a measure of mechanical dyssynchrony r= -0.54, p= 0.005*

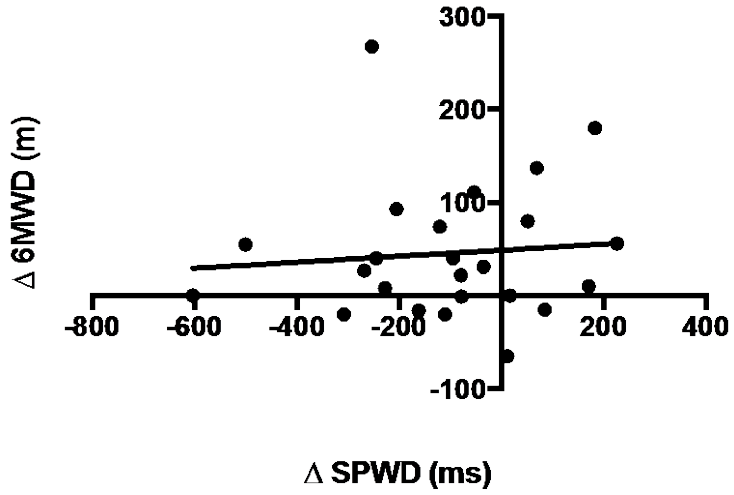


Figure 2.19 *Correlation between change in function measured by six-minutes walk distance (6MWD) and change in echocardiographic intra-ventricular septal to posterior wall delay (SPWD) as a measure of mechanical dyssynchrony r= 0.08, p= NS*

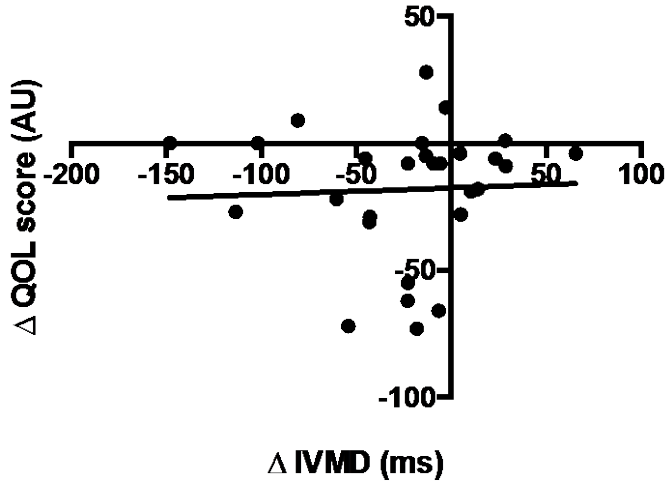


Figure 2.20 *Correlation between change in function measured by the Minnesota Living with Heart Failure Quality of Life score (QOL score) and inter-ventricular mechanical delay (IVMD) as a measure of mechanical dyssynchrony r= 0.04, p=NS.*

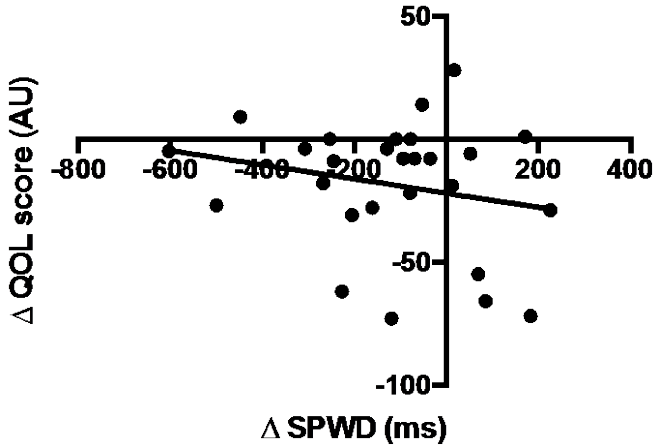


Figure 2.21 *Correlation between change in function measured by the Minnesota Living with Heart Failure Quality of Life score (QOL score) and intra-ventricular septal to posterior wall delay (SPWD) as a measure of mechanical dyssynchrony r= -0.21, p=NS*

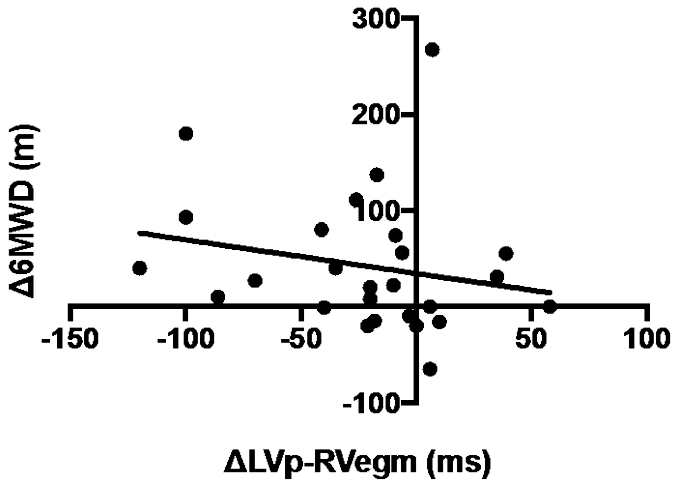


Figure 2.22 *Correlation between change in function measured by six-minutes walk distance (6MWD) and change in conduction time between onset of left ventricular pacing to the onset of right ventricular intra-cardiac electrocardiogram (LVp-RVegm), as a measure of electrical dyssynchrony r= -0.21, p= NS*

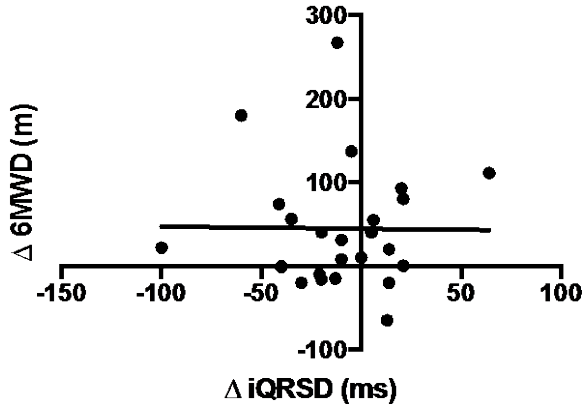


Figure 2.23 *Correlation between change in function measured by six-minutes walk distance (6MWD) and change in intrinsic QRS duration (iQRSD), as a measure of electrical dyssynchrony r= -0.01, p= NS*

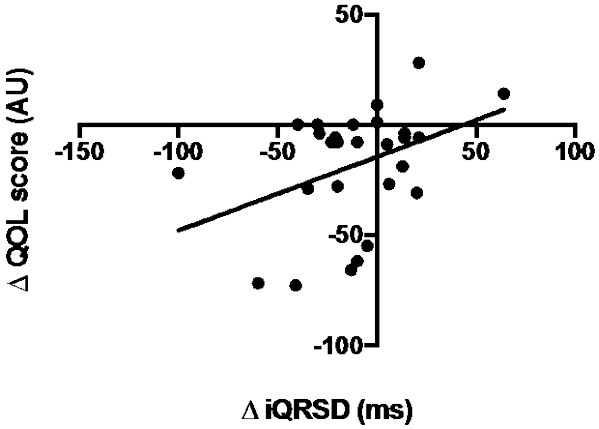


Figure 2.24 *Correlation between change in function measured by the Minnesota Living with Heart Failure Quality of Life score (QOL score) and change in intrinsic QRS duration (iQRSD), as a measure of electrical dyssynchrony r=0.24, p= 0.04*

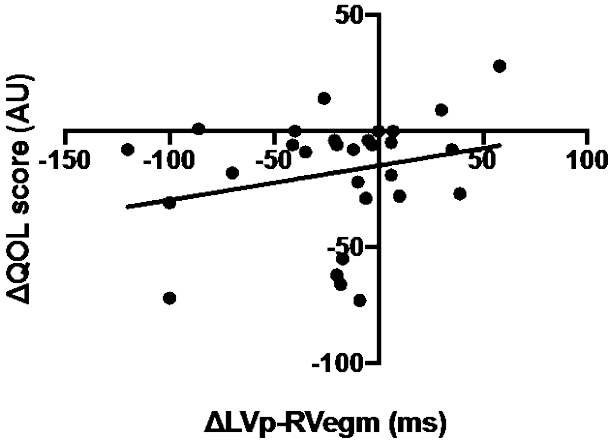


Figure 2.25 *Correlation between change in function measured by the Minnesota Living with Heart Failure Quality of Life score (QOL score) and change in conduction time between onset of left ventricular pacing to the onset of right ventricular intra-cardiac electrocardiogram (LVp-RVegm), as a measure of electrical dyssynchrony r= 0.24, p= NS*

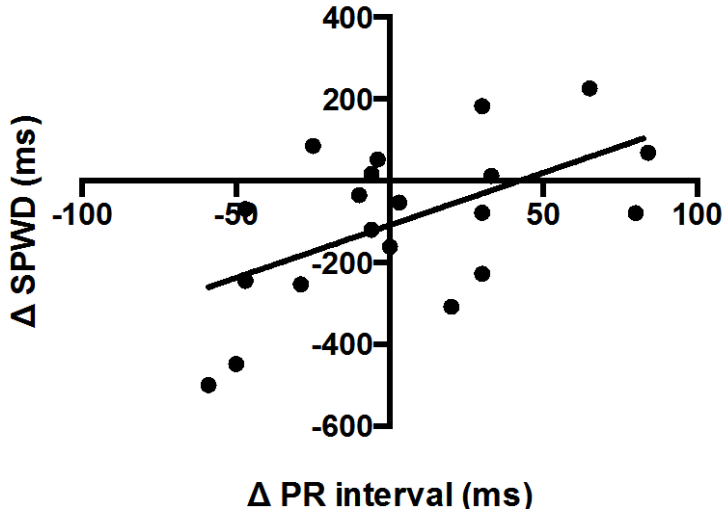


Figure 2.26 *Correlation between change in PR interval as a measure of cardiac electrical conductivity and change in septal to posterior wall delay (SPWD) as a measure of intra- ventricular mechanical dyssynchrony r= 0.56, P= 0.009*

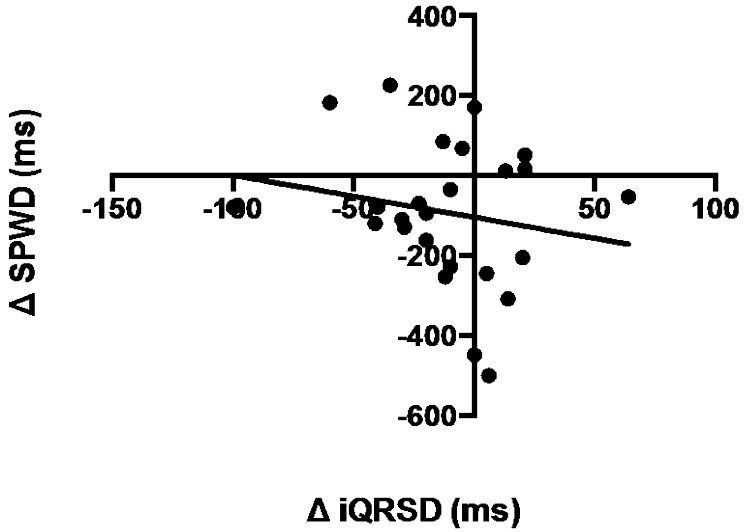


Figure 2.27 *Correlation between change in intrinsic QRS duration as a measure of cardiac electrical dyssynchrony and septal to posterior wall delay (SPWD) as a measure of intra-ventricular mechanical delay r= -0.18, p= NS*

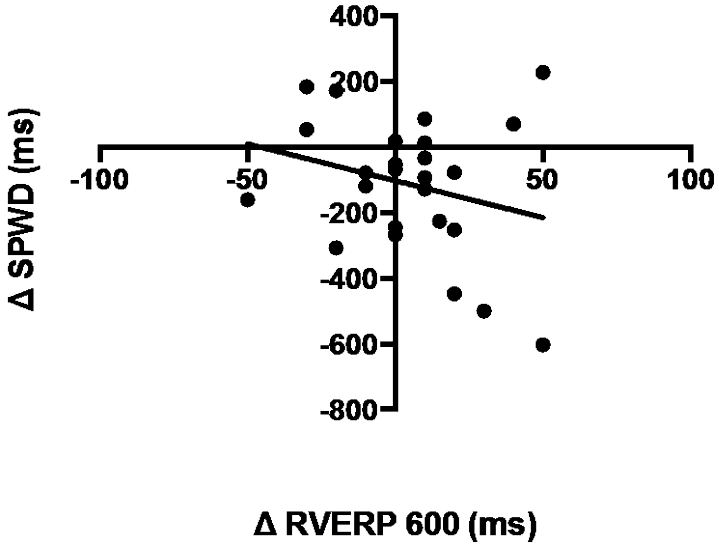


Figure 2.28 *Correlation between change in right ventricular effective refractory period (RVERP) at cycle length of 600ms as a measure of cardiac excitability and change in left ventricular septal to posterior wall delay (SPWD) as a measure of intra-ventricular mechanical dyssynchrony r= -0.26, p=NS*

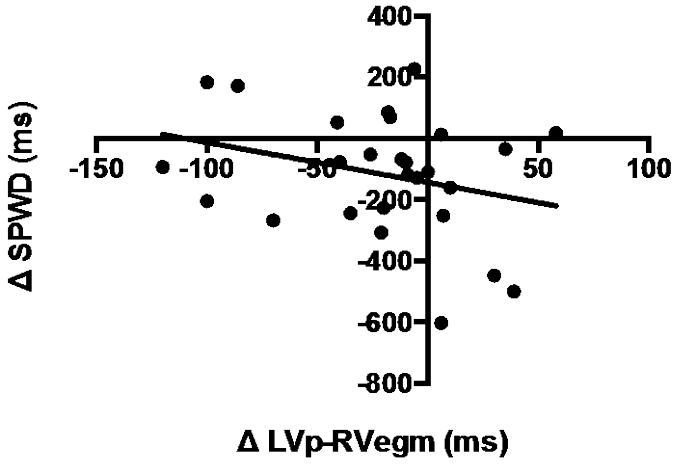


Figure 2.29 *Correlation between change in left ventricular pacing to the onset of right ventricular intra-cardiac electrogram (LVp-RVegm) as a measure of cardiac electrical dyssynchrony and change in left ventricular septal to posterior wall delay (SPWD) as a measure of intra-ventricular mechanical dyssynchrony r= -0.28, p= NS*

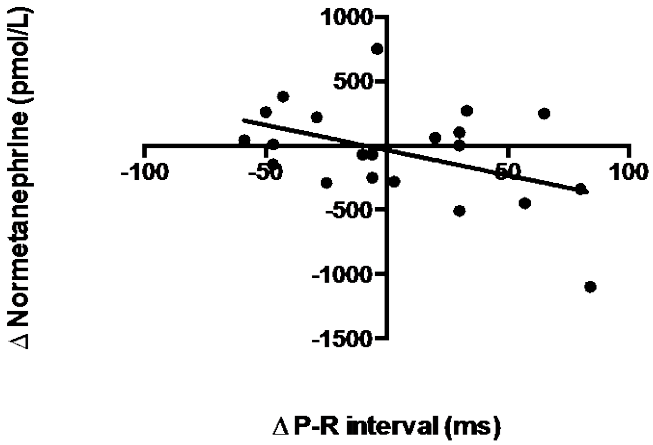


Figure 2.30 *Correlation between change in P-R interval as a measure of cardiac electrical conductivity and change in plasma normetanephrine concentration r= -0.45, p= 0.04*

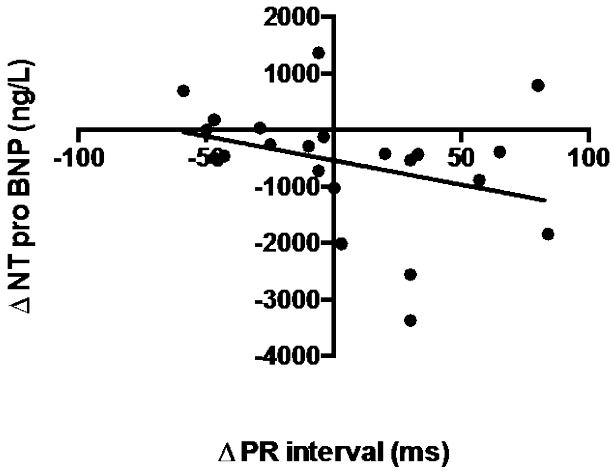


Figure 2.31 *Correlation between change in P-R interval as a measure of cardiac excitability and change in plasma NT-pro BNP concentration r= -0.44, p= 0.04*

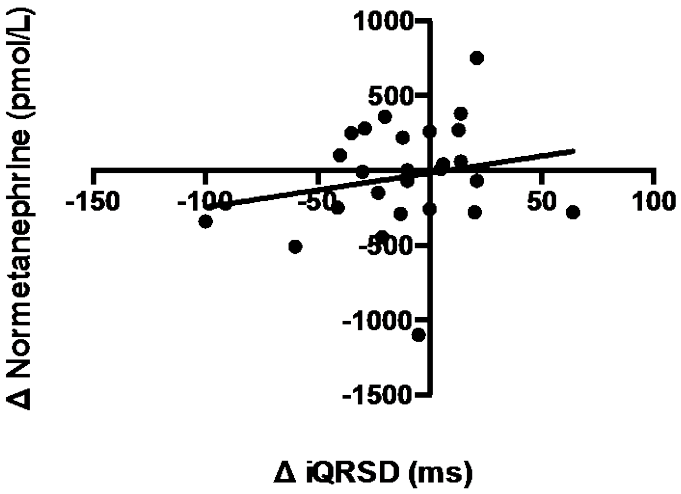


Figure 2.32 *Correlation between change in intrinsic QRS duration as a measure of cardiac electrical dyssynchrony and plasma normetanephrine concentration r= -0.17, p= NS*

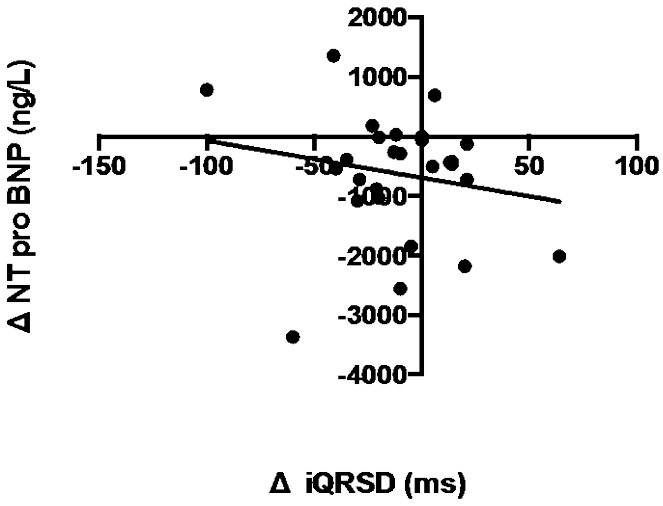


Figure 2.33 *Correlation between change in intrinsic QRS duration as a measure of cardiac electrical dyssynchrony and plasma NT-pro BNP concentration r= -0.14, p= NS*

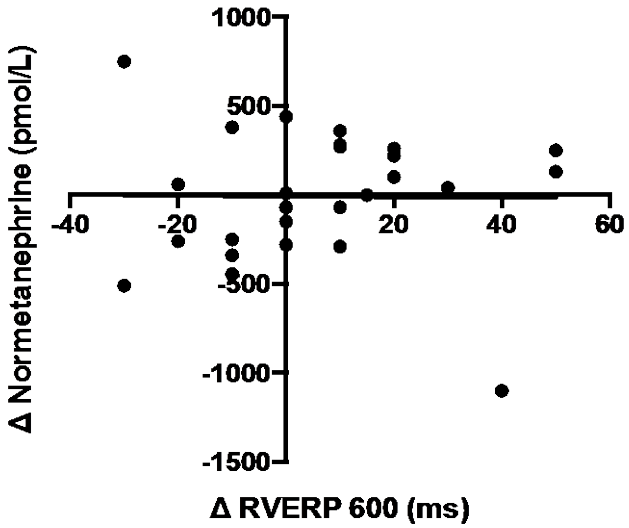


Figure 2.34 *Correlation between change in right ventricular effective refractory period (RVERP) at a cycle length of 600ms as a measure of cardiac excitability and change in plasma normetanephrine concentration r= -0.01, p= NS*

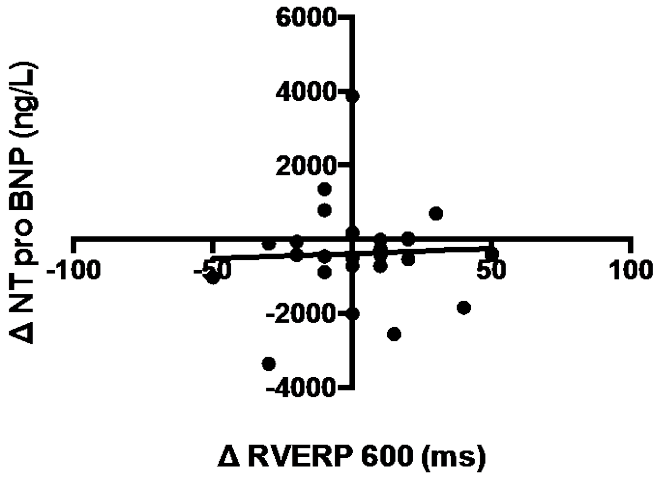


Figure 2.35 *Correlation between change in right ventricular effective refractory period (RVERP) at a cycle length of 600ms and change in plasma NT-pro BNP concentration r=0.07, p= NS*

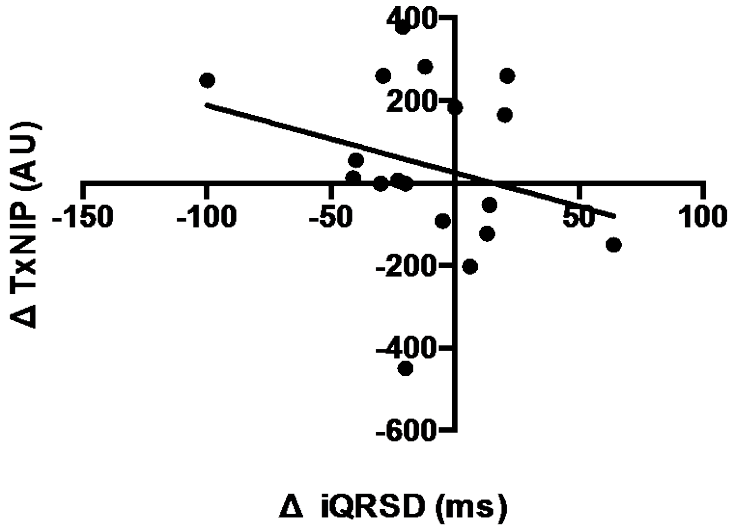


Figure 2.36 *Correlation between intrinsic QRS duration as a measure of cardiac electrical dyssynchrony and platelet thioredoxin (TXNIP) content r=-0.26, p=NS*

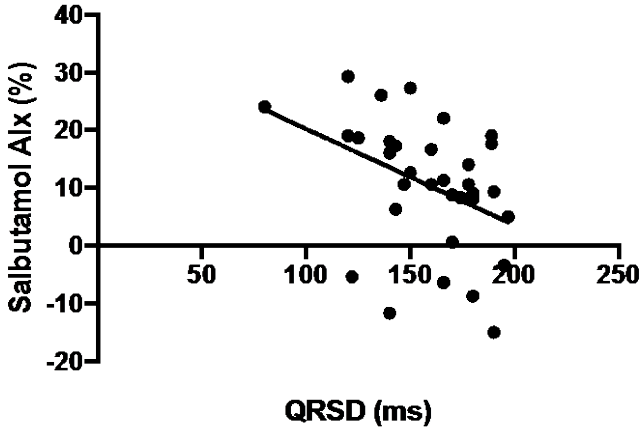


Figure 2.37 *Correlation between change in augmentation index to salbutamol before CRT implantation and the intrinsic QRS duration r= -0.40, p= 0.02*

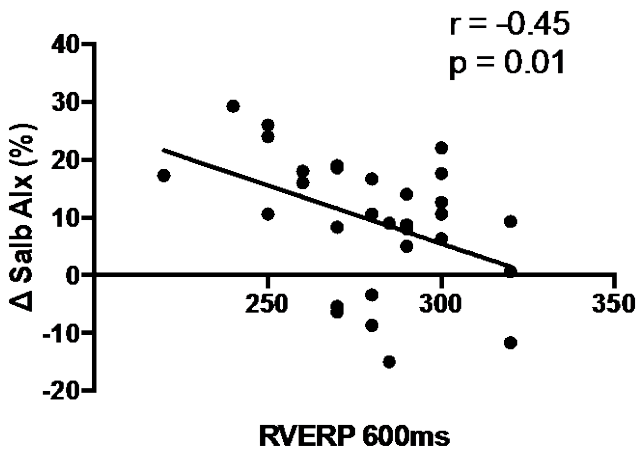


Figure 2.38 *Correlation between baseline right ventricular effective refractory period at 600ms and change in augmentation index to salbutamol prior to CRT implantation r= -0.45, p= 0.01*

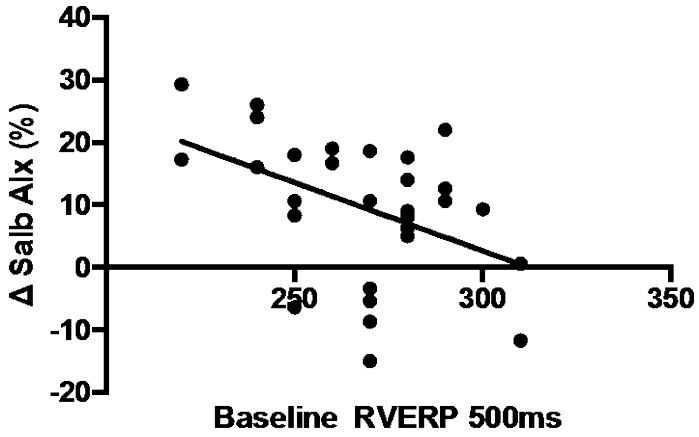


Figure 2.39 *Correlation between baseline right ventricular effective refractory period at 500ms and change in augmentation index to salbutamol prior to CRT implantation r= -0.44, p= 0.01*

