The Effects of Left Ventricular Assist Devices on Myocardial Function and Cardiac Nervous System

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Submitted for Doctorate of Philosophy (PhD), 2010

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Abstract

Myocardial recovery in patients with non-ischaemic end-stage dilated cardiomyopathy can occur following left ventricular assist device (LVAD) and drug combination therapy. The effects of LVAD and drug combination therapy on the cardiac nervous system have not been adequately studied. It is well recognised that contractile reserve is decreased in heart failure patients who have an increased level of circulating norepinephrine secondary to failure of the myocardial norepinephrine transporter system. The aims of this study are to examine prospectively the outcome of using continuous-flow Heart Mate (HM) II LVAD and drug combination therapy on the frequency and durability of recovery, the clinical effects of left ventricular unloading on cardiac sympathetic nervous system, and the impact on norepinephrine transporter activity and neurohormonal levels.

23 non-ischaemic end-stage heart failure patients, confirmed histologically, were implanted with a HM II LVAD between February 2006 and March 2009. The combination therapy with anti-failure reverse remodelling medication was commenced once patients were off inotropes. The first objective was to determine a cut-off point where the contribution of the continuous-flow HM II LVAD was minimal thus allowing safe and reliable assessment of the underlying left ventricular function without causing left ventricular reloading. The impacts of LVAD and drug combination therapy on myocardial function and cardiac nervous system were assessed by studying: 1) contractile reserve using a 6–minute walk exercise test; 2) norepinephrine transporter using serial nuclear $^{123}$I-mtaiodobenzylguanidine ($^{123}$I-MIBG) imaging; 3) the changes in catecholamine levels using liquid chromatography mass spectrometry. Immunohistochemistry was utilised to assess norepinephrine transporter fibre concentration from the left ventricular apex biopsies taken at implantation in an attempt to correlate with myocardial recovery.

Experimental and clinical studies identified 6000 rpm as an efficient and a safe “low” speed to study the underlying myocardial function. Out of the 23 patients, 15 had recovered and had significant improvement in contractile reserve (the
ejection fraction has increased in the recovered patients by $7.73 \pm 4.37\%$ as compared to $1.13 \pm 4.20\%$ in non-recovered patients ($p=0.012$). Recovered patients have also exhibited significant improvement in $^{123}$I-MIBG uptake parameters which were correlated significantly with changes in catecholamine levels. Further, recovered patients had higher percentage of norepinephrine transporter immunoreactive nerve fibers per total area in the left ventricular apex as compared to non-recovered patients and the concentration correlated positively with recovery.

In conclusion, LVAD and drug combination therapy has a positive impact on myocardial function and the cardiac nervous system which were more enhanced in the recovered subpopulation.
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**Abbreviations List**

6MW Six-minute Walk
AC Adenyllyl Cyclase
ACC American College of Cardiology
ACE-I Angiotensin Converter Enzyme Inhibitor
AF Atrial Fibrillation
ALP Alkaline Phosphotase
ALT Alanine Transaminase
AngII Angiotensin II
ANP Atrial Natriuretic Peptide
AR(s) Adrenergic Receptor(s)
AT$_1$ Type-1 Angiotensin II Receptor
AT$_2$ Type-2 Angiotensin II Receptor
ATP Adenosine Triphosphate
βARK1 β-adrenergic receptor kinase
BiVAD Biventricular Assist Device
BMI Body Mass Index
bmp Beats per minute
BNP Brain Natriuretic Peptide
BSA Body Surface Area
BTR Bridge to Recovery
BTT Bridge to Transplantation
BV Blood Volume
Ca$^{2+}$ Calcium ions
cAMP  cyclic Adenosine Monophosphate
cGMP  cyclic guanosine monophosphate
CABG  Coronary Artery Bypass Grafting
CaM  Calmodulin
CHGA  Chromogranin A
CHGB  Chromogranin B
CI  Cardiac Index
CNBD  Cyclic nucleotide-binding domain
CO  Cardiac Output
CPB  Cardiopulmonary Bypass
CR  Contractile Reserve
CRT  Cardiac Resynchronisation Therapy
CRT-D  Cardiac Resynchronisation Therapy Device
CSA  Cross Sectional Area
CT-1  Cardiotrophin-1
CVA  Cerebrovascular Accident
Da  Dalton
DA  Dopamine
DAG  2-Diacylglycerol
DBH  Dopamine β-hydroxylase
DBP  Diastolic Blood Pressure
DCM  Dilated Cardiomyopathy
DM  Diabetes Mellitus
DOPA  Dihydroxyphenylacetic Acid
ΔQ  Difference in Flow between Systole and Diastole
DSE  Dobutamine Stress Echocardiography
DT  Destination Therapy
EC  Excitation-Contraction
ECM  Extracellular Matrix
EF  Ejection Fraction
eNOS  Endogenous Nitric Oxide Synthase
EPI  Epinephrine
EPI 166  Epinephrine with molecular weight 166 Da
ET-1  Endothelin-1
<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<td>ET-3</td>
<td>Endothelin-3</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FS</td>
<td>Fractional Shortening</td>
</tr>
<tr>
<td>$G_{\alpha_s}$</td>
<td>G-protein stimulatory $\alpha$-subunit</td>
</tr>
<tr>
<td>$G_{\alpha_i}$</td>
<td>G-protein inhibitory $\alpha$-subunit</td>
</tr>
<tr>
<td>H/M</td>
<td>Heart Mediastinum Ratio</td>
</tr>
<tr>
<td>HCN</td>
<td>Hyperpolarisation-activated cyclic nucleotide-gated channels</td>
</tr>
<tr>
<td>HF</td>
<td>Heart Failure</td>
</tr>
<tr>
<td>HM I LVAD</td>
<td>HeartMate I LVAD</td>
</tr>
<tr>
<td>HM II LVAD</td>
<td>HeartMate II LVAD</td>
</tr>
<tr>
<td>HO-CM</td>
<td>Hypertrophic Cardiomyopathy</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>$^{132}$I-MIBG</td>
<td>$^{132}$I-Metaiodobenzylguanidine</td>
</tr>
<tr>
<td>IABP</td>
<td>Intra Aortic Balloon Pump</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass Correlation Coefficient</td>
</tr>
<tr>
<td>ICD</td>
<td>Implantable Cardioverter Defibrillator</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischaemic Heart Disease</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin 1</td>
</tr>
<tr>
<td>IL-2</td>
<td>Interleukin 2</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin 10</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible Nitric Oxide Synthase</td>
</tr>
<tr>
<td>INTERMACS</td>
<td>International Registry for Mechanical Assisted Circulatory Support</td>
</tr>
<tr>
<td>IP</td>
<td>Implantable Pneumatic VAD</td>
</tr>
<tr>
<td>IP$_3$</td>
<td>Inositol Triphosphate</td>
</tr>
<tr>
<td>IP$_3$R</td>
<td>Inositol Triphosphate Receptor</td>
</tr>
<tr>
<td>IVAD</td>
<td>Intracorporeal Ventricular Assist Device</td>
</tr>
<tr>
<td>keV</td>
<td>kiloelectron volt</td>
</tr>
<tr>
<td>l/min</td>
<td>Litres / minute</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid Chromatography</td>
</tr>
<tr>
<td>LCMS</td>
<td>Liquid Chromatography Mass Spectrometry</td>
</tr>
</tbody>
</table>
LTCC: L-type Ca\(^{2+}\) Channels
LV: Left Ventricle / Left Ventricular
LVAD(s): Left Ventricle Assist Device(s)
LVAS: Left Ventricle Assist System
LVEDD: Left Ventricle End-Diastolic Diameter
LVEDP: Left Ventricle End-Diastolic Pressure
LVEF: Left Ventricle Ejection Fraction
LVESD: Left Ventricle End-Systolic Diameter
MAO: Monoamine Oxidase
MAP: Mean Arterial Blood Pressure
MBq: Mega-Becquerel
MCS: Mechanical Circulatory Support
MET: Metanephrine
MD: Muscular Dystrophy
MHC: Myosin Heavy Chain
MHD: Mental Health Dimension of SF36 QOL questionnaire
MI: Myocardial Infarction
MIBG: Metaiodobenzylguanidine
Mins: Minutes
mmHg: Millimetre Mercury
MMP(s): Matrix Metalloproteinase(s)
MR: Mitral Regurgitation
MS: Mass Spectrometry
mVO\(_2\): Maximal Oxygen Uptake
NE: Norepinephrine
NET: Norepinephrine Transporter
NF\(_\kappa\)B: Nuclear Factor Kappa B
NH: Neurohormonal
NO: Nitric Oxide
NorMET: Normetanephrine
NR: Not –reported
NS: Non-significant
NRec: Non-Recovered Group
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>PA</td>
<td>Pulmonary Artery</td>
</tr>
<tr>
<td>PCWP</td>
<td>Pulmonary Capillary Wedge Pressure</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PDU</td>
<td>Pump Drive Unit</td>
</tr>
<tr>
<td>PHD</td>
<td>Physical Health Dimension of SF36 QOL questionnaire</td>
</tr>
<tr>
<td>PI₃K</td>
<td>Phosphoinositide 3-Kinase</td>
</tr>
<tr>
<td>PIP₂</td>
<td>Phosphotidylinositol biphosphate</td>
</tr>
<tr>
<td>PIP₃</td>
<td>Phosphatidylinositol Triphosphate</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein Kinase A</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>PLB</td>
<td>Phospholamban</td>
</tr>
<tr>
<td>PLC₉</td>
<td>Phospholipase C₉</td>
</tr>
<tr>
<td>PLM</td>
<td>Phospholemman</td>
</tr>
<tr>
<td>PP</td>
<td>Pulse Pressure</td>
</tr>
<tr>
<td>PPM</td>
<td>Post-Partum Cardiomyopathy</td>
</tr>
<tr>
<td>PVAD</td>
<td>Paracorporeal Ventricular Assist Device</td>
</tr>
<tr>
<td>PW</td>
<td>Pulsed Wave</td>
</tr>
<tr>
<td>QOL</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>RAAS</td>
<td>Renin-Angiotensin-Aldosterone System</td>
</tr>
<tr>
<td>RAP</td>
<td>Right Atrial Pressure</td>
</tr>
<tr>
<td>Rec</td>
<td>Recovered Group</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operating Characteristics</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>rpm</td>
<td>rotations per minute</td>
</tr>
<tr>
<td>RV</td>
<td>Right ventricle</td>
</tr>
<tr>
<td>RVAD</td>
<td>Right Ventricular Assist Device</td>
</tr>
<tr>
<td>RyR</td>
<td>Ryanodine Receptor</td>
</tr>
<tr>
<td>RyR2</td>
<td>Isoform 2 of RyR</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>S.D.</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SERCA</td>
<td>Sarcoendoplasmic Reticulum Ca²⁺ ATPase</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic Nervous System</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
</tr>
</tbody>
</table>
SR  Sarcoplasmic Reticulum  
SV  Stroke Volume  
SvO$_2$  PA Oxygen Saturation  
TAH  Total Artificial Heart  
TGF-β  Transforming Growth Factor β  
TH  Tyrosine Hydroxylase  
TIMP(s)  Tissue Inhibitors of MMPs  
TNFα  Tissue Necrosis Factor α  
Trp  Transient Receptor Potential Channels  
Tx  Transplantation  
Tyr  Tyrosine  
V-ATPase  Vacuolar-ATPase  
VAD(s)  Ventricular Assist Device(s)  
VE  Vented Electric VAD  
VIP  Vasoactive Intestinal Peptide  
Vmax$_f$  Peak Velocity in Forward Direction  
VMAT  Vesicular Monoamine Transporters  
VO$_2$  Peak Oxygen Consumption  
VTI$_f$  Forward Velocity Time Integral  
W/O  Washout Rate  

Studies / Trials Acronyms

CAPRICORN  Carvedilol Post-Infarct Survival Control in Left Ventricular Dysfunction  
CHRISTMAS  Carvedilol Hibernation Reversible Ischaemia Trial: marker of success trial  
CIBIS  Cardiac Insufficiency Bisoprolol Study  
COMPANION  Comparison of Medical Therapy, Pacing and Defibrillation in Heart Failure  
COPERNICUS  Carvedilol Prospective Randomized Cumulative Survival  
COMET  Carvedilol or Metoprolol European Trial  
CONSENSUS  Cooperative New Scandinavian Enalapril Survival Study
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIG</td>
<td>The Digitalis Investigators Group</td>
</tr>
<tr>
<td>HOPE</td>
<td>Heart Outcomes Prevention Evaluation</td>
</tr>
<tr>
<td>MADIT-2</td>
<td>Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy</td>
</tr>
<tr>
<td>MDC</td>
<td>Metoprolol in Dilated Cardiomyopathy Study</td>
</tr>
<tr>
<td>MERIT-HF</td>
<td>Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure</td>
</tr>
<tr>
<td>RALES</td>
<td>The Randomised Aldactone Evaluation Study</td>
</tr>
<tr>
<td>RECOVER</td>
<td>Research into Etanercept: Cytokine antagonism in Ventricular Dysfunction</td>
</tr>
<tr>
<td>REMATCH</td>
<td>The Randomised Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure</td>
</tr>
<tr>
<td>RENAISSANCE</td>
<td>Randomised Etanercept North America Strategy to Study Antagonism of Cytokines</td>
</tr>
<tr>
<td>RENEWAL</td>
<td>Randomised Etanercept Worldwide Evaluation</td>
</tr>
<tr>
<td>RESOLVD</td>
<td>Randomised Evaluation of Strategies for Left Ventricular Dysfunction</td>
</tr>
<tr>
<td>SAVE</td>
<td>Survival and Ventricular Enlargement Trial</td>
</tr>
<tr>
<td>SCD-HeFT</td>
<td>Sudden Cardiac Death in Heart Failure Trial</td>
</tr>
<tr>
<td>SOLVD</td>
<td>Studies of Left Ventricular Dysfunction</td>
</tr>
<tr>
<td>USCP</td>
<td>United States Carvedilol Programme</td>
</tr>
<tr>
<td>Val-HeFT</td>
<td>The Valsartan Heart Failure Trial</td>
</tr>
<tr>
<td>VEST</td>
<td>Vesnarinone Trial</td>
</tr>
</tbody>
</table>
CHAPTER 1 - Introduction
1.1 Heart Failure

“...his heart is flooded...body parts are all together weak...obstruction in his abdomen...not in a condition to leap the Nile...stomach is swollen...chest is asthmatic...”

Scripts from the Edwin Smith and the Ebers Egyptian papyri, written 2500 – 3000 BC and discovered in Thebes in 1862 referring to a clinical picture of a failing heart and signs of congestion in an Egyptian priest

“...pulse had all kinds of irregularities...he was so weak that every breath involved great effort...unable to lie on either side...for never for one moment could he breathe freely...was forced to sit upright in order to breathe at all; if by chance he did lie on his back or side, the suffocation was awful...danger of asphyxia...at all times, asleep or awake, he was menaced by suffocation...incision at the elbow proved fruitless...stomach was visibly enlarged to a great size...feet swelled up ...the circulation of blood in the arteries had finally stopped...”

A biography of the Byzantine emperor Alexius I Comnenus (reigned from 1081 to 1118) written by his daughter Anna before his fatal illness of heart failure at age 62

Although descriptions of heart failure (HF) exist from ancient Egyptians, Greece, India, and the Romans (Jon F.Lutz, 1988; Saba MM et al., 2006), it remains a clinical syndrome that largely defies definition. Many definitions have been proposed based on either physiologic or clinical attributes (Tan LB et al., 1996; Ventura HO et al., 1992; Wagner S & Cohen K, 1977). In 1985, Poole Wilson was the first to suggest that HF is “a clinical syndrome caused by an abnormality of the heart that is recognised by a characteristic pattern of haemodynamic, renal, neural and hormonal responses.”

Years later, the Heart Failure Task Force (Hunt SA et al., 2005) adapted this concept and defined heart failure as a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood.
1.2 Epidemiology and Aetiology of Heart Failure

The incidence and prevalence of HF increase steeply with age reaching over 1% per annum in those aged 85 years and over. The average age at first diagnosis is 76 years. There are more than 6 million people diagnosed with this disease in the USA and 6.5 million cases in Europe. In the UK the incidence is 140 per 100,000 men and 120 per 100,000 women with approximately 120,000 new cases each year. Although the incidence is higher in men, evidences suggest higher mortality in women with the condition.

HF has a poor prognosis, with about 40% of patients dying within the first year of diagnosis. Coronary artery disease acts as the commonest primary aetiology in 36 – 57% of cases in HF (Cowie MR et al., 1999; Ho KK et al., 1993; McKee PA et al., 1971; Remes J et al., 2009; Adams KF et al., 2005; Lloyd-Jones DM et al., 2006). Other aetiological factors include cardiomyopathies characterised by progressive ventricular dilatation (i.e. dilated cardiomyopathy (DCM)) and restrictive cardiomyopathy, hypertension, valvular and congenital heart diseases, arrhythmias, alcohol and drugs, high output failure such as anaemia and thyrotoxicosis, pericardial heart disease, primary right heart failure, bacterial and viral myocarditis, collagen diseases such as rheumatoid arthritis and progressive systemic sclerosis, neuromuscular diseases such as muscular dystrophy, amyloidosis, sarcoidosis and peri-partum cardiomyopathy.

As of 2004, the total annual cost was £716 million to the NHS; 60% for hospital inpatient care; 17% for primary care; 23% for outpatient care including outpatient investigations and drugs. The annual cost in the USA exceeds $60 billion accounting for approximately just over 5% of the total healthcare budget. In addition to the economic burden the management of this disease places on health care systems of the developed countries, HF has an immense negative impact on the psychological and sociological status of patients and their relatives and quality of life of the patients. HF has also placed patients at increased risk of re-hospitalisation, social isolation, depression and anxiety.
Symptoms of HF range from mild breathlessness and reduced exercise tolerance to signs of reduced cardiac output and impaired myocardial contractility. Symptoms have been classified by the New York Heart Association (NYHA) as demonstrated in table 1.1.

Table 1.1: NYHA classification of HF

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No limitation is experienced by any activities</td>
</tr>
<tr>
<td>II</td>
<td>Slight, mild limitation of activity; the patient comfortable at rest or with mild exertion</td>
</tr>
<tr>
<td>III</td>
<td>Marked limitation of any activity; patient comfortable only at rest</td>
</tr>
<tr>
<td>IV</td>
<td>Any physical activity brings on discomfort and symptoms at rest</td>
</tr>
</tbody>
</table>

Cardinal manifestations of HF include dyspnoea, orthopnoea, paroxysmal nocturnal dyspnoea, fatigue, limitation to exercise tolerance, anorexia, and fluid retention, which may lead to pulmonary congestion and peripheral oedema.

Fatigue and exercise intolerance are partly related to abnormalities in skeletal muscle with premature muscle lactate release, impaired muscle blood flow, deficient endothelial function, and abnormalities in skeletal muscle structure and function (Piña IL et al., 2003). Reduced cerebral blood flow, when accompanied by abnormal sleep pattern, may occasionally lead to somnolence and confusion in chronic HF (Watson RDS et al., 2000) contributing to the fatigue symptoms. Paroxysmal nocturnal dyspnoea results from increased left ventricular filling pressures and is considered an important predictive factor of HF (Watson RDS et al., 2000). Oedema is a sign of a right heart failure which is normally associated with right hypochnodrial pain secondary to liver distension, ascites, and rarely, bowel oedema (Watson RDS et al., 2000).
In 2001, the American College of Cardiology (ACC) introduced a new classification for HF that emphasizes the evolution and the progression of the disease through four different stages (table 1.2) (Hunt SA et al., 2005).

**Table 1.2: ACC staging classification of HF**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Patients at high risk for developing heart failure but no functional or structural heart disorders</td>
</tr>
<tr>
<td>B</td>
<td>A structural heart disorder but no symptoms at any stage</td>
</tr>
<tr>
<td>C</td>
<td>Previous or current symptoms of heart failure in the context of an underlying structural heart problem, but managed with medical treatment</td>
</tr>
<tr>
<td>D</td>
<td>Advanced disease refractory to standard treatment, requiring hospital-based support, a heart transplant or palliative care</td>
</tr>
</tbody>
</table>

**Table 1.2: ACC staging system of HF focusing on its evolution and progression.**

This staged classification is considered to be a departure from the traditional NYHA classification as it underpins the fact that established risk factors and structural abnormalities are necessary for the development and the progression of HF. Studies have shown that advanced HF accounts for approximately 5% of symptomatic HF with a 1-year mortality ranging from 33% - 50% (Brophy JM et al., 1994;The CONSENSUS Trial Study Group, 1987b;The SOLVD Investigators, 1991;Adams KF & Zannad F, 1998;Shah M et al., 2001).

The ACC classification can be linked to the clinical features in the NYHA classification as follows: Stage A encompasses “pre-heart failure” and it does not have a corresponding NYHA class. It is recognised that intervention with treatment at Stage A could presumably prevent progression to overt symptoms. Stage B would correspond to NYHA Class I, Stage C to NYHA Class II and III, and Stage D overlaps with NYHA Class IV.
HF may progress from Stage A to Stage D in a given patient but does not usually follow the path in reverse. In contrast, a patient with NYHA class IV symptoms might have quick improvement to class III with diuretic therapy alone or even to class I with the use of a combination of mechanical circulatory support and pharmacological therapy “the ethos of the present study”. This staged HF classification promotes a way of thinking about the disease that is similar to thinking about cancer — that is, HF is a **progressive** disease with a scope of identifying and screening of patients who are at risk.
1.3 Pathogenesis and Pathophysiology of HF

HF is commonly a disorder of left ventricular (LV) remodeling, which results from an index event (i.e. initial insult). This may be clinically silent, such as the expression of a genetic mutation, or obvious, such as the loss of a large mass of contractile tissue from acute myocardial infarction.

Myocardial Remodeling

Remodeling is a central feature in the progression of HF (Wilson S. Colucci, 1997). It is associated with an increase in the LV muscle mass, an increase in ventricular volume, and a change in shape of the ventricle (Jay N. Cohn, 2005; Bernard Swynghedauw, 1999; Bozkurt B, 1999). If the process is initiated by an acute myocardial infarction, remodeling is asymmetric because of the localised necrosis. However, if the results from a more generalized disease, such as cardiomyopathy, the ventricle remains symmetric but with different shape and volume (see later for the LV geometrical changes in HF).

At the cellular level, the histologic changes that occur in the remodeled ventricle include myocyte hypertrophy (Cleutjens JP et al., 1995; Katz AM, 1994; Gaballa MA & Goldman S, 2002; Yacoub MH, 2001), myocyte slippage (Olivetti G et al., 1990), interstitial growth (Hirohata S et al., 1997), myocyte lengthening, (Terracciano CMN et al., 2004) and myocyte apoptosis (Narula J et al., 1996)

Mechanisms inducing remodeling are not fully established, but thought to be secondary to mechanical stretch (Chien KR, 1999; Hunter JJ & Chien KR, 1999). Furthermore, the cellular and molecular actors initiating the remodeling process remain incompletely understood.

Until the early eighties, the stimulus for myocyte growth has been viewed as a haemodynamic response to the index event. However, this view has now been largely abandoned and its application is limited to the management of decompensated “end-stage” HF patients where positive inotropic drugs and vasodilators remain widely used.
The more recent neurohormonal (NH) model describes the neuroendocrine activation process as the **hallmark** in the progression of HF and that inhibition of neurohormones is likely to have long-term benefits with regard to morbidity and mortality. This transitional change in the understanding of the pathophysiology of HF has resulted in improved medical and non-medical management of the disease.

The second axis that has been shown to contribute to remodeling is inflammation with the activation of several inflammatory markers associated with certain changes that occur within the extracellular matrix (ECM) (Birks EJ & Yacoub MH, 1997). Figure 1.1 is a schematic representation of the mechanisms involved in myocardial remodeling.

**Figure 1.1: Mechanisms of remodeling**

![Diagram of Mechanisms of Remodeling](image)

**Fig 1.1:** A general overview of the mechanisms involved in myocardial remodeling. Adapted from (Francis GS & Wilson Tang WH, 2003; Wilson S.Colucci, 1997).

Both NH model and inflammatory second axes with the involvement of the ECM will be discussed next.
1.3.1 The Neurohormonal Model

Neurohormonal controls play an essential role in the haemostatic regulation of salt and water metabolism, circulatory function and blood pressure, through the activity of the sympathetic nervous system (SNS), renin angiotensin aldosterone system, natriuretic peptides, and endothelin.

1.3.1.1 Cardiac Neuronal Anatomy and Role of Norepinephrine

Sympathetic fibers leave the spinal cord at segments T1 to L2-3. Preganglionic sympathetic fibers consist of small myelinated fibers that come off the spinal roots as white rami communicantes and synapse in the paravertebral ganglia. Adrenergic fibers that innervate the heart originate in the left and right stellate ganglia. The left stellate innervates the right ventricle, whereas the right stellate innervates the anterior and lateral portions of the heart. Parasympathetic innervation originates from the medulla and follows through the right and left vagus nerves which then divide into superior and inferior cardiac nerves (figure 1.2).

Figure 1.2: The anatomy of cardiac neuronal system

Figure 1.2: A schematic diagram of the anatomy of the sympathetic and the parasympathetic cardiac neuronal system. From (Olshansky B et al., 2008).
In 1984, Cohn et al were the first to demonstrate a link between sympathetic activation and mortality in HF (Cohen JN et al., 1984). Plasma norepinephrine (NE) has since been shown to be associated with progressive ventricular dilatation and the severity of cardiac dysfunction (Cohen JN, 1995; Cohen JN, 2002; Francis GS et al., 1990).

In HF patients there is a specific increased activity in cardiac sympathetic efferent neuronal activity rather than an increase in the generalised sympathetic activity which initially supports but then ultimately harms the failing heart (Davis D et al., 1988; Esler M et al., 1997; Kaye D & Esler M, 2005). This contention is based on several observations. First, the heart exhibits substantial (Swedberg K et al., 1984) and selective adrenergic activation (Hasking G et al., 1986) compared with most other organs. Second, adrenergic activation is regionally regulated within the failing human heart (Bristow MR et al., 1992). Third, cardiac adrenergic activation is a better predictor of outcome than systemic sympathetic markers (Eisenhofer G et al., 1996; Hasking G et al., 1986; Rundqvist B et al., 1997; Kaye DM et al., 1994). Finally, recent data indicated that cardiac adrenergic activation is the first neurohormonal abnormality that can be detected in the progression of heart failure from asymptomatic LV dysfunction to the clinical syndrome (Eisenhofer G et al., 1996; Hasking G et al., 1986; Rundqvist B et al., 1997; Kaye DM et al., 1994).

The release of NE from sympathetic nerves is controlled by the pre-synaptic α2A- and α2C- adrenergic receptors (AR) (Philipp M et al., 2002; Hein L et al., 1999) with α2C AR having the most affinity for NE (Bünemann M et al., 2001). Following the interaction with α- and β- receptors, NE is taken up by the catecholamine transporter of which there are two types; neuronal (uptake-1) and non-neuronal (uptake-2) transporters (Eisenhofer G, 2001). Uptake-1 transporters are located at the cardiac sympathetic nerve endings at the pre-synaptic level and include the norepinephrine transporter (NET) whilst the uptake-2 system is located on the myocyte and its activity is very low in the human heart (Figure 1.3).
Figure 1.3: Catecholamine synthesis requires tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH), as well as cytochrome b-561 as an electron shuttle. From (Fung MM et al., 2008).

Once synthesised, NE transport and vesicle formation is aided by vesicular monoamine transporters (VMAT), NET, chromogranins A and B (CHGA, CHGB), and the vacuolar-ATPase (V-ATPase). NE is then released in a nonexocytotic mechanism by tyramine.

The released NE acts on α- and β- AR at the postsynaptic level. In normal individuals 92% of synaptic NE is removed from the synapse via NET (figure 1.4). This is reduced to 85% in HF patients (Eisenhofer G, 2001).
Figure 1.4: Quantitative illustration of neuronal uptake

**Blood stream**

Control subjects

- DHPG
- NE
- COMT
- NMN
- MHPG

Cardiac failure

- DHPG
- NE
- COMT
- NMN
- MHPG

Sympathetic nerve ending

- NE
- DOPA
- DA
- DOPAC
- TYR
- DOPAC
- DA
- DOPAC
- TYR

**Cardiac myocyte**

- NE
- DOPA
- DA
- DOPAC
- TYR
- DOPAC
- DA
- DOPAC
- TYR

Fig 1.4: Quantitative illustration of the processes of neuronal and extraneuronal uptake, synthesis, vesicular-axoplasmic exchange, metabolism, release, spillover, and turnover of NE in sympathetic nerves of the normal (left) and failing (right) human heart. From (Eisenhofer G, 2001).

Initially, it was uncertain whether the rise in NE in HF is secondary to enhanced release of NE from the pre-synaptic membrane (Mancia G, 1990) or a defect in the NET system at the presynaptic level (Hasking G et al., 1986). Studies have later confirmed that although NE release is increased, it was the failure of the uptake system that was responsible for the abundance of the NE molecule at the synaptic level (Allman KC & Lahiri A, 2002; Lotze U et al., 1999; Eldadah BA et al., 2004; Böhm M et al., 1995). The role of NET is described in details in chapter 5 entitled “Mechanical left ventricular unloading results in normalisation of the cardiac norepinephrine transporter uptake mechanism”.

Abundance of NE in the synapse leads to increased sympathetic drive which initially acts as a method of compensatory inotropic mechanism (Eisenhofer G,
such that it helps to restore cardiovascular haemostasis. This has been referred to as “asymptomatic left ventricular dysfunction” phase (McDonagh TA et al., 1997;Thomas KG & Redfield MM, 1997a) which is associated with an initial increase in mean arterial blood pressure and tachycardia (Thomas KG & Redfield MM, 1997b), both are usually concealed from the patient and the physician. Shortly afterwards, the asymptomatic left ventricular dysfunction develops into an overt or symptomatic heart failure which is characterised by progressive neurohumoral activation and LV remodeling (Fu YC et al., 2004; Shah M et al., 2001). The net result is the development of arrhythmias, myocardial apoptosis and necrosis, tissue hypoxia, increased cardiac oxygen consumption and myocardial oxygen wastage, reduced coronary blood flow, and loss of contractile reserve (Fukuoka S et al., 1997; Gudjonsson T & Rahko PS, 2002b; Liang C, 2003; Opie LH, 2002).

Animal studies have shown that acute intravenous infusions of isoproterenol or NE result in acute contraction band lesions attributed to relative hypoxia, increased sarcolemmal permeability, calcium overload, elevation of cyclic AMP (cAMP), activation of \( \alpha \)- and \( \beta \)- ARs, and formation of oxidative catecholamine metabolites (Todd GL et al., 1985; Mann DL et al., 1992). Chronic catecholamine administration in rats causes interstitial fibrosis, reduces \( \beta \)-adrenergic receptor mediated inotropic responses, promotes myocyte apoptosis, and induces pump dysfunction primarily through LV dilatation, all features of remodeling (Osadchii OE et al., 2007; Brouri N et al., 2004).

Excessive NE exerts its toxic effects directly through five different mechanisms: cardiac ARs (Gerald W Dron II, 2002; Lefkowitz RJ et al., 2000), renin-angiotensin-aldosterone system (Packer M, 1992), calcium handling (Arai M et al., 1993), natriuretic peptides (Rodeheffer RJ, 2004), and endothelin (Henrion D & Laher I, 1993). The following five subsections discuss the involvement of each of these mechanisms in the pathophysiological process of HF.
1.3.1.2 Adrenergic Receptors (ARs)

Total adrenergic signalling in the heart is the sum of systemic epinephrine release from the medulla of the adrenal glands and local myocardial NE release from cardiac sympathetic nerves. The adrenergic hypothesis of HF is represented in figure 1.5.

**Figure 1.5: The adrenergic hypothesis of heart failure**

![Diagram](image)

**Fig 1.5:** The adrenergic hypothesis of HF. Increased cardiac adrenergic drive contributes to 2 hallmarks of the natural history of HF: impaired exercise response and progressive myocardial dysfunction. Adapted from (Bristow MR, 1993).

Both NE and epinephrine exert their biological actions via the activation of 9 different AR subtypes, 3 alpha1-receptors (α1A, α1B, and α1D), 3 alpha2-receptors (α2A, α2B, and α2C), and 3 beta-receptors (β1, β2, and β3). All have 7 transmembrane domains and signal via the interaction of the heterotrimeric G-proteins.

Of the β-AR subtypes, β1-AR is the most abundant in the cardiac tissue with a ratio to β2-AR of 70:30 (Bristow MR et al., 1986). It exerts its potent positive inotropic, chronotropic, and lusitropic effects by stimulating the adenylyl cyclase (AC) / protein kinase A (PKA) signaling pathway through the overexpression of the stimulatory α-subunit of G-protein (G_{s}).
The targets of PKA are illustrated in figure 1.6 and include:

1) the L-type calcium channels (LTCC) and ryanodine receptors (RyR): activation of each results in intracellular leak in calcium ions \((\text{Ca}^{2+})\) and an increase in \(\text{Ca}^{2+}\) entry into the sarcoplasmic reticulum (SR) (Lehnart SE et al., 2005; Zhao XL et al., 1994);

2) the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which initiate and modulate the rhythmic activity in cardiac pacemaker cells (Ludwig A et al., 1998);

3) phospholamban (PLB), which accelerates \(\text{Ca}^{2+}\) reuptake by sarcoplasmic reticulum (SERCA) hence accelerating cardiac relaxation (Sulakhe PV & Vo XT, 1995);

4) troponin I and myosin binding protein-C, which reduce myofilament sensitivity to \(\text{Ca}^{2+}\) and hence the relaxation of myofilaments is accelerated (Sulakhe PV & Vo XT, 1995);

5) phospholemman (PLM), relieving its inhibitory influence and resulting in the stimulation of the sodium pump (Despa S et al., 2005).

**Figure 1.6: β-AR signalling**

![Figure 1.6: β-AR signalling](image)

**Fig 1.6:** The major intracellular effects of the sympathetic transmitters NE and epinephrine is mediated by formation of cAMP, which increases the activity of the cAMP-dependent PKA. ATP, adenosine triphosphate; CNBD, cyclic nucleotide-binding domain; \(\text{G}_{\alpha_4}\) and \(\text{G}_{\alpha_6}\), G protein alpha-subunit subtypes. From (Triposkiadis F et al., 2009).
Associated with β-AR activation is the autoregulation process of receptor desensitisation. This process operates as a safety device to prevent overstimulation of receptors in the face of excessive β-AR agonist (Malcolm Johnson, 2006). Therefore, abundance of NE in the synaptic cleft results into four different alterations in the β-AR: i) **downregulation** of the receptor secondary to either reduced mRNA levels or enhanced receptor degradation (Hadcock JR & Malbon CC, 1991; Hausdorff WP et al., 1990; Ali Ahmed, 2003); ii) **impairment** of the remaining β-AR i.e. receptor uncoupling from adenylate cyclase (Bristow MR et al., 1982; Brodde OE, 1991); iii) **internalisation** of the uncoupled receptors (Malcolm Johnson, 2006); and iv) **phosphorylation** of the internalised receptors (Malcolm Johnson, 2006).

Another mechanism of action of NE on β-AR is the self-phosphorylation by the specific β-adrenergic receptor kinase (βARK1). Once phosphorylated, the inhibitor protein β-arrestin binds to it and inhibits its function by up to 70% (Benovic JL et al., 1987; Lohse MJ et al., 1990a; Lohse MJ et al., 1990b; Ungerer M et al., 1993). Gene-targeted animal studies have shown that in HF βARK1 activity is increased and that partial inhibition of βARK1 enhances β-AR signaling (Rockman HA et al., 1998; Koch WJ et al., 1995; Akhter SA et al., 1997).

Furthermore, overexpression of $G_{\alpha}s$ following NE binding results in the development of progressive myocardial fibrosis, apoptosis (modulated by $Ca^{2+}$-activated calmodulin kinase II pathway and inhibited by reactive oxygen species products such as superoxide dismutase/catalase-mimetics and catalase), and cardiomyocyte hypertrophy with functional deterioration – cardinal changes in DCM (Engelhardt S et al., 1999; Iwase M et al., 1996; Iwase M et al., 1997; Colucci WS, 1998; Colucci WS et al., 2000b; Communal C & Colucci WS, 2005).

At the post synaptic membrane, the concentrations of $\alpha_{1A^-}$ and $\alpha_{1B^-}$-ARs is equivalent to 20% of the total β-AR (Woodcock EA et al., 2008). Both $\alpha_{1A^-}$ and $\alpha_{1B^-}$-ARs subtypes couple to the $G_{q}$ family of heterotrimeric G-proteins, which, in turn, activate phospholipase $C_b$ ($PLC_b$). The latter hydrolyzes phosphatidylinositol bisphosphate (PIP$_2$) to generate the second messengers inositol trisphosphate (IP$_3$) and 2-diaeylglycerol (DAG). IP$_3$ contributes to the regulation of intracellular $Ca^{2+}$ responses and interacts with perinuclear inositol trisphosphate receptors (IP$_3$R).
distinguishing growth related gene transcription. DAG activates protein kinase C (PKC) as well as some of the transient receptor potential (Trp) channels. Trp activation results in an increase in the permeability to Ca\(^{2+}\) which enters the cell and activates calcineurin to initiate downstream growth signaling pathways. The \(\alpha_1\)-AR also transactivates epithelial growth factor receptors, resulting in formation of phosphoinositide 3-kinase (PI\(_3\)K) and phosphatidylinositol trisphosphate (PI\(_{3}\)P), activation of the Akt pathway, and initiation of cell-survival signaling pathways. (figure 1.7).

**Figure 1.7: \(\alpha\)-AR signalling**

![Diagram of \(\alpha\)-AR signalling](image.png)

**Fig 1.7:** Agonist-induced stimulation of \(\alpha_1\)-ARs activates G\(_{q}\) family and PLC\(_{b}\), resulting in hydrolysis of PIP\(_2\), to generate IP\(_3\) and DAG which in turn activates PKC to initiate a series of phosphorylations that alter channel activity and induce transcriptional changes. From (Triposkiadis F et al., 2009).

Studies have shown that over stimulation of \(\alpha\)-AR for example due to the abundance of NE results in eccentric cardiac hypertrophy, increased cardiac myocyte cross-sectional area, intrinsic myocyte contractile dysfunction, striking changes in cardiac gene expression recapitulating those seen in the haemodynamically overloaded heart, and a unique predisposition to cardiomyocyte apoptosis, which is the mechanism for progression to dilated cardiomyopathy (Bishopric NH et al., 1987;Long CS et al., 1989;Milano CA et al., 1994;Adams JW et al., 1998;D'Angelo DD et al., 1997;Satoh N et al., 2000).
1.3.1.3 Renin-Angiotensin-Aldosterone System

In 1989, Anand et al have demonstrated that HF is associated with marked elevations of each of the components of the Renin-Angiotensin-Aldosterone System (RAAS) (Anand IS et al., 1989). Normally, renin is released from the juxtaglomerular apparatus in response to sympathetic stimulation and reduced renal blood flow (Hall JE et al., 1990). In HF, the RAAS is overstimulated causing an increase in renin production. The resulting angiotensin II (AngII) has direct myocardial toxic effects by stimulating cytokines, growth factors and fibroblast activity (Boateng SY et al., 2009;Francis GS et al., 1993;Sadoshima J et al., 1993;Tan LB et al., 1991) and acts directly as a vasoconstrictor (Sadoshima J & Izumo S, 1993). Plasma aldosterone levels may be elevated as high as 20-fold in patients with HF, primarily due to increased production by the adrenal glands following stimulation by the high plasma AngII concentrations. In addition to its electrolyte and metabolic effects, aldosterone promotes collagen accumulation and cardiac fibrosis altering the filling characteristics of the LV and impairing contractile function (Izawa H et al., 2005;Swedberg K et al., 1990;Weber KT et al., 1993;Weber KT & Brilla CG, 1991), exerts a negative effect on endothelial function via prostacycline (Johnson FK et al., 2005), and increases plasminogen activator inhibitor-1 levels (Chun TY & Pratt JH, 2005).