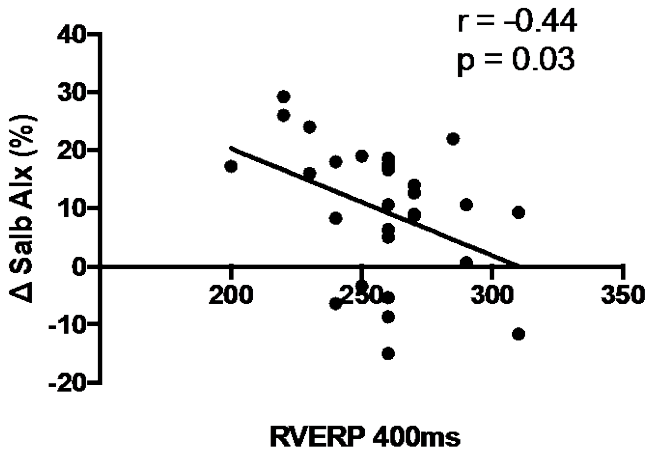


Figure 2.40 *Correlation between baseline right ventricular effective refractory period at 400ms and change in augmentation index to salbutamol prior to CRT implantation r= -0.44, p= 0.03*

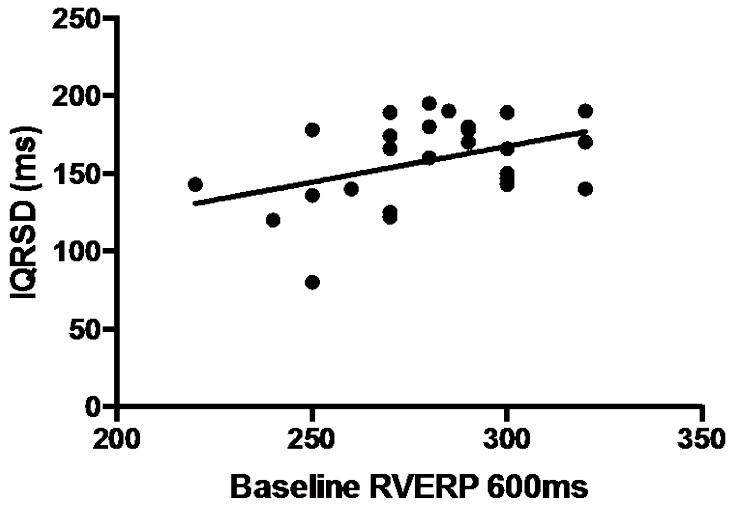


Figure 2.41 *Correlation between baseline right ventricular effective refractory period at 600ms and intrinsic QRS duration r= 0.41, p= 0.02*

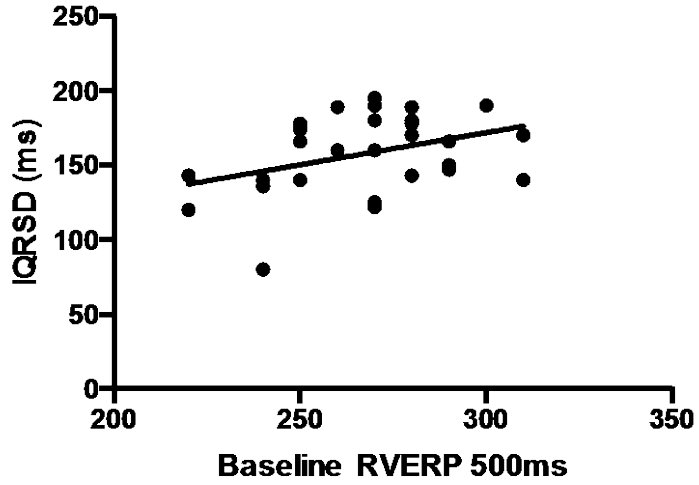


Figure 2.42 *Correlation between baseline right ventricular effective refractory period at 500ms and intrinsic QRS duration r= 0.37, p= 0.04*

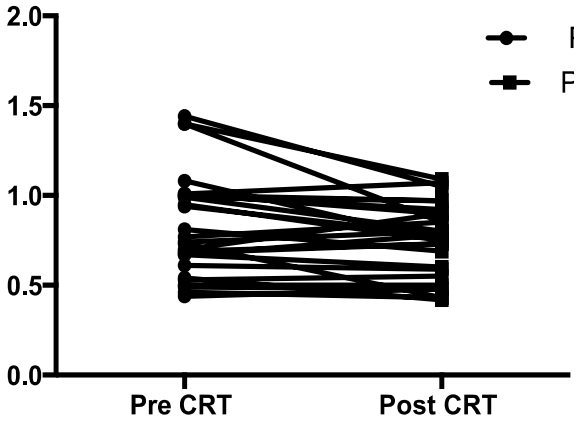


Figure 2.43 *Paired t-test of symmetric dimethyl arginine levels pre-CRT implant and 6 months after, p= 0.013*

CHAPTER 3

DISCUSSION

3.1The patient population tested.

The indications for CRT had been traditionally based on QRS duration, NYHA functional class and left ventricular ejection fraction. Based on CARE-HF(Cleland , Daubert et al. 2005) and COMPANION, (Bristow, Saxon et al. 2004) CRT was recommended in patients with left ventricular ejection fraction of 35% or less with wide QRS and NYHA class III or ambulatory class IV. Following results from the RAFT study(Tang , Wells et al. 2010), the NHYA class was expanded to include patients in class II, and indeed, 2013 European Society of Cardiology Guidelines on cardiac pacing and cardiac resynchronization therapy(Brignole, Auricchio et al. 2013) concluded that there was no significant heterogeneity in morbidity and mortality among patients in NHYA II-IV with moderate to severe left ventricular systolic dysfunction considered for CRT and therefore decided to adopt the term 'symptomatic heart failure' rather than NYHA class. Furthermore, the REVERSE(Linde, Abraham et al. 2008) and MADIT-CRT(Moss, Hall et al. 2009) trials have demonstrated improvement in left ventricular remodelling as well as in heart failure hospitalisations in patients with NYHA class I-II. Even in select patients with left ventricular ejection fraction of more than 35% but less than 50%, CRT has been recommended. For example, the results of BLOCK –HF trial(Curtis 2013) showed that in patients with LVEF less that 50% who require pacing due to atrioventricular block, biventricular pacing reduced all cause mortality, heart failure hospitalisation and left ventricular remodelling compared to right ventricular pacing alone, even in patients with NHYA functional class I-III. Relating these to our patient population, 23 (70%) were in NYHA class III-IV, 7 (21%) were in NYHA class III while 3 (9%) were in NYHA class I. Of the 3 patients that reported NYHA class I symptoms, only one had mild left ventricular systolic dysfunction and received CRT (consistent with BLOCK-HF trial) especially as this particular patient also had Lamin-A gene mutation and indeed had multiple episodes of ventricular tachyarrhythmia on

follow-up. 52% of our cohort had ischemic aetiology of heart failure a similar proportion to the 55% in the COMPANION trial. Our patients were also generally older (mean age 71.2 years) compared to some of the landmark trials like COMPANION (66 years) and the RAFT (66.1 years). 42% of our patients had diabetes mellitus (comparable to 41% in the COMPANION but higher than the 32.8% in the RAFT trial). Although the earlier landmark trials like COMPANION and CARE-HF did not include patients with permanent atrial fibrillation, the RAFT trial cohort included 12.8% of patients with permanent atrial fibrillation; 15% of our cohort had permanent atrial fibrillation. The (considerable) extent of pharmacological treatment in our cohort is also generally similar to that in the COMPANION trial. 91% of our patients were either on an ACE inhibitor or an ARB antagonist while 55% were on an aldosterone antagonist compared to 90% and 55% respectively for the COMPANION cohort.

Experienced operators implanted the devices across the three recruiting sites. The left ventricular pacing lead was deployed in all cases intravenously (with the exception of one patient that ended up with epicardial lead placement due to coronary sinus dissection) into a coronary sinus tributary, preferably, the posterolateral or lateral branch. The anterior cardiac vein was avoided. There were no other major adverse peri-procedural events.

3.1.1 The general efficacy of CRT in the cohort: overall efficacy.

Paired analyses of the echocardiographic measurements, most clinical parameters and some biochemical parameters showed significant improvements at 6 months in the cohort in general indicating that the cohort received ‘good’ CRT. This is very important because if the converse were to be true, then it would be difficult to proceed with testing the hypotheses.

3.1.2 Echocardiographic changes:

Our cohort exhibited a +7.0 absolute increase in LVEF from 31.0 ± 6.0 % at baseline to 38.0 ± 10.0% at 6 months which is somewhat similar to the findings from the CARE-HF (Cleland , Daubert et al. 2005) which recorded a mean absolute improvement in LVEF of 6.7% at 18 months. Similarly, we noted a reduction in inter-ventricular mechanical delay of -23.7ms while the CARE-HF reported a mean reduction of -21.0ms at both 3 and 18 months. The MIRACLE study(Abraham, Fisher et al. 2002) recorded a somewhat lower, albeit significant absolute increase of + 4.6% in LVEF at 6 months.

3.1.3 Improvement in Clinical parameters

In agreement with most CRT studies, our cohort showed significant improvements in NYHA functional class, quality of life score and 6-minute walk distance. We observed a mean decrease of 0.8 in NYHA class, which compares well with a mean decrease of 1.0 in the MIRACLE-ICD trial(Young, Abraham et al. 2003). Other studies have also reported significant improvements in NYHA functional class following CRT(Abraham, Fisher et al. 2002, Cleland , Daubert et al. 2005).

We found that our cohort had an improvement of -17.8 points as measured by the Minnesota Living with Heart Failure (MLHF) questionnaire. The MIRACLE study reported an improvement of -18 points in their group(Abraham, Fisher et al. 2002), the MIRACLE-ICD(Young, Abraham et al. 2003) reported -17.5 while the MUSTIC study showed an improvement of -17.4 points(Cazeau, Leclercq et al. 2001). On the other hand, there was no significant difference in MLHF quality of life score in the REVERSE trial(Linde, Abraham et al. 2008) (-8.4 ± 17.1 in CRT-ON vs. -6.7 ± 15.9 in CRT-OFF; p= 0.26). The REVERSE group differed from ours and the others mentioned above in that

it consisted of patients who were either asymptomatic or had only mild heart failure symptoms. Consequently, the baseline MLHF quality of life score in the REVERSE group was 27.0 ± 20.1 compared to our baseline of 41.9 ± 25.6 (higher values indicate worse quality of life).

With regards to 6-minute walk distance, we observed a significant improvement of 42.5m in our study group. In the MIRACLE study(Abraham, Fisher et al. 2002), 6-minute walk distance was significantly improved by 39m following CRT while in the MUSTIC trial(Cazeau, Leclercq et al. 2001), it was increased by 49.2m. Again, similar to the effect on the MLHF quality of life score, there was a trend towards improvement in 6-minute walk distance of 12.7m in the REVERSE study of mildly symptomatic or asymptomatic heart failure patients(Linde, Abraham et al. 2008).

There was no significant improvement in exercise peak oxygen consumption (VO_2 max) in our group. This agrees with the results of the landmark MIRACLE ICD trial(Young, Abraham et al. 2003), but is in contrast to the result obtained in the MIRACLE study, with an absolute but statistically significant median increase in VO_2 max of 1.1ml/kg/min. VO_2 max was also statistically improved in the MUSTIC study(Cazeau, Leclercq et al. 2001) but only by 8% and by an absolute value of 1.2ml/kg/min. Furthermore, the MUSTIC study(Cazeau, Leclercq et al. 2001) was a cross-over study with two study groups having alternate periods of active and inactive CRT pacing. In the first study group with CRT-ON, there was no significant improvement in VO_2 max with absolute increase of 0.6ml/kg/min. When these patients in MUSTIC were followed up for 12 months, the improvement in VO_2 max was no longer statistically significant(Linde, Leclercq et al. 2002). A different and smaller study of thirteen patients however showed that in spite of significant improvements in NYHA class, quality of life score, 6 minute walk distance and duration of exercise

testing, CRT failed to improve VO_2 max(Livanis, Flevari et al. 2003). This may reflect type II error.

In retrospect, power calculations were carried out for changes in VO_2 max, based on actual number of patients who completed the study. Taking into account the variability in VO_2 max at baseline, the current study had 80% power to detect the 8% increase in VO_2 max post CRT reported in MUSTIC. Therefore, the study was somewhat underpowered in this regard.

The question arises as to how CRT insertion can induce substantial improvement in another objective functional parameter such as LVEF, and yet have little or no effect on VO_2 max. Perhaps one explanation might be that in patients with severe chronic heart failure, exercise tolerance may be substantially limited by extra-cardiac factors such as muscle wasting. However, the study design did not permit analysis of this issue to be performed.

Overall, therefore, the effects of CRT on functional status were reasonably consistent with those of previously reported studies. **In Table 3.1** the current results are compared with those of previously published analogous data.

3.1.4 Changes in biochemical parameters

Our cohort demonstrated a significant improvement in plasma NT pro-BNP concentrations at 6 months in keeping with the results of some previous studies(Cleland , Daubert et al. 2005, Hoogslag, Hoke et al. 2013). Interestingly, Hoogslag et al found that a reduction in NT pro-BNP of ≥15% at 6 months post CRT implant showed better concordance with

echocardiographic response than with clinical response. In addition, they found that patients with substantial reduction in NT pro-BNP demonstrated better long-term outcomes than those without such changes(Hoogslag, Hoke et al. 2013). In our study, there was a 21% reduction in NT pro-BNP at 6 months. Given that changes in NT pro-BNP concentrations are widely accepted as indicators of improvement/deterioration of heart failure, these results can be taken as further evidence that the CRT procedure "worked".

One possible biochemical impact of CRT would be amelioration of inflammatory activation, either predominantly within the myocardium and/or within the systemic circulation. There is an abundance of evidence for inflammatory activation in patients with heart failure, and although tumour necrosis factor-α and interleukin-6 have been implicated(Torre-Amione, Kapadia et al. 1996, Deswal, Petersen et al. 2001), the precise inflammatory pathways involved remain poorly delineated.

Our cohort had a baseline hs-CRP concentration of 2.4 (1.4-6.1) mg/mL with no subsequent significant change at 6 months. Michelucci et al (2006) divided their CRT cohort into groups with and without adverse cardiac events and found that although both groups recorded significant improvements in clinical parameters, only the group with no clinical adverse events had a significant reduction in hs-CRP at 6 months(Michelucci, Ricciardi et al.) Furthermore, their hs-CRP responder group had a somewhat lower baseline hs-CRP concentrations than the non-responder group. Other studies have shown that baseline hs-CRP > 3.0mg/mL is predictive of future adverse events and lack of response to CRT(Kamioka, Suzuki et al. 2012, Cai, Hua et al. 2014). In summary, there is no good evidence from previous studies that CRT decreases plasma hs-CRP concentrations, and the current results are consistent with these data.

Although there was a trend towards reduction in plasma concentrations of metanephrine and normetanephrine, (see **Figures 3.1 and 3.2)** the changes were not statistically significant. Previous studies have documented positive modulatory effects of CRT on autonomic tone especially by increasing heart rate variability (Adamson, Kleckner et al. 2003, Livanis, Flevari et al. 2003, Fantoni, Raffa et al. 2005). However, this effect was not associated with reduction in plasma catecholamine levels at 3 months(Adamson, Kleckner et al. 2003). Seifert et al (2007) however found a significant decrease in plasma norepinephrine concentrations when they followed up twenty-two patients for 12 months after CRT insertion(Seifert, Schlegl et al. 2007). Overall the current data are equivocal as regards evidence of amelioration of sympathetic stimuli by CRT. This is a major issue, because catecholamine stimulation represents a substantial contributor to cardiovascular dysfunction in chronic heart failure. (Mann, Kent et al. 1992, Osadchii, Norton et al. 2007)

Activation of matrix metalloproteinases, particularly MMP-2 and MMP-9 would in theory be particularly important, because these proteins have been implicated as initiators of glycocalyx shedding, and as such, of worsening of tissue oedema and of white cell infiltration into tissues. However, in our study, CRT did not significantly alter MMP-2 and-9 concentrations. This is partially in contrast to a previously published report. (Hessel, Bleeker et al. 2007) demonstrated a significant reduction in plasma concentrations of MMP-9 but no change in plasma MMP-2 concentrations following CRT. Tolosana et al(Tolosana, Mont et al. 2010) on the other hand found that higher baseline levels of MMP-2 predicted non-response to CRT on univariate analysis. This particular study however did not report the effect of CRT on MMP-2. Recent data from our laboratory (currently unpublished) found that mean MMP-2 concentration was 337.1 ± 97.0ng/ml for acute heart failure and 215.9 ± 27.3ng/ml in normal subjects. While MMP concentrations do not necessarily predict enzymatic activity, it does appear that at least expression of

MMP-2 and MMP-9 was not elevated substantially above normal values in the CRT population currently evaluated. Supposing that the previous report of MMP-9 suppression post CRT insertion (Hessel, Bleeker et al. 2007) actually reflect an anti-inflammatory action, the detection of this might well depend on extent of use of heart failure drugs with anti-inflammatory effects, such as ACE inhibitors and mineralocorticoid antagonists, which was not stated in the reported study. In our current series, such therapy was extensive at baseline.

Glycocalyx shedding reflects inflammatory activation and consequent erosion of this cell-protecting carbohydrate-rich layer. In the current study, we were aware that inflammatory activation, particularly, but not entirely, via MMP-2 and-9 effect, can result in erosion of the glycocalyx and release of its components, including syndecan-1 (SD-1) into the blood stream. In the current study, baseline concentrations of SD-1 were 55ng/ml (IQR 39.2-79.2), compared with a mean of 20.1ng/ml in normal subjects (Tromp, van der Pol et al. 2014). It is therefore likely that there was some activation of glycocalyx shedding though to a lesser extent than in acute heart failure (Neves, Meneses et al. 2015) On the other hand, SD-1 concentrations did not change following CRT, indicating that CRT is likely to exert no effects on either endothelial activation or vascular permeability.(Curry and Adamson 2012)

3.2.THE PRIMARY HYPOTHESIS

Our primary null hypothesis was that improved clinical status following CRT is independent of changes in peripheral vascular dysfunction. From our findings, this null hypothesis holds.

With a sample size of 33 patients, and paired analysis, the study was powered to detect approximately a 1.25 SD difference in augmentation index in response to salbutamol (a marker of eNOS activation) at α =0.05, β =0.8 level, Therefore, the potential that a substantial improvement in eNOS-related arterial function was masked by type II error is quite small. The null hypothesis is retained because in spite of significant improvements in measures of left ventricular contractile functions, as evidenced by reduction in LVESV, IVMD, SPWD and increase in LVEF, there was no improvement in augmentation index response to inhaled salbutamol. In addition, all the other measures of peripheral vascular function with the notable exception of symmetric dimethyl arginine concentration (to be discussed below), did not improve with CRT.

3.2.1 Lack of impact on arterial distensibility or reactivity:

There was no significant change post-CRT in either baseline augmentation index (AI_X), or in endothelium-independent NO response (using nitroglycerin) or endothelium-dependent NO response (using salbutamol). However, there was a trend for effects of both salbutamol and GTN on AIx to increase (by about 20%), see **Figures 3.3 and 3.4.** Several factors could contribute to this apparent absence of significant effects. First, our baseline augmentation index of 19.9 ± 7.8% before the implantation of CRT was only marginally greater than previously reported normal values of 15 ± 16% by McEniery et al(McEniery, Wallace et al. 2006). This could be a consequence of our cohort being extensively treated pharmacologically for heart failure and for dyslipidemia before receiving CRT, thus limiting the effects of heart failure on arterial compliance. For instance, ninety-one percent of our cohort was on either an angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker, and ACEi are known to significantly reduce AI_X (Jiang, Li et

al. 2005). Statins also cause significant reduction in AI_X via eNOS stimulation(Sahebkar, Pecin et al. 2016) and 55% of patients were on a statin.

The lack of effect with CRT on baseline AI_X may be that CRT itself may exert opposing effects on AI_X: by increasing stroke volume into non-compliant vessels with already depressed Windkessel effect, there would be an increase in the velocity of both the forward and the reflected waves in the peripheral vasculature. In addition, considering that 76% of the patients were on β-adrenoceptor antagonists, the use of β-adrenoceptor antagonists could potentially blunt the effect of salbutamol on AI_X, thus obscuring the effect of CRT. However, at baseline, there was no significant difference in AI_X response to salbutamol in patients receiving and not receiving β-adrenoceptor antagonists. As this is an important issue, we re-evaluated the actual power of the study to detect relatively small increases in AIx responses, generating operating characteristic curves for both responses to GTN and to salbutamol using actual data from baseline. See **Figure 3.5.** The results indicated that with a mean AIx of 20 and standard deviation (SD) of 7.8, and half SD of about 4, to detect 20-24, which is an increase of 0.5SD, n= 30 has 76% power and n= 40 has 87% power. Our calculated sample size was 33, hence our study is adequately powered.

The possible effect of CRT on peripheral vascular endothelial function has been previously evaluated by other investigators using changes in flow-mediated dilatation (FMD) of the brachial artery with varying results. (Akar, Al-Chekakie et al. 2008)observed that CRT induced only a trend towards improvement in FMD, (p=0.12). In a very recent study of 19 patients receiving CRT, Warriner et al(Warriner, Lawford et al. 2016) similarly observed that post CRT, but there was no significant improvement in FMD irrespective of clinical and echocardiographic response. On the other hand, Santini et al(Santini, Capria et al.

2013) found that in a cohort of 57 patients who received CRT, FMD was improved significantly irrespective of CRT–response status of patients. FMD was not utilised in the current study because of concerns that the results are less reproducible than those of AIx determination, and because of the impracticality of evaluating components of specifically NO-dependent and eNOS-dependent responses. However, it can be summarised from the three above-mentioned FMD-based studies that there is no consensus that CRT improves FMD. Among the FMD-based studies, both Akar et al (2008) and Warriner et al (2016) suggested "heterogeneity" of response to CRT at the level of FMD: patients with low baseline FMD were more likely to improve post CRT. This sort of analysis is questionable for two reasons:

1. The overall analysis of the designated end-point showed no significant change, and
2. This type of sub-analysis risks the possible impact of 'regression towards the mean'

Overall, therefore, the finding that there is no significant change in AIx response to salbutamol in the current study is consistent with the majority of previous findings based on FMD recording.

3.2.2 Effects on ADMA and SDMA

We found that plasma concentrations of ADMA, a potent competitive inhibitor of NOS, were not significantly altered by CRT intervention, indicating that CRT does not appear to have a beneficial effect with regards to NOS-catalysed NO generation. If a putative increase in AIx response to salbutamol were engendered by reduction in ADMA concentrations, responses to nitroglycerin would have remained unchanged. It is also important to recognize that CRT therefore has not affected ADMA kinetics, which is largely determined by redox-related metabolic clearance.

On the other hand, there was a significant reduction, post-CRT in the concentrations of SDMA, a weak NOS inhibitor but a potent pro-inflammatory modulator(Schepers, Barreto et al. 2011). Although SDMA is predominantly renally-cleared (unchanged)(Ronden, Houben et al. 2012), the significant reduction in SDMA levels post-CRT is not due to improved renal clearance. This is because, despite a significant inverse correlation of SDMA with eGFR at baseline, there was no significant change in eGFR at 6 months. It is therefore more likely that activity of the enzyme, protein arginine methyltransferase (PRMT) type II, which generates SDMA in response to catabolic stimuli, may have decreased following CRT, indicating some form of reduction in inflammatory activation. Whatever the significance of reduction in SDMA concentrations may be, this must be a specific, rather than a generalized reduction in inflammatory activation, given that hs-CRP and MMP-2 and –9 concentrations did not change.

3.2.3 Platelet expression of thioredoxin interacting protein (TXNIP)

TXNIP is a critical regulator of redox stress and activator of inflammatory cascades.

Contrary to our expectations, CRT did not result in reduction of platelet expression of TXNIP in our cohort in spite of its being known to be shear stress dependent(Yamawaki, Pan et al. 2005, Spindel, Burke et al. 2014). These findings raise a number of issues. For example, is there significant restoration of arterial laminar flow following CRT, as has been previously assumed? Does suppression of TXNIP expression by ACE inhibitors (widely used in this cohort) preclude further reduction by restoration of laminar flow? Is the reciprocal relationship between platelet TXNIP expression and responsiveness to NO (Sverdlov, Chan et al. 2013) unilaterally driven by NO? In any case, there is no suggestion that CRT impacted TXNIP kinetics. It remains possible that vascular TXNIP may respond differently.

3.2.4 Effects of CRT on platelet physiology.

One of the potential benefits of CRT is that increased NO generation and/or signaling, might also in theory impact platelet aggregability and anti-aggregatory effects of NO donors respectively. However, in the current study, neither aggregation response to ADP nor anti-aggregatory effect of the NO donor sodium nitroprusside changed. Thus CRT has no major effects on NO signaling at the platelet level

3.2.5 Outcome: Primary Hypothesis

In summary therefore, with regard to our primary hypothesis, CRT, in spite of inducing significant improvement in left ventricular contractile function in our cohort, did not result in any significant change in peripheral vascular function, most markers of inflammatory activation or in platelet nitric oxide signaling, thereby indicating a disconnect between improved mechanical contractility and both inflammation and vascular endothelial function.

3.3 THE SECONDARY HYPOTHESES: OUTCOME

3.3.1 Does the extent of reduction in mechanical dyssynchrony predict functional improvement?

We prospectively nominated improvement in VO_2 max as the functional parameter to be correlated with reduction in both SPWD and IVMD, the two parameters of mechanical dyssynchrony and with reduction in both LVp-RVegm and intrinsic QRS duration, as markers of electrical dyssynchrony. Unfortunately, VO_2max did not increase significantly, making any correlation attempt of doubtful statistical legitimacy. However, other, more subjective, markers of functional improvement reached statistical significance, and therefore can in theory be utilised for the above correlations studies in place of changes in VO_2max. We chose to use changes in both quality of life score ($p= 0.001$) and in 6-minute walk distance ($p=0.005$) for substitute analyses. In fact, these post hoc analyses showed that improvement in 6-minute walk distance was associated with significant reduction in inter-ventricular mechanical delay but not with intra-ventricular mechanical delay. There were however, no significant correlations between any of these measures of mechanical dyssynchrony and the observed improvement in quality of life scores. In contrast to these findings, there was no correlation between 6-minute walk distance and intrinsic QRSD or LVp-RVegm, the parameters used to measure electrical dyssynchrony, however, improvement in quality of life score was associated with significant reduction in intrinsic QRS duration but not with LVp-RVegm. While these findings may suggest a disconnect between mechanical and electrical dyssynchrony, given that these were not our initial choices for comparators, the conclusions from these analyses are less than definitive.

Our findings should be compared with those of previous investigations. In a double-blind randomised single centre study, patients with both mechanical and electrical dyssynchrony

were compared to patients with only electrical dyssynchrony but no mechanical dyssynchrony at baseline and 6 months after CRT insertion. Mechanical improvement was defined by multiple criteria. Both groups had improvement in NYHA functional class and VO_2 max, which were slightly better in the group with mechanical dyssynchrony. However, there was no significant difference in LVEF and LVESV at 6 months of follow-up in either groups. Within this study, patients with no mechanical dyssynchrony who were randomised to only ICD showed a significant deterioration in VO_2max. Therefore, the authors suggested that 'no positive response' does not necessarily mean 'no benefit', Diab et al(Diab, Hunter et al. 2011). In spite of their observed overall improvement in VO_2max in patients with mechanical dyssynchrony, there was a disconnect between the extent of improvement in VO_2max and that in measures of mechanical dyssynchrony, suggesting that benefit does not completely result from amelioration of mechanical dyssynchrony.

3.3.2 Change in function versus change in electrical dyssynchrony.

The significant finding here is a positive correlation between improvement in quality of life score and reduction in intrinsic QRS duration (as a measure of electrical dyssynchrony) but not with LVp-RVegm. A previous study which categorised CRT recipients at 6 months post implantation as either responders or non-responders using improvement in 6-minute walk distance, NYHA functional class and quality of life score as parameters found that although both responders and non-responders had similar baseline intrinsic QRS duration, only the responders had significant reduction in intrinsic QRS duration. (Molhoek, VAN Erven et al. 2004)

The mechanism underlying this correlation has not been explored but it may be that such improvement in electrical conduction from the left to the right ventricle indicates better intra-myocyte signalling as opposed to simple mechanical expulsion of blood from the heart. Of course, while reduction in mechanical dyssynchrony is expected to be immediate

following CRT implantation, improvement in electrical conduction takes time and denotes reversal of mal-adaptive changes that had occurred in the dyssynchronous heart. Furthermore, while the parameters of mechanical dyssynchrony at 6 months post CRT were assessed with biventricular pacing ON, the electrical parameters were assessed with biventricular pacing OFF and thus the electrical parameters are more indicative of intrinsic intra-cardiac changes than the mechanical dyssynchrony parameters. Also, there was no significant correlation between changes in iQRSD and SPWD in our patients suggesting separate underlying mechanisms. In canine model, improvement in electrical activation times following CRT was found to be associated with better haemodynamic profile(Strik, Rademakers et al. 2012).

3.3.3 Intrinsic cardiac intervals and measures of mechanical dyssynchrony

We observed that reduction in PR interval (that was prolonged at baseline) was significantly associated with reduction in SPWD. Although to the best of our knowledge, no studies have shown that CRT improves intrinsic atrio-ventricular conduction, a recent sub-analysis from the COMPANION trial has shown that CRT-D reduced all cause mortality more so in patients with prolonged baseline PR interval irrespective of LBBB status compared with optimal medical treatment (p=0.001) while only a trend towards reduction in all cause mortality in patients with normal baseline PR interval was observed (p=0.07).(Lin, Buhr et al. 2017) CRT however has been shown to induce atrial remodelling in the form of reduction in left atrial volume index, and that patients with no atrial remodelling and no ventricular remodelling had worse outcomes whereas patients with only atrial remodelling had favourable outcomes similar to that seen in patients with both atrial and ventricular remodelling.(Kloosterman, Rienstra et al. 2016). Although we did not measure left atrial volume index, the observed reduction in baseline intrinsic PR interval is likely an indicator of improved atrial and ventricular remodelling following CRT.

3.3.4 Change in cardiac excitability

3.3.4.1 Change in cardiac excitability versus change in neurohumoral activation.

Because of the parallel relationship between action potential duration and right ventricular effective refractory period at multiple cycle lengths(Lee, Liem et al. 1992), we used RVERP as a surrogate for cardiac excitability. We found no relationship between cardiac excitability using RVERP and the catecholamine metabolites and also none with NT pro-BNP levels. An outstanding confounder to this is the fact that CRT did not result in any significant changes in RVERP or in the levels of catecholamine metabolites. Chronic catecholamine infusion is known to shorten ventricular effective refractory period in experimental animals(Qin, Liu et al. 2013). We envisaged that CRT would result in reduction in plasma levels of catecholamine metabolites with subsequent prolongation of RVERP, however, none of these occurred in our patients. With regards to relationship between RVERP and NT-proBNP, although we are not aware of any previous report on this, it is known that elevated levels of NT-proBNP is associated with increased propensity to arrhythmogenesis by inhibiting Ca^{2+} uptake by SERCA as well as increasing Ca^{2+} sparks by RyR2 channels, all via increased sympathetic discharge.(Thireau, Karam et al. 2012) Thus, one would have expected that the significant reduction in NT-proBNP observed in our study should result in some alterations in RVERP, but this did not occur. This is premumably due to the absence of reduction in plasma levels of catecholamine metabolites, suggesting that NT-proBNP, if acting independent of sympathetic modulation, may not affect cardiac refractoriness or excitability.

A previous study (Tarquini, Guerra et al. 2009) has shown that while CRT induces reduction in NT pro-BNP concentrations and renin-angiotensin system activation in patients with reverse left ventricular remodelling, there was no suppression of

inflammatory activation (assessed with TNF-α, adinopectin and norepinephrine) following CRT.

3.3.2.2 Change in cardiac excitability versus change in redox stress.

We did not observe any association between change in cardiac excitability (assessed with RVERP) and redox stress using TXNIP. The expected correlation between change in TXNIP and change in RVERP was hampered by the observation that CRT did not result in alteration of either RVERP or platelet content of TXNIP.

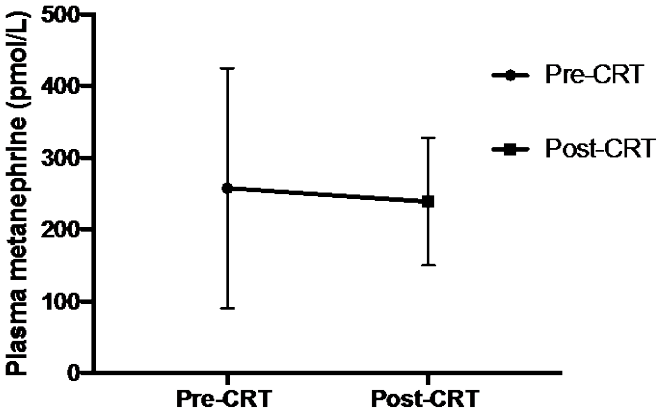
3.3.5 Summary of correlations performed.

The correlations performed in this study has been summarised and shown in Table 2.4 *in* Chapter 2.

3.4 TABLES AND FIGURES FOR CHAPTER 3

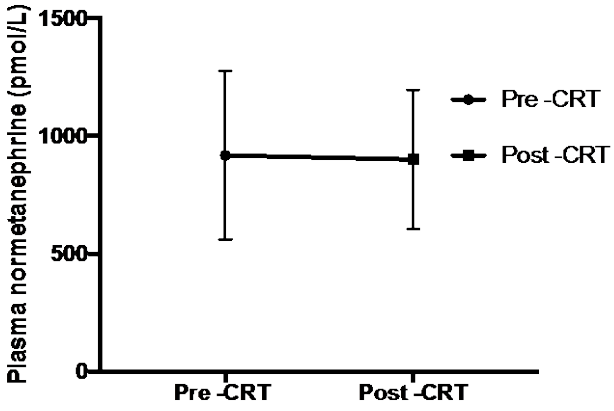
Table 3.1 Comparison of efficacy of CRT in our study cohort with previously published landmark trials

Parameters	MUSTIC	PATH-CCF	MIRACLE	MIRACLE-ICD	CURRENT STUDY
6 MWD (m)	23% (+49) increase, p<0.001	25% (+44) increase p<0.001	Increased, (+39) p=0.005	No change	Increased, (+42.5) p= 0.005
QOL score (AU)	Improved, 32% (-16.9 points) p<0.001	Improved, (-18.3) p<0.001	Improved, (-18) p=0.001	No change	Improved, (-17.8) p=0.001
VO_2max(ml/kg/min)	8% (+0.9) increase, p<0.03	24% (+1.84) increase, p=0.002	Improved, (+1.1) P<0.001	No change	6.5% (+0.3) increase, p=NS
NYHA	NA	Improved P< 0.001	Improved, p<0.001	P=0.05	Improved, p< 0.001
LVEF (%)	NA	NA	Improved, (+4.6) p<0.001	Improved, (+3.8) p=0.02	Improved, (+7.0) p<0.001



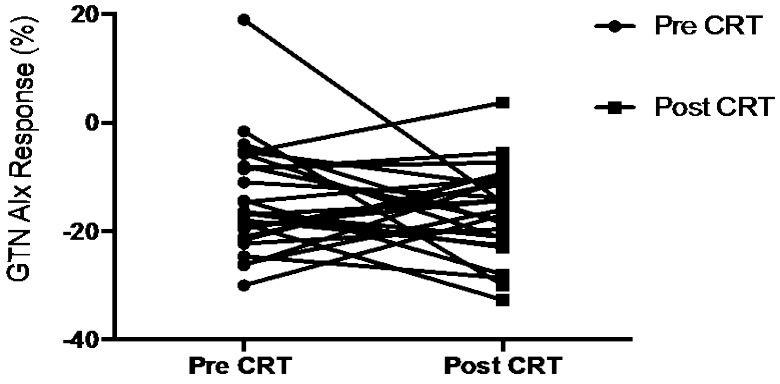
Mean	257.9	239.3
Std. Deviation	167.3	88.86

Figure 3.1 Plasma concentrations of metanephrine before CRT implant and 6 months after, n= 28, p=NS. Paired t-test



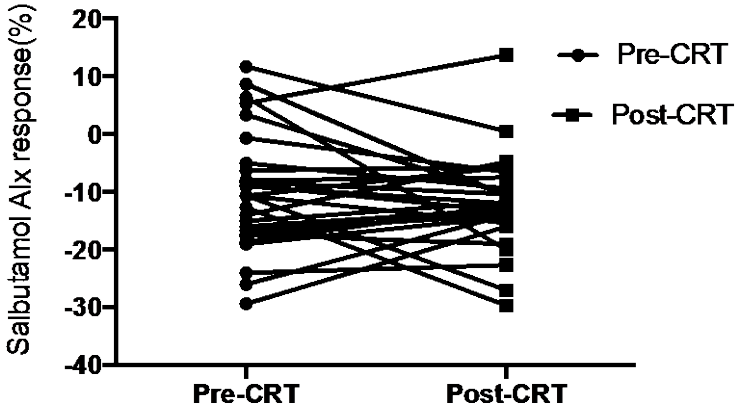
Mean	918.8	900.7
Std. Deviation	356.6	295.6

Figure 3.2 Plasma concentrations of normetanephrine before CRT implant and 6 months after, n= 28, p=NS. Paired t-test



Mean	-14.13	-16.66
Std. Deviation	10.05	8.183

Figures 3.3 Augmentation index response to sublingual GTN prior to CRT implant and 6 months after, n=27, p= NS. Paired t-test.



Mean	-9.875	-11.93
Std. Deviation	10.3	8.214

Figure 3.4 Augmentation index response to inhaled salbutamol prior to CRT implant and 6 months after, n=28, p= NS. Paired t-test

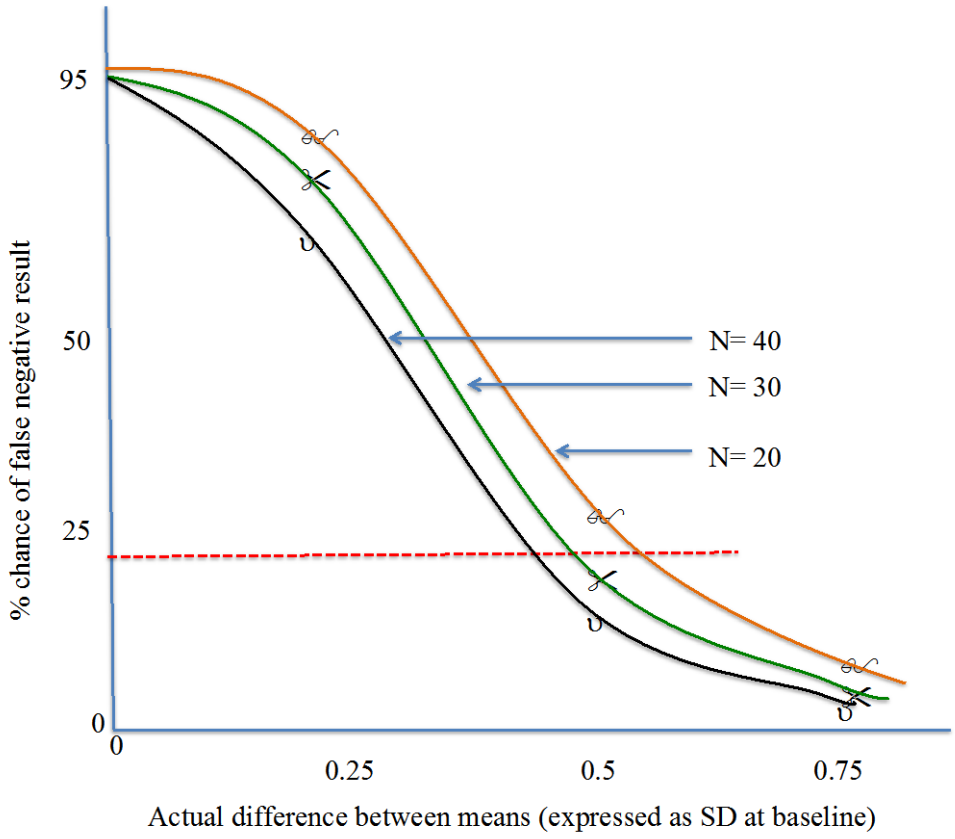


Figure 3.5 Series of operating characteristic curves showing in particular the relationships between size of investigated series and probability of Type II error. The type II level of 20% (beta=0.80) is highlighted because this represents a conventional limit of sample size calculation. Curves are shown for n= 20, n=30 and n=40.

CHAPTER 4

4.1 PUBLICATIONS and MANUSCRIPTS

4.1.1Published Manuscript

Statement of Authorship

Title of Paper	Does cardiac resynchronization therapy restore peripheral circulatory homeostasis?
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Ajaero, C. N., Chong, C.-R., Procter, N. E. K., Liu, S., Chirkov, Y. Y., Heresztyn, T., Chan, W. P. A., Arstall, M. A., McGavigan, A. D., Frenneaux, M. P., and Horowitz, J. D. (2018) Does cardiac resynchronization therapy restore peripheral circulatory homeostasis?. ESC Heart Failure, 5: 129–138. doi: 10.1002/ehf2.12211.

Principal Author

Name of Principal Author (Candidate)	Chukwudiebube Ajaero
Contribution to the Paper	Design of study, recruitment of patients, acquisition and analyses of all data. Wrote manuscript and coordinated submission to the journal
Overall percentage (%)	85%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	**Date** 19/3/18

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	John Horowitz
Contribution to the Paper	Design of study, oversight of data, critical editing of manuscripts and was the corresponding author.
Signature	**Date** 19/3/18

Name of Co-Author	Cher-Rin Chong
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ORIGINAL RESEARCH ARTICLE

Does cardiac resynchronization therapy restore peripheral circulatory homeostasis?

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Abstract

Aims To evaluate whether peripheral circulatory 'remodelling' as measured by changes in vascular compliance and in markers of nitric oxide signalling contributes to patient response to cardiac resynchronization therapy (CRT).

Methods and results Effects of CRT were evaluated in 33 patients pre-procedure and 6 months post-procedure. Peak oxygen consumption, 6 min walk distance, New York Heart Association class, and quality of life score were evaluated. Augmentation index and its interactions with nitric oxide (NO) were evaluated by applanation tonometry. Platelet NO responsiveness and content of thioredoxin-interacting protein were assessed. Plasma concentrations of N-terminal proBNP, asymmetric and symmetric dimethylarginine (SDMA), high sensitivity C-reactive protein, catecholamines, and matrix metalloproteinases-2 and -9 were assessed. Despite significant improvement in 6 min walk distance ($P = 0.005$), New York Heart Association class ($P < 0.001$), quality of life ($P = 0.001$), and all echocardiographic parameters post-CRT, there were no significant changes in augmentation index measurements, thioredoxin-interacting protein content, and platelet NO response. Significant falls in N-terminal proBNP ($P = 0.008$) and SDMA ($P = 0.013$; independent of renal function) occurred. Falls in SDMA predicted reduction in high-sensitivity C-reactive protein ($P = 0.04$) and increases in peak oxygen consumption ($P = 0.04$). There were no correlations between changes in echocardiographic parameters and those in vascular function.

Conclusions These data suggest that the beneficial effects of CRT over 6 months are independent of any change in peripheral NO-related signalling. However, there is evidence that suppression of inflammation occurs, and its magnitude predicts extent of clinical improvement.

Keywords Augmentation index (AI_X); Symmetric dimethylarginine (SDMA); Thioredoxin-interacting protein (TXNIP); Cardiac resynchronisation therapy (CRT); Nitric oxide (NO) signalling; Left ventricular dyssynchrony

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Introduction

Over the last two decades, as a result of several landmark trials demonstrating mortality and morbidity benefits with cardiac resynchronization therapy (CRT) with or without defibrillation insertion,[1–3] CRT has become a standard of care in select patients with systolic heart failure and concomitant left ventricular dyssynchrony. Substantial limitations of CRT include not only the 30–40% of recipients classified as 'non-responders'[2] but also significant heterogeneity in the commonly used criteria of response and poor agreement between these different clinical and echocardiographic parameters of response.[4] There has been considerable interest in understanding the mechanisms underlying benefits associated with

CRT and specifically whether these arise purely from mechanical correction of dyssynchrony. Of particular interest is the putative effect of CRT on peripheral vascular function. Two small studies have shown consistent improvement in microvascular cutaneous reactive hyperaemia post-CRT insertion,[5,6] while studies of the effects of CRT on large conduit vessels utilizing flow-mediated [i.e. nitric oxide (NO)-dependent] dilatation (FMD) have shown conflicting results.[7–9] In this present study, we sought to evaluate the effect of CRT on peripheral vascular function and associated markers of inflammation, as well as vascular and platelet NO signalling, we also examined the effects of CRT on parameters of inflammatory and neurohumoral activation.

Objectives of the study were as follows:

(1) To evaluate whether the optimal outcome post-CRT is engendered not only by reversal of left ventricular systolic dysfunction but also by amelioration of peripheral circulatory dysfunction.
(2) To evaluate the effects of CRT on neurohumoral activation.

Methods

Thirty-three patients with conventional indications for CRT were prospectively evaluated before and 6 months after CRT insertion. Clinical characteristics were noted, including New York Heart Association functional class status, and quality of life was evaluated using the standard (and well-validated) Minnesota Living with Heart Failure Questionnaire.

Exercise capacity was evaluated both by 6 min walk distance and by cardiopulmonary exercise testing.

Six minute walk distance

The protocol for the 6 min walk distance has been described.[10] This test was performed indoors in a dedicated and marked hallway that is 30 m long with a hard floor. Cones were used to delineate the turn-around points. The procedure was duly explained, and patients were asked to walk as far as possible and as fast as they could for a timed period of 6 min. They were allowed to slow down, to rest or to lean against the wall if necessary, at their own discretion, and to resume walking again at their possible fastest pace. Running or jogging was not allowed. The test was terminated if a patient developed chest pain, became unduly dyspnoeic, or looked ashen with significant diaphoresis. If any patient ordinarily mobilized with a walking aid, he or she was allowed to use the same during the test. The maximum distance walked at 6 min was recorded for each patient.

Cardiopulmonary exercise testing

Cardiopulmonary exercise testing was performed according to established guidelines.[11] Briefly, a bicycle ergometer (Model: ergoline/100/200 GmbH, Germany) and linked to ExpAir Medisoft S.A Belgique 1.31.02 software were utilized in all cases. Before performing each test, the equipment was calibrated both for airflow and volume, including low and high flows (with calculated volumes within ±3%) using a 3 L syringe and also for gases with carbon dioxide set at 4% and oxygen at 20%. A semi-automated progressive incremental (ramp) protocol in which the pre-test was set at 0 W while the workload started from 10 W and increased by 10 W every minute was used. Patients pedalled for a minute on the pre-test setting of 0 W before loading commenced. They were encouraged to exercise as long as possible, ideally for up to 8 to 12 min, and especially aiming to achieve a respiratory exchange ratio of 1 and above, with cycling rate kept at 60–70 revolutions per minute. Volitional exhaustion was the usual endpoint although exercise was terminated if a patient developed chest pain, acute ischaemic changes on the electrocardiogram, or hypotension. After unloading, the patient pedalled for a further 1 min. Measurements of oxygen consumption (VO_2), carbon dioxide output, ventilator equivalent, and respiratory exchange ratio were automatically acquired and finally averaged and displayed at 10 s interval. The highest reading of the three last averages at peak exercise was chosen as the peak oxygen consumption (VO_2 max).

Echocardiographic measurements

All echocardiographic measurements were performed according to the American Society of Echocardiography and the European Association of Cardiovascular Imaging guidelines.[12] A Phillips echocardiogram machine model iE33, 2009, Bothell WA, 98041 USA was used for image acquisition, and analyses were performed using Echopac Software Only BT 11 Version 113, 2013 General Electric Co. M-mode echocardiographic analysis was used to assess left ventricular interventricular dyssynchrony. Septal to posterior wall delay was calculated as the time difference between the onset of the QRS to that of the peak of deformation of the interventricular septum and the left ventricular posterior. Although extent of dyssynchrony was analysed as a continuum, septal to posterior wall delay of 130 ms or more was considered diagnostic of clinically significant of intraventricular mechanical dyssynchrony.[13] Left ventricular volumes including end-diastolic, end-systolic, and stroke volumes were measured in two dimensions and ejection fraction calculated by the modified Simpson's method in biplane using 2D images.

Vascular endothelial function

Vascular endothelial function was assessed by changes in augmentation index (AI_X) using radial artery applanation tonometry as previously described.[14] Briefly, patients were first rested in a supine position for 30 min. Using a commercially available pulse waveform analyser, the SphygmoCor system (AtCor Medical, Sydney, Australia, model CvMS V9), baseline AI_X was computed as the average of three readings. Sublingual glycerine trinitrate (GTN 300 μg) was administered and AI_X remeasured every 5 min for 20 min. The difference between the lowest value of AI_X with GTN and the baseline AI_X (that is, the maximum fall in AI_X with GTN) was utilized as a measure of endothelium-independent NO signalling. Subsequently, 400 μg of inhaled salbutamol was administered, and measurements were repeated every 5 min for 20 min. The difference between the lowest AI_X with salbutamol and the baseline (the maximum fall in AI_X with salbutamol) represents a measure of endothelium-dependent NO signalling.[15] Using the acquired radial artery waveform, a validated, generalized transfer function was used to generate the corresponding central aortic pressure waveform from which AI_X values were calculated. All measurements were indexed to a heart rate of 75 b.p.m., and only high fidelity tracings were used.

Platelet expression of thioredoxin-interacting protein

Platelet expression of thioredoxin-interacting protein (TXNIP) was quantified by immunofluorescence as previously described.[16] Briefly, blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and spun at 250 g for 10 min at room temperature. Slides were smeared, air-dried, and fixed with 4% paraformaldehyde and stored in −70°C freezer, and immunofluorescence staining performed within 6 months.

For immunofluorescence staining, the slides were allowed to warm to room temperature and then washed three times in phosphate-buffered saline (PBS) for 5 min per wash. A 100 μL of diluted goat's serum 1:5 with PBS (as a blocking solution) was added and incubated at room temperature for 30 min. Primary antibody was prepared by diluting rabbit anti-Vitamin D3 Up-regulated Protein (VDUP) TXNIP, (Invitrogen Corporation, Carlsbad, CA, USA) with 1% (w/v) bovine serum albumin in PBS in 1:50 ratio. The blocking solution was discarded without washing the slides, and then, 100 μL of the primary antibody was added and incubated overnight at 2–4°C. The primary antibody was discarded the next day, and the slides were washed three times in PBS for 5 min per wash. Two conjugated 2° antibodies: dilute phycoerythrin-labelled CD41 antibody (Becton, Dickinson and Company, USA), in 1:50 with PBS, serving as a platelet marker, and dilute fluorescein isothiocyanate-labelled anti-rabbit polyclonal antibody (Becton, Dickinson and Company, USA), in 1:100 with PBS were prepared while shielded from light. A 100 μL of each of the 2° antibodies was added to the slide and incubated at room temperature for 60 min. The slides were again washed three times in PBS for 5 min per wash, dried with lens tissue, and one drop of Dako fluorescent mounting medium was added, covered with a slip, and allowed to sit at room temperature for 10 min. Image acquisition was performed with Carl Zeiss Microscope, Germany, using ×400 magnification, multichannel for rhodamine and green fluorescent protein and exposure time set at 5000 ms. The intensities of TXNIP staining for each platelet were obtained utilizing image analysis software (AxioVision 40 version 4.8.2, Carl Zeiss Microscopy, Germany)

Subsequent analysis or counting was performed by randomly identifying 100 platelets per slide and average TXNIP obtained.