A possible common pathogenic mechanism that would cause the effects seen by the increased levels of AngII and aldosterone in heart failure is the enhanced release and the reduced uptake of NE at nerve endings (Sunners C & Raizada MK, 1986;Weber MA & Rurdy RE, 1982). Type-1 AngII (AT1) receptor is responsible for most of the effects of AngII. It has a pathogenic role through promotion of myocyte growth and fibrosis in cardiac hypertrophy and HF (Varo et al., 2000). Stimulation also results in vasoconstriction through IP3 and Ca2+ release, myocardial cell growth through PKC release, positive inotropy, catecholamine release and increased aldosterone secretion with fibrosis. Nuclear factor kappa B (NF-κB), a ubiquitous nuclear transcription factor which promotes the expression of many inflammatory genes, may also be activated (Ruiz-Ortega et al., 2000). Type-2 AngII (AT2) receptors mediate antigrowth effects which might be useful during AT1 blockade (Ohkubo et al., 1997).
1.3.1.4 Calcium Handling in Heart Failure

In the heart, excitation-contraction (EC) coupling is the central mechanism by which electrical activation is translated into cardiac contraction and its initiation in ventricular myocytes is highly localized to the T-tubule network. T-tubules is a complex system of interconnected membrane structures continuous with the extracellular space which is integrally involved in multiple cellular processes including membrane transport, excitability, and cellular signalling (Kenneth B Margulies, 2002). T-tubules are also the site of most junctional complexes formed between sarcolemma containing LTCC and junctional SR.

SR controls Ca\(^{2+}\) uptake, storage and release. At rest calsequestrin, a Ca\(^{2+}\) binding protein holds Ca\(^{2+}\) in the SR. Following excitation, Ca\(^{2+}\) induced Ca\(^{2+}\) release (CICR) occurs through the RyR channel of which only the RyR2 isoform is expressed in cardiac tissue (Fleischer & Inui, 1989;Otsu et al., 1990;Arai et al., 1992). Myocyte relaxation is initiated by SERCA (Nagai et al., 1989;Lytton et al., 1989;Lompre et al., 1994;Colyer, 1993;Arai et al., 1992) and its function is attenuated by the regulatory protein PLB (Tada & Katz, 1982) as depicted in figure 1.6.

In HF, several proteins are changed with respect to their expression, phosphorylation status, and function leading to remodelling of EC coupling. Dysfunctional Ca\(^{2+}\) handling in HF leads to impaired cardiac contraction and relaxation (Arai et al., 1993;Richard et al., 1998;Wickenden et al., 1998;Morgan et al., 1990). Investigators have demonstrated that Ca\(^{2+}\) transport proteins are either affected or decreased in hypertrophy and severe HF (Arai et al., 1993;Kiss et al., 1995;Lehnart et al., 2005). In the failing ventricular myocytes T-tubules are depleted and dilated and associated with changes in the density of a variety of proteins in both surface and T-tubular sarcolemma but with preservation of the protein composition of junctional complexes (Kostin S et al., 1998;Balijepallia RC et al., 2003). This subcellular remodelling contributes to abnormal EC coupling in HF. In addition, the action potential is initially prolonged in HF mainly due to Ca\(^{2+}\) flux (which acts as a compensatory mechanism) but later becomes maladaptive due to decreased Ca\(^{2+}\) response (Wickenden AD et al., 1998).
Animal studies have shown that over-expression of other Ca\textsuperscript{2+} handling proteins such as calmodulin (CaM) or calsequestrin may induce hypertrophy and hyperplasia (Gruver et al., 1993; Jones et al., 1998; Sato et al., 1998). In contrast, PLB-knockouts cannot inhibit SERCA2a (the SERCA cardiac isoform) and therefore show positive inotropism and lusitropism, but are not hypertrophied (Luo et al., 1994). Over-expression of SERCA2a causes similar results also without hypertrophy (He et al., 1997).

As mentioned above and depicted in figure 1.6, heart failure is associated with over activation of the AC/PKA signaling pathway which in turn results in excessive Ca\textsuperscript{2+} leakage, and enhanced Ca\textsuperscript{2+} uptake by the SR therefore accelerating cardiac relaxation. The physiological outcome is prolonged Ca\textsuperscript{2+} transients during both release and re-uptake and reduced the capacity to restore lower resting Ca\textsuperscript{2+} during diastole (Arai et al., 1993; Gwathmey et al., 1987; Morgan et al., 1990).

1.3.1.5 Natriuretic Peptides

The initial discovery of Atrial Natriuretic Peptide (ANP) in the early 1980s led to the discovery of Brain Natriuretic Peptide (BNP), the most important natriuretic peptide to be released by cardiac myocytes in response to bio-mechanical stretch. BNP is released as a prohormone which is then cleaved into a biologically inactive N-terminal fragment and a bioactive C-terminal fragment. BNP causes vasodilatation and suppression of RAAS (Sudoh et al., 1988; Davis et al., 1992; Struthers, 1994), natriuresis (Torda et al., 1989; Fregoneze et al., 1989), and attenuation of hypertrophic signaling pathways in cardiac myocytes (Struthers, 1994; Fleischer et al., 1997). As such, BNP can be considered as an endogenous anti-remodeling hormone. In HF, BNP levels are elevated secondary to the mechanical stretch induced by remodeling. Therefore, it has been a useful marker for the diagnosis and the potential treatment of HF and has been recently shown to be a strong prognostic indicator for both asymptomatic and symptomatic patients with HF (Maisel A & Mehra MR, 2005; Pfister R et al., 2004; Rodeheffer RJ, 2004).
1.3.1.6  Endothelins

Produced in many tissues, endothelins modulate vasomotor tone, cell proliferation and hormone production and are important in vascular physiology and disease. Discovered in late 1980’s, there are three endothelin peptides; endothelin-1 (ET-1) has hormone or growth factor like properties in many mammalian cells, inducing expression of proto-oncogenes, myosin heavy chains, α-actin and troponin in cardiac myocytes; endothelin-2 (ET-2) is produced mainly in the gut but has no unique physiological functions; endothelin-3 (ET-3) is of unknown origin but is thought to act mainly on neurological tissue.

ET-1 is the only endothelin to be produced in endothelial cells and the most potent endogenous vasoconstrictor identified (100 times greater than NE). ET-1 has a positive inotropic effect, induces myocyte hypertrophy, and stimulates fibroblastic proliferation. It is elevated in HF and promotes sympatho-adrenergic overstimulation by potentiating NE-induced effects on vascular smooth muscle cells (Henrion D & Laher L, 1993). Upregulation of ET-1 is important in the development of cardiac hypertrophy and the amount locally produced might relate to the degree of hypertrophy and HF (Lerman et al., 1991). Shubeita et al showed that ET-1 can induce molecular and cellular changes generating cardiac hypertrophy that include initiation of the growth program through activation of early genes, reactivation of embryonic genes and stimulation of myosin light chain production (Shubeita et al., 1990). It is also possible that ET-1 may lead to the stimulation of adrenal aldosterone production thereby worsening the effects of HF (Rosolowsky & Campbell, 1990).

In 2005, Backs et al have identified the relationship between ET-1 and NET (Backs J et al., 2005). In addition to promoting NE-induced effects at the post-synaptic level in HF, the group demonstrated that ET-1 inhibits NET uptake mechanism at the pre-synaptic membrane through the endothelin A receptor and hence enhances sympathetic overstimulation.
1.3.2 Inflammatory Axis

The interest in understanding the role of inflammatory mediators in HF arises from the observation that many aspects of the syndrome of HF can be explained by the known biological effects of proinflammatory cytokines. When expressed at sufficiently high concentrations, tissue necrosis factor α (TNFα), interleukin 1 (IL-1) and interleukin 6 (IL-6) are sufficient to mimic some aspects of the so-called HF phenotype: LV remodeling and dysfunction, pulmonary oedema, cardiomyopathy, reduced skeletal muscle blood flow, endothelial dysfunction, anorexia and cachexia, activation of foetal gene program, and cardiac myocyte apoptosis (Bozkurt B et al., 1998; Thaik CM et al., 1995).

Thus, the "cytokine hypothesis" (Seta Y et al., 1996) for HF states that heart failure progresses, at least in part, as a result of the toxic effects exerted by endogenous cytokine cascades on the heart and the peripheral circulation. It is important to note that the “cytokine hypothesis” does not imply that cytokines cause HF, per se, but rather that the overexpression of cytokine cascades contributes to the disease progression.

Experimental studies showed that direct injection of TNFα would induce hypotension, metabolic acidosis, haemoconcentration, and death within minutes (Pagani FD et al., 1992) and that the negative inotropic effects induced by the circulating TNFα is completely reversible once the TNFα-infusion is stopped (Bozkurt B et al., 1998).

Plasma TNFα concentrations are elevated in patients with HF (Novo G et al., 2009) in correlation with NYHA functional class (Torre-Amione G et al., 1996; Kapadia SR et al., 2000) and prognosis (Rauchhaus M et al., 2000). From the Vesnarinone Trial (VEST) study, Deswal et al studied 1200 patients with advanced HF with a mean follow-up of 55 weeks to demonstrate that high TNFα levels at baseline were associated with increased mortality (Deswal A et al., 2001). Investigators have speculated five possible sources for the elevated levels of TNFα in HF (Torre-Amione G et al., 1999):
i) activation of the immune system,
ii) the failing heart may be a source itself such that TNFα mRNA and protein were present in the failing heart but not detectable in the non-failing heart,
iii) decreased cardiac output leads to elaboration of TNFα by underperfused metabolic tissues,
iv) increased bowel permeability as a result of mesentric congestion in end-stage HF leads to bacterial translocation and endotoxin release (a potent stimulus for TNFα production), and
v) polymorphism in the TNFα gene locus might be responsible for the increased TNFα expression.

Recently, Selmeczy et al have shown that abundance of NE secondary to NET deficiency in NET knockout mice resulted in elevated TNFα (Selmeczy Z et al., 2003). Administration of desipramine caused a reduction in TNFα and induced interleukin-10 (IL-10) production.

TNFα modulates myocardial function through two different pathways: i) an immediate pathway that is manifested within minutes and mediated by the activation of the neutral sphingomyelinase pathway (Oral H et al., 1997); and ii) a delayed pathway that requires hours to days to develop and is mediated by nitric oxide (NO) (Gulick TS et al., 1989;Ballingand JL et al., 1993). The effects of TNFα in HF are represented in table 1.3.

Table 1.3: The effects of TNFα in heart failure

<table>
<thead>
<tr>
<th>Effects of TNFα in HF</th>
<th>Modulators of the effects of TNFα in HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis</td>
<td>↑Fas, ↓BcL2</td>
</tr>
<tr>
<td>Cardiotoxicity</td>
<td>↑iNOS</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>↑O₂⁻, ↑iNOS</td>
</tr>
<tr>
<td>Vascular dysfunction</td>
<td>↑Bax, ↓BcL-2, ↑iNOS</td>
</tr>
<tr>
<td>Proteolysis</td>
<td>↑Ubiquitin proteosome activity</td>
</tr>
</tbody>
</table>

Table 1.3: Increased expression of TNFα stimulates apoptosis, cardiotoxicity, vascular dysfunction, oxidative stress, and proteolysis. Adapted from (Berry C & Clark AL, 2000).
Progressive LV dysfunction in human and animal cardiomyopathy is associated with loss of nearly one-third of cardiomyocytes as a result of apoptosis (Narula J et al., 2006; Narula J et al., 1996). Apoptosis is initiated by two pathways, the adrenergic and the reactive oxygen species–tumor necrosis factor–caspase signaling pathways (Communal C et al., 1998; Fu YC et al., 2004). The activation of Fas, a transmembrane cell surface protein, has also been highlighted as an important mechanism in the development of apoptosis in HF (Sheppard R et al., 2005).

High TNFα and IL-1 concentrations are accompanied by NO generation (Gross & Levi, 1992). NO is a free radical gas that functions as a biological messenger in physiological solutions and can diffuse easily across membranes to interact with other enzymes leading to the production of messengers such as cyclic guanosine monophosphate (cGMP). It is synthesised by NO synthase of which there are 3 types – endothelial, neuronal and inducible (Loscalzo & Welch, 1995). Current evidence suggests that endothelial NO synthase (eNOS) predominates in the normal heart in contrast to the failing heart in which inducible NOS (iNOS) predominates (Drexler et al., 1998).

iNOS is Ca\(^{2+}\) independent, present in cardiac myocytes, vascular smooth muscle cells and infiltrating inflammatory cells and regulated at transcriptional level, thus taking hours to produce (Loscalzo & Welch, 1995). Increased expression in HF has been shown to parallel the induction of myocardial ANP (Habib et al., 1996; Haywood et al., 1996). Once induced by IL-1 and TNFα, iNOS is activated by NFκB (Barnes PJ & Karin M, 1997). Transcription is also increased by AngII but inhibited by transforming growth factor β (TGF-β) (Ikeda et al., 1995; Ikeda et al., 1996). The activated iNOS produces large amounts of NO for prolonged periods (Moilanen & Vapaatalo, 1995).

Once produced, NO exerts negative inotropic effects over a time course (Schulz et al., 1995), increases ventricular relaxation affecting diastolic function (Paulus et al., 1994), modulates NET activity in a cGMP independent manner (Kaye DM et al., 1997; Kaye DM et al., 2000a), induces apoptosis in myocytes and fibroblasts (Narula J et al., 1996), and generates reactive oxygen species which in turn result in oxidative stress on the myocardium and apoptosis (Colucci WS, 1997). In 2
communications, Dhall et al have shown that oxidative stress is increased in the myocardium during the transition from compensated hypertrophy to failure (Dhalla AK et al., 1996; Dhalla AK & Singal PK, 1994).

IL-1, released from activated T lymphocytes is activated in HF. It stimulates cardiomyocyte hypertrophy by inhibiting transcription of the alpha-actin gene promoter in vitro (Patten M et al., 1996) and activating foetal gene program (Colucci WS, 1997). The latter is characterised by increased expression of ANP and downregulation of adult, muscle-specific genes such as SR Ca2+ adenosine triphosphatase (Thaik CM et al., 1995). Similar to TNFα, its negative inotropic effects are mediated through the production of NO (Panas D et al., 1998).

IL-6 is proinflammatory, proteolytic (Goodman MN, 1994), increases endothelial vasoactive peptides (Xin X et al., 1995), and enhances the catabolic actions of TNFα (Tsujinaka T et al., 1996). Its production is also increased in HF (Torre-Amione G et al., 1996; Testa M et al., 1996). Similar to TNFα, high levels of circulating IL-6 was associated with increased mortality (Deswal A et al., 2001). Triggers for IL-6 production from cells within the vascular wall and in leucocytes are hypoxia, SNS activation, endothelin and TNFα (Berry C & Clark AL, 2000). The proteolytic effect of TNFα is thought to be mediated through IL-6.

Circulating interleukin-2 (IL-2) and IL-10 levels can also be elevated in patients with DCM (Marriott JB et al., 1998) such that IL-2 may be elevated as a result of abnormal lymphocyte function, and the anti-inflammatory cytokine IL-10 may be increased as a counter-regulatory response.

Li et al demonstrated a direct relation between cardiotrophin-1 (CT-1), an inflammatory cytokine induced following myocardial infarction, and NE release and uptake (Li W et al., 2003). The group studied the effects of CT-1 on cultured sympathetic neurons and showed that CT-1 suppresses NE uptake by decreasing tyrosine hydroxylase and NET mRNA.

To date, anti-TNFα therapy has shown to be ineffective in HF patients. Two large trials of anti-cytokine medication, Etanercept, in HF were recently stopped (Anker & Coats, 2002). The Randomised Etanercept North American Strategy to Study
Antagonism of Cytokines (RENAISSANCE) and Research into Etanercept: Cytokine Antagonism in Ventricular Dysfunction (RECOVER) trials and their combined analysis – Randomised Etanercept Worldwide Evaluation (RENEWAL) examined the effects of Etanercept, a fusion protein directed against TNFα (Coletta A et al., 2002). A total of 1123 patients were recruited into the RECOVER study and 925 into RENNAISSANCE, of these 25% were NYHA class II, 70% class III and 4% were in class IV; 62% of patients had ischaemic heart disease. The mean treatment period was 12.7 months in the RENNAISSANCE study and 5.7 months in the RECOVER study. There was no significant difference between placebo and etanercept in terms of the primary endpoint in either study.

Analysis of hazard ratios for death/worsening heart failure showed that in the RECOVER study patients on the higher dose appeared to show slightly better results than those on the lower dose (0.87 versus 1.01). In the RENNAISSANCE study the hazard ratio was increased in the etanercept treatment group. There was also a trend towards an increased risk of death in the etanercept group for both studies combined (hazard ratio of 1.10) and an indication of worse outcome in patients with non-ischaemic heart disease and in those aged less than 65 years. A smaller study, the anti-TNF alpha therapy against chronic heart failure (ATTACH), that examined the effect of Infliximab (antibody against TNF) was also stopped early due to increased adverse events in the treatment group (Coletta A et al., 2002).

Questions, however, persist about the administered doses and HF classes of the patients involved. Thus, although high dose anti-TNFα therapy may not be useful in HF, the effect of lower doses on the cytokine hypothesis of HF has not yet been adequately established (Anker & Coats, 2002).
1.3.3 Involvement of the Extracellular Matrix in Remodeling

The ECM plays a key role in both systolic and diastolic myocardial performance and cell signaling since it is an important determinant of the structural characteristics of the myocardium and consists of different components such as collagens, proteoglycans, glycoproteins (e.g. fibronectin), peptide growth factors, and proteases. The ECM is in dynamic equilibrium, regulated by a family of metabolic zinc dependent proteases termed matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPS) (Dollery et al., 1995; Li et al., 1998). There is an increasing appreciation that through changes in the nature and quantity of the ECM, non-myocyte could play an essential role in determining the response of the myocardium to pathologic stimuli (Weber KT & Brilla CG, 1991). ECM is important in myocardial remodeling as it alters architectural structure and mechanical properties exerting profound functional effects (Rossi et al., 1998; Weber et al., 1988; Gunja-Smith et al., 1996; Spinale et al., 1991).

Different mechanisms have been shown to activate MMPs. However, the most relevant in the pathogenesis of HF is TNF-induced activation of MMPs associated with the loss of fibrillar collagen. The outcome is LV dilatation (Sivasubramanian N et al., 2001; Li YY et al., 2000). The dissolution of the fibrillar collagen weave that surrounds the individual cardiac myocytes and links the myocytes together would be expected to allow for rearrangement (slippage) of myofibrillar bundles within the ventricular wall (Weber KT, 1989). However, long-term stimulation (i.e., 8 to 12 weeks) with TNF in TNF-transgenic mice resulted in an increase in fibrillar collagen content that was accompanied by decreased MMP activity and increased expression of TIMPs. Taken together, these observations suggest that sustained myocardial inflammation provokes time-dependent changes in the balance between MMP activity and TIMP activity. That is, during the early stages of inflammation, there is an increase in the ratio of MMP activity to TIMP levels that fosters LV dilation. However, with chronic inflammatory signaling, there is a time-dependent increase in TIMP levels, with a resultant decrease in the ratio of MMP activity to TIMP activity and a subsequent increase in myocardial fibrillar collagen deposition.
Pathologic myocardial hypertrophy also involves interstitial fibrosis, which may both alter the mechanical properties of the myocardium (e.g. impair relaxation) and restrict the delivery of nutrients to myocytes (Conrad CH et al., 1995). Conversely, a reduction or alteration in the quantity or quality of certain structural proteins could compromise the integrity of the extracellular skeleton and lead to chamber dilatation. Many factors present in the failing myocardium can influence the composition of the ECM for example, NE, acting on β-AR, can stimulate fibroblast DNA and protein synthesis (Calderone A et al., 1995), and endothelin can affect the synthesis and degradation of collagen in the myocardium (Guarda E et al., 1993).

In addition to these structural defects, studies have shown the involvement of the both intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in the pathogenesis of HF (Terracio L et al., 1991). Immunohistochemical studies have shown that both ICAM-1 and VCAM-1 to be persistent in HF and myocarditis suggesting myocardial cell damage is partly induced through different immunological pathways (Ino T et al., 1997).
1.3.4 Alteration in LV Chamber Geometry

The net outcome of the involvement of the inflammatory cytokines and the ECM is alteration in the ventricular chamber geometry. There are four patterns of LV geometry based on the relative wall thickness and the LV mass index (figure 1.8).

Figure 1.8: Patterns of LV geometry

Figure 1.8: Four different patterns of LV geometry based on relative wall thickness and LV mass index. Adapted from (Lang RM et al., 2005; Opie LH et al., 2006)
Normal Geometry

Buckberg described the normal myocardium to consist of a single, continuous tissue that wraps around itself, spiraling up from the apex of the heart in a clockwise and anticlockwise fashion, to form a helix with elliptically shaped ventricles (Gerald D.Buckberg, 2002) (figure 1.9).

Figure 1.9: The helical structure of the heart

![The helical heart is seen in (a). The apical view in (b) defines the clockwise and counterclockwise spirals. From (Gerald D.Buckberg, 2002).](image)

These spiral rotations produce an oblique muscle fiber orientation. Interestingly, the predominant motion of the heart is not constricting and dilating, but rather shortening and narrowing and hence when fibers shorten 15%, the net production is a 60% ejection fraction (EF) as depicted in figure 1.10. Because of its elliptical shape and defined apex, the ventricle is subjected to a relatively low level of lateral stress.
Figure 1.10: The relationship between fiber angle and ejection fraction is compared for contractile shortening of 15%. The transverse, or circular, arrangement allows a 30% EF, which becomes 60% with a spiral orientation. From (Gerald D. Buckberg, 2002; Buckberg GD et al., 2001a).
LV chamber geometry in HF

In HF the geometry is altered depending on the initial insult. In HF secondary to anterior MI, the heart dilates because the apex is lost. Apical dilatation can also occur from valvular insufficiency with ventricular stretching caused by volume overload. In the non-ischemic failing heart, the muscle itself becomes damaged and the spherical shape replaces the apical contour. From (Buckberg GD et al., 2001b).

With HF, the helical apex is lost and becomes replaced by a sphere. The structural consequence is that the oblique apical loop fibers become more transverse, so that the fiber orientation of the apical loop begins to resemble the basal loop. Subsequently, the EF decreases and a 15% shortening produce only a 30% EF (Gerald D.Buckberg, 2002).

1.3.5 Role of neurohormonal activity and inflammation in acute decompensated heart failure

Chronic HF patients usually decompensate and present as de novo cardiac decompensation (Summers RL & Amsterdam E, 2009). The precipitating factors include non-compliance with diet and medication, uncontrolled hypertension, cardiac ischaemia, arrhythmias, pulmonary embolism, and other systemic illness.

Studies have shown that patients with decompensated chronic HF have increased NH activity as reflected by elevated levels of NE, renin activity, endothelin, and aldosterone (Aronson D & Burger AJ, 2003b; Aronson D & Burger AJ, 2003a). These mediators are associated with vasoconstriction, fluid retention, and cardiac arrhythmias. In addition, increased levels of cytokines have been documented in those patients and have been significantly correlated with prognosis (Chin, 2003; Mueller C et al., 2006).
In summary, HF is a complex syndrome that is associated with neurohormonal activation. The pathophysiology of HF is initiated by over-stimulation of the cardiac SNS. The synaptic NE abundance in HF is secondary to excessive release from the afferent neurons and the failure of the NET uptake mechanism at the presynaptic membrane. Activation of the inflammatory axis and different pathways of the ECM will result into the pathological and remodeling features seen in HF. At the clinical level, cardiac sympathetic dysfunction has been correlated to impaired myocardial contractile reserve, impaired contractility, and reduced exercise tolerance (Ohshima S et al., 2005; L'abbate A & Sambuceti G, 2001).
1.4 Management

Historically, prolonged bed rest was used to reduce heart rate, blood pressure, cardiac output and cardiac size, thus promoting recovery of the myocardial function (Burch GE & DePasquale NP, 1966).

Over the past four decades management of HF has become more advanced and highly dependent on disease stage (Young JB, 2004). Mild to moderate disease is managed symptomatically using medications that include diuretics, β-blockers, angiotensin converting enzyme inhibitors (ACE-I), angiotensin II antagonist, and aldosterone antagonist (spironolactone). However, for advanced HF, cardiac transplantation has been the only effective treatment and remains the gold standard treatment (Korewicki J, 2009).

1.4.1 Medical Management

Modern therapeutic strategies usually involve reduction of myocardial work with stabilisation and improvement of myocyte function using a combination of pharmacological, non-pharmacological and surgical management. Advances in medical therapy to alleviate symptoms and favor prognosis have occurred in recent years with increased use of β-blockers, ACE-I, angiotensin II antagonist, and aldosterone antagonist.

1.4.1.1 β-blockers

Formerly, adrenergic blockade using β-blockers was contraindicated in HF due to the short-term adverse effects on the myocardial function (Epstein SE & Braunwald E, 1966; Gaffney TE & Braunwald E, 1963). Over the past two decades major randomised trials have confirmed that early initiation of β-blockade therapy is safe and improves the outcome of HF patients (Table 1.4).
Table 1.4: Summary of large randomised trials with β-blockers and the ACC/AHA guidelines recommended doses.

<table>
<thead>
<tr>
<th>β-blocker</th>
<th>Trial</th>
<th>Patients (n)</th>
<th>Benefit</th>
<th>Initial Dose (mg)</th>
<th>Maximum Dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metoprolol</td>
<td>MDC</td>
<td>383</td>
<td>All-cause mortality or morbidity was 34% lower in the metoprolol as compared to the placebo group. The change in EF from baseline to 12 months was significantly greater with metoprolol than with placebo (p &lt; 0.0001).</td>
<td>5–10 bd</td>
<td>100 bd</td>
</tr>
<tr>
<td>Metoprolol CR/XL</td>
<td>MERIT-HF</td>
<td>3,991</td>
<td>All-cause mortality was 34% lower in the metoprolol CR/XL group than in the placebo group (7.2% versus 11.0%, p = 0.00009).</td>
<td>12.5–25 od</td>
<td>200 od</td>
</tr>
<tr>
<td></td>
<td>USCP</td>
<td>1,094</td>
<td>All-cause mortality was 65% lower in the carvedilol than in the placebo group (3.2 versus 7.8%, p &lt; 0.001).</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAPRICORN</td>
<td>1,959</td>
<td>All-cause mortality was lower in the carvedilol than in the placebo group (12% versus 15%; p = 0.03).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carvedilol</td>
<td>COPERNICUS</td>
<td>2,289</td>
<td>Carvedilol reduced the combined risk of death or hospitalization for a cardiovascular reason by 27% (p = 0.00002) and the combined risk of death or HF hospitalisation by 31% (p = 0.000004).</td>
<td>3.125 bd</td>
<td>25 bd</td>
</tr>
<tr>
<td></td>
<td>COMET</td>
<td>3,029</td>
<td>All-cause mortality was lower in the carvedilol than in the metoprolol group (34% versus 40%, p = 0.0017).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bisoprolol</td>
<td>CIBIS</td>
<td>641</td>
<td>Bisoprolol reduced HF hospitalisation (p &lt; 0.01) and improved the functional status.</td>
<td>1.25 od</td>
<td>10 od</td>
</tr>
<tr>
<td></td>
<td>CIBIS II</td>
<td>2,647</td>
<td>All-cause mortality was 34% lower with bisoprolol than in the placebo group (11.8% versus 17.3%, p &lt; 0.0001).</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CIBIS III</td>
<td>1,010</td>
<td>This study demonstrated that it may be as safe and efficacious to initiate treatment for CHF with bisoprolol as with enalapril.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.4: Large randomised trials with β-blockers. MDC, Metoprolol in Dilated Cardiomyopahty Study; MERIT-HF, Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure; USCP, US Carvedilol Program; CAPRICORN, Carvedilol Post-Infarct Survival Control in Left Ventricular Dysfunction; COPERNICUS, Carvedilol Prospective Randomized Cumulative Survival; COMET, Carvedilol or Metoprolol European Trial; CIBIS, Cardiac Insufficiency Bisoprolol Study. Adapted from (Mann DL & Bristow MR, 2005).
At the cellular and molecular levels, β-blockers have been shown to prevent the toxic effects of NE, improve myocardial oxygen supply to demand ratio by lengthening diastolic coronary flow, reduce contractile responses to increased sympathetic activity, increase the use of more energy efficient substrates, reduce LV volumes and hence improve systolic function, increase functional capacity and exercise tolerance, reduce re-infarction rate post-MI and overall mortality, improve tachycardia, reduce the progression to symptomatic HF, reduce the production of aldosterone, and induce molecular remodeling through induction of the foetal gene program (Wild DM & Kukin M, 2007; Bristow MR, 1997; Hall SA et al., 1995; Lowes BD et al., 2002; Lowes BD et al., 1999; Bristow MR et al., 1998; Doughty RN et al., 1997; Eichhorm EJ, 1992; Eichhorm EJ et al., 2009; Lechat P et al., 1998; Metra M et al., 1994; The Metoprolol in Dilated Cardiomyopathy (MDC) Trial Study Group, 1998; Colucci WS, 1997; Lechat P et al., 2001).

These beneficial effects on LV remodeling might be responsible for the improvement in symptoms and reduction in mortality seen with the use of β-blockade therapy (Cleland JGF et al., 1999).

Differences exist in adrenergic receptor selectivity. Both metoprolol and bisoprolol (second generation) are β₁ specific blockers that increase the density of β-receptors and cardiac NE concentrations (Bristow MR, 1997; Heilbrunn SM et al., 1989). In the MDC trial, the need for cardiac transplantation but not death was reduced in those treated with metoprolol (Waagstein F et al., 1993). In the CIBIS trial, there was a 20% reduction in mortality and 30% fewer admissions for HF (CIBIS Investigators and Committees, 1994). The follow-on CIBIS-II trial showed a 32% reduction in hospitalisation (similar to the 34% from metoprolol in the MERIT-HF study and 35% from carvedilol in the COPERNICUS trial) (Leizorovicz A et al., 2002; Sallach JA & Goldstein S, 2003; Domanski MJ et al., 2003; MERIT-HF Trial, 1999; Packer M et al., 2001a).

Carvedilol, a third generation β-blocker and a slightly β₁ selective antagonist but non-selective at higher doses (Bristow MR, 2000), reduces cardiac NE concentration (Ruffolo RR Jr & Feuerstein GZ, 1997), decreases cardiac adrenergic drive (Ruffolo RR Jr & Feuerstein GZ, 1997), has anti-oxidant properties which will reduce the oxidative stress observed in remodeling and apoptosis (Feuerstein GZ et al.,
1997; Feuerstein GZ et al., 1993; Yue TL et al., 1992; Nakamura K et al., 2002). Carvedilol does not up-regulate cardiac β-receptors (Gilbert EM et al., 1993) and when administered in conjunction with digoxin, diuretics and ACE inhibitor, there was a significant reduction in mortality rate to 3.2% as compared to 7.8% in the placebo treated group and in the rate of hospitalisation by 26% - 38% (Colucci WS et al., 1996; Packer M et al., 1996b; Packer M et al., 1996a).

According to Bristow, carvedilol is tolerated by around 92% of those with mild-moderate HF (Bristow MR et al., 1998). In meta-analyses, carvedilol has been associated with greater increase in EF than metoprolol (Gilbert EM et al., 1996; Metra A et al., 2000; Packer M et al., 2001a; Packer et al., 2001; Metra et al., 2000; Francis & Cohn, 1990; Doughty et al., 1997; Lowes et al., 1999; Gilbert et al., 1996). In addition, carvedilol was shown to reduce LV mass and mitral regurgitation whilst improving cardiac geometry (Bristow MR, 1997; Bristow MR et al., 1998; Hall SA et al., 1995; Lowes BD et al., 1999).

Recently, two trials have provided further insight into the benefits of carvedilol in HF. The COMET trial addressed the heterogeneity of β-blockers with respect to the outcome and showed over 5-years period, carvedilol reduced mortality by 17% in HF patients as compared to metoprolol (Poole-Wilson PA et al., 2003). The second study, the Carvedilol Hibernation Reversible Ischaemia Trial: marker of success trial (CHRISTMAS), investigated possible mechanisms of β-blocker induced improvement of myocardial function (Cleland JGF et al., 2003). Investigators hypothesised that carvedilol induced improvement in EF is associated with the extent of hibernating or reversibly ischaemic myocardium such that the majority (66%) of those who benefited had evidence of hibernating myocardium or reversible ischaemia in two or more segments of myocardium. Carvedilol was shown to improve function in hibernating segments through reduction in heart rate, conserving energy and improving myocyte efficiency, increasing duration of diastolic blood flow, switching energy substrate from free fatty acids to the more efficient glucose and enhancing the protective effects of its antioxidant actions (Feuerstein GZ & Ruffolo RR Jr, 1995).
1.4.1.2  Renin Angiotensin Aldosterone System blockers

Development of ACE-I was the first major pharmacological step in achieving RAAS blockade (Ondetti MA et al., 1977). Initially they were used in hypertension but it emerged that their mechanism was greater than simply reducing systemic vascular resistance. They reduce production of AngII and bradykinin breakdown with beneficial effects on vascular tone, atherosclerosis and thrombosis (Chobanian AV et al., 1990; Ridker PM et al., 1993).

It has become clear that ACE inhibition leads to arterial and venous vasodilatation and unloads the LV thereby improving cardiac pump function, leading to improvement in clinical condition, exercise tolerance and prognosis (The CONSENSUS Trial Study Group, 1987a). Addition of an ACE-I inhibitor to a regime of rest, salt restriction and a diuretic has therefore become an established approach to clinical management of post-MI patients with LV dysfunction and symptoms of HF (ACE Inhibitor Myocardial Infarction Collaborative Group, 1998; Garg R & Yusuf S, 1995).

Although there were differences in the characteristics of the studied population, both Studies of Left Ventricular Dysfunction (SOLVD) and Survival and Ventricular Enlargement (SAVE) trials have suggested that ACE-I treatment reduced the risk of cardiovascular events and hospitalisation (Yusuf S et al., 1992). The SOLVD trial studied the effects of enalapril in patients with low EF with or without symptoms of HF. Those treated with enalapril had reduced remodeling with a significant reduction in the risk of developing HF (Greenberg BH et al., 1995; Konstam MA et al., 1992). In the SAVE trial, the effects of captopril was studied in MI survivors with LV dysfunction with no symptoms of HF. It showed that long-term captopril treatment is associated with improved survival and reduced morbidity and mortality, reduced post MI LV remodeling and reduced risk of death from cardiovascular causes by 21%, risk of heart failure by 37%, and recurrent MI by 25% (Pfeffer MA et al., 1992; St John SM et al., 1994). In contrast results from the Cooperative New Scandinavian Enalapril Survival Study (CONSENSUS II), also designed to evaluate enalapril post-MI, failed to show improved survival during the six months follow-up period (CONSENSUS Trial Study Group, 1987).
The results of both SOLVD and SAVE trials led to the Heart Outcomes Prevention Evaluation (HOPE) study in which ramipril was administered to those at high risk of cardiovascular disease but without LV dysfunction or symptoms of HF. The outcomes were that ramipril prevented events related to ischaemia and atherosclerosis in addition to those already identified relating to HF and LV dysfunction. Further, risk of death, MI, cerebrovascular accidents (CVA), coronary revascularisation, HF, new cases of diabetes mellitus and its complications were substantially reduced (McQueen MJ et al., 2005).

These findings suggested that reducing tissue activity of the RAAS allowed the cardiovascular and renal systems to avoid the deleterious effects of AngII and aldosterone activities which were discussed in section 1.3.1.3 and included growth, hypertrophy, proliferation, collagen deposition and tissue remodeling. In particular, there was enough evidence that ACE inhibition prevented the adverse long-term consequences of cardiac remodeling, with beneficial reductions in HF and mortality (Lonn EM et al., 1994;Pfeffer MA et al., 1988;Cohn JN et al., 2000;Sutton MG & Sharpe N, 2000).

The vascular protective effects include direct anti-atherogenesis through increased production of prostacyclin, anti-proliferative and anti-migratory effects on smooth muscle cells, neutrophils and mononuclear cells, improvement of endothelial function, protection from plaque rupture, anti-platelet effects, enhancing endogenous fibrinolysis, anti-hypertensive effects and improved arterial compliance and tone (Lonn EM et al., 1994). Rutherford et al have also shown that the need for revascularisation is reduced in patients receiving long-term captopril therapy for ischaemic heart disease (IHD) induced HF (Rutherford JD et al., 1994). The cardioprotective effects included restoration of balanced oxygen supply and demand through haemodynamic alterations, reduction of LV mass and sympathetic stimulation, and beneficial effects on reperfusion injury (Lonn EM et al., 1994;Pfeffer MA et al., 1988). Coronary vasoconstriction may also benefit through reduced production of AngII, inhibition of bradykinin breakdown and increased production of NO (Perondi R et al., 1992). ACE inhibition was also shown to stop the reduction in SERCA2a seen in cardiac hypertrophy, normalise SERCA2a protein levels and Ca^{2+} uptake, normalise myocyte relaxation and decline in intracellular
Ca$^{2+}$ concentration, and improve both SR dependent and independent Ca$^{2+}$ cycling (Boateng et al., 1998; Boateng et al., 2001; Zierhut et al., 1996; Bruckschlegel et al., 1995; Anger et al., 1995) although the precise signalling mechanisms remain unclear (Boateng et al., 1998).

In addition to the effects on Ca$^{2+}$ handling proteins, ACE-I were found to reverse cardiac sympathetic nerve terminal abnormalities seen in HF. Finally, Kawai et al have demonstrated a significant improvement in cardiac NE uptake and the reversal of cardiac sympathetic nerve terminal abnormalities seen in HF with ACE inhibition (Kawai H et al., 1999; Kawai H et al., 2000).

1.4.1.3 Angiotensin II Antagonists

It is well recognised that ACE inhibition does not fully block AngII production since other pathways allow continued residual AngII activity (Urata H et al., 1990). Therefore, blocking AT receptors is considered to be a more beneficial target to inhibit the effects of AngII.

Recent evidence suggests that AT$_1$ blockers have similar effects as ACE inhibitors on cardiac remodeling and HF. However, pharmacological differences exist in that they can inhibit the action of AngII generated through not only by ACE but also from other pathways (Urata H et al., 1990; Liu YH et al., 1997; Pitt B et al., 2000; Liu et al., 1997; Pitt et al., 2000; Urata et al., 1990). Many AT$_1$ receptor blockers are available that might shift activity toward stimulation of the beneficial (AT$_2$) receptors.

Interestingly, activation of AT$_2$ receptors mediate antigrowth effects (Ohkubo N et al., 1997). Therefore blocking the deleterious actions of AngII at the AT$_1$ receptor may make the favourable actions of AngII on the AT$_2$ receptor unopposed. Although evidence is conflicting, this may counter-regulate the detrimental effects of the AT$_1$ receptor during cardiac hypertrophy and HF (Horiuchi M et al., 1999). No therapeutic agents are currently available for specific action on the AT$_2$ receptors.

Experimental studies have revealed that combination therapy of ACE-I and AT$_1$ receptor blockers have benefits in excess of single agent therapy both in HF and remodeling post-MI (Nakamura Y et al., 2009; Kim S et al., 2001). In clinical practice, Hamroff et al demonstrated that HF patients treated with losartan in
addition to ACE-I had improved exercise tolerance (Hamroff G et al., 1999). Another study, the Randomised Evaluation of Strategies for Left Ventricular Dysfunction trial (RESOLVD) studied the combined effects of candesartan and enalapril in symptomatic HF (The CONSENSUS Trial Study Group, 1987a). RESOLVD investigators demonstrated that the combined therapy was well tolerated by most, prevented increases in LV systolic and diastolic volumes seen when single agent therapy is used, resulted in the most significant reduction of aldosterone and BNP levels, and prevented cardiac remodeling. The Valsartan Heart Failure Trial (Val-HeFT) examined the effects of adding valsartan to standard HF therapy and similarly demonstrated a significant reduction in mortality and morbidity (Cohn JN & Tognoni G, 2001).