Asymmetric dimethylarginine and symmetric dimethylarginine

For asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) concentration estimation in plasma, 10 mL of blood was collected in heparinized tubes and immediately put in ice, before centrifugation at 4°C at 1800 ***g*** for 15 min. Plasma was collected in Eppendorf tubes and stored at −70°C until analysed. Plasma ADMA and SDMA concentrations were determined by high performance liquid chromatography, as described previously.[17] Briefly, this involved extraction, derivatization with fluorescent derivatizing reagent (AccQ-Fluor™), and chromatography.

Matrix metalloproteinase-2 and matrix metalloproteinase-9

Matrix metalloproteinase-2 was estimated from blood samples collected in EDTA tubes, while matrix metalloproteinase-9 was estimated from blood samples collected in heparinized tubes. For both, collected blood was immediately put in ice and was centrifuged for 15 min at 1800 g at 4°C within 30 min of collection. Platelet-poor plasma was collected and stored at −70°C until analysed. Assays were performed with R&D Quantikine quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Ltd, Minneapolis USA) according to the manufacturer's instructions. Plasma concentrations of matrix metalloproteinase-2 and matrix metalloproteinase-9 were determined by ELISA (R&D Systems Ltd) according to manufacturer's instructions.

Platelet aggregometry test; responsiveness to nitric oxide

Platelet response to NO was assessed *in vitro* in whole blood using a 4-channel impedance aggregometer (Chrono-Log Corporation, model 700) according to a previously described protocol, which required temporary interruption of therapy with $P2Y_{12}$ receptor antagonists.[18] Briefly, 9 mL of blood was collected into plastic tubes containing 1 mL of acid citrate anticoagulant. The blood was allowed to stand at room temperature for 20 min before testing. A 500 μL of normal saline was pre-warmed to 37°C in a 4-channel impedance aggregometer after which 450 μL of whole blood was added to the saline and both pre-warmed for 5 min. All tests were run at 37°C and a stirring speed of 900 rpm. Platelet aggregation was induced with adenosine 5′-diphosphate (ADP) (2.5 μM), and responses were measured as an increase in impedance (in Ohms), at 7 min. When NO donor sodium nitroprusside (10 μM) was added to samples 1 min before adenosine 5′-diphosphate, the resultant inhibition of aggregation was evaluated as a percentage relative to control.

N-terminal proBNP levels

These were analysed by the South Australia Pathology laboratory at the Queen Elizabeth Hospital. Samples were collected in heparinized tubes and analysed for N-Terminal proBNP with the Elecsys proBNP system (Roche diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim). The Sandwich principle was used: this involves a first incubation with a biotinylated monoclonal NT-proBNP-specific antibody and a monoclonal NT-proBNP-specific antibody labelled with a ruthenium complex. A second incubation was performed with the addition of streptavidin-coated microparticles, and the microparticles were subsequently captured on the surface of the electrode magnetically. A photomultiplier finally measured the chemiluminescent emission induced by application of voltage to the electrode.

Plasma metanephrine and normetanephrine

Analyses for catecholamine metabolites were performed with fresh samples collected in K_3EDTA bottles and analysed utilizing liquid chromatography-tandem spectrometry. The upper limits of the normal ranges for plasma concentrations were $<$500 pmol/L for free metanephrine and $<$900 pmol/L for free normetanephrine.

High sensitivity C-reactive protein

Samples were collected in K_3EDTA bottles and analysed by the immuno-turbidimetric quantification method on Beckman Coulter AU analysers (Beckman Coulter, Inc., Brea, CA, USA) according to the manufacturer's recommendations.

Ethics approval

The study complies with the Declaration of Helsinki, and approval for the study was granted by the Ethics and Human Research Committee of The Queen Elizabeth Hospital. All participants provided prior written informed consent.

Statistical analyses

The power of the study was calculated on the basis of variability in responsiveness of AI_X to salbutamol and was approximately 80% to detect a 5% variation in response at $P < 0.05$.

All data are expressed as mean ± standard deviation unless stated otherwise. The effects of cardiac resynchronization therapy on clinical, biochemical, endothelial, and echocardiographic parameters were assessed using paired *t*-test for normally distributed variables and Wilcoxon matched-pairs signed-rank test for non-parametric data. Each patient served as his/her control. Interactions between changes in left ventricular contractility and endothelial functions as well as the interactions between changes in function and neurohumoral parameters were correlated using Pearson correlation coefficients for normally distributed data and Spearman correlation for non-parametric data. Relationship between categorical changes in SDMA and changes in high-sensitivity C-reactive protein concentrations and in VO_2 max were evaluated by non-paired *t*-tests. A two-tailed *P* value < 0.05 was considered statistically significant.

All data were analysed with Prism 6 for Mac OS X version 6.0h October 2015.

Results

The baseline characteristics of patients are listed in *Tables 1* and *2*. The mean QRS duration was 158.7 ± 23.6 ms. Approximately half of the patients had an ischaemic aetiology of heart failure, and the majority were in New York Heart Association functional class III–IV pre-CRT implantation. All patients were also extensively treated for heart failure, and the majority were receiving β-adrenoceptor antagonists. One patient died 2 months after CRT insertion, while three patients declined the 6 month follow-up visit. Among the whole cohort, there was significant improvement post-CRT in all echocardiographic parameters and in all clinical parameters, with the exception of VO_2 max, as shown in *Figures 1* and *2* and *Table 3*. There was no significant change overall in vascular and platelet functions except for a statistically significant

Table 1 Baseline characteristics of patients

Age (years)	71.2 ± 9.7
Female, *n* (%)	10 (30.3)
Weight (kg)	87.1 ± 18.6
Height (cm)	172.5 ± 8.1
BMI (kg/m^2)	29.3 ± 6.1
Ischaemic aetiology of CHF, *n* (%)	17 (52)
History of current/past smoking, *n* (%)	19 (57.6)
NYHA class	
• I, *n* (%)	3 (9)
• II, *n* (%)	7 (21)
• III, *n* (%)	19 (58)
• IV, *n* (%)	4 (12)
Comorbidities	
• Hypertension, *n* (%)	21 (64)
• Diabetes, *n* (%)	14 (42)
• COPD, *n* (%)	4 (12)
• Atrial fibrillation, *n* (%)	5 (15)
Medications	
• ACE inhibitor	22 (67)
• Angiotensin receptor blocker	8 (24)
• β-adrenoceptor blocker	25 (76)
• Aldosterone Antagonist	18 (55)
• Digoxin	9 (27)
• Frusemide	24 (73)
• Statin	18 (55)
• Aspirin	17 (52)
• Clopidogrel	7 (21)
• Perhexiline	5 (15)
Clinical assessment	
Systolic BP (mmHg)	126.4 ± 17.1
Diastolic BP (mmHg)	71.2 ± 9.2
Heart rate (b.p.m.)	69 ± 13.6
6MWD (m)	321.74 ± 104.62
VO_2 max (mL/min/kg)	13.8 ± 4.7
QOL score	41.9 ± 25.6
Biochemistry	
NT-proBNP (ng/L)	1814.0 (1091–3073)
eGFR (mL/min/1.73 m^2)	56.4 ± 22.2
Plasma metanephrine (pmol/L)	252.1 ± 158.3
Plasma normetanephrine (pmol/L)	996.5 ± 396.2
Plasma MMP-2 (ng/mL)	217.5 ± 51.2
Plasma MMP-9 (ng/mL)	27.7 ± 9.1
high-sensitivity C-reactive protein (mg/L)	2.4 (1.4–6.1)
Ventricular function	
LVESV (mL)	137.4 ± 55.3
LVEDV (mL)	192.8 ± 67.4
EF (%)	29.8 ± 6.1
SPWD (ms)	120.0 ± 195.0
IVMD (ms)	43.9 ± 44.0

6MWD, 6 min walk distance; ACE, angiotensin-converting enzyme; BMI, body mass index; BP, blood pressure; CHF, chronic heart failure; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; EF, ejection fraction; eGFR, estimated glomerular filtration rate; IVMD, interventricular mechanical delay; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; MMP, matrix metalloproteinase; NT-proBNP, N terminal proBNP; NYHA, New York Heart Association; QOL, quality of life; SPWD, septal to posterior wall delay; VO_2 max, peak oxygen consumption during exercise.
Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile values.

Table 2 Endothelial function/no signalling pre-CRT

Baseline AI_X (%)	19.9 ± 7.8
GTN AI_X change (%)	−13.9 ± 10.0
Salbutamol AI_X change (%)	−11.3 ± 10.2
ADMA (μM)	0.6 ± 0.07
SDMA (μM)	0.73 (0.60–1.0)
Platelet SNP response (%)	33.9 ± 26.5
Platelet TXNIP (AU)	136.9 ± 111.2

ADMA, asymmetric dimethyl arginine; AI_X, augmentation index; CRT, cardiac resynchronization therapy; GTN, glycerine trinitrate; SDMA, symmetric dimethyl arginine; SNP, sodium nitroprusside; TXNIP, thioredoxin-interacting protein.
Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile values.

reduction in plasma concentrations of SDMA (paired *t*-test, $P = 0.013$).

As shown in *Figure 3A–C*, although a statistically significant inverse correlation existed at baseline between estimated glomerular filtration rate and SDMA concentration, at 6 months, significant decreases in plasma levels of SDMA had occurred without any change in estimated glomerular filtration rate. It is therefore unlikely that changes in renal function had contributed to the observed fall in SDMA levels. A categorical reduction in SDMA concentrations was predictive of both a reduction in high-sensitivity C-reactive protein concentrations ($P = 0.04$: *Figure 3D*) and an increase in VO_2 max ($P = 0.04$: *Figure 3E*). With regard to neurohumoral and inflammatory activation, there was a statistically significant reduction in plasma levels of NT-proBNP (paired *t*-test, $P = 0.008$) but no significant change in any other marker of NO signalling or of inflammatory activation. It must be noted, however, that in the absence of significant changes, effects of both salbutamol and GTN on AI_X tended to increase (by about 20%). On univariate analysis, there was no significant correlation between change at 6 months in left ventricular end-systolic volume and changes at 6 months in baseline AI_X, GTN-mediated fall in AI_X, salbutamol-mediated fall in AI_X, or with platelet NO response.

Discussion

In this study, the first point to be noted is perhaps the fact that the cohort on the whole 'responded' to CRT, in the sense that there was significant improvement in parameters of left ventricular ejection fraction, as well as significant improvement in symptomatic status as shown in *Table 3*. Therefore, we have a cohort in whom there was effective mechanical cardiac resynchronization; otherwise, it would have been futile for us to proceed with the pursuit of understanding of any further mechanistic effect of CRT. The lack of statistically significant improvement in VO_2 max was similar to the results of some previously reported CRT studies[19,20] although CRT has also been shown to improve VO_2 max.[2] In addition, there was a significant reduction in the plasma concentrations of N-terminal proBNP, a similar finding to the CARE-HF trial.[2] In this regard, a *post hoc* analysis of the MADIT-CRT trial[21] found that reduction in BNP following CRT was associated

Figure 1 Effects of cardiac resynchronization therapy (CRT) on clinical parameters in individual patient. (A) New York Heart Association (NYHA) functional class (n = 29: this is partially obscured graphically by identical changes in 20 patients). (B) 6 min walk distance (6MWD). (C) Peak oxygen consumption (VO_2 max). (D) Quality of life (QOL) score. All analyses were performed via paired t-tests.

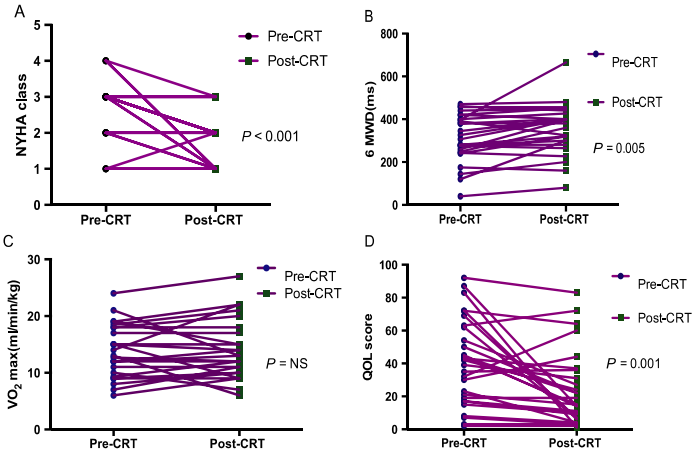
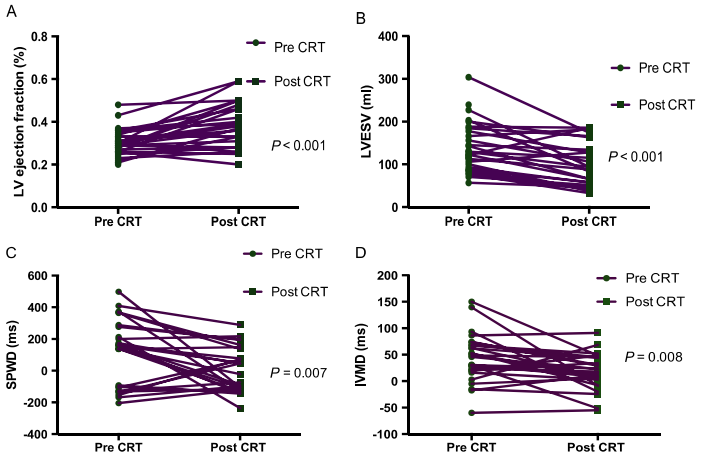


Figure 2 Effects of cardiac resynchronization therapy (CRT) on echocardiographic parameters in individual patients. (A) Left ventricular (LV) ejection fraction. (B) Left ventricular end-systolic volume (LVESV). (C) Septal to posterior wall delay (SPWD). (D) Interventricular mechanical delay (IVMD). All analyses were performed via paired t-test.



with significant decrease in hospitalized heart failure and mortality.

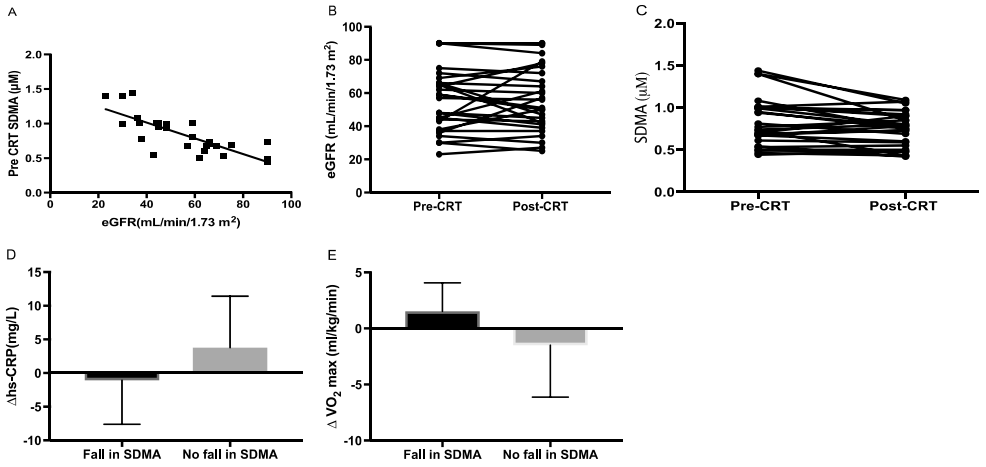
In spite of these salutary effects of CRT in our cohort, there was no significant change in conventional markers of peripheral vascular endothelial function as assessed by AI_X with radial artery applanation tonometry. Specifically, there were no significant changes either in baseline AI_X or in endothelium-independent and endothelial-dependent AI_X, as assessed with GTN and salbutamol, respectively. A potential confounder to this apparent lack of effect on vascular

Table 3 Effects of cardiac resynchronization therapy: analyses are limited to patients in whom 6 months data post-CRT were available

Parameters	Pre-CRT	Post-CRT	*P*-value
Clinical			
NYHA	2.7 (0.8)	1.9 (0.7)	<0.001
VO_2 max (mL/min/kg)	13.8 (4.67)	14.1 (5.3)	NS
6MWD (M)	314.5 (112.8)	357.0 (117.0)	0.005
QOL score	40.7 (25.4)	22.9 (22.3)	0.001
Vascular and Platelet			
Baseline AI_X (%)	20.3 (8.2)	20.3 (8.1)	NS
GTN response [AI_X change (%)]	−14.1 (10.0)	−16.6 (8.1)	NS
Salbutamol response [AI_X change (%)]	−9.9 (10.5)	−11.9 (8.3)	NS
SNP response (%)	30.5 (21.8)	25.2 (19.7)	NS
TXNIP (AU)	144.8 (122.6)	188.5 (126.6)	NS
ADMA (μM)	0.66 (0.08)	0.65 (0.09)	NS
SDMA (μM)	0.83 (0.28)	0.74 (0.20)	0.013
Biochemistry			
Plasma metanephrine (pmol/L)	257.8 (167.2)	239.2 (88.8)	NS
Plasma normetanephrine (pmol/L)	918.8 (356.6)	900.7 (295.6)	NS
NT-proBNP (ng/L)	1862 (1091–3185)	1469 (774–2841)	0.008
MMP-2 (ng/mL)	217.2 (52.5)	219.8 (49.9)	NS
MMP-9 (ng/mL)	27.5 (9.6)	26.5 (10.0)	NS
High-sensitivity C-reactive protein (mg/L)	2.4 (1.4–7.1)	3.5 (1.6–11.0)	NS
Echocardiographic			
LV ejection fraction (%)	31.0 (6)	38 (10)	<0.001
LVESV (mL)	136.6 (57.5)	98.9 (52.1)	<0.001
SPWD (ms)	119.1 (201.2)	1.74 (141.4)	0.007
IVMD (ms)	43.6 (44.6)	19.9 (33.9)	0.008

6MWD, 6 min walk distance; ADMA, asymmetric dimethyl arginine; AI_X, augmentation index; CRT, cardiac resynchronization therapy; GTN, glycerine trinitrate; IVMD, interventricular mechanical delay; LV, left ventricular; LVESV, left ventricular end-systolic volume; MMP, matrix metalloproteinase; NS, non-significant; NT-proBNP, N terminal proBNP; NYHA, New York Heart Association; QOL, quality of life; SDMA, symmetric dimethyl arginine; SNP, sodium nitroprusside; SPWD, septal to posterior wall delay; VO_2 max, peak oxygen consumption; TXNIP, thioredoxin-interacting protein.

Figure 3 Implications of changes in plasma symmetric dimethyl arginine (SDMA) concentrations regarding renal excretion of SDMA, and variations in high-sensitivity C-reactive protein concentrations and peak oxygen consumption (VO_2 max) post-cardiac resynchronization therapy (CRT). (A) Correlation between baseline estimated glomerular filtration rate (eGFR) and SDMA concentrations($r = -0.80$, $P < 0.001$). (B) Changes in eGFR post-CRT in individual patients (P = NS). (C) Changes in SDMA concentrations post-CRT in individual patients (P = 0.013). (D) Changes in high-sensitivity C-reactive protein concentrations (P = 0.04). (E) Changes in VO_2 max values (P = 0.04).



endothelial function is the possibility that CRT may indeed have opposing effects on AI_X: by increasing stroke volume into non-compliant vessels with already depressed Windkessel effect, there would be an increase in the velocity of both the forward and the reflected waves in the peripheral vasculature. It is also possible that extensive pharmacological treatment of our cohort for heart failure and dyslipidemia may have resulted in pre-CRT improvement of vascular endothelial function. Statins, for instance, are known to cause a reduction in aortic AI_X,[22] Angiotensin-converting enzyme inhibitors (ACE-I) also significantly reduce AI_X,[23] and 91% of our cohort were receiving ACE-I or angiotensin receptor blocker. It is also possible, given the observation of a (non-significant) 20% improvement on vascular responsiveness to salbutamol and GTN post-CRT, that a small improvement in these parameters might have been obscured via type II error. Indeed, the probability of detecting a change of less than 0.5 SD of baseline values would have been less than 80%. In the case of responses to salbutamol, for example, an absolute improvement of less than 5% might have gone undetected. In addition, the use of β-adrenoceptor antagonists would potentially blunt the effect of salbutamol on AI_X, thus obscuring the effect of CRT. Interestingly, the study cohort included 24% of patients who were not receiving β-adrenoceptor antagonists presumably because of contra-indications and/or prior adverse effects. However at baseline, there was no significant difference in AI_X response to salbutamol between patients receiving and not receiving β-adrenoceptor antagonists.

The lack of effect of CRT on either basal or NO-stimulated AI_X was somewhat different from the results of previous studies that utilized FMD as a criterion of NO-mediated arterial vasomotion. All of these have shown some evidence of improvement in FMD. Akar *et al*.[7] observed that although patients with worse FMD of the brachial artery responded better to CRT and that these responders had improvement in FMD at 90 days, the overall improvement was not statistically significant ($P = 0.12$).

In a subsequent study of 57 patients, Santini *et al*.[8] found that in a CRT cohort, significant improvement in FMD occurred in both responders and non-responders at 3, 6, and 12 months although with somewhat greater improvement in the responder group than in the non-responder group, with these results being only of borderline statistical significance. A recent study by Warriner *et al*.[9] also found that lower FMD predicts response to CRT at 12 months. However, in their cohort, CRT did not result in significant improvement in FMD in either the responder group or the non-responder group. It is not completely clear to what extent the reported differential FMD responses in the Santini study represent the phenomenon of regression to the mean. In any case, we are unable to make direct comparisons in the current study. We did not measure FMD, essentially because AI_X is a more reproducible parameter than FMD.[24,25] Importantly, in our study, CRT did not affect ADMA concentrations also indicating that NO generation via endothelial NO synthase activation was likely to be unaffected. In one previous study, high ADMA concentrations tended to predict poor responses to endothelial NO synthase activation by FMD.[26] A further caveat concerning the current findings is that evaluation of changes in platelets and blood may not be fully representative of changes within the myocardium.

It might have been expected that CRT would tend to normalize patterns of arterial distention and thus decrease expression of TXNIP given that the latter is shear stress-dependent.[27] However, no change in platelet TXNIP content occurred. In retrospect, there have been no previous direct studies of effects of CRT on arterial shear stress, and it may be that reversal of LV dyssynchrony has minimal effects on this parameter. Furthermore, platelet TXNIP expression is suppressed by NO signalling, such as are induced by ACE inhibitor therapy.[28]

A notable exception to the overall negative data in this study was the observed fall in plasma SDMA concentrations (*Figure 3*). This is most unlikely to be a 'false positive' given first, the $P = 0.013$ value. SDMA, unlike ADMA, is only a very weak inhibitor of NO synthase, but it is a substantial mediator of inflammatory change.[29] This could not be explained by improved renal function, an important consideration because SDMA is excreted (mainly) unchanged in the urine.[30] A fall in SDMA concentrations independent of alterations in renal function post-CRT may have reflected either decreased protein catabolism (representing the mechanism of formation of SDMA via the enzyme, protein arginine methyltransferase type II). Importantly, although there was no significant fluctuation in other inflammatory markers in the cohort as a whole, patients with a reduction in SDMA levels also had significant reductions in high-sensitivity C-reactive protein relative to those without a fall in SDMA, which further supports the concept that amelioration of inflammation activation may occur post-CRT. Interestingly, a fall in SDMA concentrations also predicted increases in VO_2 max (*Figure 3E*)

In summary, in an extensively medically treated cohort of patients, CRT did not significantly alter markers of NO signalling. It is therefore unlikely that changes in NO signalling significantly contribute to the salutary effects of CRT within 6 months. However, there is some evidence that CRT may lead to suppression of inflammatory activation within the systemic circulation.

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laboratory bench ran smoothly and also thank Adrian Elliott for initial assistance with performing cardiopulmonary exercise tests.

Conflict of interest

None declared.