1.4.1.4 Aldosterone Antagonist

As discussed above, ACE inhibition will inhibit aldosterone production, a transient effect of ACE-I.

The Randomised Aldactone Evaluation Study (RALES) showed beneficial effects from spironolactone administration in HF resulting in 30% increased survival due to reverse remodeling by reversal of matrix fibrosis (Zannad F et al., 2000). It also showed that low doses could oppose the effect of aldosterone in promoting cardiac fibrosis. Only those patients with a reduction in serum profibrotic markers following administration had improved survival benefit and the largest benefit was found in those groups with the highest baseline serum collagen markers. Two further trials have shown significant benefit with aldosterone antagonism in heart failure patients and concluded that the benefits may be partially related to enhanced NE uptake and decreased NE release from nerve terminals (Pitt B et al., 1999; Pitt B et al., 2003).
1.4.1.5 Digitalis

The use of digitalis has been plagued by controversy since its initial use by Withering in 1775 (Harding JP & Jessup M, 2005). In addition to its well-known positive inotropic effect, digoxin has been shown to lessen autonomic dysfunction.

The Digitalis Investigation Group (DIG) has illustrated that the incidence of both death and hospitalization has reduced when digoxin was added to diuretics and ACE-I (The Digitalis Investigation Group, 1997). The effectiveness of digoxin therapy in men with heart failure and a LV EF of \( \leq 45\% \) may be optimal when the serum digoxin concentration is in the range of 0.5 to 0.8 ng/ml (Rathore SS et al., 2003) suggesting that lower doses of digoxin may be safer. Digoxin may also be less safe in women (Rathore SS et al., 2002). The most recent DIG trial examined the effects of digoxin on all cause mortality and hospitalisation in HF patients with EF \(<45\%\) (Collins JF et al., 2003). The outcome was improved symptoms, but no significant effect on mortality.

1.4.1.6 Inotropes

Although their use is controversial, inotropes have been extensively kept as the last non-surgical therapeutic option in the treatment of patients with acute HF. The most commonly used intravenous inotropes include adrenergic agonists, endogenous catecholamines, and phosphodiesterase inhibitors. Intravenous inotropic therapy for acute HF has various regimens: short-term, long-term, or intermittent infusion, as described below (Brater DC, 1998):

- Short-term inpatient therapy consists of infusion lasting several hours to a few days in combination with diuretics until patients are clinically stabilized.
- Prolonged continuous infusion via an indwelling intravenous catheter may become necessary in patients with advanced HF who cannot be weaned from intravenous to oral therapy (1) to enable them to be discharged from the hospital, (2) to serve as a bridge to a more definitive intervention (eg, revascularization, assist device placement, or cardiac transplantation), or (3) to serve as a palliative care.
Periodic intermittent infusion has been proposed in an attempt to improve clinical outcomes, for example, as a pulse infusion (4–6 hours) for several days per week or as a single longer infusion (24–72 hours) once weekly (Brater DC, 1998; Hunt SA et al., 2005). This strategy has been controversial. The American College of Cardiology/American Heart Association (ACC/AHA) 2005 guideline on chronic HF (Hunt SA et al., 2005) recommends against intermittent intravenous positive inotropic therapy for HF, even in its advanced stages.

Inotrope usage is associated with extensive injury to the myocardium. In one study, approximately 14% of patients were treated with inotropes where only 15% had preserved LV systolic function following treatment (Adams KF et al., 2003). A retrospective observational analysis has shown that short-term inotrope therapy (dobutamine or milrinone) was associated with significantly higher in hospital mortality compared with short-term vasodilator therapy (nitroglycerin or nesiritide) (Abraham WT et al., 2005). These findings have also been confirmed by other investigators (Boehar N et al., 2008; Shin DD et al., 2007) and raise concerns that inotrope therapy for acute HF may be harmful, especially when used in patients who do not have low blood pressure or low cardiac output.
1.4.2 ICD implantation and resynchronisation therapy

Approximately 25% of patients with HF have abnormal electrical activation of the LV leading to dyssynchronous contraction between the walls of the LV (Kass DA, 2003). The result is less efficient ventricular emptying and in many cases mitral regurgitation. Dyssynchrony also increases net end-systolic volume and wall stress and induces regional redistribution of stress. Chronically, this leads to hypertrophy of the stressed region with increased metabolic demand coupled with higher glucose metabolism and blood flow requirements (Kass DA, 2003). In addition, 50% of patients with severely reduced LV EF (<30%) die suddenly, mostly as a result of ventricular arrhythmias (Ehlert FA et al., 2010).

The Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT) showed that an implantable cardioverter defibrillator (ICD) reduced the risk of death by 23% in patients with mild to severe symptomatic HF and reduced EF who were receiving optimum antiarrhythmic therapy (Bardy GH et al., 2005a). These findings are supported by similar large reductions in death with a cardiac resynchronisation-defibrillator device (CRT-D) in patients with severe symptomatic HF and a low LV EF in the COMPANION study (Comparison of Medical Therapy, Pacing and Defibrillation in Heart Failure) (Bristow MR et al., 2004a) and with an ICD in survivors of myocardial function with a reduced LV EF in the MADIT-2 trial (Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy) (Moss et al., 2002). A major limitation of ICD is its cost as it has been estimated that the number of ICD’s needed to be implanted to save 1 life is 18 (Sanders GD et al., 2005).

Cardiac-resynchronisation therapy (CRT) received Food and Drug Administration (FDA) approval for use in selected patients with severe systolic dysfunction in 2001. A number of pivotal prospective trials have consistently shown that CRT improves EF, quality of life (QOL), and functional status in symptomatic patients with an EF of less than 35% and a prolonged QRS duration (mean range, 155 to 209 msec) (Bax JJ et al., 2005). In addition, a systematic review in 2007 demonstrated that CRT decreased the rate of hospitalization by 37% and lowered the rate of death from any cause by 23% in HF patients (McAlister FA et al., 2007).
In 2009, Bax and Gorcsan reported that 30% of patients who received CRT do not benefit (Bax JJ & Gorcsan J, 2009) as they demonstrated a positive clinical response as shown by an increase in exercise capacity and improvement in QOL measures. These improvements, however, were not accompanied by improvements in LV systolic function. This observation has led to two related areas of investigation: i) alternative measures to detect mechanical dyssynchrony (i.e. disparity in the timing of regional ventricular contraction) apart from the electrical delay that is manifested by a wide QRS duration, and ii) selection of patients who are likely to have more consistent benefit from CRT. Evidence of mechanical dyssynchrony has been shown to be an independent predictor of clinical events and worsened survival in patients with HF and has correlated better than the QRS duration with the long-term benefit of CRT (Bax JJ & Gorcsan J, 2009).
1.4.3 Surgical Management

Despite the advances in medical management of HF, there remain circumstances in which surgical procedures are the only or best treatment option.

1.4.3.1 Cardiac Transplantation

The first successful human-to-human heart transplant was performed in 1967 (Barnard CN, 1967). Early results were not very promising due to allograft rejection and the procedure was dedicated to highly specialised centres. It wasn’t until the introduction of cyclosporine A in the early eighties that cardiac transplantation has become the therapeutic procedure of choice for patients with end-stage HF (Hunt SA, 1998). Unfortunately, transplantation is limited by the small number of donor hearts available such that transplant numbers have decreased worldwide and meet less than 10% of the need for cardiac replacement therapy. Figure 1.11 represents the trend in cardiac transplantation since the registry has started in 1982.

Figure 1.11: The ISHLT Registry of adult heart transplants

![Figure 1.11: The ISHLT Registry of adult heart transplants](image)

**Fig 1.11:** The ISHLT registry of all adult heart transplants since 1982 until 31 December 2007. From (ISHLT Registry, 2009).
It is also worth noting that transplantation is inapplicable in older patients or those with co-morbid medical conditions that could preclude them from consideration for transplantation. Other problems associated with transplantation include rejection and coronary graft atherosclerosis. Even following successful transplantation, 1-year survival is only around 79% with a subsequent attrition rate of 4% per annum and 10-year survival <50% (Hosenpud et al., 1998). Although these results are better than medical therapy, they remain far from ideal.

To address this problem alternative supportive surgical strategies have evolved including coronary artery revascularisation, mitral valve surgery, left ventricular reduction, cardiomyoplasty, and mechanical circulatory support (Bolling SF et al., 2001; Garrido MJ & Oz MC, 2002; Gregoric I et al., 2002; Slaughter MS & Ward HB, 2000; Zeltsman D & Acker MA, 2002).

1.4.3.2 Revascularisation

From 25 multi-center trials (where approximately 44,000 HF patients were studied) coronary artery disease was reported to be the cause of HF in nearly 65% of the patients (Cohn JN et al., 1986; CONSENSUS Trial Study Group, 1987; DiBianco R et al., 1987; Studies of Left Ventricular Dysfunction Investigators (SOLVD), 1991; Cohn JN et al., 1991; Packer M et al., 1991; Studies of Left Ventricular Dysfunction Investigators, 1992; Packer M et al., 1993; Feldman AM et al., 1993; Singh SN et al., 1995; Packer M et al., 1996a; Packer M et al., 1996c; Digitalis Investigation Group, 1997; Cohn JN et al., 1998; Pitt B et al., 1999; Torp-Pedersen C et al., 1999; Colucci WS et al., 2000a; Packer M et al., 2001b; Beta-Blocker Evaluation of Survival Trial Investigators, 2001; Cohn JN & Tognoni G, 2001; Abraham WT et al., 2002; Bristow MR et al., 2004b; Bardy GH et al., 2005b; Cleland JGF et al., 2005; Taylor AL et al., 2004; Jones RH et al., 2009a).

It is well established that coronary artery bypass grafting (CABG) increases survival, improves angina, and improves QOL in patients with HF secondary to IHD (Alderman EL et al., 1983; Edmond M et al., 1994; CASS Investigators, 1984). The use of arterial revascularisation and off-pump techniques has produced additional benefits (Bittner HB & Savitt MA, 2002) and studies have shown that viable myocardium, as assessed using stress echocardiography or positron emission
tomography (PET), is a prerequisite for successful coronary revascularisation (Pitt M et al., 2001; Kalra DK & Zoghbi WA, 2002; Allman KC et al., 2002).

1.4.3.3 Mitral Valve Surgery

Functional mitral regurgitation is a significant complication of end-stage HF, and may affect all patients as a pre-terminal or terminal event. Mitral regurgitation develops secondary to an alteration in the annular-ventricular apparatus (Chen FY et al., 1998) and altered ventricular geometry (Boltwood CM et al., 1983), resulting in incomplete leaflet coaptation.

In ischaemic HF this can be attributed to papillary or lateral wall muscle dysfunction, whereas in non-ischaemic HF patients, mitral regurgitation can be ascribed to annular dilatation and chordal tethering, all as a result of remodeling. Left ventricular adaptations to mitral regurgitation include increase in pre-load, wall tension, diastolic volume, and stroke volume (SV).

Historically, the surgical approach to treat functional mitral regurgitation in HF was mitral valve replacement. However, studies have shown that interruption of the annulus-papillary muscle continuity is associated with very high mortality (Stevenson LW et al., 1987; Blondheim DS et al., 1991; Pitarys CJ II et al., 1990; Pinson CW et al., 1984). In 1983, Tyrone David reported the importance of preserving the annulus-papillary muscle continuity (David TE et al., 1983) such that preserving the mitral valve apparatus enhances and maintains LV function and geometry with an associated decrease in wall stress (Goldman ME et al., 1987; Tischler MD et al., 1994). There is now compelling evidence that mitral valve annuloplasty using an undersized remodeling ring with or without mitral valve repair is more superior to mitral valve replacement (Smolens IA et al., 2000; Badhwar V & Bolling SF, 2002; Bolling SF, 2002; Carpentier A et al., 1980; Enriquez-Sarano M et al., 1995). The aim of the annuloplasty is to reduce the mitral annulus to correct for the zone of coaptation. Either procedures has been shown to re-establish the ellipsoid shape and somewhat the normal geometry to the base of the LV (Bolling SF et al., 1995; Bach DS & Bolling SF, 1996; Bach DS & Bolling SF, 1995; Romano MA &

*Coaptation generally requires a large leaflet area greater than 2.5 times than the area of the mitral valve orifice (Bolling SF et al., 2001).
Bolling SF, 2004), to improve coronary flow reserve and velocity especially in those with restricted coronary flow reserve (Akasaka T et al., 1998), and to reduce the activated cytokines and pro-inflammatory markers seen in HF (Smolens IA et al., 2000). Furthermore, operative mortality is reduced to less than 5% and one year survival exceeds 80% (Smolens IA et al., 2000; Bishay ES et al., 2000; Bitran D et al., 2001). In patients with mild to moderate mitral regurgitation secondary to ischaemic HF, Tolie et al have suggested that revascularisation alone is sufficient to enhance improvement in mitral regurgitation with perioperative mortality of 2% and 50% 5-year survival (Tolis GA Jr et al., 2002). It is worth noting that mitral insufficiency may still occur with adequate coaptation suggesting that there is a subvalvular malfunction and hence the optimal surgical intervention in these situations is mitral valve replacement (Calafiore AM et al., 2001).

1.4.3.4 LV Volume Reduction

In 1996, Batista was the first to state that all mammalian hearts share the same ratio of mass to diameter, regardless of the size of the heart \( m = 4r^3 \), where \( m \) and \( r \) represent the mass of the heart and the radius of the LV chamber, respectively (Batista RJ et al., 1996). The group proposed that for those hearts that do not comply with this relationship, an operative procedure, LV myoreduction surgery, should be performed to restore the ratio to normal. With this procedure, Batista has demonstrated a reduction in the LV volume from 200 mls to approximately 90-100 mls, intra-operative mortality of 5%, a 30-day mortality of 22%, and a 2-year survival of 55%. The Cleveland group reported perioperative mortality of 3.2% and a 2-year survival rate of 68% (McCarthy PM, 1999). This procedure, however, has not been met with great success in the United States or worldwide due to the high incidence of procedure failure (McCarthy JF et al., 1998; Konertz W et al., 1999).

From the Surgical Treatment for Ischemic Heart Failure (STICH) trial, Jones et al concluded that adding surgical ventricular reconstruction surgery to CABG has resulted in a significant reduction in the LV volumes as compared with CABG alone, however, there was no greater improvement in symptoms, exercise tolerance, reduction in death rate or hospitalisation for cardiac causes (Jones RH et al., 2009b).
In 1985 Professor Dor and his group introduced an endoventricular circular patch in an attempt to restore the natural geometry and the dynamics of the LV (Dor V et al., 2001) in ischaemic HF with anterior infarct that involves the apex. The idea is based on the fact that ventricular contraction starts at the apex and moves to the left-ventricular outflow tract. By excluding the infarcted apical septum and replacing it with a patch, a more ellipsoid ventricle is created (De Bonis M & Alfieri O, 2006). The left ventricle is opened through the infracted apex and the transitional zone between scar tissue and viable myocardium is identified. A circular purse-string suture is applied. Finally, the apex is closed either directly or using a patch. Care must be taken not to undersize the ventricular cavity. This approach has been limited to ischaemic dilated cardiomyopathy and its use was contraindicated in patients with: i) systolic pulmonary artery pressure more than 60 mm Hg (when not associated with severe mitral regurgitation); ii) severe right ventricular dysfunction as assessed by tricuspid anulus plane systolic excursion technique; and iii) regional asynergy without dilation of the ventricle (Menicanti L & Di Donato M, 2002). Other relative contraindicating factors include NYHA class IV, EF less than 20%, age more than 70 years, the need for urgent intervention, and the need for an additional mitral procedure (especially mitral valve replacement).

1.4.3.5 External Compression

In 1984, Professor Carpentier was the first to introduce external compression as dynamic cardiomyoplasty technique. Recently, studies have shown that positioning a mesh graft around the heart had:

i. stopped further dilatation in the LV (Oz MC, 2001),
ii. induced a significant reduction in wall stress and myocyte stretch (Oz MC, 2001; Konertz WF et al., 2001),
iii. resulted in improved functional mitral regurgitation (Chaudhry PA et al., 2001),
iv. enhanced ejection fraction (Konertz W et al., 2001),
v. caused an improvement in end-systolic and end-diastolic volumes by an average of 15.6 mls and 18.8 mls, respectively (Starling RC et al., 2007),
vi. resulted in a significant improvement in NYHA classification from 2.7 ± 0.1 to 1.8 ± 0.2, p<0.001 (Bredin E et al., 2009),
vii. caused an increase in exercise tolerance as assessed by the 6-minute walk test (Bredin E et al., 2009), and

viii. caused a significant reduction in BNP levels from 0.14 ± 0.04 ng/ml to 0.06 ± 0.03 ng/ml, p<0.05 (Bredin E et al., 2009).

Examples of external compression devices are the Acorn device which comes in six different sizes to avoid overcompression. Another device is the Myosplint which consists of an implantable transventricular 1.4 mm polyethylene braided splint coated with expanded polytetrafluoroethylene and two polyestercoated epicardial pads. The splint is positioned to bisect the dilated LV, effectively creating two smaller LV chambers. The epicardial pads are adjusted to reduce the LV diameter. Based on Laplace’ Law the wall tension is thereby decreased. Although early clinical results are promising, the need for a non-randomised clinical trial remains to be awaited (Fukamachi K & McCarthy PM, 2005).

1.4.3.6 Mechanical Circulatory Assistance

Mechanical circulatory assistance is an important adjunct to the management of patients with severe end-stage heart failure. This is a life saving approach in patients who fail to improve or stabilise with intravenous inotropes or vasodilators, intra-aortic balloon pump (IABP) support and mechanical ventilation (Delgado DH et al., 2002).

Patients requiring mechanical circulatory assistance generally fall into four categories, those with:

i) cardiogenic shock resulting from acute MI,
ii) post-surgical myocardial dysfunction,
iii) acute cardiac failure from myocarditis,
iv) decompensating chronic HF.

Although this approach falls under the surgical management category, it will be discussed separately in section 1.5.
1.5 Mechanical Circulatory Support

Ventricular assist device (VAD)s are mechanical circulatory support (MCS) devices that assist the circulation. They are rapidly evolving and are being inserted into an increasing number of patients with advanced heart failure. VADs are not only life saving in decompensating chronic HF patients that would otherwise die before a donor heart became available, but also improve secondary organ function for transplantation, reduce pulmonary hypertension and allow for improvement of nutritional status.

The future use of these devices, particularly as survival continues to increase, is likely to extend to their wider use as destination therapy i.e. when the device is inserted lifelong as an alternative to transplantation.

There is also now compelling evidence that with LV unloading using left ventricular assist devices (LVAD)s recovery of patients’ myocardial function can also occur, allowing device removal and avoiding the need for transplantation, immunosuppression and its associated complications and leaving the patient with an excellent QOL. This also means that the precious resource of a donor organ can be used for another needy individual. This indication, known as “bridge to recovery” (BTR), is a newer and expanding indication and will be the focus of this thesis.

1.5.1 Overview of LVADs

In 1953, Gibbon was the first to perform an open-heart surgery using cardiopulmonary bypass (CPB) circuit (Gibbon, 1954; Frazier, 2000). By the mid 1960s, a shift had occurred from CPB circuits towards more dedicated cardiac assist devices.

In 1971, De Bakey reported the first successful clinical application of a pneumatically driven VAD in a 37-year-old woman who could not be weaned from CPB following valve replacements (DeBakey M, 1971). She was supported for 10 days with the paracorporeal circuit from left atrium to right axillary artery and was then weaned successfully and subsequently discharged from hospital. Seven years later, Norman et al reported a case of bridging to transplantation using an
intracorporeal pneumatic abdominal LVAD for 5 days when the patient survived the support but died after transplantation (Norman JC et al., 1978).

The total artificial heart (TAH), developed by Liotta and DeBakey Baylor-Rice research team (DeBakey M, 2000), was first used as a temporary support device by Cooley et al in 1969 in a 47-year-old man who could not be weaned from CPB following repair of a LV aneurysm (Cooley D et al., 1969). The world’s first permanent TAH implant occurred in 1982 with the Jarvik-7 in a 61-year-old man with advanced DCM who survived 112 days on support (Kirklin JK & Frazier OH, 2006).

Indication for TAH include: biventricular failure, LV failure with intractable arrhythmia, LV failure with prior mechanical heart valve replacement and LV failure with severe anatomical damage such as ventricular septal defect, or ventricular rupture. The CardioWest TAH is approved for use in the USA. It is pneumatically driven and is implanted in the orthotopic position. Its use has been assessed in five centres and the outcome was a significant improvement in the rate of survival to cardiac transplantation and survival after transplantation (Copeland JG et al., 2004). The AbioCor, another TAH has obtained conditional FDA approval as a destination device (Rohinton J Morris, 2008).

VADs can be classified according to their principle of operation. First generation devices are pulsatile devices that generate pulsatile flow. They consist of multiple moving components and are subdivided into extracorporeal pneumatic (e.g. Abiomed AB5000 and Thoratec), intracorporeal pneumatic (e.g. Thoratec HeartMate I IP) or electromechanical (e.g. Novacor and Thoratec HeartMate I VE). Second generation devices are rotary pumps with one moving part that generate axial continuous flow. Examples include HeartMate II, MicroMed-Debakey, and Jarvik2000. Implantation of second generation devices is easier with greater reliability and reduced complications as compared to the first generation devices (Song et al., 2003). Third generation devices are based on magnetic levitation technology with no bearing parts such as the HeartWare VAD.

All these devices are operated using an external power source with a driveline that usually exits the right upper abdominal quadrant and connects to the external
controller and batteries. The exit site of the drive line is an important source of infection. The future direction is to operate LVADs through a transcutaneous energy transfer system to avoid the need for an external driveline.

MCS devices provide a spectrum of short or long-term circulatory support. LV support can be partial or complete and additional RV (RVAD) or even biventricular support (BiVAD) can be tailored to haemodynamic needs. The technology is being refined for application to broader patient groups with increased safety and lower cost (Frazier, 2003).

Key features of long-term mechanical circulatory support are reliability, durability, biocompatibility, functionality and affordability. Blood-biomaterial interaction, the coagulation system and the immune response are all challenging biological barriers to the development of long term MCS (Dee K et al., 2002; Didisheim P, 1994; Koster A et al., 2000; Loebe M et al., 2001; Menconi MJ et al., 1995; Schuster M et al., 2001; Spanier T et al., 1996). These have been partially defeated by developing and using specific materials and specific blood-contacting corrosion resistance surfaces which have certain structural strength and almost no toxicity levels (Burgreen GW et al., 2004). Other key features include reduced risk of infection and low incidence of device malfunction requiring part or all of the device to be replaced.

Tables 1.5, 1.6, and 1.7 represent the characteristics of different types of VADs.
### Table 1.5: Different Pulsatile VADs

<table>
<thead>
<tr>
<th>Pump Type</th>
<th>Device Description</th>
<th>Clinical Experience</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thoratec Paracorporeal (PVAD)</strong></td>
<td>A positive displacement pump that generates a 65 ml of SV.</td>
<td>Approved in USA, Europe, Japan, Canada and Australia for Bridge-to-Transplant (BTT) and postcardiotomy recovery. Can be used as an LVAD, RVAD, or BiVAD.</td>
</tr>
<tr>
<td></td>
<td>It has volume capacity of 318 mls, weighs 417 grams and has a thick percutaneous lead (20 mm).</td>
<td>INR needs to be maintained between 2.5 and 3.5.</td>
</tr>
<tr>
<td></td>
<td>The blood pumping chamber has two mechanical valves to ensure unidirectional flow and can produce a beat rate of 40-110 with a flow rate of 1.3-7.2 l/min using alternating positive and negative air pressures.</td>
<td>Until March 2005, 2900 patients were implanted with the PVAD. Worldwide, survival from implant to either transplantation or recovery was 64.8% when used as an LVAD, 56.6% when used as a BiVAD, and 31.2% when used as an RVAD (<a href="http://www.thoratec.com">www.thoratec.com</a>).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Been used for BTR in 3.8% with LVAD, 5.6% with BiVAD, and 8.3% with RVAD. The 5-year transplant-free survival was 77% for this subgroup (<a href="http://www.thoratec.com">www.thoratec.com</a>).</td>
</tr>
<tr>
<td><strong>Thoratec Intracorporeal (IVAD)</strong></td>
<td>Implantable version of PVAD with slightly reduced weight (399 grams) and volume (252 mls). Percutaneous lead is also thinner (9 mm). Has a smooth polished titanium housing for implantability, and optical sensor that determines whether the pump is full or empty.</td>
<td>Approved by the FDA in August 2004.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Similar to PVAD, INR needs to be maintained between 2.5 and 3.5.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Until March 2005, 86 patients had IVAD of which 63 were for BTT (<a href="http://www.thoratec.com">www.thoratec.com</a>).</td>
</tr>
</tbody>
</table>
HeartMate I (HM I)

2 models
1) Implantable Pneumatic (IP)
2) Vented Electric (VE) which has been modified to the XVE model

Made of titanium with polyurethane diaphragm.

Has a low-speed torque motor that operates a pusher plate mechanism. Can be powered pneumatically and electrically (through two external batteries) and operates on two modes: auto and fixed

Porcine valves (25 mm) are suspended in the inflow cannula and the outflow Dacron graft (diameter 20 mm) to ensure unidirectional blood flow.

The device pumps between 4 and 10 l/min with a maximum SV of 83 mls.

It has a unique blood pumping surface which consists of titanium microspheres and a fibrillar textured surface that promotes “pseudointima” that resists thrombogenesis, although it has also been shown to be immunologically active (Ankersmit HJ et al., 1999; Spanier TB et al., 1999).

Ejection occurs when the pump chamber is filled. However, timing is not linked to the native cardiac cycle so changes in left ventricular-LVAD phase relations can occur, with LVAD ejection occurring either ‘synchronously’ with LV systole or ‘asynchronously’.

Dependent upon whether the LV generates sufficient pressure to open the aortic valve, blood flow may either be in series (LV - LVAD – aorta), or in parallel (LV & LVAD – aorta, through the aortic valve and device outflow conduit respectively).

The IP model was first implanted in 1986 and since then 5000 devices have been used where 65% experienced a successful outcome as BTT.

The electric version was first introduced in 1991.

Anticoagulation is limited to aspirin.

REMATCH trial started in 1998 as a multicentre study and compared the outcome of using the HM I LVAD with optimal medical management. The outcome was improved 1-year survival (52% versus 25%, p=0.002) and an improved QOL (Long JW, 2001; Long JW et al., 2005).

Also been used as BTR (Harefield Bridge to Recovery Protocol) in combination with pharmacological therapy. Explantation rate was 73% and 4-year survival exceeded 90% (Birks EJ et al., 2006). See text for study details and the BTR protocol.

Since FDA approval in 2002 for destination therapy (DT), 246 patients were implanted of which 67% were discharged home with longest duration of support of 86 days to date.
**Novacor LVAS**

Uses an implanted pump drive unit (PDU) which is implanted in a pocket in the left upper abdominal quadrant.

It produces a SV of 70 mls and has a symmetrical dual pusher plate and two porcine valves to control a unidirectional flow of blood.

The sac-type blood pump has a smooth blood-containing surface coupled to a pulsed-solenoid energy converter that drives the blood pump to match the physiological requirements.

INR needs to be maintained between 2.0 and 3.0.

Since 1984 to date, more than 1700 have been implanted with the majority for BTT. Maximum duration of support was 6.1 years (Wheeldon DR et al., 2002).

BTR and DT represent a much smaller experience.

**Abiomed 5000**

Houses an Angioflex® membrane and two proprietary tri-leaflet valves. The pump fills with blood by gravitational force and by vacuum assistance from the drive console.

The cannulae and drive console are the same as those used for the ABIOMED BVS 5000 (see below).

Can provide flow rates of up to 6 l/min.

Can be used as an LVAD, RVAD or BiVAD.
BVS 5000

Houses two polyurethane chambers: an atrial chamber that fills with blood through gravitational force and a ventricular chamber that pumps blood by air-driven power. Produces up to 5 l/min.

Atrial chamber is vented outside the patient. Ventricular chamber is connected to the power console by a 0.25-inch pneumatic line. Two trileaflet valves separate the atrial and ventricular chambers.

It was the first external/outside the body (extracorporeal) VAD on the market and has been used in more than 700 institutions throughout the world.

It was also the first FDA approved device for the support of all patients with reversible heart failure.

Table 1.5: Descriptive summaries of the properties of some pulsatile first generation LVADs. These devices have one way valves to ensure unidirectional flow of blood. Due to the numerous components in each device, for example the HeartMate I XVE has 38 moving components, pulsatile LVADs are prone to mechanical wear and tear.
<table>
<thead>
<tr>
<th>Pump Type</th>
<th>Device Description</th>
<th>Clinical Experience</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jarvik 2000</td>
<td>This device is placed in the LV with no inlet cannula. No valves, internal compliance chamber, or an externalised vent. Operates in a range of 8000-12000 rotations per minute (rpm) generating flow of up to 8 l/min. Uses a brushless direct-current motor which is contained within the titanium housing and creates electromagnetic force necessary to rotate the impeller. Power consumption is only 3-7 watts and supplied by a 12 volts battery. Power cable can come out abdominally or post auricular through a skull-mounted pedestal and hence reduce risk of infection.</td>
<td>INR to be kept between 2.5 and 3.5. To date implanted in over 100 patients; 80% received the device as BTT and 20% as DT. Has also been used as BTR in a small number of patients using the Harefield BTR protocol (George RS et al, <em>in press</em>). Associated with low rates of infection and device failure. Following completion of a pilot US study, approval was granted in July 2005 for it to undergo clinical trials in the USA as a BTT and for use as DT. In Europe it is already in use in both settings. Haemolysis has been reported with this device.</td>
</tr>
<tr>
<td>Micromed-Debakey</td>
<td>1/10th the size of pulsatile device and weighs 93 grams. Has ceramic bearings. Can provide up to 10 l/min of blood flow.</td>
<td>INR to be maintained between 2.5 and 3.5. 326 implanted worldwide for BTT purposes (<a href="http://www.micromedtech.com">www.micromedtech.com</a>). Current trials remain ongoing in both BTT and DT settings.</td>
</tr>
</tbody>
</table>
INCOR® (Berlin Heart) Wighs 200 grams and its volume is 82 mls. Diameter 30 mm and length 12 cms.

Rotational speed 5000-10000 rpm. Typical power consumption of 4W.

Axially active and radially passive without producing any actual physical contact. There is no direct mechanical contact between it – as the only movable part in the INCOR® pump - and its static components. This prevents any mechanical friction and, as a result, no frictional heat. This means no wear and tear at all to the parts and, consequently, a potentially infinite product life span for the INCOR® heart support system.

Has sensors linked to the magnetic bearing to prevent any suction through the pump in the left ventricle. Sensors detect the residual pulsality linked with the device and the pump then automatically reduces its rotation speed and allows a renewed filling of the ventricle. The originally selected pump performance is then restored slowly and in a controlled way.

INR to be kept between 2.8 and 3.2

Its use was first reported in 2005 in 15 patients in NYHA class IV (Schmid C et al., 2005). No early bleeding complications were reported. None of the patients had developed drive line infections.
HeartMate II (HM II)

Small, lightweight device (350 grams) designed to accommodate a broad patient population - body surface area (BSA) as low as 1.2 m$^2$.

It represents 1/7$^{th}$ the size and ¼ the weight of the first generation HM I VAD.

Flexible inflow and outflow conduits to accommodate anatomical changes. Consists of an internal blood pump (a 12 mm diameter straight tube made of titanium alloy).

It is implanted in the pre-peritoneal position. In automatic mode of 9000-11000 revolutions/min, it can produce a flow of 4-7 l/min. The maximum flow that can be produced is 10 l/min.

The tube incorporates the hydraulic components of the pump: the inlet stator, a pump rotor (incorporates a pump magnet), and an outlet stator. Helical blades curve around a central shaft. The blades introduce a radial / tangential velocity. The outlet stator vanes convert the radial velocity of the blood flow to an axial flow.

Shares the same hardware platform and system components as the HM XVE LVAD.

INR to be kept between 2.0 and 3.0

Following the successful completion of its phase I pilot study in 2004, Thoratec was granted approval to begin a phase II pivotal trial in February 2005. The HM II Pivotal Trial is a multi-centre evaluation of the HM II LVAD for advanced-stage HF patients (Butler KC & Farrar DJ, 2005; Frazier OH et al., 2004a; Pagani FD et al., 2005).

To date, more than 1000 patients have been implanted with the device.

Been successfully used for DT (Slaughter MS et al., 2009b).

Using Harefield Bridge to Recovery Protocol the device was used as BTR (results to be published)

Table 1.6. Descriptive summaries of the properties of some of the non-pulsatile devices along with their clinical use. The second generation axial flow pump devices are being increasingly used now. These are continuous flow rotary pumps with only one moving part, the rotor, which is considered virtually silent compared to first generation devices and is associated with reduced risk of device failure.
### Table 1.7: Third generation device

<table>
<thead>
<tr>
<th>Pump Type</th>
<th>Device Description</th>
<th>Clinical Experience</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeartWare</td>
<td>One moving part, the impeller, with no mechanical bearing. The impeller spins at rates between 2,000 and 4,000 rpm to generate up to 10 l/min of blood flow. The total size is equivalent to 50 cubic centimeters making it implantable in the pericardial space. The inflow cannula is integrated with the device itself, ensuring proximity between the heart and the pumping mechanism. The impeller is suspended within the pump housing through a combination of passive magnets and a hydrodynamic thrust bearing. This hydrodynamic suspension is achieved by a gentle incline on the upper surfaces of the wide impeller blades (wide blades minimise risk of pump induced haemolysis and thrombus). When the impeller spins, blood flows across these inclined surfaces, creating a &quot;cushion&quot; between the impeller and the pump housing. At no point there is contact between the impeller and the housing chamber. Reliability is enhanced through the use of dual motor stators allowing a seamless transition between dual and single stator mode if required.</td>
<td>INR to be kept between 2.0 and 3.0. Use as BTR was first reported in 2008 (Wood C et al., 2008) HeartWare remains under investigation and although it has been awarded the CE trademark, its use in USA has not been approved by the FDA as yet.</td>
</tr>
</tbody>
</table>

Table 1.7: HeartWare VAD, a third generation device with magnetic levitation technology.
1.5.2 Clinical Application of VADs

1.5.2.1 Bridging to transplantation

Even with medical treatment, those with advanced HF have a very poor prognosis often worse than malignancy with 40-60% annual mortality (Gheorghiade et al., 2000;Swedberg et al., 1999) unless patients are transplanted. But due to limited donor organ supply-demand mismatch (as discussed above), MCS devices provide the only practical alternative to heart transplantation (Deng & Naka, 2002). It is estimated that as many as 60,000 patients with HF could be helped in the USA alone (Kenneth L Franco, 2001;W Piccione, 2001).

According to the United Network for Organ Sharing (UNOS) in USA the death rates for advanced HF patients on the waiting list have decreased from 43% in 1990 to 17% in 1999 (Deng MC, 2002). This significant reduction may be contributed by the increasing use of MCS devices in urgently listed patients.

In the UK, indications for VAD implantation for the purpose of BTT is limited to patients who:

i) are appropriate candidates for cardiac transplantation (transpulmonary pressure gradient <14 mm Hg, mVO₂<14 ml/kg/min, CI<2.1 l/min/m², no active infection or malignancy, no recurrent pulmonary embolism), or

ii) would become appropriate after a period of VAD support

iii) had rapidly deteriorating heart function and clearly would not survive to transplant despite the provision of an “urgent” category nationally.

The presence of a MCS device per se does not increase the risk of subsequent transplantation (Frazier et al., 2001;Jaski et al., 2001;Massad et al., 1996;Taylor et al., 2003;Birks et al., 2002). Transplantation results may even be improved if this kind of pre-operative support is used (Goldstein et al., 1998;Aaronson et al., 2002;Bank et al., 2000). However, the use of LVAD as a BTT is associated with drive line infections and increased HLA antibodies. The latter would reduce the chance of transplantation. Further, LVAD patients would have had at least one sternotomy which adds to the surgical complications at the time of transplantation.
Support on the device allows renal function, nutritional status and pulmonary vascular resistance to improve before transplantation, which usually takes several weeks or months. Transplantation should only be considered once these improvements have occurred.

In a prospective, multicenter clinical trial conducted at 24 centres in the United States, 280 transplant candidates (232 men, 48 women; median age, 55 years; range, 11-72 years) unresponsive to inotropic drugs, IABP, or both, were treated with the HM I pulsatile LVAD (Frazier OH et al., 2001). Average support was 112 days (range, < 1-691 days), with 54 patients (approximately 20%) supported for more than 6 months. 71% of patients (198/280) survived with 67% (188/280) receiving heart transplant, and 4% (10/280) had the device removed electively. There was also a significant improvement in metabolic parameters (bilirubin decreased from 1.2 mg/dL to 0.7 mg/dL (p = 0.0001) and creatinine decreased from 1.5 mg/dL to 1.1 mg/dL (p = 0.0001)). Adverse events associated with LVAD support were low.

In 2007, Sharples et al reported a 44% survival rate to transplantation in the UK having studied the population between 2002 and 2004 (Sharples LD et al., 2007). In contrast, Park et al had a survival rate to transplantation of 85.7% (Park SJ et al., 2000). On average the reported survival rates to transplantation ranged between 60% to 75% (Navia JL et al., 2002; El-Banayosy A et al., 2000; Sun BC et al., 1999; DeRose JJ Jr et al., 1997). These data are all from the first-generation pumps.

Data from the second-generation pumps, which would be expected to have a better outcome owing to their smaller size, easier surgical implantation etc, are now becoming available. Miller et al published a prospective multicentre study of 133 NYHA class IV patients on a transplant waiting list who underwent HM II LVAD implantation as BTT (Miller LW et al., 2007). All patients were receiving inotropic support (except for 11% intolerant because of arrhythmia) and 41% were also IABP dependent. After 180 days, 100 (75%) patients had reached the principal outcome of transplantation, recovery or survival on ongoing support with eligibility for transplantation. Patients on HM II support had improved NYHA class, 6MW functional status and QOL. The overall survival was 81% at 6
months and at 1 year the overall survival of patients who underwent transplantation or continued to receive MCS while remaining a candidate for transplantation was estimated to be 70%. These 133 patients, enrolled from March 2005 to May 2006, represented the primary cohort. Enrolment continued following this and Pagani et al published the results of 281 of 469 patients enrolled by April 2008 that had completed study end points or had at least 18 months of follow-up with ongoing device support (Pagain FD et al., 2009). Of these, 79% had either received a transplant, been explanted due to myocardial recovery, or remained alive on a VAD at 18 months. Of the 55.8% (n = 157) that received a transplant, post-transplant survival was 96% at 30 days and 86% at 1 year. During the study period 78% of patients were discharged from the hospital with a VAD, after a median post surgery stay of 25 days.

Data has also started to emerge from third-generation devices. VentrAssist have published data describing a success rate of 82% in 33 patients at a follow-up point of 154 days with these results maintained at 365 days with 60.6% receiving transplantation and 21.2% remained eligible for transplantation (Esmore D et al., 2008). Patients who have received the Duraheart VAD as BTT had a 78% 2-years survival rate to transplantation (Nojiri C et al., 2008). Fifty patients have received the HeartWare device with an 85% 1-year actuarial survival to transplantation (Wieselthaler GM et al., 2008).