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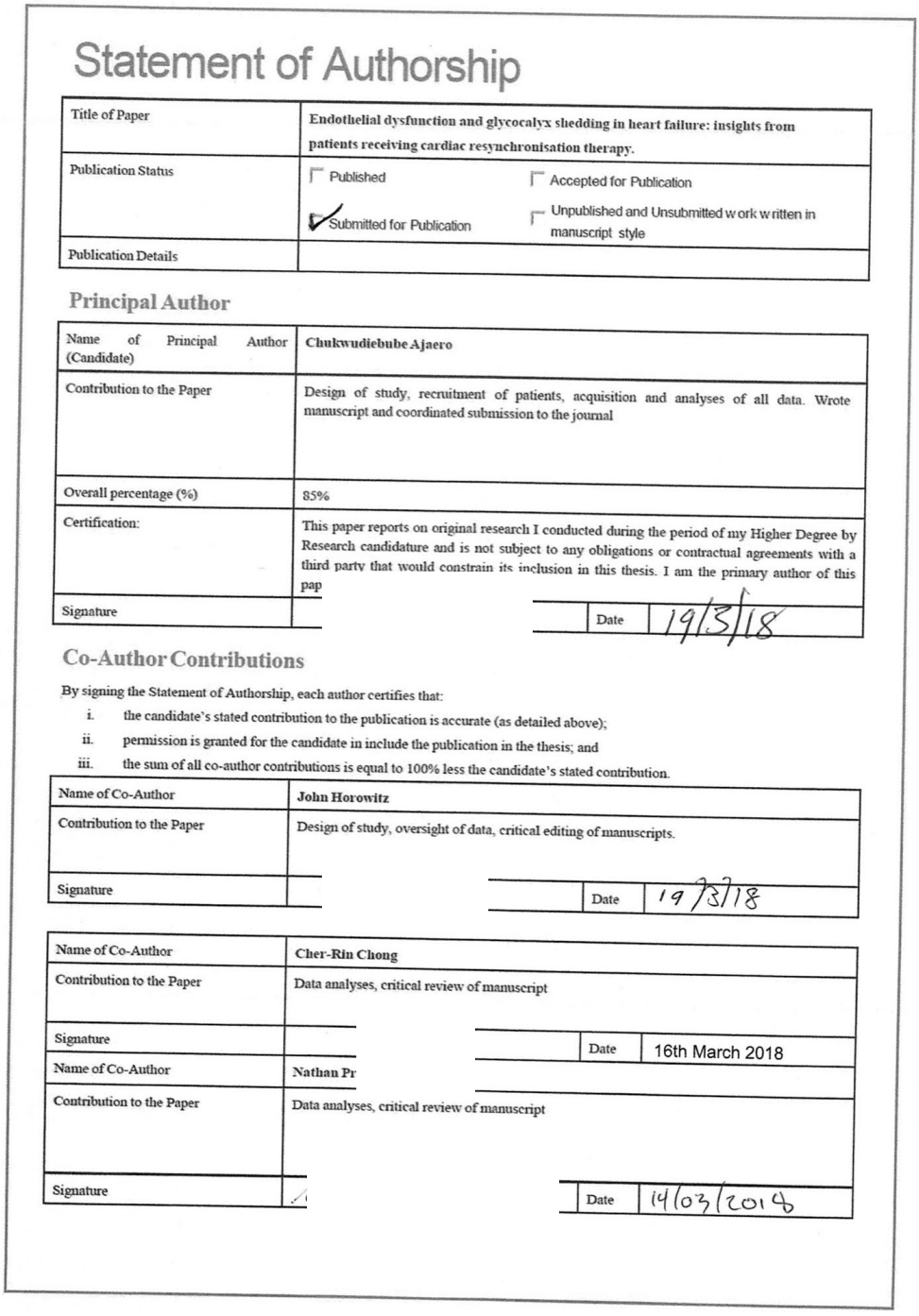
4.1.2 Unpublished Manuscripts

4.1.2.1 Manuscript 1

Title:

Endothelial dysfunction and glycocalyx shedding in heart failure: insights from patients receiving cardiac resynchronisation therapy



Statement of Authorship

Title of Paper	**Endothelial dysfunction and glycocalyx shedding in heart failure: insights from patients receiving cardiac resynchronisation therapy.**
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	

Principal Author

Name of Principal Author (Candidate)	**Chukwudiebube Ajaero**
Contribution to the Paper	Design of study, recruitment of patients, acquisition and analyses of all data. Wrote manuscript and coordinated submission to the journal
Overall percentage (%)	85%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this pap
Signature	Date 19/3/18

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	**John Horowitz**
Contribution to the Paper	Design of study, oversight of data, critical editing of manuscripts.
Signature	Date 19/3/18

Name of Co-Author	**Cher-Rin Chong**
Contribution to the Paper	Data analyses, critical review of manuscript
Signature	Date 16th March 2018
Name of Co-Author	**Nathan Pr**
Contribution to the Paper	Data analyses, critical review of manuscript
Signature	Date 14/03/2018

Name of Co-Author	**Safei Liu**		
Contribution to the Paper	Data analyses, critical reviewi of manuscript		
Signature		Date	14/3/18
Name of Co-Author	**Yuliy**		
Contribution to the Paper	Supervision of data acquisition and analysis, critical review of manuscript		
Signature		Date	15/03/18
Name of Co-Author	**Tam**		
Contribution to the Paper	Some data analyses, critical review of manuscript		
Signature		Date	15/3/18
Name of Co-Author	**W P**		
Contribution to the Paper	Study design, data analyses, critical review of manuscript		
Signature		Date	20/3/18
Name of Co-Author	**Mar**		
Contribution to the Paper	Study design, critical review of manuscript		
Signature		Date	20/3/18
Name of Co-Author	**Andrew**		
Contribution to the Paper	Study design, supervision of data acquisition and analyses, critical review of manuscript		
Signature		Date	13/3/18

Manuscript details	Endothelial dysfunction and glycocalyx shedding in heart failure: insights from patients receiving cardiac resynchronisation therapy.		
Name of Co-Author	**Michael Frenneaux**		
Contribution to the Paper	Study design, critical review of manuscript		
Signature		Date	14 March 2018

Brief Title: Glycocalyx shedding in systolic heart failure

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Disclosures.

There was no relationship with industry

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Abstract

Objectives:

To determine (a) whether chronic heart failure with reduced ejection fraction (HFrEF) is associated with increased glycocalyx shedding; (b) whether glycocalyx shedding in HFrEF with left ventricular dyssynchrony is related to inflammation, endothelial dysfunction and/or redox stress and is ameliorated by cardiac resynchronisation therapy.

Background:

Glycocalyx shedding has been reported to be increased in heart failure and is a marker of increased mortality. Its role in dyssynchronous systolic heart failure and the effects of cardiac resynchronisation therapy (CRT) are largely unknown.

Methods :

Twenty-six patients with dyssynchronous HFrEF were evaluated before and 6 months after CRT insertion. Echocardiographic septal to posterior wall delay (SPWD) assessed intra-ventricular mechanical dyssynchrony, and quality of life, integrity of nitric oxide (NO) signalling, inflammatory and redox-related biomarkers were measured. Glycocalyx shedding was quantitated via plasma levels of the glycocalyx component, syndecan-1.

Results:

Syndecan-1 levels pre-CRT were inversely correlated with LVEF ($r=-0.45$, $p=0.02$) and directly with SPWD ($r=0.44$, $p=0.02$), QOL ($r=0.39$, $p=0.04$), plasma NT-proBNP ($r=0.43$, $p=0.02$), and the inflammatory marker, symmetric dimethylarginine (SDMA) ($r=0.54$, $p=0.003$). On multivariate analysis, syndecan-1 levels were predicted by SPWD and SDMA ($\beta=0.42$, $p=0.009$ and $\beta=0.54$, $p=0.001$, respectively). No significant correlation was found between syndecan-1 levels and other markers of endothelial dysfunction/inflammatory activation. Following CRT there was no significant change in syndecan-1 levels.

Conclusions:

In patients with dyssynchronous HFrEF, markers of glycocalyx shedding are associated with magnitude of mechanical dyssynchrony and elevation of SDMA levels and inversely with LVEF. However, CRT does not reverse this process.

Keywords: Glycocalyx shedding, cardiac failure, resynchronization therapy, endothelial function, symmetric dimethylarginine

List of abbreviations:

CRT: cardiac resynchronisation therapy

SPWD: Septal to posterior wall delay.

QOL : Quality of life score

LVEF: Left ventricular ejection fraction

NT-proBNP: N-terminal pro brain natriuretic peptide

AI_X : Augmentation index

NO: nitric oxide

SDMA: Symmetric dimethylarginine

TXNIP: thioredoxin interacting protein

Introduction

In spite of several ground-breaking advances in the understanding and management of heart failure over the last few decades, HFrEF remains common in most Western societies with substantial associated morbidity, mortality and economic burden (Krum and Abraham 2009). This underscores the need for further understanding of the pathophysiological mechanisms of heart failure. It is known that chronic heart failure is associated with immune and inflammatory activation (Candia, Villacorta et al. 2007), as well as with vascular endothelial dysfunction (Katz, Hryniewicz et al. 2005) with its associated potential for disturbance in laminar blood flow (Ferrari, Bachetti et al. 1998). There is increasing evidence that shedding of the vascular endothelial glycocalyx may represent a link between these two processes (Kolarova, Ambruzova et al. 2014), largely via activation of matrix metalloproteinases (Mulivor and Lipowsky 2009) and resultant induction of endothelial dysfunction. However, the extent of endothelial glycocalyx shedding in patients with severe HFrEF and concomitant left ventricular dyssynchrony, and its putative association with nitric oxide signalling and inflammatory activation, remain largely unexplored. Importantly, a direct correlation between glycocalyx shedding and loss of laminar blood flow has been observed (Lipowsky 2012).

Over the last two decades, cardiac resynchronisation therapy (CRT) has emerged as the standard of care in patients with HFrEF associated with concomitant dyssynchronous ventricular contractions, especially with left bundle branch block. CRT offers symptomatic benefit to approximately two-thirds of recipients; several landmark trials have shown that CRT, with or without defibrillator insertion, not only improves 'soft' clinical end-points, but also reduces risk of mortality and reduces heart failure hospitalisations (Bristow, Saxon et al. 2004, Cleland, Daubert et al. 2005). The basis for this benefit of CRT remains uncertain but it is thought that reduction in dyssynchrony contributes to improvement in clinical status (Pouleur, Knappe et al.). Nonetheless, large

multi-centre prospective trials have suggested that no echocardiographic measure of dyssynchrony is sufficient by itself to predict response to CRT (Chung, Leon et al.).

Recently, efforts to understand the mechanisms of benefit associated with CRT have begun to address factors beyond improvement in left ventricular contractile efficiency, turning to possible effects on peripheral vascular function (Enomoto, Yamabe et al. 2011, Yufu, Shinohara et al. 2015, Warriner, Lawford et al. 2016). It is now known that the integrity of the endothelial glycocalyx layer plays a prominent role in maintenance of vascular endothelial function by modulating vascular permeability, coagulation, vascular tone, and complement activation (Curry and Adamson). Increased glycocalyx shedding occurs in conditions of increased shear and oxidative stress (Lipowsky 2012). Release into plasma of syndecan-1, a marker of glycocalyx shedding and thus of endothelial dysfunction, has been found to be increased in patients with heart failure (Neves, Meneses et al. 2015). This elevation of plasma syndecan-1 concentrations is also a correlate of increases in the combined end point of all-cause mortality and hospitalised heart failure in patients with heart failure with preserved ejection fraction (Tromp, van der Pol et al. 2014). The role of glycocalyx shedding in patients with systolic heart failure and concomitant left ventricular dyssynchrony remains unknown.

Objectives of the current study

1. To confirm activation of glycocalyx shedding in chronic dyssynchronous HFrEF and to identify the clinical determinants of the extent of this putative change with particular regard to severity of heart failure and extent of left ventricular dyssynchrony.

2. To evaluate the potential interactions of glycocalyx shedding with concomitant inflammation, endothelial dysfunction and redox stress, and in particular to determine whether glycocalyx shedding is associated with:
    a. Impaired function of the NO signalling cascade
    b. Increased expression of the inflammatory activator, thioredoxin-interacting protein (TXNIP), the expression of which is increased by non-laminar flow (Wang, Nigro et al. 2012).
3. To determine whether CRT, possibly by ameliorating non-laminar flow, reduces the extent of glycocalyx shedding.

Methods

Patients with conventional indications for CRT (n=26) were prospectively evaluated before and six months after CRT implant. Patients' quality of life was evaluated using the Minnesota Living with Heart Failure Questionnaire, with higher scores indicating more impaired quality of life.

Echocardiographic measurements

Radial artery applanation tonometry: determination of augmentation index.

Platelet expression of thioredoxin-interacting protein.(TXNIP)

Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA)

Matrix metalloproteinase-2 and matrix metalloproteinase-9

Platelet aggregometry test; responsiveness to NO

N-Terminal proBNP levels

Plasma metanephrine and normetanephrine

Methodology for all these listed above have been described in details in Chapter 2

Measurement of plasma levels of syndecan-1.

For syndecan-1 assay, blood was collected in EDTA tubes and immediately put on ice and centrifuged for 15 minutes at 1800g at 4ºC within 30 minutes of collection. Plasma was collected and stored at -70ºC until analysed. Analysis was performed utilizing Human sCD138 ELISA kit (Diaclone SAS, France version 7, 2015) according to manufacturer's instructions. .

Ethics approval.

The study complied with the *Declaration of Helsinki* and approval for the study was granted by the Ethics and Human Research Committee of The Queen Elizabeth Hospital and all participants provided written informed consent.

Statistical analyses

All data are expressed as mean ± SD unless stated otherwise. Univariate correlations between syndecan-1 levels and endothelial/NO- signalling, echocardiographic parameters and neurohumoral activation were performed using Pearson correlation coefficients for normally distributed data and Spearman correlation for non-parametric data. Backward stepwise multiple regression analysis was performed utilising parameters with significant univariate correlations with baseline syndecan-1 levels. The interaction of CRT with glycocalyx shedding was assessed by Wilcoxon matched-pairs signed rank test. Apart

from the multiple regression analyses performed using SPSS 23 version 11.8.2 (2013 Citrix systems), all other analyses were performed using Prism 6 for Mac OS X version 6.0h October 2015.

A p-value < 0.05 was considered statistically significant.

Results

The baseline characteristics of patients are listed in Table 1. Half of the patients had an ischaemic basis for heart failure, the majority had class III-IV symptomatic status and all had substantial evidence of dyssynchrony both electrically and mechanically. All patients were also extensively treated medically for systolic heart failure prior to CRT implantation. The biochemical and physiological data for this group are summarized in **Table 2.**

In general, renal function was well preserved, as was platelet response to the NO donor, sodium nitroprusside. Median syndecan-1 levels were clearly elevated beyond the previously reported range for normal subjects (34.1 ± 8.0 ng/ml (Çekiç, Kırcı et al. 2015).

Univariate correlates of Syndecan-1 at baseline:

Syndecan-1 levels correlated negatively and significantly with left ventricular ejection fraction (r=-0.45, p=0.02). There was also a statistically significant positive correlation between syndecan-1 levels and septal to posterior wall delay (r=0.44, p=0.02) and a statistically significant positive correlation between syndecan-1 and NT-proBNP (r=0.43, p= 0.02). There was a trend towards a significant positive correlation with plasma normetanephrine (r=0.37, p=0.09). A direct correlation between syndecan-1 levels and degree of impairment of quality of life measured with the Minnesota Living with Heart

Failure Questionnaire (with higher values indicating greater impairment) was also observed (r=0.39, p=0.04). These relationships are depicted in **Figure 1**.

With regard to vascular endothelial function as assessed with applanation tonometry and NO signalling, there was no significant correlation between syndecan-1 levels and baseline augmentation index (r=-0.23, p=NS), nor with change in augmentation index in response to salbutamol(r=-0.12, p=NS). There was also no significant correlation between levels of syndecan-1 and those of asymmetric dimethylarginine (ADMA) (r=0.28, p=0.15), inhibition of ADP-induced platelet aggregation with sodium nitroprusside (r=-0.33, p=0.12), nor with platelet thioredoxin-interacting protein (TXNIP) levels (r=0.22, p=NS). On the other hand, there was a strong and significant positive correlation between syndecan-1 and symmetric dimethylarginine (SDMA), (r=0.54, p=0.003).

Multivariate determinants of syndecan-1 at baseline

Backwards stepwise multiple linear regression analysis was performed utilising parameters that exhibited significant univariate correlations with syndecan-1 levels (ejection fraction, SPWD, SDMA concentrations and NT-pro BNP concentrations, together with baseline eGFR (p=0.10 on univariate analysis).

The results, summarized in **Table 3**, revealed that baseline SPWD and SDMA concentrations were both directly and significantly related to syndecan-1 levels. Importantly, eGFR was not significantly related to syndecan-1 levels.

Effects of CRT

There was no significant change in syndecan-1 levels between baseline and 6 months post-CRT median (IQR) 55 ng/ml (39.2-75.2) vs 59 ng/ml (31.7-79.6) respectively, p=0.45. There was also no significant correlation between changes in LVEF post-CRT and those in syndecan-1 levels at 6 months, (r=0.31, p=0.13). Similarly, no correlations

were found between changes in syndecan-1 levels and other measures of vascular biology and NO signalling including change in baseline augmentation index (r=-0.16, p=NS), change in AI_X with salbutamol (r=-0.35, p=NS), platelet ADP-aggregation inhibition with sodium nitroprusside (r=-0.01, p=NS), ADMA (r= -0.14, p=NS), SDMA (r=0.13, p=NS), nor platelet TXNIP content, (r=0.05, p=NS). In addition, there were no significant correlations between changes in syndecan -1 and NT-proBNP (r=0.04, p=NS), nor with changes in SPWD (r=0.06, p=NS).

Discussion

In this study, we sought to determine whether glycocalyx shedding, as measured by release of syndecan-1 into plasma, plays a major part in the pathophysiology of severe systolic heart failure associated with extensive left ventricular dyssynchrony. The bases for these evaluations were: (1) endothelial glycocalyx shedding may be triggered by inflammatory activation, particularly involving matrix metalloproteinases (Lipowsky and Lescanic 2013), and (2) in turn may precipitate endothelial dysfunction and abnormal responsiveness of blood vessels to shear stress: all of which have been reported in systolic heart failure (Ferrari, Bachetti et al.).

To date, there have been 2 reported clinical studies relevant to the current observations. Tromp et al (Tromp, van der Pol et al. 2014) performed an evaluation of syndecan-1 levels in a large cohort of patients with chronic heart failure, many of whom had preserved ejection fractions. Levels of syndecan-1 were not elevated beyond the normal range (median 20.1ng/ml), and these exhibited correlations on multivariable regression analyses with markers of fibrosis and remodelling, but not with extent of LV systolic dysfunction. No correlations with extent of dyssynchrony were sought. A Brazilian study (Neves, Meneses et al. 2015) of patients with acutely decompensated heart failure (with mean LVEF of 41.5 ±14.4%) revealed far greater mean syndecan-1 levels (approximately

130ng/ml), which were correlated with extent of acute kidney injury and with in-hospital mortality rate. The current study is therefore the first to indicate that chronic systolic heart failure is associated with evidence of increased glycocalyx shedding.

In the current study, univariate analyses suggested that the extent of syndecan-1 elevation was directly related both to the degree of impairment of LVEF and also to the degree of mechanical dyssynchrony. As regards correlations with biochemical/physiological markers, to our surprise there was no correlation between syndecan-1 levels and any parameters of integrity of NO signalling, given that in canine models, the integrity of the endothelial glycocalyx layer had been found to correlate with flow-mediated NO synthesis(Mochizuki, Vink et al. 2003). To the best of our knowledge, no previous study had evaluated this putative association in human studies. Therefore it appeared that variability in NO signalling in such patients may have primarily reflected factors such as treatment modalities (Zhang, Xie et al. 1997, Kalinowski, Dobrucki et al. 2003). In support of this possibility, syndecan-1 levels noted in the Brazilian cohort of patients with acutely decompensated heart failure(Neves, Meneses et al. 2015) were much higher than in the currently evaluated patients. In our centre, we have also observed that in patients with acute takotsubo cardiomyopathy, plasma syndecan -1 levels were much higher (97 ± 65ng/ml) (Nguyen, Liu et al. 2016) than in our cohort of patients with chronic HFrEF, who were already receiving optimal medical treatment.

Syndecan-1 levels also exhibited direct univariate correlations with NT-proBNP and SDMA and this is consistent with greater cardiomyocyte distension and increased inflammation respectively(Hall 2005, Schepers, Glorieux et al. 2009).

On multivariate analyses, there were persistent significant associations with degree of mechanical dyssynchrony and with SDMA levels; the latter remained significant after taking eGFR values into account, which is important given the substantial influence of

renal function on SDMA clearance (Kielstein, Salpeter et al. 2006). On the other hand, TXNIP expression, which might have been expected to be a direct correlate, given its association with non-laminar flow (Wang, Nigro et al. 2012), exhibited no significant association with syndecan-1 levels. Again this may well reflect suppression of TXNIP expression by ACE inhibitor therapy (Sverdlov, Chan et al. 2013).

CRT did not reduce syndecan-1 levels 6 months post procedure in spite of the fact the cohort as a whole exhibited significant improvement in LVEF as well as in clinical symptoms. Thus the lack of effect on syndecan -1 levels may indicate that CRT is relatively ineffective in restoring laminar flow, and/or that the deposition of fibrous tissue leads to ongoing inflammatory activation, which perpetuates glycocalyx shedding

As regards the two significant correlates of syndecan-1 levels on multivariate analyses, (that is, with dyssynchrony and SDMA levels), only association, rather than causation, can be identified definitely at this stage. However, syndecan-1 release appears to engender fibrotic change in tissues (Tromp, van der Pol et al. 2014), and indeed conducting system fibrosis is likely to be critical to development of mechanical dyssynchrony (Sugiura, Okada et al. 1970). As regards SDMA, this is generated by protein catabolism and cleared largely, but not entirely, unchanged in the urine. Since it has also been suggested that SDMA may represent a surrogate for glomerular function (Kielstein, Salpeter et al. 2006), it is important that its association with syndecan-1 levels was independent of eGFR. Since it has recently emerged that SDMA contributes to inflammatory activation (Schepers, Barreto et al. 2011), the association with syndecan-1 may reflect the inflammatory origin of glycocalyx shedding, but SDMA may also contribute to development of myocardial inflammation and fibrosis in this group of patients.

Study Limitations

The study was limited mainly by its small size, with possible risk of Type 2 error, for example, regarding effect of CRT insertion, which might have expected to lower syndecan-1 levels, and also relationship with extent of systolic dysfunction. Importantly, the patients were extensively treated medically and this may have affected findings. Finally, as mentioned earlier, we are unable to draw any definite conclusions regarding cause- and- effect relationships, but it does appear that reducing mechanical dyssynchrony by CRT does not reverse glycocalyx shedding.

Conclusions:

In this study, we demonstrated for the first time that in patients with severe left ventricular systolic dysfunction and concomitant mechanical dyssynchrony, the activation of glycocalyx shedding and that the extent of this process correlated directly with the degree of left ventricular mechanical dyssynchrony. Furthermore, independent of renal function, glycocalyx shedding is also associated with significantly higher plasma levels of the inflammatory marker, SDMA. Larger studies and perhaps with patients with less advanced stages of heart failure are required to shed more light on the possible effects of CRT in patients with systolic heart failure and concomitant left ventricular dyssynchrony.

Clinical Perspectives:

Patients under consideration for cardiac resynchronisation therapy (CRT) usually constitute a group with severe systolic heart failure refractory to conventional pharmacotherapy. In the current study, we provide the first clinical evidence that such CRT candidates suffer from acquired damage to the glycocalyx, or outer layer of endothelial cells. This will cause increased capillary permeability, predisposing towards development of congestion irrespective of severity of hemodynamic changes. Interestingly, glycocalyx damage increased directly with the extent of mechanical dyssynchrony, but was not reversed within 6 months of CRT insertion

Translational outlook:

Glycocalyx 'shedding' is a well-described process activated by pro-inflammatory enzymes such as matrix metalloproteinases. In animal models, inhibition of these enzymes, for example by low dose doxycycline, reverses damage to the glycocalyx, and ameliorates circulatory disturbances. The results of the current study should therefore increase therapeutic focus on peripheral circulatory hemostasis in severe heart failure, and potential for institution of treatments to protect the glycocalyx in such patients.

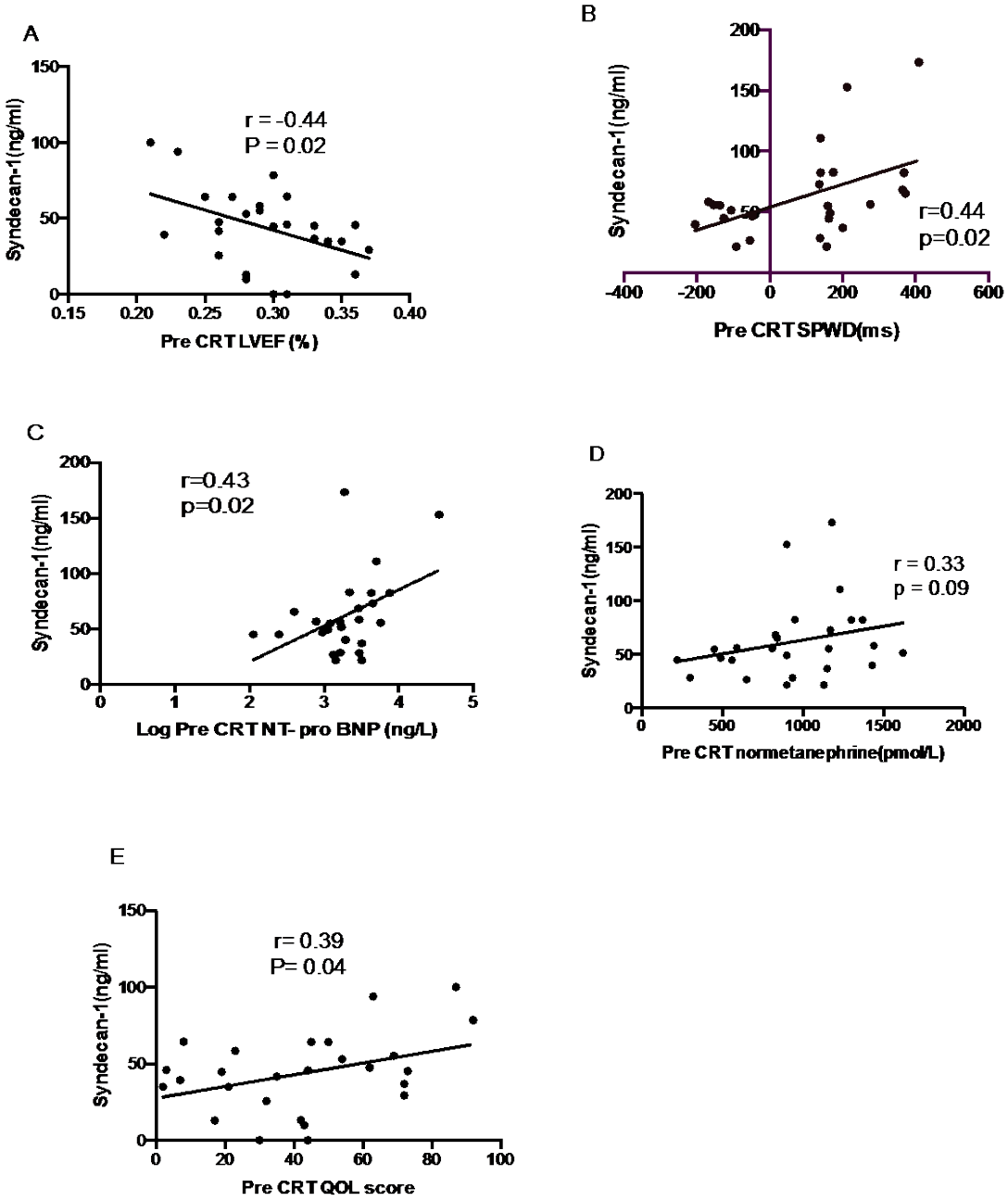

Figure 1: Univariate correlates of clinical, echocardiographic and biochemical plasma concentrations of syndecan-1 at baseline:

A: with LVEF

B: with SPWD

C: with NT-proBNP

D: with plasma normetanephrine

E: with QOL score

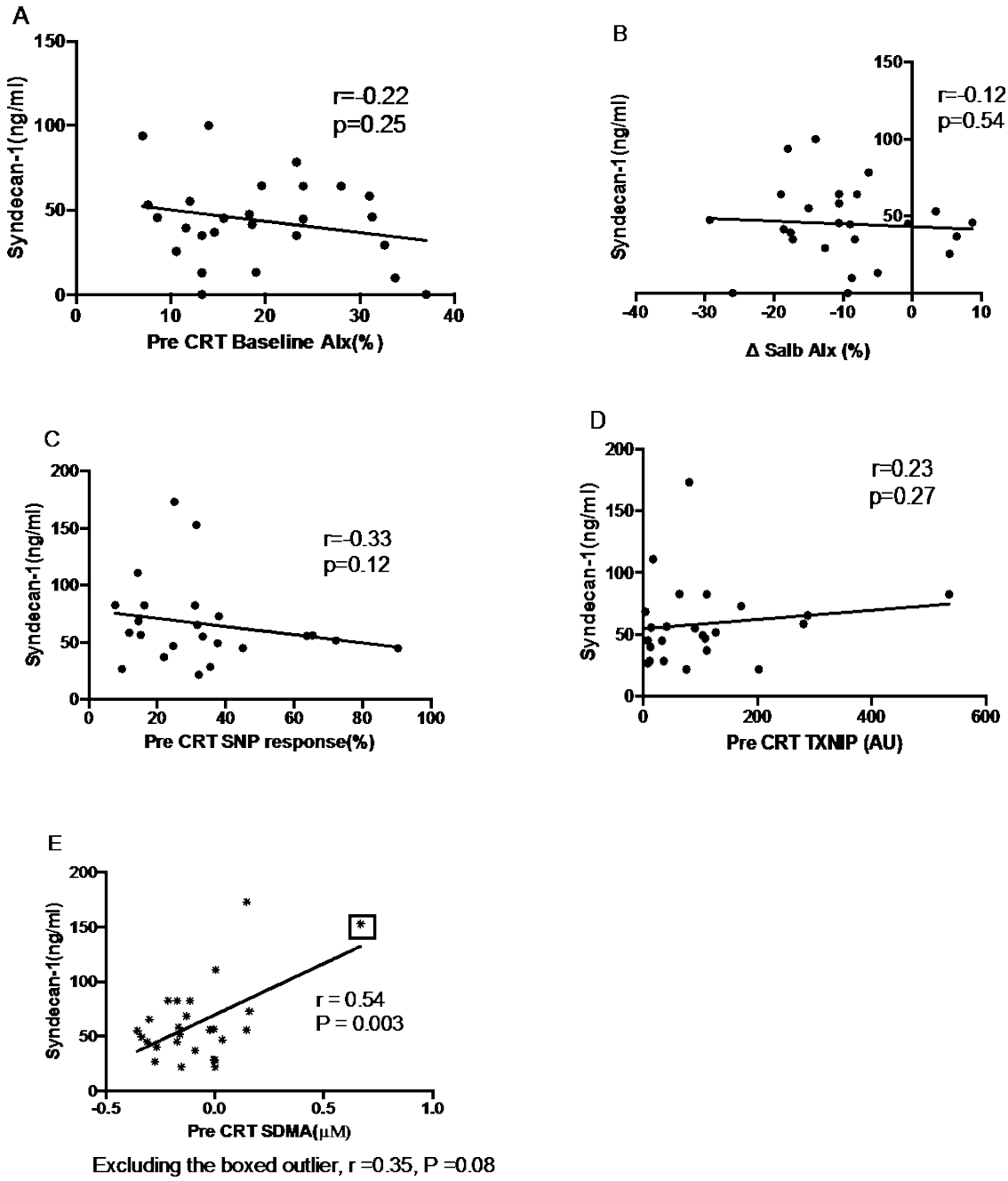

Figure 2: NO and redox-signaling correlates of plasma syndecan-1 levels at baseline

A: Baseline AIX

B: AIX change with salbutamol

C: Platelet aggregation inhibition with sodium nitroprusside(SNP)

D: with TXNIP

E: with SDMA

Table 1: Baseline patient characteristics

Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile ranges

Age (years)	72.1± 7.5
Female, n (%)	9 (34.6%)
Ischemic aetiology n (%)	13 (50.0%)
BMI (kg/m^2)	29.8 ± 6.52
Intrinsic QRS duration (ms)	159 ± 24
Comorbidities	
Hypertension, n (%)	17 (65.3)
Diabetes, n (%)	12 (46.1)
Atrial fibrillation, n (%)	3 (11.5)
Clinical evaluations	
Systolic BP (mmHg)	125 ± 16
Diastolic BP (mmHg)	69 ± 7.9
NYHA class	
I & II, n (%)	8 (30.8)
III & IV, n (%)	18 (69.2)
Quality of Life Score	42.6(25.9)
Medications: n (%)	
• ACE inhibitor	17 (65.3)
• Angiotensin receptor blocker	7 (26.9)
• Beta Blocker	20 (76.9)
• Aldosterone Antagonist	14 (53.8)
• Digoxin	7 (26.9)
• Frusemide	19 (73.0)
• Statin	15 (57.6)
Echocardiographic measurements	
LVESV (ml)	138 ± 57
Ejection fraction, (%)	29.4 ± 4.3
SPWD, (ms)	139 (-99-206)

BMI: Body mass index; NYHA: New York Heart Association; LVESV: Left ventricular end-systolic volume; SPWD: Septal to posterior wall delay;

Table 2: Biochemical and physiological group data

Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile ranges

Biochemical/ Neurohumoral measurements	
eGFR (mL/min/1.73m^2)	**53.7 ± 21.5**
NT-pro BNP (ng/L)	**1895 (1180-3459)**
Plasma Metanephrine (pmol/L)	**205 (150-297.5)**
Plasma Normetanephrine (pmol/L)	**942 ± 366**
Plasma MMP-2 (ng/ml)	**220 ± 54.1**
Plasma MMP-9 (ng/ml)	**26.4 ± 9.05**
Endothelial function/NO signaling	
Baseline AIx (%)	19.4 ± 8.7
GTN AIx change (%)	-13.2 ± 9.9
Salbutamol AIx change (%)	-9.1 ± 10.26
ADMA (µM)	0.65 ± 0.07
SDMA (µM)	0.75 (0.59-1.0)
Platelet SNP response (%)	33.4 ± 21.5
Platelet TXNIP (AU)	108 (14.3-123)
Syndecan-1 (ng/ml)	55.2 (39.2-79.2)

MMP: matrix metalloproteinase; Aix: Augmentation index; ACE: Angiotensin coverting enzyme; ADMA: Asymmetric dimethyl arginine; SDMA: symmetric dimethyl arginine; SNP: Sodium nitroprusside; TXNIP: Thioredoxin interacting protein

Table 3: Multivariate regression analysis of determinants of syndecan-1 levels at baseline

Variable	*Standardised coefficient beta*	*P- value*
Baseline EF (%)	-0.263	0.136
Baseline eGFR (mL/min/1. 73 m2)	-0.163	0.437
Pre CRT SPWD (ms)	0.421	0.009
SDMA	0.545	0.001
NT-pro BNP	-0.072	0.886

4.1.2.2 Manuscript 2

Title:

Outcome in electrical remodelling parameters following cardiac resynchronization therapy among patients with ischemic and non-ischemic aetiology of heart failure and the effect on clinical functional parameters.

Statement of Authorship

Title of Paper	**Outcome in electrical remodelling parameters following cardiac resynchronization therapy among patients with ischemic and non-ischemic aetiology of heart failure and the effect on clinical functional parameters.**
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	NA

Principal Author

Name of Principal Author (Candidate)	**Chukwudiebube Ajaero**
Contribution to the Paper	Design of study, recruitment of patients, acquisition and analyses of all data. Wrote manuscript and will coordinated submission to the journal
Overall percentage (%)	85%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 19/3/18

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	**Andrew McGavigan**
Contribution to the Paper	Study design, supervision of data acquisition and analyses, critical review of manuscript .
Signature	Date

Name of Co-Author	**John Horowitz**
Contribution to the Paper	Design of study, oversight of data, critical editing of manuscripts.
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Contribution to the Paper	D manuscript
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ABSTRACT:

Aim

The beneficial effects of cardiac resynchronization therapy (CRT) in heart failure are largely considered to be due to improved mechanical contractility. The contributory role of electrical remodelling is less clear. We sought to evaluate the impact of electrical remodelling in these patients.

Methods.

33 patients with conventional indications for CRT and with ischemic (ICM) (n=17) and non-ischemic (NICM) (n=16) aetiologies for heart failure were prospectively recruited. Functional parameters of peak exercise oxygen consumption (VO_2max) and Minnesota quality of life (QOL) score, echocardiographic measures of LV functions and parameters of electrical remodelling, e.g. intrinsic QRS duration (iQRSD), intracardiac conduction times of LV pacing to RV electrocardiogram (LVp-RVegm), were measured at CRT implant and after 6 months.

Results

Only two electrical parameters predicted functional or symptomatic improvement. LVp-RVegm reduction significantly correlated with improvement in VO_2max ($r= -0.42$, $p= 0.03$ while reduction in iQRSD significantly correlated with improvement in QOL score ($r=0.39$, $p= 0.04$). The extent of changes in LVp-RVegm and iQRSD was significantly greater in NICM than in ICM patients ($p=0.017$ and $p=0.042$ for heterogeneity). There was also significant differential impact on QOL score in the NICM relative to the ICM group ($p=0.003$) but none with VO_2max. On multivariate analysis, only non-ischemic aetiology was a significant determinant of reduction in iQRSD.

Conclusion

CRT induces potentially beneficial reduction in LVp-RVEgm and iQRSD, which are seen selectively in NICM rather than ICM patients. The extent of improvement in these markers is predictive of some functional and symptomatic measures of CRT efficacy.

Keywords

1. Electrical remodelling
2. Cardiac resynchronization therapy
3. Intracardiac conduction time.
4. Intrinsic QRS duration
5. Cardiopulmonary exercise testing
6. LV pace to RV electrocardiogram.

Condensed Abstract:

We evaluated electrical remodelling following CRT in patients with ischemic and non-ischemic cardiomyopathy by utilising intra-cardiac conduction times and intrinsic QRS duration. Significant improvement in these parameters was observed in patients with non-ischemic cardiomyopathy. Electrical parameters also correlated with functional and clinical measures.

What's New?

- Cardiac resynchronisation therapy results in improvement in global electrical remodelling, as measured with intrinsic QRS duration only in patients with non-ischemic cardiomyopathy.
- Left ventricular intra-myocardial conduction time is improved only in patients with non-ischemic cardiomyopathy.
- Functional and clinical parameters correlate with improvement in parameters of electrical remodelling.

INTRODUCTION

Results from previous landmark studies have clearly shown that cardiac resynchronization therapy (CRT) reduces morbidity and mortality in patients with chronic systolic heart failure and associated prolonged QRS duration(Bristow, Saxon et al. 2004, Cleland, Daubert et al. 2005). To date, prolonged QRS duration, especially of left bundle branch block morphology, remains the best predictor of response to CRT (Poole, Singh et al. 2016). Indeed, in patients with evidence of mechanical dyssynchrony on echocardiogram but with narrow QRS duration, CRT is futile and may be associated with harm(Thibault, Harel et al. 2013). In spite of this, the benefit of CRT on improved left ventricular volume and systolic function has been considered to be largely mediated by reversal of adverse left ventricular mechanical remodelling(Linde, Abraham et al. 2008) rather than through positive electrical remodelling.

Although there are limited data reporting changes in individual electrical parameters following CRT, the majority have focused on relatively simple tools such as intrinsic QRS complex duration and morphology and QT dispersion and have yielded inconsistent results(Aslani, Khajei et al.). The effects of CRT on other parameters of electrical remodelling are not well characterized, nor are the relationships between electrical remodelling and changes to functional status and mechanical function.

Furthermore, it is well recognized that CRT is associated with reduction in the occurrence of ventricular arrhythmias, especially in patients who achieve echocardiographic improvement in LV function and volumes(Di Biase, Gasparini et al. 2008). Although the mechanism is at present unknown, positive electrical remodelling may play a role due to alterations in intra-cardiac electrical conduction and refractoriness.

Finally, whether electrical remodelling plays a role in the observation that patients with non-ischemic aetiology of heart failure (NICM) have greater improvement in left ventricular mechanical remodelling following CRT compared to those with an ischemic basis of heart failure (ICM)(Chen, Duan et al. 2014) remains to be fully explored. There are significant differences between patients with (NICM) and (ICM). Functional LBBB rather than fixed block has been noted to be more prevalent in patients with (NICM) than in those with (ICM) (Auricchio, Fantoni et al. 2004), perhaps making patients with (NICM) more amenable to improvement with CRT in electrical parameters and consequently to improvement in mechanical function than those with (ICM).

In this study, we prospectively evaluated changes in intra-cardiac electrical parameters following CRT, utilizing markers of excitability and local and global electrical conduction in patients with both ischemic and non-ischemic aetiologies of heart failure.

OBJECTIVES

1. To evaluate the effects of CRT on reversal of electrical remodelling.
2. To test the null hypothesis that extent of reversal in electrical remodelling is independent of a history of ischemic (ICM) heart disease.
3. To test the hypothesis that extent of electrical remodelling predicts functional and/or symptomatic improvement post CRT.

METHODS

Patient selection:

As described previously in Chapter 2

Functional status assessment:

a. Cardio-pulmonary exercise testing:

b. Quality of life score

The self-administered Minnesota Living with Heart Failure Questionnaire assessed quality of life score with higher scores indicating lower functional status.

Measures of electrical remodelling:

Parameters utilized included the intrinsic QRS duration as an overall marker of global electrical dyssynchrony, left ventricular pacing to the onset of right ventricular intra-cardiac electrocardiogram (LVp-RVegm), right ventricular pacing to the onset of left ventricular intra-cardiac electrocardiogram (RVp-LVegm), calculations of Q-LVegm and left ventricular intra-cardiac electrocardiogram to the end of QRS complex (LVegm-QRSend) as markers of inter-ventricular electrical conduction times.

As regards specific methodology:

a. **Intrinsic QRS duration**
b. **Left ventricular pacing to right ventricular intra-cardiac electrocardiogram (LVp-RVegm)**
c **Right ventricular pacing to left ventricular intracardiac electrocardiogram (RVp-LVegm)**

Q-LVegm:

RVegm-LVegm:

d. **Left ventricular intra-cardiac electrocardiogram to the end of QRS complex (LVegm-QRSend)**

None of the patients had any of the leads repositioned during the study period thereby ensuring constant landmarks for the measurements.

Echocardiographic measurements:

All echocardiographic measurements were performed according to the American Society of Echocardiography guidelines.(Balady, Arena et al. 2010) A Phillips echocardiogram machine model iE33, 2009, Bothell WA, 98041 USA was used for image acquisition, and analyses were performed using Echopac Software Only BT 11 Version 113, 2013 General Electric Co. Measurements included left ventricular end-diastolic and end-systolic volumes, from which ejection fraction was calculated with Simpson's rule for biplane evaluation.

The study complies with the *Declaration of Helsinki* and the Ethics and Human Research Committee of The Queen Elizabeth Hospital granted approval for the study. All participants provided prior written informed consent.

Statistical analyses

All data are expressed as mean ± SD unless stated otherwise. Interval changes in functional and electrophysiological parameters were assessed using paired t- test for normally distributed variables, and Wilcoxon matched-pairs signed rank test for non-parametric data. Each patient served as his/her control. Interactions between changes in functional status and changes in

electrical parameters were correlated using Pearson correlation coefficients for normally distributed data and Spearman correlation for non-parametric data. Categorical variables were analysed with chi-square and Fisher's exact test. A two-tailed P value <0.05 was considered statistically significant.

All data were analysed with Prism 7 for Mac OS X version 6.0h October 2016 apart from backward stepwise multivariate analysis performed on SPSS version 11.8.2, year 2013.

RESULTS

Baseline Characteristics

Table 1 shows the patients' baseline demographics. 17 patients (52%) had underlying ischemic aetiology of heart failure and all patients were extensively treated medically. The (NICM) group had higher baseline LVEF of 0.33 ± 0.06 versus 0.26 ± 0.04, p =0.001, and significantly lower left ventricular volumes, while diabetes and statin therapy were more frequent among the (ICM) patients. The mean baseline intrinsic QRS duration was 158 ± 27.2ms. There were no significant differences in baseline electrical parameters in both groups. No patient had right bundle branch block on the ECG. Of the initial 33 patients, 1 died a month after CRT insertion and 3 did not return for follow-up

Changes in mechanical and clinical parameters

At 6 months, there were significant improvements in LVEF from 31±6 to 38 ±10 per cent ($p<0.001$), in inter-ventricular mechanical dyssynchrony as assessed with IVMD 43.6± 44.6 to 19.9±33.9ms ($p=0.012$) and in symptomatic status as measured with the QOL score

40.7±25.4 to 22.9 ±22.3 (p =0.001) in the whole cohort (**Table 2**). Notably, although at baseline the (NICM) group had better LV systolic function than the (ICM) group, at 6 months post CRT insertion, there were significant improvements in LVEF and LVESV in both groups and no significant differences between groups for LVEF or LVESV changes.

Changes in electrical parameters

In terms of electrical parameters, at 6 months, there was a trend towards reduction in intrinsic QRS duration in the whole cohort (159 ± 23.7 to 148 ± 29.4ms, p =0.08) and this was driven by significant reduction in iQRSD in (NICM) patients (165 ± 20.3 to 141 ± 28.6ms, p =0.01) (**Table 3**). There was no significant change in iQRSD in the (ICM) cohort from 153 ± 25.6 to 154 ± 28.4ms. Consistent with these data, the decrease in iQRSD in (NICM) patients was statistically greater than that in the (ICM) group (p = 0.01). Figure 1

A similar pattern of results was found with the electrophysiological parameter of LVp-RVegm. In the whole cohort, there was significant reduction in LVp-RVegm from 117 ± 44.5 to 97.0 ± 45.0ms (p=0.019) and this was driven by the (NICM) group (121 ± 31.4 to 86.4 ±29.0ms, p=0.005) with no significant change in the (ICM) group (112 ± 56.2 to 109 ± 56.5ms, p= NS). The between-group difference was also statistically significant (p=0.04). For LVegm-QRSend calculations, there was significant reduction in the whole cohort from 77.5 ±53.3 to 53.9 ± 35.5ms, p= 0.024 and this was somewhat more marked in the (NICM) (81.5 ±60.4 to 48.6 ± 44.3ms,) than (ICM) group (70.6 ± 51.6 to 63.5 ± 30.9ms), none of these differences reached statistical significance.

No significant change was observed in RVp-LVegm at 6 months in the whole cohort (86.9± 39.6 to 93.3ms, p=NS) and no significant change in RVegm-LVegm. These data are shown in **Table 3**.

A positive correlation was observed between change in iQRSD and change in LVegm-QRSend but with no significant change in Q-LVegm at 6 months, (Figure 2, A-D). Although there was no significant improvement in VO_2 max at six months, there was a significant negative correlation in individual patients between change in VO_2 max and LVp-RVegm (r = -0.42, p=0.03). There was also significant positive correlation between changes in QOL score and those in the iQRSD (r = 0.39, p=0.04 **Figure 3 A/B**)

Importantly, there was no significant correlation (r=0.18, p=0.37) between extent of electrical remodelling as measured by iQRSD and that of mechanical remodelling, as measured by IVMD.

Multivariate determinants of reduction in intrinsic QRS complex duration:

Multivariate analysis of changes in iQRSD performed on pre-defined variables of age, diabetes, gender, IVMD and aetiology showed that only non-ischemic aetiology was a significant determinant of reduction in iQRSD with β= 0.44, P =0.023 (**Table 3 and Figure 4**)

DISCUSSION.

In this study, we were able to demonstrate firstly that our cohort in general had effective cardiac resynchronization at 6 months as shown by significant improvements in echocardiographic and functional parameters. In addition to these, we also observed overall significant improvements in the electrical parameters of iQRSD, LVp-RVegm, and LVegm-QRSend. Importantly, these changes tended to be accentuated in the (NICM) group rather than the (ICM) group. On

multivariate analysis, the only significant predictor of extent of reduction in IQRSD was (NICM) aetiology of heart failure. These findings are consistent with the fact that patients with (NICM) generally respond better to CRT than patients with (ICM) aetiology(Chen, Duan et al. 2014).

There was no significant difference in mechanical remodelling, as measured by changes in IVMD between (ICM) and (NICM) patients. There are two potential explanations for the selective reduction in iQRSD and LVp-RVegm in (NICM) patients: either the absence of extensive myocardial scarring in such patients facilitates potential changes in electrical conduction, and/or the absence of extensive myocardial scarring is a pre-requisite for CRT-associated improvements in myocardial energetics as postulated by Lidner et al (Lindner, Vogt et al. 2005) who found that CRT induces selective increase in regional myocardial oxygen consumption and blood flow in (NICM) patients but not in (ICM) which leads to facilitated intra-ventricular electrical conduction. Furthermore, there was no significant correlation between extent of electrical and of mechanical remodelling. Therefore, functional improvement correlates of the extent of electrical remodelling cannot be regarded as surrogates reflecting the beneficial effects of amelioration of mechanical dyssynchrony. It is possible that this better response in the (NICM) may partly be due to their having significant improvement in electrical remodelling. A sub-analysis of the CARE-HF trial(Gervais, Leclercq et al. 2009) found that QRS duration at 3 months post CRT implant but not QRS duration at baseline, predicted mortality and hospitalized heart failure, again highlighting the benefits associated with improvement in electrical remodelling. Similarly, a retrospective analysis of 337 patients by Iler et al,(Iler, Hu et al. 2008) found after adjustments for confounders that wider QRS duration after CRT implant but not at baseline independently predicted mortality or heart transplantation. Reduction in intrinsic QRS duration in our (NICM) group may be an indicator that the electrical dyssynchrony in this group reflects functional rather than anatomic block in the left bundle.

Consistent with this notion, Auricchio et al (Auricchio, Fantoni et al. 2004) have demonstrated that patients with systolic heart failure and left bundle branch block could have a functional block that potentially could be ameliorated with pacing or an anatomic block that is fixed. Interestingly, two-thirds of their patients with functional block had non-ischemic aetiology of heart failure.

Although, QRS duration and morphology remain the best tools at present to select patients for CRT insertion, recent evidence has cast doubt on the reliability of a simple dichotomization. For instance, Sassone et al (Sassone, Gambetti et al. 2015) retrospectively evaluated 243 patients and found that in patients with left bundle branch block who underwent CRT insertion, the effect of baseline intrinsic QRS duration on echocardiographic responsiveness and event free survival followed a U-shaped curve with worse outcomes for QRS duration less than 120-130ms or greater than 180ms. Importantly, this analysis includes both (ICM) and (NICM) patients. A much greater QRS duration may therefore reflect more extensive and potentially irreversible conduction blocks that not only affect the His-Purkinje system but also the myocardium. Hence we evaluated other measures of electrical remodelling with the subsequent novel finding of significant reduction in conduction times from the left ventricle to the right ventricle with left ventricular-only pacing, which was accentuated in the (NICM) group only. Given that no significant change occurred in conduction times from the right ventricle to the left ventricle with right ventricular-only pacing, it is likely that the enhanced conduction time with left ventricle-only pacing is due to improvement in left ventricular intra-myocardial electrical conduction rather than improvement in the His-bundle conduction. This would agree with previous findings by Auricchio et al(Auricchio, Fantoni et al. 2004) who noted that one-third of their heart failure patients with LBBB had intact transseptal conduction times and near- normal left ventricular endocardial activation times. The authors therefore concluded that in these patients,

the LBBB is mainly due to left ventricular intra-myocardial conduction delay. A rigorous evaluation of canine models of left bundle branch block by Strik et al(Strik, Rademakers et al. 2012) also found that both epicardially and endocardially implanted CRTs improve intramural electrical activation in the left ventricle, again making it less likely that improvement in His-Purkinje conduction post CRT represent the principal basis for improved electrical remodelling.

In our study group, we also found that with pacing briefly turned off at 6 months, CRT significantly reduced the difference between intrinsic QRS duration and the time interval from the onset of QRS complex to the onset of the left ventricular intra-cardiac electrocardiogram, (LVegm-QRSend), which was driven by reduction in intrinsic QRS duration without affecting the time interval between the onset of RV intra-cardiac electrocardiogram and the onset of LV intra-cardiac electrocardiogram or Q-LVegm. In other words, the time taken to initiate left ventricular depolarization remains unaltered, but once initiated, it takes less time to complete ventricular contraction. This finding also supports the hypothesis of improvement in left ventricular intra-mural conductivity rather than His-bundle conduction or right ventricular conduction. This could also explain why there was no improvement in the RVegm-LVegm which measures time interval for spontaneous depolarization of both ventricles. When all these measurements of electrical remodelling are considered together, we can conclude that in our cohort, CRT did not result in improvement in RV and/or His-bundle conduction but in improvement in left ventricular intra-myocardial conduction. Interestingly, Knuuti et al (Knuuti, Sundell et al. 2004) in their study of ten patients with NICM found that CRT improved regional left ventricular myocardial oxidative metabolism but had no effect on the right ventricular myocardial oxidative metabolism at rest.

The consistent finding of improvement in electrical parameters in the (NICM) group in our study may contribute to the selective reduction in all-cause mortality of CRT-D over CRT-P only in patients with ischemic aetiology of heart failure but not in patients with (NICM) (Kutyifa, Geller et al. 2014), perhaps indicating that CRT-P resulted in improvements in electrical remodelling in the (NICM) group only and thus mitigating the need for high voltage therapy. Furthermore, the recent DANISH study(Køber, Thune et al. 2016) found no mortality benefit of ICD with or without CRT over conventional medical treatment with or without CRT-P in patients with non-ischemic basis of heart failure.