In addition to the improved survival rate to transplantation the clinical outcome following transplantation had also improved. In 2006, Drakos et al reported the outcome of 278 patients who underwent cardiac transplantation from 1993 to 2002 (Drakos SG et al., 2006). Pre-transplant MCS was required in 72 patients and was not required in the remaining 206 patients. One-month and 1-year survival after transplantation was similar between the two groups (MCS, 92% and 85%, respectively versus no MCS, 97% and 92%, respectively). At 1 year of follow-up 56% of MSC patients were free from rejection as compared to 52% of patients who did not require MCS (p=0.60). Post transplant events such as hospital stay, intensive care unit stay, extubation time, acute allograft dysfunction, reoperation rates, acute renal and hepatic dysfunction, infections, arrhythmias, thromboembolic and neurologic complications, and the development of cardiac
allograft vasculopathy were similar between the two groups. The incidence of chronic renal insufficiency was lower in the MCS group (15.3% versus 37.9%, p<0.001). Similarly, those who were bridged to transplantation had significantly lower incidence of acute renal failure as compared to those who did not receive a MCS prior to transplantation (5.6% versus 16.7%, p<0.05) (Bank AJ et al., 2000). Right sided HF was also lower (31.6% versus 52.6%, p<0.05). The same group reported a lower incidence of re-operation, rejection, disability post transplantation, and infection (Bank AJ et al., 2000).

In 2008, using SF36 QOL questionnaire, we reported that BTT patients had a significantly higher mental health dimension as compared to those who were transplanted without pre-transplant MCS (71.4 ± 21.1 versus 39.4 ± 44, p=0.01) (George RS et al., 2008b). These findings raise an important question of the significance of LVAD in affecting patients QOL outcome post transplantation.

Patients supported with LVAD for BTT experienced multiple complications (Dembitsky WP et al., 2004). In 2003, John et al reported that the incidence of post-transplantation rejection was greater in LVAD recipients who were sensitized by LVAD use prior to transplantation, but lower in unsensitised recipients not supported in this way (John et al., 2003). In contrast, Birks et al reported a lower rate of rejection in LVAD supported patients bridged to transplantation (Birks et al., 2002). Device failure is another problem that can be life threatening and a limiting factor of long periods of use of an LVAD. Navia et al reported device failure in 7.6% of patients implanted with HM I VE LVAD (Navia JL et al., 2002). In our centre, Birks et al reported cumulative probability of device failure in 6% of patients at 6 months, 12% at 1 year, 27% at 18 months, and 64% at 2 years (Birks EJ et al., 2004b). This is far less of a problem, however, with the newer generation devices.

Another major limitation of using VAD as BTT is the cost associated with the procedure. As expected the total cost for LVADs exceeded that of orthotopic heart transplant (DiGiorgi PL et al., 2005; Sharples LD et al., 2006). Interestingly, Bank et al reported that the average daily cost was similar between those who were bridged to transplantation and those who did not receive a MCS device (Bank AJ et al., 2000).
1.5.2.2 Destination therapy

Technological improvements now allow consideration of long-term LVAD implantation as an alternative to cardiac transplantation in those who do not show signs of recovery or for whom transplantation is contraindicated (Birks et al., 2004; Rose et al., 2001).

DT refers to the use of MCS devices as the final means by which a patient will be treated (Clark & Zafirelis, 2000). The results of the Randomised Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure trial (REMATCH) had effectively demonstrated the efficiency of permanent device therapy. This study compared the use of the pulsatile HM I VE LVAD with optimal medical management in those with end-stage heart failure and ineligible to receive cardiac transplantation (Rose et al., 1999; Rose et al., 2001). The trial began randomisation in 1998 and was the first to demonstrate the positive impact of MCS on QOL and survival in end stage HF (Rose et al., 2001). In total, 129 patients were enrolled from 20 centres, and received either optimal medical therapy or permanent therapy with HM I VE LVAD (Rose et al., 2001). All patients were in NYHA class IV for at least 60 of 90 days despite maximal medical treatment and they were ineligible for cardiac transplantation for any of the following single or combined reasons: age > 65 years, insulin-dependent diabetes with end-organ damage, chronic renal failure or significant irreversible comorbidity. Median age was 69 years. Those who received the MCS benefited from 48% reduced all cause mortality. At 1 year, survival was 52% (74% if < 60yrs) as compared to 25% in the medically managed group. The 2-years survival was 23% as compared to 8%. Overall the median survival was 408 days for MCS patients as compared to 150 days in the medical treatment group. Terminal HF caused most deaths in the medical therapy group, in contrast to sepsis (41% deaths) and device failure (17% deaths) in the MCS group. QOL and NYHA class improved at follow up with MCS, but adverse events were twice those of the medical group, mainly comprising infection, bleeding and device malfunction (Rose et al., 2001). There was a significant improvement in survival for LVAD patients enrolled during the second half of the trial (January 2000 to July 2001) compared with the first half (May 1998 to December 1999) (Park SJ et al., 2005)
reflecting improvements in patient management and device modifications even throughout the period of the trial. The 1-year survival in the second half of the trial was 59% versus 44% in the first half (p=0.029) and the 2-year survival was 38% versus 21%. Both the Minnesota Living with Heart Failure QOL scores (Stevenson LW et al., 2004) and NYHA functional class (Richenbacher WE et al., 2003) have improved significantly over the course of the trial. Although survival was better in the LVAD group, the risk of CVA was higher in the LVAD group as compared to the medically treated group (0.19 versus 0.052) (Lazar RM et al., 2004) and device related sepsis such as drive line infections caused substantial morbidity and mortality (Holman WL et al., 2004). The REMATCH investigators concluded MCS as an acceptable alternative treatment if a patient is ineligible for transplantation and that 270 deaths/yr/1000 patients could be prevented (Rose EA et al., 2001).

In 2005, Long et al reported their experience with the modified HM I XVE LVAD (see table 1.5 for the new features of the modified version) in four high volume centers (Long JW et al., 2005). The group reported an improved 90% and 61% 30-day and 1-year survival, respectively. The death rates due to sepsis and device failure were 8.3 times and 2.2 times lower than the REMATCH trial, respectively. Overall, patients were 2.1 times less likely to experience an adverse event and there was a reduction of 66%, 63%, 89% and 92% in neurological dysfunction, sepsis, site infection, and for combined and suspected device failure respectively. In a non-randomised trial, Rogers et al showed that patients treated with the Novocor first generation LVAD had better survival rates as compared to medically treated patients, 46% versus 22% at 6 months and 27% versus 11% at 12 months (Rogers JG et al., 2007). In this trial, 85% of patients receiving LVAD had either no symptoms or minimal HF symptoms at the last assessment. None of the medically treated group reported any improvement in NYHA functional class. QOL measures had also improved in the LVAD group.

Introduction of axial flow and centrifugal devices have improved LVAD survival, haemodynamics, end-organ function, quality of life and functional capacity (Miller LW et al., 2007) and reduced VAD-related complications. In Dec 2009, Slaughter et al randomised patients with advanced heart failure who were
ineligible for transplantation to receive either the continuous-flow HM II LVAD or the pulsatile-flow HM I LVAD (Slaughter MS et al., 2009b). The trial was conducted in 38 centers with a randomisation ratio of 2:1. The investigators reported that both devices improved the QOL and functional capacity. Those who received the HM II LVAD had a significantly higher probability of survival from stroke and device failure at 2 years as compared to those who received the HM I LVAD (46% versus 11%, p<0.001).

To determine which end-stage heart failure patient who is ineligible for transplantation would have a better outcome, Lietz et al devised a scoring system that used 9 pre-implant parameters to identify 90-day in-hospital mortality after LVAD implantation as DT, survival to hospital discharge, and 1-year survival (Lietz K et al., 2007). The group determined that a score of 0-8 represent a low operative risk, a score of 9-19 represent a medium risk, and a score of 20-31 represent a high risk.

Cost analysis of the REMATCH trial showed that sepsis, pump infection and perioperative bleeding were the main sources of increased implantation costs (Oz et al., 2003). The greatest expense was in non-survivors. The cost of implantation and readmission in those who survived more than 1 year was comparable to other life saving organ replacement procedures (Oz et al., 2003;Showstack et al., 1999).
1.5.2.3 Bridging to recovery

Levin et al termed the process of reversal of the adverse changes in HF as “reverse remodeling” (Levin HR et al., 1995). Over the years there has been ample evidence supporting the notion of reverse remodeling following LVAD implantation at the molecular, cellular and clinical levels.

1.5.2.3.i LVAD and reverse remodelling – molecular and cellular levels

The exact mechanisms of myocardial recovery remain unclear (Caforio et al., 1990). Whether reverse remodelling is due to a “U-turn” of the pathological mechanisms that occur in remodelling or the generation of new pathways remains unclear. However, the following changes have been reported during unloading with an LVAD (Hetzer et al., 2000;Hummel et al., 1994;Altemose et al., 1997;Wallukat & Wollenberger, 1987;Wallukat et al., 1995;Loebe et al., 1997b;Muller et al., 1997):

i) normalisation of deranged Ca\textsuperscript{2+} transport,

ii) reduction in cytokines and neurohumeral activity,

iii) improved glucose utilisation,

iv) improvements in HF related gene expression,

v) up-regulation of β-adrenergic receptors, improved myocyte contractility, and

vi) reduction in β\textsubscript{1}-adrenoceptor auto-antibodies.

The effects of unloading on myocyte size and structural proteins

Hematoxylin and eosion (H&E) staining of longitudinal and cross-sectional myocardial samples taken from patients with end-stage HF demonstrated an increase in the size of cardiomyocytes with loading followed by reduction in myocyte size after long-term LVAD support (figure 1.12) (Dipla K et al., 1998a;Khan N et al., 2003a;Xydas S et al., 2006;Zafeiridis A et al., 1998a).
Figure 1.12: Effects of LVAD support on myocyte size

![Image of myocardial longitudinal and cross-sectional samples pre- and post-LVAD support](image1.png)

Figure 1.12: Myocardial longitudinal and cross-sectional samples taken from a patient pre- and post-LVAD support. The paired samples demonstrate reduction in myocyte size after long-term LVAD support. From (Khan N et al., 2003a).

Interestingly, Bruckner et al showed a significant correlation between the length of LVAD support and the percentage change in myocyte size (figure 1.13) (Bruckner BA et al., 2001).

Figure 1.13: Correlation between length of LVAD support and myocyte size

![Image of scatter plot showing correlation](image2.png)

Figure 1.13: There is significant correlation between duration of LVAD support and the percentage reduction in myocyte size. From (Bruckner BA et al., 2001).
Enlargement of isolated myocytes in HF might be more pronounced in the long axis dimension as compared to the width or depth (Yacoub MH, 2001). Figure 1.14 depicts isolated ventricular myocytes from patients at the time of insertion of an LVAD (middle horizontal panel) showing severe hypertrophy as compared to normal controls (top horizontal panel). Bottom panel shows ‘normalization’ of the size of the myocytes post left ventricular assist device (Yacoub MH, 2001).

**Figure 1.14: Cardiac myocyte size in normal, HF and post LVAD support**

![Cardiac myocyte size comparison](image)

**Figure 1.14: LVAD normalises the size of the myocytes.**

In addition to the changes seen in myocyte size, LVAD support has resulted in decreased apoptosis through complex mechanisms. Some of these mechanisms include an increase in mRNA levels for apoptosis-inhibiting proteins FasEx06del and Bcl-XL (Cascino I et al., 1996;Schumann H et al., 1997), reduced apoptotic DNA fragmentation (Bartling B et al., 1999;Milting H et al., 1999), and upregulation of genes associated with cell growth, DNA repair and apoptosis (Chen Y et al., 2003).

As previously described, remodeling is associated with induction of specific gene programmes involving several groups of gene and enhanced secretory activity of the fibroblasts which leads to both reparative and reactive fibrosis which is related to changes in total collagen content, an increase in collagen I and III concentration.
and a change in collagen cross-linking in the cardiac ECM. The responses of collagen concentration and cross-linking to LVAD support remains controversial. Klotz et al demonstrated an increase in collagen cross-linking and the ratio of collagen type I to III after 145±33 days of LVAD support and hence an increase in myocardial stiffness (Klotz S et al., 2005b). Xydas et al, on the other hand showed a decrease in collagen deposition from time of LVAD implantation to time of transplantation in 17 patients who were bridged to transplantation (Xydas S et al., 2006). Similarly, Bruckner et al demonstrated that long-term mechanical circulatory support significantly reduced collagen content and fibrosis (Bruckner BA et al., 2001). Bruggink et al, suggested a biphasic pattern for the collagen turnover and the volume of ECM with LVAD support (Bruggink AH et al., 2006). They showed that the initial response of the cardiac ECM response to LVAD (within 200 days of support) was an increase in Types I and III collagen turnover and after 400 days of support there was a reduction in both the collagen and the volume of the ECM. Therefore, it could be assumed that the pattern of ECM and collagen response to LVAD is correlated to the length of support.

Using real time polymerase chain reaction, Felkin et al. demonstrated that patients in deteriorating HF requiring LVAD support have elevated MMP1 and MMP8 mRNA levels compared to patients with stable heart failure undergoing elective heart transplantation (Felkin LE et al., 2006). In addition TIMP 1-4 mRNA levels were also higher in the deteriorating patients requiring LVAD support with only TIMP4 reaching statistical significance (Felkin LE et al., 2006). Li et al looked at the protein expression of MMPs and TIMPs after LVAD support and showed that MMP-1 and -9 were decreased, TIMP-1 and -3 were increased, and there was no change in MMP-2 and -3 and TIMP-2 and -4 after LVAD support (Li YY et al., 2001).

Using real time polymerase chain reaction paired myocardial samples collected at implantation and explantation following myocardial recovery were analysed. We described that LVAD support was associated with a specific pattern of changes in the mRNA content of some sarcomeric, non-sarcomeric, and membrane-associated genes (Birks EJ et al., 2005). This was also confirmed at the protein
level (Latif N et al., 2007) and paralleled improvements in haemodynamic function in LVAD patients showing clinical myocardial recovery.

Dystrophin is another structural protein that provides support for the myocyte and the cardiomyocyte membrane by linking N-terminus with the dystrophin-associated protein complex and sarcolemmal at the C-terminus. Studies have shown that mutations in the N-terminus of dystrophin are responsible for x-linked dilated cardiomyopathy (Ortiz-Lopez R et al., 1997; Towbin JA et al., 1993). Vatta et al demonstrated that with prolonged unloading with an LVAD most patients with reduced N-terminus expression before implantation exhibited an increase in expression (Vatta M et al., 2002).

**Reverse remodelling and adrenergic receptors**

LV unloading has been associated with restoration of cardiac β-AR signalling (Pandalai PK et al., 2006), reversal of the downregulation of β-AR (Ogletree-Hughes ML et al., 2001), an increase in β-AR responsiveness (Dipla K et al., 1998a) especially to inotropic stimulation by the SNS (Ogletree-Hughes ML et al., 2001), and an increase in β-AR density in both left and right ventricles (Klotz S et al., 2005a). The primary mechanisms behind these improvements is diminished myocardial G-protein coupled receptor kinase-2 activity and reduction in PKA hyperphosphorylation of the RyR2/Ca^{2+} release channels (Pandalai PK et al., 2006; Klotz S et al., 2005a). LVAD support has also been associated with an increase in α_{1}-AR density and alteration in distribution (Grigore A et al., 2005).

**Reverse remodelling and calcium regulation**

Terracciano et al, studied the effects of LVAD and myocardial recovery on SR Ca^{2+} content and EC coupling by studying isolated cardiomyocytes from the LVAD core at the time of LVAD implantation and from myocardial biopsy samples at the time of explantation in patients who recovered and at the time of transplantation in those who did not recover and were transplanted (Terracciano CMN et al., 2003; Terracciano CMN et al., 2004). They showed that recovered patients had an increase in their SR Ca^{2+} content and an increased gain in EC coupling resulting in larger Ca^{2+} transient and stronger cell contraction (figure 1.15).
Figure 1.15: SR Ca\textsuperscript{2+} content from myocytes isolated from LVAD cores and tissue from explanted (recovery) and transplanted hearts (without recovery). Cells were voltage-clamped at their resting membrane potentials. The SR Ca\textsuperscript{2+} content was significantly higher in the recovered patients and was associated with an improved current density.

The group speculated that the increased levels of SR Ca\textsuperscript{2+} could be due to one of the following: 1) increased SR Ca\textsuperscript{2+} uptake, 2) reduced SR Ca\textsuperscript{2+} leak as a consequence of a more stable ryanodine receptor complex, 3) or possibly, altered function of the sodium-calcium (Na\textsuperscript{+}/Ca\textsuperscript{2+}) exchanger.

Interestingly this study showed that reduction in cell capacitance and cell size were not necessarily associated with clinical recovery (Terracciano CMN et al.,
Further studies of the effects of LVAD on the Na\(^+\)/Ca\(^{2+}\) exchanger suggested that unloading increases the function of the exchanger (Terracciano CM et al., 2007), an important player which is over expressed in heart failure (Stagg MA et al., 2004).

**Reverse remodelling and neurohormonal levels**

Studies have shown that LVAD support lessened neurohormonal activation. Quantitative real time polymerase chain reaction revealed that LVAD support resulted in normalisation of ANP and BNP mRNA levels in a significant correlation fashion with reversal of cardiomyocyte hypertrophy (Kuhn M et al., 2004). Others have shown that LVAD support has induced significant reduction in NE, plasma renin activity, AngII levels, epinephrine, and vasopressin (Estrada-Quintero T et al., 1995;James KB et al., 1995).

1.5.2.3.ii LVAD and reverse remodelling – clinical application

In 1996, Dr Frazier was the first to report that the LV structure and function change with LVAD support (Frazier OH et al., 1996). His observation was confirmed when several groups studied the LV’s response to LVAD support and identified sufficient degree of myocardial recovery for device explantation (Frazier OH et al., 1996;Levin HR et al., 1996;Loebe M et al., 1997;Müller J et al., 1997;Mancini DM et al., 1998;Frazier OH & Myers TJ, 1999;Farrar DJ et al., 2002;Dandel M et al., 2005). In addition, examination of patients who had been transplanted showed that some reversibility of HF had been achieved at structural, cellular, molecular and functional levels although the mechanisms remained unknown (Altemose et al., 1997;Bartling et al., 1999;Bruckner et al., 2001;Heerdt et al., 2000;Hetzer et al., 2000;Li et al., 2001;Takeishi et al., 2000;Zafeiridis et al., 1998). Also patients who had had their device removed early due to infection or malfunction were observed to have good myocardial function that was maintained (Frazier & Myers, 1999;Hetzer et al., 1999).

Studies that have demonstrated myocardial recovery have all shown significant improvement in the ventricular dimensions with LVAD support. At 30 days of
Until recently, the explantation rate, however, remained small and ranged from 5-24% (Dandell M et al., 2005;Frazier OH & Myers TJ, 1999;Frazier OH et al., 2004b;Hetzer R et al., 2000;Hetzer R et al., 2001;Mancini DM et al., 1998;Mann DL & Willerson JT, 1998). In addition, HF has shown to recur again in some patients within the first two years of explantation (Hetzer R et al., 2000;Mancini DM et al., 1998) mainly in those who had spent more time on MCS support (Hetzer et al., 1999;Loebe et al., 1997b). One interpretation for this phenomenon was that excessive LV unloading might have resulted in cardiac atrophy (Loebe et al., 1997a;Madigan et al., 2001).

To enhance the degree of myocardial recovery some investigators have used additional pharmacological therapy including ACE inhibition to ameliorate neurohormonal activation and β-blockade to improve cardiac function and influence any underlying β-receptor autoimmune disease (Hetzer et al., 2000;Wallukat et al., 1996;Muller et al., 2000).

**Harefield Bridge-to-Recovery Protocol and Rationale**

The Harefield strategy was developed in an attempt to induce greater and more predictable recovery and allow elective explantation with reduced risk of re-dilatation and HF (Hon & Yacoub, 2003;Yacoub, 2001;Yacoub et al., 2001b;Yacoub et al., 2001a). The Harefield strategy combined mechanical unloading with pharmacological agents to assist unloading and induce maximal regression of pathological hypertrophy through reverse remodeling of the myocytes and cytoskeleton and normalisation of cellular metabolic function in non-ischaemic DCM patients.