Interestingly, the improvement in LVp-RVegm in our (NICM) group was also significantly correlated directly with change in VO_2 max at 6 months although no overall change occurred in VO_2 max itself. No study, to the best of our knowledge has shown this relationship, although improved ventricular activation has been shown to result in improved haemodynamics(Strik, Rademakers et al. 2012). Further re-enforcing the postulate that improvement in electrical remodelling could lead to improvement in functional status is our finding of a direct correlation between improvement in QOL score and reduction in intrinsic QRS duration at 6 months, in spite of the fact that in our cohort, change in QOL did not correlate with changes in measures of mechanical dyssynchrony. Our findings would therefore suggest that CRT-induced reversal of electrical remodelling could potentially lead to improvement in functional status independent of improvement in mechanical effects. The mechanisms underlying this observation remain uncertain, but a previous study found that reduction in intrinsic QRS duration 12 months post CRT implant was associated with improvement in NYHA functional status.(Sebag, Martins et al. 2012)

LIMITATIONS

Our study is limited by the small number of participants with the resultant potential for 'false negative' results by virtue of Type II error.

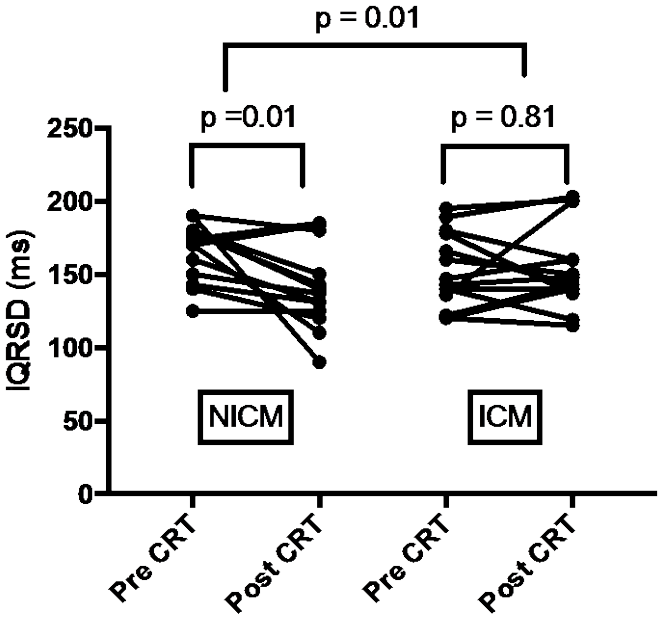
The (NICM) group also had better left ventricular systolic functions at baseline, which might contribute, to some of the observed findings. However both groups responded well echocardiographically and clinically. In addition both groups had similar baseline electrical parameters thus suggesting a disconnect between 'severity' of mechanical and electrical remodelling..

CONCLUSION

CRT reverses electrical remodelling and selectively improves left ventricular intramyocardial electrical conduction in patients with non-ischemic aetiology of heart failure. There seems to be a substantial disconnect between 'severity' of mechanical and electrical changes. Ultimately, improvements in electrical remodelling are associated with improvement in functional status independent of changes in echocardiographic mechanical dyssynchrony.

Acknowledgments

The authors wish to thank Dr Michael Frenneaux for his expert opinions



Mean	165.4	140.5	152.6	154.2
Std. Deviation	20.32	28.67	25.62	28.44

Figure 1: Changes in intrinsic QRS duration in the non-ischemic (NICM) and the ischemic (ICM) groups

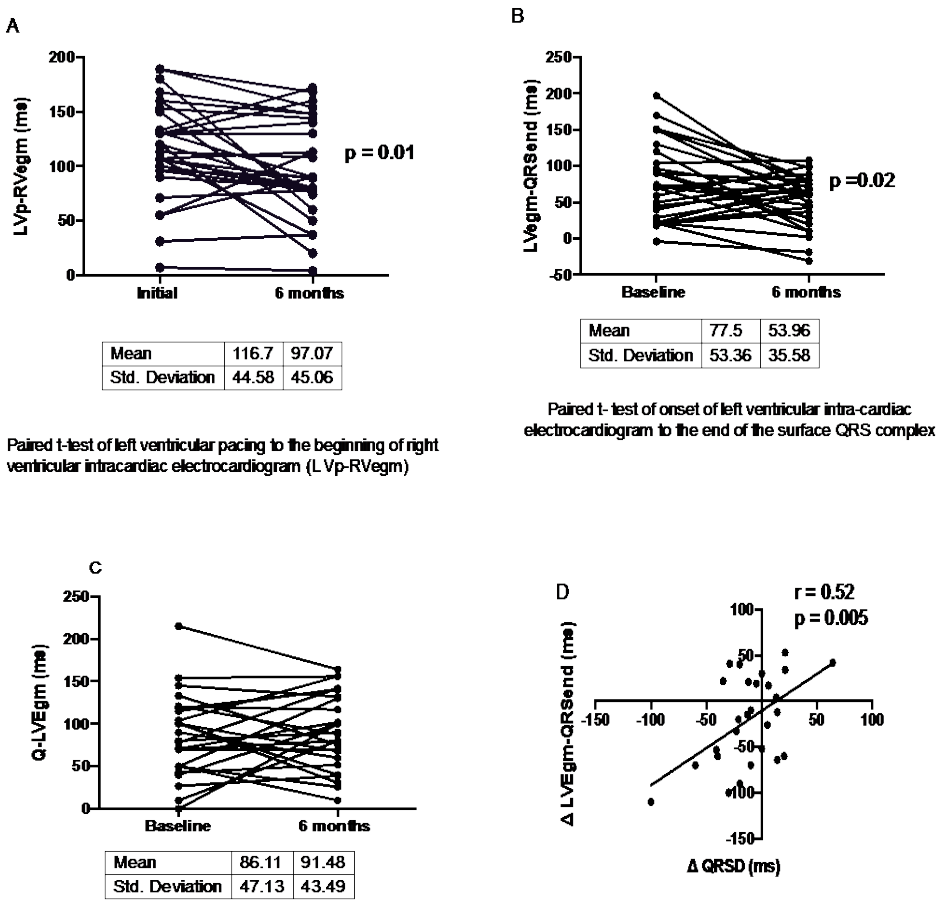


Figure 2: Electrical changes 6 months post CRT implant.

A: LVp-RVegm, paired t-test: p =0.01

B: LVegm-QRSend, paired t-test: p = 0.02

C: Q-LVegm, paired t-test: p = 0.54

D: Correlation between change in intrinsic QRS duration and change in LVegm-QRSend: r = 0.52, p = 0.005

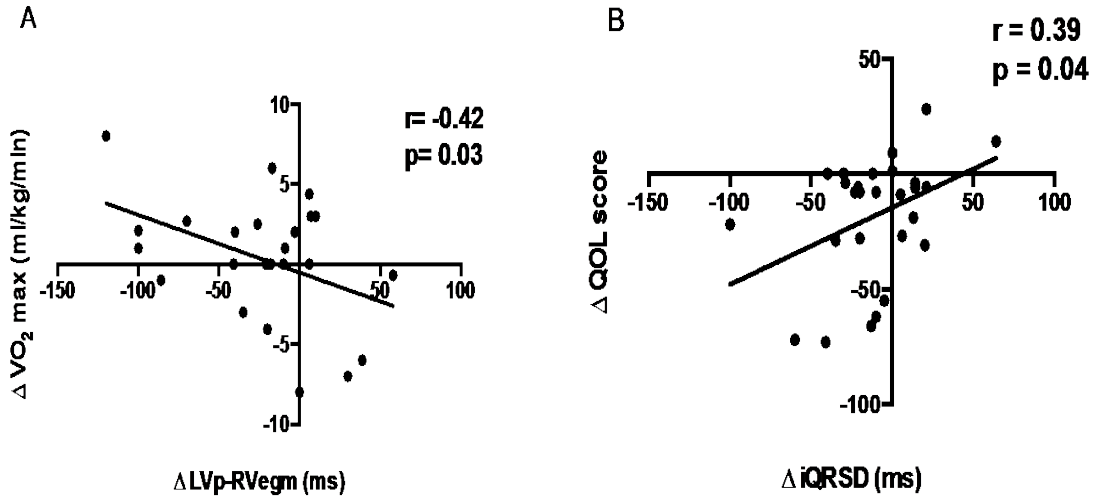


Figure 3: Correlations between electrical changes and functional status 6 months post CRT implant:

A: LVp-RVEgm and VO_2 max: r= -0.42, p= 0.03

B: iQRSD and QOL: r=0.39, p= 0.04

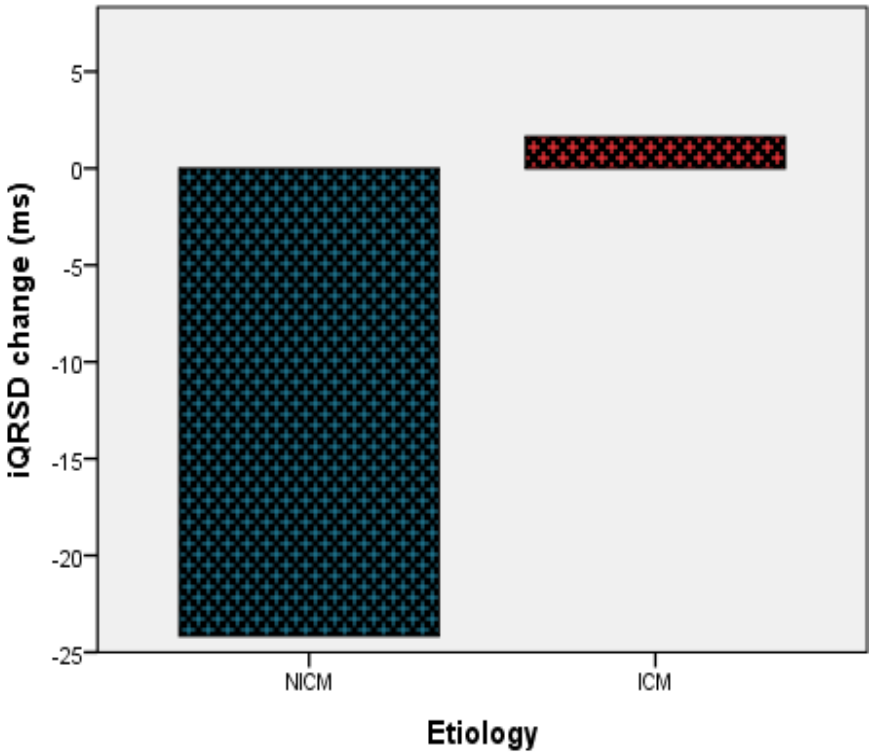


Figure 4: Effect of aetiology on change in iQRSD from baseline to 6 months.

iQRSD (ms):intrinsic QRS duration; NICM: non-ischemic cardiomyopathy; ICM: ischemic cardiomyopathy.

Table 1: Baseline characteristics of patients

Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile values

	ALL	ICM	NICM	Unpaired t – test, p-value
Age (years)	71.2 ±9.7	71.8 ± 10.2	71 (68.3-77.5)	0.64
Female, n (%)	10 (30.3)	3 (17.6)	7 (43.7)	0.10
BMI (kg/m^2)	29.3 ± 6.1	28 ± 6.45	30.7 ± 5.6	0.21
Comorbidities: • **Hypertension, n (%)** • **Diabetes, n (%)** • **Atrial fibrillation, n (%)** • **eGFR (ml/min/1.73m^2)**	 21 (64) 14 (42) 5 (15) 56.2 (21.9)	 12 (70.5) 10 (58.8) 2 (11.7) 53.1 (22.9)	 9 (56.2) 4 (25) 3 (18.7) 59.7 (21.0)	 0.39 0.04 0.57 0.39
Medications: • **ACE inhibitor** • **Angiotensin receptor blocker** • **Beta Blocker** • **Aldosterone Antagonist** • **Digoxin** • **Furosemide** • **Statin** • **Aspirin** • **Amiodarone**	 22 (67) 8 (24) 25 (76) 18 (55) 9 (27) 24 (73) 18 (55) 17 (52) 2 (6.0)	 11 (64.7) 5 (29.4) 13 (76.4) 8 (47.0) 4 (23.5) 13 (76.4) 13 (76.4) 10 (58.8) 2 (11.7)	 11 (68.7) 3 (18.7) 12 (75.0) 10 (62.5) 5 (31.2) 11 (68.7) 5 (31.2) 7 (43.7) 0 (0.0)	 >0.99 0.68 >0.99 0.49 0.61 0.70 0.01 0.49 0.48
CLINICAL ASSESSMENT **Systolic BP (mmHg)** **Diastolic BP (mmHg)** **Heart rate (bpm)** **VO$_2$max (ml/min/kg)** **QOL Score**	 126± 17.1 71.2 ± 9.2 69 ± 13.6 13.8 ± 4.7 41.9 ± 25.6	 125 ± 18.6 69.3 ± 9.0 70.4 ± 11.6 12.0 ± 4.0 45.6 ± 26.0	 130 ± 16.8 73.8 ± 8.9 67.5 ± 15.7 15 ± 4.7 38 ± 25.5	 0.44 0.16 0.54 0.07 0.40
VENTRICULAR FUNCTION **LVESV (ml)** **LVEDV (ml)** **EF (%)** **IVMD (ms)**	 137±55.3 193±67.4 29.8±6.1 43.9 ± 44.0	 166 ± 53.0 224 ± 64.5 26 ± 4.0 34.1 ± 45.7	 108 ± 42.8 161 ± 55.9 33 ± 6.0 53.0 ± 41.7	 0.002 0.006 0.001 0.24
ELECTRICAL PARAMETERS **QRSD (ms)** **LVp-RVegm (ms)** **RVp-LVegm** **LVegm-QRSend (ms)** **RVegm-LVegm** **Q-LVegm**	 158 ± 27.2 118 ± 43.3 88.5 ± 38.6 72.0 ± 53.1 57.9 ± 35.1 88.6 ± 45.7	 156 ± 24.9 115 ± 52.4 96.8 ± 39.5 67.1 ± 47.4 62.2 ± 39.5 89.2 ± 48.9	 161 ± 29.6 121 ±31.4 79.2 ± 36.7 64.6 ± 53.3 52.9 ± 29.8 87.9 ± 43.3	 0.62 0.69 0.20 0.89 0.47 0.94

BMI: Body mass index; ***VO$_2$****max: peak oxygen consumption during exercise; QOL: Minnesota Living with Heart Failure questionnaire; LVp-RVegm: onset of left ventricular pacing to onset of right ventricular intracardiac electrogram; RVegm-LVegm: onset of right ventricular intracardiac*

electrocardiogram to onset of left ventricular intracardiac electrocardiogram; RVp-LVegm: onset of right ventricular pacing to the onset of left ventricular intracardiac electrocardiogram; LVP-QRSend: onset of left ventricular pacing to the end of QRS complex; RVERP: right ventricular effective refractory period.

Table 2: Effects of cardiac resynchronization therapy on clinical and echocardiographic parameters in the whole cohort.

Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile values

Parameters	ALL			NICM			ICM			P for heterogeneity
	Pre-CRT	Post-CRT	P-value	Pre-CRT	Post-CRT	P-value	Pre-CRT	Post-CRT	P-value	
CLINICAL										
VO_2max (ml/min/kg)	13.8 (4.67)	14.1 (5.3)	0.80	15.4 (4.8)	15.9 (5.4)	0.63	12.0 (3.9)	11.8 (4.3)	0.85	
QOL score	40.7 (25.4)	22.9 (22.3)	0.001	35.2 (23.8)	15.4 (12.3)	0.003	46.5 (26.7)	31.0 (27.9)	0.20	0.66
ECHOCARDIOGRAPHIC										
LV EF (%)	31.0 (6.0)	38.0 (10.0)	<0.001	34.5 (5.8)	42.2 (10.2)	0.006	26.9 (3.3)	34.0 (9.0)	0.013	0.87
LVEDV (ml)	193 (69.8)	152 (66.7)	<0.001	145 (121.7-318.7)	109 (37.6)	<0.001	227 (64.8)	197 (60.8)	0.11	0.88
LVESV (ml)	137 (57.5)	98.9 (52.1)	<0.001	106 (45.9)	64.2 (31.5)	<0.001	167.2 (52.4)	133 (45.5)	0.046	0.57
IVMD (ms)	43.6 (44.6)	19.9 (33.9)	0.012	54.6 (42.6)	29.0 (25.0)	0.08	30.8 (45.0)	9.54 (40.9)	0.07	

VO2max: peak oxygen consumption during exercise; QOL: Minnesota Living with Heart Failure questionnaire; LVEF: left ventricular ejection fraction; LVED: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volme; IVMD: interventricular mechanical delay; NICM: non-ischemic cardiomyopathy; ICM: ischemic cardiomyopathy

Table 3: Electrical changes following cardiac resynchronization therapy.

Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile values

Parameters	ALL			NCM			ICM			P for heterogeneity
	Baseline	6months	p-value	Baseline	6months	p-value	Baseline	6months	p-value	
iQRSD (ms)	159 (23.6)	148 (29.4)	0.082	165 (20.3)	140 (28.6)	0.012	153 (25.6)	154 (28.4)	0.81	0.017
LVp-RVEgm (ms)	117 (44.5)	97.0 (45.0)	0.019	121(31.4)	86.4 (29.0)	0.005	112 (56.2)	109 (56.5)	0.76	0.042
LVEgm-QRSend (ms)	77.5 (53.3)	53.9 (35.5)	0.024	81.5 (60.4)	48.6 (44.3)	0.064	70.6 (51.6)	63.5 (30.9)	0.55	0.21
Q-LVEgm (ms)	86.1 (47.1)	91.4 (43.4)	0.54	90.4 (43.9)	92.3 (46.5)	0.9	82.0 (51.2)	90.6 (42.2)	0.4	
Rvegm-Lvegm (ms)	55.1 (34.4)	57.8 (33.6)	0.48	55.2 (29.5)	52.5 (25.1)	0.87	55.0 (39.5)	63.1 (40.7)	0.25	
RVp-LVEgm(ms)	86.9 (39.6)	93.3 (37.9)	0.3	79.2(36.6)	78.6(32.9)	0.93	95.1(42.3)	109 (37.4)	0.17	

iQRSD: Intrinsic QRS duration; LVp-RVegm: onset of left ventricular pacing to onset of right ventricular intracardiac electrogram; RVegm-LVegm: onset of right ventricular intracardiac electrocardiogram to onset of left ventricular intracardiac electrocardiogram; RVp-LVegm: onset of right ventricular pacing to the onset of left ventricular intracardiac electrocardiogram; LVP-QRSend: onset of left ventricular pacing to the end of QRS complex; NICM: non-ischemic cardiomyopathy; ICM: ischemic cardiomyopathy.

Table 4: Multivariate determinants of iQRSD at 6 months following CRT

Parameter	Standardized coefficients beta	P-Value
Diabetes	-0.23	0.25
Gender	0.27	0.18
Age	0.06	0.71
IVMD (ms)	0.14	0.44
Aetiology	0.44	0.02

IVMD: interventricular mechanical delay

4.1.2.3 Manuscript 3

Title:

Relationship between integrity of vascular nitric oxide signalling and intra-cardiac conduction and refractoriness in patients undergoing cardiac resynchronisation therapy.

Statement of Authorship

Title of Paper	**Relationship between integrity of vascular nitric oxide signalling and intra-cardiac conduction and refractoriness in patients undergoing cardiac resynchronisation therapy**
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	NA

Principal Author

Name of Principal Author (Candidate)	**Chukwudiebube Ajaero**
Contribution to the Paper	Design of study, recruitment of patients, acquisition and analyses of all data. Wrote manuscript and coordinated submission to the journal
Overall percentage (%)	85%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this pa
Signature	Date 19/3/18

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	**John Horowitz**
Contribution to the Paper	Design of study, oversight of data, critical editing of manuscripts.
Signature	Date 15/3/18

Name of Co-Author	**Yuliy Chirkov**
Contribution to the Paper	Data analyses, critical review of manuscript
Signature	Date 15/03/18
Name of Co-Author	**Tamila Heresztyn**
Contribution to the Paper	Data analyses, critical review of manuscript
Signature	Date 15/3/18
Name of Co-Author	**Andr**
Contribution to the Paper	Study design, supervision of data acquisition and analyses, critical review of manuscript
Signature	Date 13/3/18

Name of Co-Author	**W P Chan**
Contribution to the Paper	Data analyses, critical review of manuscript
Signature	Date 20/03/18
Name of Co-Author	**Ma**
Contribution to the Paper	Study design, critical review of manuscript
Signature	Date 20/3/18

ABSTRACT:

Introduction:

Prolonged QRS duration is both an indicator and predictor of response to cardiac resynchronisation therapy (CRT). However the full mechanism(s) underlying benefits from CRT remain uncertain, and it has been suggested that improved peripheral vascular function may play a role. We prospectively assessed correlation between electrical parameters and vascular and platelet nitric oxide (NO) signalling pre and 6 months post-CRT implant.

Methods and Results

33 patients for routine implantation of biventricular pacemakers were evaluated prior to and 6 months after the implant. Baseline clinical characteristics were analysed. NO signalling was assessed by platelet responsiveness to sodium nitroprusside (SNP), plasma concentration of asymmetric dimethyl arginine (ADMA) and augmentation index (A_{IX}) using radial artery applanation tonometry. Electrical parameters included intrinsic QRS duration and right ventricular effective refractory period (RVERP).

At baseline, there was an inverse correlation between the magnitude of endothelium dependent NO-signalling and QRS duration (r= -0.40, p =0.01) and RVERP (r= -0.44, p=0.01) but no correlations with endothelium-independent NO-signalling or platelet response to SNP. RVERP also correlated positively with QRS duration (r=0.37, p=0.04) and with ADMA (r=0.38, p=0.04). However, post-CRT, despite a trend towards reduction in QRSD (159±23.7 to 148±29.4, p=0.08), there were no significant changes in NO-signalling parameters and in RVERP.

Conclusion:

In patients with dyssynchronous heart failure, prolongation in QRS duration and higher RVERP are associated with reduced endothelium-dependent NO-signalling. However, CRT did not result in improvement in peripheral NO signalling.

KEYWORDS

Nitric oxide signalling

Biventricular pacemaker

Right ventricular effective refractory period

Augmentation index

QRS duration

BACKGROUND

Cardiac resynchronisation therapy (CRT) has been shown to reduce morbidity and mortality in heart failure(Abraham, Fisher et al. 2002) and although it reduces mechanical dyssynchrony, the precise mechanism of benefit is not fully known.

Normalisation of shear stress in the peripheral vasculature, coronary bed and myocardium post CRT would in theory lead to increased nitric oxide (NO) production via nitric oxide synthase (NOS) and decreased NOS 'uncoupling' due to diminished superoxide production(Davies 2009). Therefore, CRT might improve peripheral vascular function, for example, by potentiating NO(S) signalling. However, no definitive data on this exists and assessment of NO signalling may depend on whether endogenous tone, response to agents releasing endogenous NO or exogenous NO donors are utilised.

Nitric oxide (NO) is known to play important roles in cardiac excitation-contraction coupling. The generation of NO in vivo is facilitated by the nitric oxide synthase (NOS) enzyme of which there are three isoforms (Balligand, Ungureanu-Longrois et al. 1994, Balligand, Kobzik et al. 1995, Xu, Huso et al. 1999). In the healthy heart, sub-cellular compartmentalisation of NOS isoforms occurs with endothelial NOS (eNOS or NOS-3) being located in the sarcolemmal caveolae in proximity with the L-type Calcium channel (LTCC), whereas the neuronal NOS (nNOS or NOS-1) co-localises with the ryanodine (RyR) receptors and xanthine oxidase reductase (XOR) on the sarcoplasmic reticulum.(Barouch, Harrison et al. 2002) There is substantial variability in the physiologic effects of the various NOS isoforms within the myocardium, both on an inotropic and electrical excitability/conductivity basis. Specifically, increasing nNOS expression within the myocardium appears to induce positive inotropic responses, which are dependent on increased calcium mobilisation via RyR interaction. (Janssen and Periasamy 2007) On the other hand, studies in eNOS$^{-/-}$ mice suggest that eNOS exerts predominantly negative inotropic effects but accelerates intra-ventricular conduction, thus shortening action potential

duration.(Wang, Kohr et al. 2008) and therefore limiting arrhythmogenesis..(Bai, Namekata et al. 2005) This may be particularly relevant in the case of activation of β_2-adrenoceptors during chronic heart failure given that these are coupled to eNOS.(Iaccarino, Cipolletta et al. 2002)

Finally, in systolic heart failure, the third NOS isoform, (indicible NOS {iNOS}) is over-expressed within the myocardium, essentially inducing negative inotropic effects(Thoenes, Forstermann et al. 1996) Simultaneously, myocardial expression of both nNOS and eNOS is increased in heart failure. Although impact of nonspecific NOS inhibition has been assessed clinically in acute heart failure, (Nordhaug, Steensrud et al. 2004) the effects of increased NO release and the roles of the various NOS isoforms in chronic heart failure remain unclear. CRT insertion remains a means for improving clinical status in chronic heart failure without altering pharmacotherapy. In this setting, the current study was designed both to investigate (i) potential correlations between peripheral vascular NOS signaling (predominantly reflecting eNOS activation) and pre-CRT integrity of myocardial conduction and refractoriness, and (ii) potential impact of CRT on markers of peripheral NO-mediated vascular homeostasis. The implied β-adrenergic inhibitory effect of NO (Drexler, Kästner et al. 1998) as well as its parasympathetic stimulatory effect (Mohan, Heaton et al. 2002) can be mediated theoretically either through the generation of 3',5'-cyclic guanosine monophosphate (cGMP) via the activation of soluble guanylyl cyclase (Senzaki, Smith et al. 2001) or through soluble guanylate cyclase-independent *S*-nitrosylation of proteins. (Xu, Eu et al. 1998) While cardiac lusitropy is thought to be cGMP dependent(Layland, Li et al. 2002), *S*- nitrosylation of both the LTCC and RyR receptors are considered important and stimulatory to the excitation contraction coupling in the basal state.(Xu, Eu et al. 1998) It has also been demonstrated in isolated cat papillary muscle that the effect of NO and cGMP on myocardial contractility is biphasic, with low concentrations of both being positively inotropic and higher concentrations being negatively inotropic.(Mohan, Brutsaert et al. 1996)

In chronic systolic heart failure, enhancement of NOS 1 and NOS 3 activities has been observed (Damy, Ratajczak et al. 2004, Danson, Zhang et al. 2005) as well as increased *S*-nitrosylation of LTCC and RyR receptors(Bellinger, Reiken et al. 2009). Apart from this, both NOS-1 and NOS-3 also undergo extensive sub-cellular translocations (Damy, Ratajczak et al. 2004) which potentially lead to alteration in their functions, such as oxidation of soluble guanylyl cyclase (sGC) with subsequent reduced generation of cGMP.(Tsai, Liu et al. 2012) In addition to all these, a third NOS isoform- the inducible (iNOS) or NOS-2 is expressed substantially in heart failure (Thoenes, Forstermann et al. 1996) and plays a role in excitation-contraction coupling, with inhibitory effects to the flow of myocardial Ca^{2+} transients. The resultant effects of these include hypo-responsiveness to β-adrenergic stimulation, and decreased calcium transients. It can therefore be construed from these that variable NO signalling potentially affects electrophysiological parameters including conduction times in the myocardium. In spite of all these, the literature is still sparse on the effects of NO signalling on electro-pathophysiologic parameters in heart failure.

Objectives:

1. The primary objective of the current study was to determine whether CRT alters peripheral vascular endothelial homeostasis.
2. The secondary objective was to investigate the correlation, if any, between integrity of NO signalling and that of cardiac electrical parameters

METHODS

The tabular summary of evaluations is show below. Baseline evaluations were performed within two weeks prior to CRT implant, with right ventricular effective refractory period (RVERP) performed immediately after CRT insertion. All parameters were assessed again 6 months post CRT insertion.

Table 1: Summary of Evaluations Performed

Purpose	**Method(s) of evaluation**
Electrical parameters: ▪ Extent of dyssynchrony ▪ Ventricular refractoriness	a. Intrinsic QRS duration b. Right ventricular effective refractory period (RVERP)
Peripheral vascular compliance assessed by augmentation index (AI_X) using radial artery applanation tonometry.	a. Resting AI_X b. Via NOS activation using inhaled salbutamol c. 'Direct' NO donor using sublingual nitroglycerin
Platelet NO signalling	Inhibition of ADP-induced platelet aggregation with sodium nitroprusside
Biochemical marker of NOS functional integrity	Measurement of plasma levels of asymmetric dimethyl arginine

Thirty-three patients with moderate to severe systolic heart failure and electrical ventricular dyssynchrony were evaluated prior to insertion of cardiac resynchronisation device and six months afterwards. Clinical characteristics were noted. Peripheral NO signalling was

evaluated using radial artery applanation tonometry, platelet ADP-induced aggregation response to sodium nitroprusside and measurements of asymmetric dimethyl arginine.

Vascular endothelial function:

Platelet aggregometry test:

Asymmetric dimethyl arginine (ADMA) and symmetric dimethyl arginine (SDMA):

Electrophysiological parameters

c. **Intrinsic QRS duration:**

c. **Right ventricular effective refractory periods (RVERP)**

The details of these methodologies have been described in Chapter 2

The study complies with the *Declaration of Helsinki* and approval for the study was granted by the Ethics and Human Research Committee of The Queen Elizabeth Hospital and all participants provided written informed consent.

Statistical analyses

All data are expressed as mean ± SD unless stated otherwise. Interval changes in functional and electrophysiological parameters were assessed using paired t- test for normally distributed variables, and Wilcoxon matched-pairs signed rank test for non-parametric data. Each patient served as his/her control. Interactions between baseline endothelial function and NO signaling with electrophysiological parameters as well as the interactions between changes in function, NO signaling and electrophysiological parameters were correlated using

Pearson correlation coefficients for normally distributed data and Spearman correlation for non-parametric data. A two-tailed P value <0.05 was considered statistically significant.

All data were analysed with Prism 6 for Mac OS X version 6.0h October 2015

RESULTS

Table 2 shows the patients' demographics. The mean intrinsic QRS duration was (158 ± 27.2ms. 17 patients (52%) had an ischemic basis for their heart failure. Participants were extensively treated with standard conventional heart failure medications and up to 55% were also on a statin.

Correlations of NO-signalling with electrophysiological parameters at baseline

There was statistically significant negative correlation between endothelium dependent NOS-mediated vasomotor response and intrinsic QRS duration at baseline (r = -0.40, p=0.01), (**Figure 1A**) but not with endothelium-independent NO-mediated vasomotor response (**Figure 1B**) or with platelet response to the NO donor, SNP. There was also statistically significant negative correlation between endothelium-dependent NOS-mediated vasomotor response and RVERP at 500ms drive train (r= -0.44, p=0.01) but not with endothelium independent NO signaling nor with SNP response, (**Figure 2: A-C**) Also, at baseline, intrinsic QRS duration correlated directly with RVERP at 500ms (r=0.37, p=0.04) and there was a positive correlation between ADMA levels and RVERP at 400ms (r = 0.38, p = 0.04). **Figure 3**.

Changes following CRT

There were no significant changes in endothelium dependent NOS-mediated vasomotor response and in RVERP. Also there was no correlation between changes in endothelium

dependent NOS-mediated vasomotor response and the RVERP. These are shown in **Figure 4, A-C.**

DISCUSSION

In this study, we demonstrated that impairment of peripheral endothelium-dependent NOS function (reflecting by implication, loss of eNOS activity) predicts both increased QRS duration and right ventricular effective refractory period. Specifically,we found a significant inverse relationship between the magnitude of NOS-mediated vasomotor response and the extent of electrical dyssynchrony measured by the intrinsic QRS duration in patients with chronic systolic heart failure.We also demonstrated that reduction in NOS-mediated vasomotor response was associated with increased right ventricular refractoriness at different cycle lengths.We are not aware of any previous study evaluating these relationships. Although at first, it may seem intuitive that prolongation of ventricular effective period should be protective from arrhythmogenesis, it is important to consider the issue of 'normal' ventricular effective refractory period and also dispersion of refractoriness. A previous study that evaluated the prolongation of human RVERP with procainamide found a baseline RVERP of 237 ± 7 msec at drive trains of 500 and 450 msec, with procainamide prolonging this to 279 ± 16 msec. (Kastor, Josephson et al. 1977) Our cohort had a substantially greater RVERP at baseline (mean of 267.4 ± 23.7 msec at 500 msec drive train. This study, (Kastor, Josephson et al. 1977) also observed that procainamide not only increased RVERP, but also prolonged the QRS duration. In our own study, we found a direct correlation between RVERP and QRS duration at baseline. Interestingly, a recent study on isolated Langendorff-perfused rat heart showed that selective inhibition of the inwardly rectifying potassium current (I_{K1}) through Kir2.1 resulted in significant prolongation of ventricular action potential at 90% repolarisation, significant prolongation of ventricular effective refractory period with associated increased occurrence of ventricular

fibrillation.(Skarsfeldt, Carstensen et al. 2016) Put together, it may therefore be that the relationship of VERP to ventricular fibrillation may be a U-shaped curve.

Another important finding of our study is the direct correlation between plasma levels of ADMA and RVERP, and when considered together with the finding of increased RVERP being associated with reduced endothelium dependent NOS signalling, it seems to us that overall, low NOS leads to prolonged RVERP and that eNOS potentially attenuates RVERP. This is in agreement with the previous findings by Wang et al (Wang, Kohr et al. 2008) that eNOS -/- mice have prolonged action potential duration, increased early and delayed after-depolarisation and enhanced sensitivity to β-adrenergic stimulation, all of which create a labile environment for ventricular arrhythmias compared to wild type mice.

Furthermore, there are two potential explanations for the relationships between myocardial parameters (QRSD and RVERP) and response to salbutamol. As suggested by the RVERP and ADMA correlation, one of these is likely to be NOS inactivation. However, particularly if NOS-related NO production were impaired together with NOS 'uncoupling' as a result of redox stress, a second potential explanation would be increased 'scavenging' of endogenously released NO, as there is substantial evidence that 'scavenging' selectively impairs responses to endogenously rather than exogenously generated NO (PS)

The other major findings of this study was that 6 months post-CRT, there had been no significant change in either myocardial RVERP, or peripheral indices measured, in the face of a borderline decrease in QRS duration. Therefore, we found no evidence that the beneficial effects of CRT are mediated in whole or part by incremental vascular effects of NO.

Limitations:

The current study has several important limitations. As regards investigations at baseline, we observed a number of correlations between ventricular conduction and refractory parameters and peripheral responses to NOS activation (via salbutamol) rather than to a NO donor (GTN). Interpretation of this important set of findings does not extend to a cause-and-effect relationship. Indeed the only feasible way to explore this in patients would have been to administer an inhibitor of NOS systemically, a process which may not be safe in patients with severe heart failure. Thus we cannot be absolutely certain whether the major initiating factor for these relationships is myocardial or peripheral vascular.

From the post intervention component of the study, there is the potential for Type 2 error. If the current results are eventually supported by future analogous investigations, one would be tempted to conclude that changes in QRS duration post CRT either do not normalize arterial shear stress and/or that the implications of concomitant therapy, with drugs which stimulate NOS activity and/or NO signaling, such as statins and angiotensin converting enzyme inhibitors(Chirkov and Horowitz 2007) (Sverdlov, Chan et al. 2013) may have obscured effects of CRT in this regard.

CONCLUSION:

NOS activation in vessels but not effects of NO in vessels or platelets predicts narrower QRS complex and lower right ventricular effective refractory period, therefore NOS activation in peripheral vessels is associated with better inter-ventricular electrical conduction and limiting of excessive ventricular refractoriness.

Table 2: Baseline characteristics of patients

Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile values

Age (years)	71.2 ±9.7
Female, n (%)	10 (30.3)
Weight (kg)	87.1 ± 18.6
Height (cm)	173 ± 8.1
BMI (kg/m^2)	29.3 ± 6.1
Ischaemic aetiology of CHF, n (%)	17 (52)
History of current/past smoking, n (%)	19 (57.6)
QRS duration (ms)	158 ± 27.2
NYHA Class:	
• I, n (%)	3 (9)
• II, n (%)	7 (21)
• III, n (%)	19 (58)
• IV, n (%)	4 (12)
Comorbidities:	
• Hypertension, n (%)	21 (64)
• Diabetes, n (%)	14 (42)
• COPD, n (%)	4 (12)
• Atrial fibrillation, n (%)	5 (15)
Medications:	
• ACE inhibitor	22 (67)
• Angiotensin receptor blocker	8 (24)
• Beta Blocker	25 (76)
• Aldosterone Antagonist	18 (55)
• Digoxin	9 (27)
• Frusemide	24 (73)
• Statin	18 (55)
• Aspirin	17 (52)
• Clopidogrel	7 (21)

Table 3: Effects of CRT on Vascular, platelet and electrical parameters

Parameters	Pre-CRT	6 months post CRT	P value
ELECTRICAL MEASUREMENTS			
QRSD (ms)	159 (23.7)	148 (29.4)	0.08
RVERP 600 (ms)	279 (24.7)	283 (22.8)	NS
RVERP 500 (ms)	267 (23.7)	271 (19.0)	NS
RVERP 400 (ms)	259 (26.6)	261 (19.3)	NS
VASCULAR & PLATELET			
Baseline Aix (%)	20.3 (8.2)	20.3 (8.1)	NS
GTN response { Aix change (%)}	-14.1 (10.0)	-16.6 (8.1)	NS
Salbutamol response {Aix change (%)}	-9.9 (10.5)	-11.9 (8.3)	NS
SNP response (%)	30.5 (21.8)	25.2 (19.7)	NS
ADMA (μM)	0.66 (0.08)	0.65 (0.09)	NS
SDMA (μM)	0.83 (0.28)	0.74 (0.20)	0.01

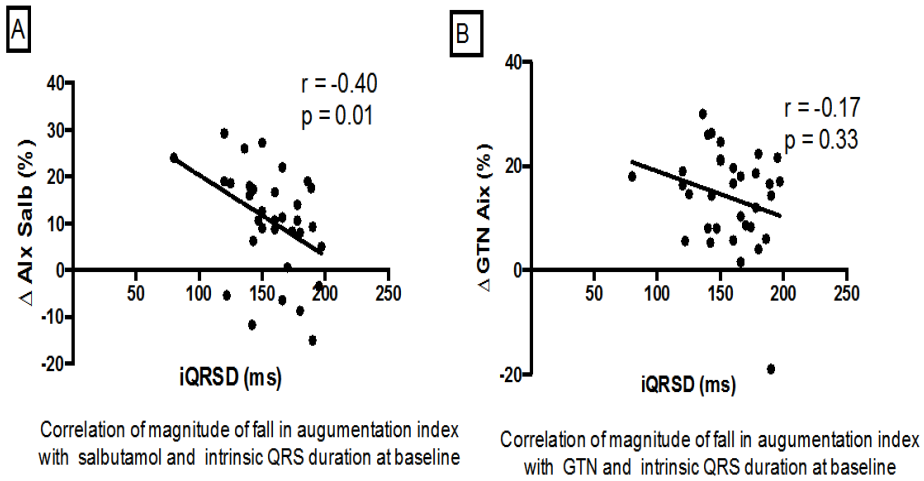
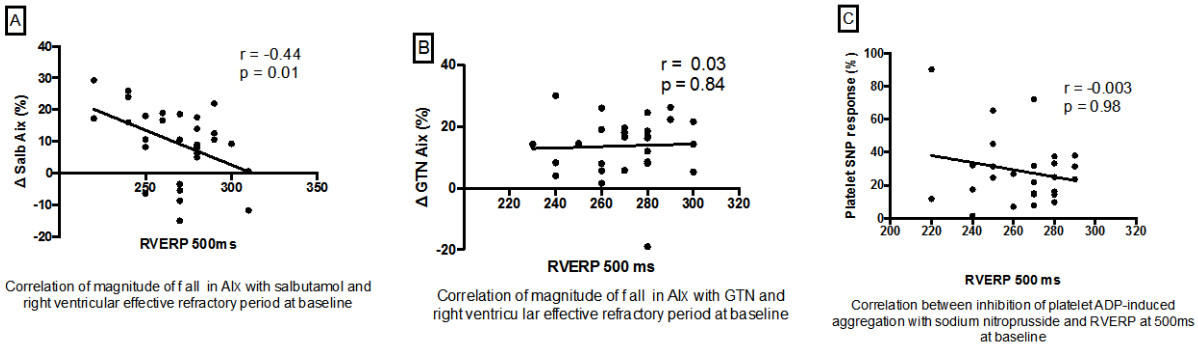


Figure 1: *Correlations between intrinsic QRS duration and (A) endothelium dependent and (B) endothelium independent nitric oxide signaling.*

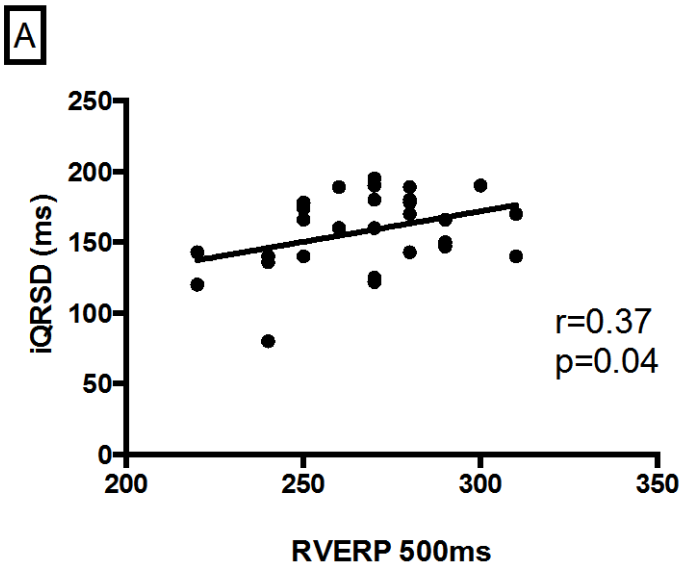


Correlation of magnitude of fall in AIx with salbutamol and right ventricular effective refractory period at baseline

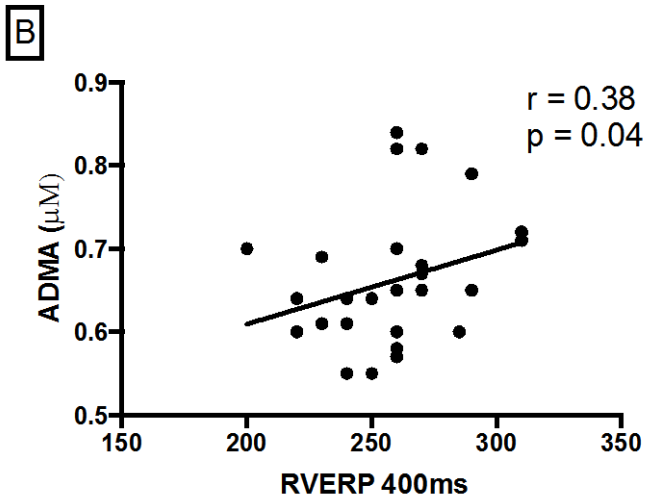
Correlation of magnitude of fall in AIx with GTN and right ventricular effective refractory period at baseline

Correlation between inhibition of platelet ADP-induced aggregation with sodium nitroprusside and RVERP at 500ms at baseline

FIGURE 2: Correlations between right ventricular effective refractory period at 500ms drive train and endothelium dependent (A) and independent (B) nitric oxide signalling and (C) platelet ADP-induced aggregation response to sodium nitroprusside.



Correlation between baseline intrinsic QRSD and right ventricular effective refractory period at at 500ms drive train.



Correlation of baseline aymemetric dimethyl arginine and right ventricular effective refractory period at 400ms

FIGURE 3: Correlations between A, right ventricular effective refractory period at 500ms and baseline intrinsic QRS duration and B, right ventricular effective refractory period at 400ms and plasma levels of asymmetric dimethyl arginine

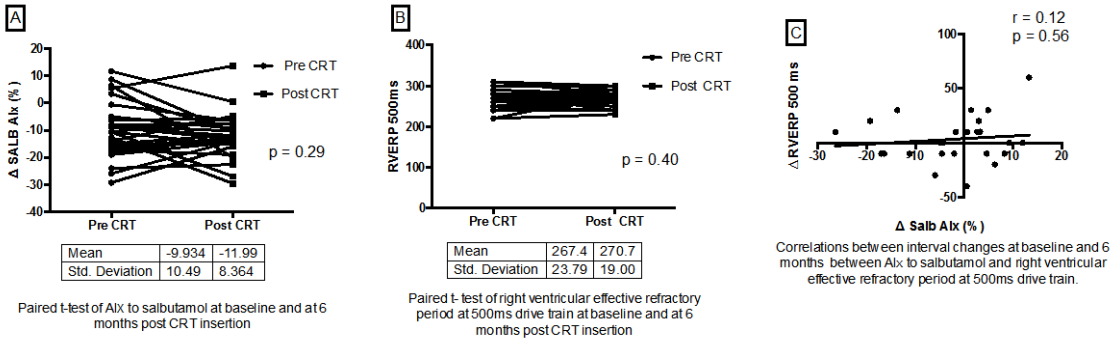


FIGURE 4: Effect of CRT on edothelium dependent NOS-mediated vasomotor response and right ventricular effective refractory period at 500ms drive train.

CHAPTER 5

SUMMARY AND FUTURE PERSPECTIVES

5.1 Summary

In this study, we evaluated whether improvement in mechanical dyssynchrony by cardiac resynchronisation therapy (CRT) would also primarily lead to improvement in peripheral vascular function in patients with dyssynchronous systolic heart failure. We envisaged that this would be the case inasmuch as CRT improved cardiac contractility. We evaluated peripheral vascular function primarily by the use of radial artery applanation tonometry to assess interval changes in baseline augmentation index, augmentation index with sublingual nitroglycerin and also with inhaled salbutamol. Because of the pivotal role nitric oxide signaling plays in vascular endothelial function, we also assessed interval changes in the inhibition of ADP-induced platelet aggregability using sodium nitroprusside (a direct nitric oxide donor) as well as changes in plasma concentrations of ADMA and SDMA. We also assessed other markers of inflammatory/neurohumoral activation such as high sensitive CRP, plasma concentrations of catecholamine metabolites matrix metalloproteinases and NT-proBNP. Furthermore, we also assessed the extent of endothelial glycocalyx shedding by measuring the plasma concentrations of Syndecan-1. Therefore, we evaluated vascular endothelial function by utilising multiple modalities. Participants were assessed at two time points: at baseline and then at 6 months post CRT insertion.

In this study, we also evaluated the effects CRT might have on redox stress. For this we assessed changes in platelet content of thioredoxin interacting protein (TXNIP) using immunohistochemical staining.

Other parameters studied were (a) the effects of reduction in measures of mechanical and electrical dyssynchrony on functional status of patients- the functional status was assessed with peak exercise oxygen consumption, 6-minute walk distance, the Minnesota Living with Heart Failure Quality of life score and the NYHA class and (b) the effects of CRT on intracardiac electrical conduction, and on right ventricular effective refractory periods.

Similar to the findings from previously published effects of CRT, (Abraham, Young et al. 2004, Bristow, Saxon et al. 2004, Cleland , Daubert et al. 2005) we found that in our cohort, CRT resulted in significant improvement in cardiac contractility as evidenced by reductions in left ventricular end-diastolic and end-systolic volumes, and increased left ventricular ejection fraction. We also found significant reduction in both intra-ventricular dyssynchrony as assessed with the septal-to-posterior wall delay and also the inter-ventricular mechanical delay. In addition to these changes, our cohort also had significant improvements in symptomatic status, which was assessed with the NYHA functional class and the Minnesota Living with Heart Failure Quality of life score. We consider these echocardiographic and symptomatic improvements as fundamentally important because they indicated that our cohort received 'effective' CRT, without which it would be difficult to proceed with further evaluation of the effects of CRT on peripheral vascular function. In addition, CRT resulted in significant reduction in plasma concentrations of NT-proBNP in our cohort, an effect that has also been documented in previous studies.

In spite of these salutary effects of CRT in our cohort, and contrary to our expectation, we found that CRT did not result in significant improvement in either resting augmentation index, or in changes in augmentation index responses to salbutamol or nitroglycerin. Further more, there was also no improvement in ex vivo platelet nitric oxide signaling as evidenced by lack of decrease in ADP-induced platelet aggregation with the addition of sodium nitroprusside, indicating that CRT did not normalise the nitric oxide/soluble guanylate cyclase axis. At baseline, plasma concentrations of the glycocalyx shedding marker, syndecan-1 correlated directly with the septal-to-posterior wall delay and inversely with left ventricular ejection fraction, but there was no significant change in plasma concentrations of syndecan-1, six months post CRT.

It is possible that the extensive background pharmacotherapy for heart failure used in our cohort may have significantly impacted the observed results, especially considering that the baseline plasma syndecan-1 concentrations in our cohort were significantly less than levels previously documented in cases of acute heart failure.(Neves, Meneses et al. 2015) Likewise, baseline plasma concentrations of metalloproteinases in our cohort were less than those observed in our laboratory in patients with acute heart failure and similar to levels observed among control subjects with no heart failure.(Spinale, Coker et al. 2000)

On the other hand, we found that CRT induced significant reduction in plasma concentrations of SDMA, a marker of inflammation, and this reduction was independent of improvement in renal excretory function. This implies a reduction in activity of the protein catalytic enzyme PRMT2, which generates SDMA.(Cha and Jho 2012) The relevance of this is not clear especially as there was no significant reduction in other markers of inflammation such as plasma concentrations of hs-CRP and the catecholamine metabolites. However, as systolic heart failure may be associated with protein wasting, leading eventually to cardiac cachexia, this reduction may be of some relevance, assuming that its consequences include limitation of protein catabolism.

There was a trend towards reduction in the intrinsic QRS duration driven by a significant reduction in patients with non-ischemic aetiology of heart failure. However, there was significant improvement in intra-myocardial electrical conduction time when pacing from the left ventricle. Interestingly, improvement in electrical conduction and electrical dyssynchrony correlated directly with VO_2max and with quality of life score.

In summary, while CRT did not exert any beneficial effects on most markers of peripheral vascular function and inflammation in our study, there was a significant reduction in SDMA, an inflammatory marker. Furthermore, improvements in electrical conduction correlated

significantly with some functional parameters of clinical improvement. There was no convincing evidence of a nitric oxide-sensitising effect of CRT.

5.2 Future perspectives:

The results of this study do not exclude the possibility that CRT has beneficial effects on peripheral vascular function. As we stated before, extensive pharmacotherapy for heart failure could have confounded the results. Furthermore, our assessments of both the NO pathway and of inflammatory change were incomplete.

Secondly, we found that at baseline, the width of the QRS complex, as well as the RVERP, correlated inversely with the effect of salbutamol on augmentation index, a marker of NOS-mediated vasodilatation.(Hayward, Kraidly et al. 2002) This has not been previously reported but this raises the possibility that QRS duration in dyssychronous heart failure could at least in part, be due to impairment of myocardial nitric oxide signaling, which in turn promotes inflammation and fibrosis, not only of the myocardium, but also of the electrical conducting tissues. It must be stated that reversal of mechanical dyssynchrony by CRT would not represent a means of normalising NO signalling. Hence this finding can only be assessed directly and definitively utilising left ventricular biopsy to quantitate NOS expression and uncoupling, perhaps in patients undergoing coronary revascularisation.

Urgency for future definitive experiments seems greatest for the findings regarding salbutamol response and syndecan-1 release pre CRT. Essentially, the issue is ‘chicken and egg’: does impairment of NO signalling induce both glycocalyx shedding and fibrosis of the His-Purkinje system? Perhaps this can only be defined by prospective studies.

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ADDENDUM AND CORRIGENDUM

P- values > 0.1 were reported as non-significant (NS) whereas p-values < 0.1 were reported in absolute numbers.