The pharmacological regimen consists of two phases. In phase I five pharmacological agents are given based on the evidence described above of their clinical benefits in HF and induction of reverse remodeling. Oral medications are commenced after stopping all inotropic therapy following implantation of the
LVAD. Digoxin is the first to be introduced and is administered as positive inotrope. Both lisinopril and carvedilol are started at low doses and uptitrated according to patient’s tolerance. Spironolactone is then started. Low doses of spironolactone exert no diuretic effect and are insignificant haemodynamically. They are also effective in the treatment of HF as shown by the RALES trial. On this basis low dose administration was incorporated into the treatment regimen to inhibit the actions of aldosterone at the cellular level. Losartan is then added as the last anti-failure medication in those who tolerated the up-titration of both carvedilol and lisinopril. Table 1.8 represents that pharmacological regimen and the maximum dosage.

Table 1.8: Phase I pharmacological therapy

<table>
<thead>
<tr>
<th>Pharmacological Agent</th>
<th>Initial Dose</th>
<th>Maximum Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digoxin</td>
<td>62.5-125 mcg</td>
<td>125 mcg</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>25 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>2.5 mg od</td>
<td>20 mg bd</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>3.125 mg od</td>
<td>25 mg bd</td>
</tr>
<tr>
<td>Losartan</td>
<td>12.5 mg od</td>
<td>100 mg od</td>
</tr>
</tbody>
</table>

Table 1.8: The five pharmacological agents administered as a phase I therapy of the Harefield BTR protocol to induce reverse remodeling. Adapted from (Birks EJ et al., 2006).

The second stage of the pharmacologic therapy is instituted after maximal regression in the LVEDD has been achieved whilst the LVAD was in situ (but reduced to a degree to unmask the underlying LV function). When constant LVEDD (<60 mm) and EF (>50%) are maintained for at least 2 weeks, according to echocardiographic assessment, carvedilol is replaced by the selective β1-blocker, bisoprolol. Following maximal bisoprolol up-titration, as per patient tolerance, an attempt to induce physiological hypertrophy of both cardiac and skeletal muscle is made using the β2 agonist, clenbuterol (Hon J et al., 2001; Hon J & Yacoub M, 2003; Petrou M et al., 1995; Petrou M et al., 1999; Soppa G et al., 2004; Tansley P et al., 2004; Wong K et al., 1998; Yacoub M et al., 2001). Clenbuterol is started at 40 μg twice daily and is up-titrated to a maximum dose of
700 μg three times daily. The dose is adjusted to maintain a resting heart rate below 100 bpm.

In 2006, Birks et al prospectively studied 15 non-ischaemic DCM patients receiving LVAD and drug combination therapy, the Harefield Bridge-to-Recovery Protocol (Birks EJ et al., 2006). This strategy resulted in sufficient myocardial recovery that allowed LVAD to be explanted in 73% of the studied patients. 1- and 4-years actuarial survival of 90.9% and 81.8%, respectively, and freedom from recurrent HF in the surviving patients of 100% and 88.9% at 1- and 4-years, respectively.

Using the above protocol these patients demonstrated an improvement in PCWP from 24.9±7 mmHg on inotropic therapy before implantation to 9.0±4.1 mmHg, p=0.004, a significant 55% increase in the CI (Birks EJ et al., 2006). To illustrate the sustainability of myocardial recovery we have showed that at three months following device explantation the right atrial pressure (RAP) was 6.2±2.1 mmHg, PCWP was 12.8±6.9 mmHg, left ventricular end-diastolic pressure (LVEDP) was 12.9±5.9 mmHg, cardiac output (CO) was 4.9±2.1 l/min, CI of 2.4±1.0 l/min/m², and pulmonary-artery oxygen saturation (sVO₂) was 69.8± 29.9%. Repeat catheterisation at one year after explantation, revealed an RAP of 5.1±3.3 mmHg, PCWP of 9.5±6.2 mmHg, LVEDP of 9.3±5.5 mmHg, CO of 4.9±2.1 l/min, CI of 2.4±1.2 l/min/m², and sVO₂ of 73.5±32%.

Similarly, exercise capacity further improved following explantation as reflected by an increase in the oxygen consumption to a mean of 26.6 mls/min/kg at 2 years following explantation. This was better than that observed after cardiac transplantation and could be due to the fact that the recovery patients retained innervation or possibly be due to the effect of temporary treatment with clenbuterol.

Before discussing the benefits of using clenbuterol, it is essential to note that the hallmark feature of myocardial recovery is the “search” for recovery. This is achieved by the continuous and vigorous assessment of the underlying / native LV function to determine which patients would recover and when.
**Monitoring recovery**

Critical to myocardial recovery is the accurate assessment of myocardial function in these patients. Previously described weaning protocols have not assessed the true myocardial response to device cessation as the device’s contribution into the circulation remained significant (Dandel M et al., 2005; Maybaum S et al., 2003; Slaughter M et al., 2001). Those groups have generally relied on assessing patients whilst the device is on, or after only reducing the speed of the device and hence reducing its power, or after only momentarily discontinuing the device (Dandel M et al., 2005; Hetzer R et al., 2001; Maybaum S et al., 2003; Slaughter M et al., 2001). This has only provided a rough estimate of the underlying myocardial function. Full device cessation on the other hand allows clinicians to assess the true capacity of the unaided myocardium to support the circulation and importantly the response to increased demand through loading and exercise.

In our previous recovery series, the pulsatile HM I LVAD was switched off for 15 minutes or more and patients were exercised using a 6MW test with continuous monitoring of several clinical, haemodynamic and echocardiographic parameters (Birks EJ et al., 2006). This approach provided a more robust method to assess the true underlying function and inotropic reserve (George RS et al., 2006; George RS et al., 2007b; George RS et al., 2007a).

**Clenbuterol in the Harefield BTR protocol**

Concerns about cardiac muscle ‘atrophy’ following chronic LVAD myocardial unloading formed the basis for some explanations of low incidence of myocardial recovery (Soloff LA, 2009). In late 1990’s, Professor Yacoub hypothesised that in order to achieve consistent successful device explantation, regression of myocardial pathological hypertrophy should be followed by the induction of physiological hypertrophy (Yacoub MH, 2001; Yacoub MH et al., 2001b).

Until mid 90’s, the pathophysiology of cardiac hypertrophy was little known (Petrou et al., 1995). Two types exist depending on the stimulus and the mechanisms (Scheuer & Buttrick, 1987; Morgan & Baker, 1991). Isoproterenol (a $\beta_1$ & $\beta_2$ agonist) and norepinephrine lead to ‘pathological’ hypertrophy, whereas
thyroxine and exercise training produce the ‘physiological’ type (Bersohn & Scheuer, 1977).

‘Pathological’ myocardial hypertrophy seen in overload, hypothyroidism and after catecholamine administration involves re-expression of proteins as foetal isoforms. RNA molecular markers of gene expression are often used to differentiate the ‘pathological’ from ‘physiological’ condition (Lipkin et al., 1988). In rat heart models they comprise sarcomeric α-actins, cardiac myosin heavy chains (MHC)s and ANF (Petrov et al., 1995). Cardiac α-actin is the usual predominant type of sarcomeric α-actin, but in pathological hypertrophy, skeletal α-actin is induced instead (Izumo et al., 1988; Carrier et al., 1992). Similarly, MHC isoform expression also changes (Waspe et al., 1990; Schwartz et al., 1992). Down regulation of SERCA2 and PLB also occurs along with increased interstitial collagen that may result in impaired cardiac function (Kiss et al., 1995; Wong et al., 1997; Stein et al., 1996; Brilla et al., 1991; Doering et al., 1988).

‘Physiological’ myocardial hypertrophy is defined as increased LV mass with normal systolic and diastolic LV function, normal relaxation times (consistent with normal expression of SERCA2 and PLB), normal morphology, normal extracellular structure (LV collagen concentration) and gene expression (SERCA2 and PLB mRNA), and LV re-expression of ANF mRNA (non-specific molecular marker of LV hypertrophy) but without contractile protein isoform switching from cardiac to skeletal α-actin to β-MHC (Petrov et al., 1995; Wong et al., 1998; Scheuer et al., 1982; Scheuer & Buttrick, 1987; Buttrick et al., 1994; Lompre et al., 1984; Doering et al., 1988).

Clenbuterol induces this type of ‘physiological’ myocardial hypertrophy which can be summarized as follows:

- ‘morphologically’ - the induction of hypertrophy associated with improved function;
- ‘functionally’ - enhanced systolic and diastolic function;
- ‘histochemically’ by prevention of increased fibrosis; and
- ‘molecularly’ by ‘physiological’ gene expression (Petrov et al., 1995; Wong et al., 1998; Hon et al., 2001).
Clenbuterol, a potent synthetic pharmacological analogue to epinephrine, is used clinically in asthma and obstructive airways disease and formerly in the meat industry to increase muscle bulk. It was abused by athletes at the 1992 Barcelona Olympic Games for its ‘anabolic’ properties, despite not being a steroid and there was considerable subsequent dispute about its mechanism of action (Beckett AH, 1992; Petrou M et al., 1995).

Research groups have reported consistent beneficial functional effects of β₂ adrenergic overexpression in mouse hearts (Liggett et al., 2000; Milano et al., 1994; Turki et al., 1996). One of these showed that the beneficial effect of β₂ cardiac receptors is prolonged (Liggett et al., 2000). In addition, β receptor mediated apoptosis has been shown to be selectively associated with β₁ rather than β₂ receptors (Zaugg et al., 2000). One study showed that overexpression of β₂ receptors potentiated the functional recovery of unloaded failing hearts, consistent with our use of the drug (Tevaeearai et al., 2002). As most negative effects of β stimulation seem to be β₁ receptor mediated, administration of β₁ antagonists in conjunction with β₂ agonists such as clenbuterol appears appropriate. Others demonstrated that clenbuterol induced nerve regeneration (Frerichs O et al., 2001) and increased nerve growth factor mRNA (an important regulator of NET – see chapter 5) (Hayes VY et al., 1995).

In 2004, Terracciano et al showed that the use of clenbuterol in combination therapy, has induced an increase in cardiac SR Ca^{2+} content and prolonged the action potential (Terracciano et al., 2004). This group from our centre has also identified that chronic administration of clenbuterol induced changes in Ca^{2+} regulation, energy metabolism and organ and cellular hypertrophy in rat hearts. Investigating the mechanisms involved at molecular and cellular level have identified clenbuterol to induce development of cellular and organ hypertrophy (cardiac hypertrophy identified by echocardiography), increase Ca^{2+} transients and SR Ca^{2+} content without changes in the rate of Ca^{2+} decline in isolated ventricular myocytes, increase expression of SERCA2a, PLB and Na+/Ca^{2+} exchanger and increase oxidative carbohydrate utilisation in the heart (Soppa et al., 2004). Rise in Ca^{2+} transients generate larger contractions and can be explained by the increased Ca^{2+} content. In HF the Ca^{2+} transients are reduced in size and have a
slower decline, acting as a possible basis for systolic and diastolic dysfunction (Beuckelmann et al., 1992). Restoring Ca\(^{2+}\) content has been shown to reverse Ca\(^{2+}\) dysregulation (Hobai & O'Rourke, 2001).

The mechanism of myocardial growth after stimulation by clenbuterol remains unclear. It has been speculated that physiological myocardial hypertrophy might be an indirect myocardial response from increased demand from clenbuterol-induced hypertrophy of skeletal muscle. This is supported by the absence of necrosis or fibrosis in clenbuterol treated hearts in contrast to the effects of the β-agonist isoproterenol (Ferrans et al., 1964). In addition, in vitro clenbuterol stimulated cardiac myocyte culture has no effect on cell morphology, in contrast to that of isoproterenol which did cause change and induced skeletal α-actin in myocytes, a recognised feature of ‘pathological’ hypertrophy (Petrou et al., 1995; Bishopric & Kedes, 1991). Other mechanisms may also be involved as there is some evidence that myocardial β\(_2\) receptors operate through a different pathway to β\(_1\) receptors (Petrou et al., 1995; Kuznetsov et al., 1995).

In animal models, it induces skeletal muscle hypertrophy (Petrou M et al., 1995). Investigators showed chronic β2 agonist administration induces slow-to-fast fibre type transition resulting in an increase in greater, faster contractions with increased stroke power and reduced contraction and relaxation times (Petrou M et al., 1999; Zeman RJ et al., 2004). It induces IGF1 through which skeletal muscle hypertrophy is mediated (Awede et al., 2002). A trend is observed to increased fast isoforms of myosin heavy chain and SERCA (Petrou M et al., 1999). It also inhibits and reverses skeletal muscle atrophy caused by denervation, disuse, endotoxaemia and cachexia (Maltin et al., 1986; Maltin et al., 1992; Maltin et al., 1993; Delday & Maltin, 1997; Choo et al., 1989; Chance et al., 1991). Atrophic skeletal muscles are more sensitive to clenbuterol and can respond to doses sufficiently low to avoid general skeletal or cardiac hypertrophy (Maltin et al., 1992). Induction of skeletal muscle hypertrophy may have been beneficial as many patients with severe HF have structural and functional cachexia of their skeletal muscles (Lipkin et al., 1988; Mancini et al., 1992). In skeletal muscle, clenbuterol induces variable degrees of hypertrophy in the same organism which may relate to heterogenous β\(_2\) receptor density in different muscles (Maltin et al.,
1993; Williams et al., 1984). The antagonist propranolol has shown varying degrees of blockade, suggesting that other mechanisms may also be involved (MacLennan & Edwards, 1989; Maltin et al., 1987).

In summary, LVAD can be utilised as BTT, DT or BTR. At the non-clinical level LV unloading using LVAD has resulted in significant changes in adrenergic receptors, Ca²⁺ handling proteins, ECM and fibrosis, apoptosis, and myocyte characteristics. The use of the Harefield BTR protocol which uses reverse remodeling drugs followed by clenbuterol in the second stage of the pharmacological regimen has enhanced myocardial recovery and made recovery more sustainable. Clinically these changes were translated into significant improvements in haemodynamic and echocardiographic parameters, exercise tolerance, and QOL. The effects of LVAD and LV unloading, however, on different clinical components of the SNS such as contractile reserve and metaiodobenzylguanidine uptake remain to be investigated.
1.6 Aims of the study

As described above, LV unloading has a significant impact on haemodynamic and echocardiographic parameters as established in HF patients. The effects of unloading on the adrenergic receptors, calcium handling proteins, ECM and fibrosis, anti-apoptotic genes and proteins have been previously studied at the molecular and cellular level. The effects of LVAD, however, on the SNS at the clinical setting and the effect on NET (the failure of which is the reason why NE is abundant in heart failure which in turn stimulates the neurohormonal pathway) remain to be investigated.

Therefore, the main aims of this thesis are:

1) to examine prospectively the outcome of using continuous flow HM II LVAD and drug combination therapy on the frequency and durability of recovery,
2) to determine the clinical effects of LV unloading on cardiac sympathetic nervous system,
3) to examine the impact of LVAD and drug combination therapy on the activity of NET and neurohormonal levels.

Objectives:

Assessment of the native LV function depends on regular monitoring of myocardial recovery whilst the support of LVAD is ceased. The first objective is to determine a cut-off point where the contribution of the HM II LVAD could be interrupted to allow assessment of the native LV function without causing reloading of the LV. Secondly, the effects of LVAD and drug combination therapy on the sympathetic nervous system will be studied at a clinical setting by assessing the contractile reserve of the myocardium following exercise. \(^{123}\)I-metaiodobenzylguanidine nuclear imaging will be used to assess the effects of LVAD and drug combination therapy on NET response. The fourth objective is to attempt to correlate the concentration of NET-fibers in end-stage heart failure using immunohistochemistry techniques with myocardial recovery and to investigate the effects of unloading on the neurohormonal levels.
CHAPTER 2 - Study Population
2.1 Study Design

2.1.1 Population characteristics

From the 1st of February 2006 until the 31st of March 2009, 36 patients were implanted with continuous flow HM II LVADs. All patients were in NYHA class IV with severe low cardiac output state, on intravenous diuretic, and inotrope dependent. According to the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS), their pre-implant profile ranged from 1 – 2. In general all implanted patients satisfied most of the domains that make up the definition of advanced heart failure described by the European Society of Cardiology (Metra A et al., 2007) – Refer to Box 1 and Box 2 in Appendix A for the descriptions of INTERMACS classification and advanced HF, respectively.

Figure 2.1 is a consort diagram representing the flow of the patients; 23 fulfilled the inclusion criteria for this study and 13 did not (table 2.1 and sections 2.1.2 and 2.1.4).

**Figure 2.1: Consort diagram of all patients receiving HM II LVAD**

![Consort diagram](image)

*Fig 2.1: Consort diagram illustrating the flow of the 36 patients implanted with HM II LVAD during the study period.*
Table 2.1 Inclusion / exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Patient has refractory symptomatic heart failure (NYHA Class IV or Stage D)</td>
</tr>
<tr>
<td>due to dilated, non-ischemic cardiomyopathy</td>
</tr>
<tr>
<td>2. Severe clinical heart failure with associated haemodynamic compromise</td>
</tr>
<tr>
<td>resistant to intensive medical therapy and requiring LVAD implantation</td>
</tr>
<tr>
<td>3. LVEF ≤ 30% and cardiomegaly at the time of LVAD implantation as documented</td>
</tr>
<tr>
<td>by radionuclide or contrast ventriculography or by echocardiography</td>
</tr>
<tr>
<td>4. ≥ 18 years of age</td>
</tr>
<tr>
<td>5. Body surface area ≥ 1.5 m²</td>
</tr>
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</table>

<table>
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<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
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<td>1. Not a heart transplant candidate</td>
</tr>
<tr>
<td>2. Evidence of active acute myocarditis</td>
</tr>
<tr>
<td>3. Hypertrophic obstructive cardiomyopathy</td>
</tr>
<tr>
<td>4. Restrictive cardiomyopathy</td>
</tr>
<tr>
<td>5. Irreversible multiorgan failure</td>
</tr>
<tr>
<td>6. Underlying bleeding disorder</td>
</tr>
<tr>
<td>7. Pregnant or lactating woman</td>
</tr>
<tr>
<td>8. Unable to follow the recovery protocol i.e. perform “off-pump” testing to</td>
</tr>
<tr>
<td>assess the native LV function</td>
</tr>
</tbody>
</table>

Table 2.1: Inclusion and exclusion criteria to enter the recovery study

2.1.2 Demographics and clinical characteristics of included patients

Demographics and clinical parameters of individual patients are included in tables 2.2 and 2.3. 21 patients had idiopathic DCM and 2 had familia DCM (refer to section 2.1.3 for the histological analysis of the underlying pathology). Table 2.4 is a summary of pre-implant demographics and clinical parameters with data being presented as means ± S.D. (minimum – maximum). Prior to implantation four patients required short term mechanical support for 40.75 ± 30.52 days. Patient 2 was intubated and the use of short-term support allowed us to assess his neurological status prior to switching to long-term support. The remaining three (patients 6, 13, and 18) were not responsive to inotropic therapy and the decision to support with short-term device was based on the fact that they had severely deranged renal function and a period of stabilisation was required prior to commitment with longer-term support devices.
Table 2.2: Pre-implantation demographics and clinical parameters of each individual patient.

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<tr>
<th>Patient Number</th>
<th>Diagnosis</th>
<th>Gender</th>
<th>Disease Duration (mths)</th>
<th>Symptoms Duration (mths)</th>
<th>Implant Age (yrs)</th>
<th>BMI (kg/m²)</th>
<th>BSA (m²)</th>
<th>PMH</th>
<th>Number of Inotropes</th>
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<td>Duration</td>
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<td>1.74</td>
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**Table 2.2:** Pre-implant demographics. Table A.1 in Appendix A includes details of the implantation date and duration of study on individual patients.

* Patient 1 and 2 were intubated prior to device implantation
† Patients 2 and 5 were haemofiltered prior to device implantation
†† Patients 1, 5, 6, 8, and 19 had IABP prior to device implantation
§ Patients 2, 6, 13, and 18 had pre-implant short term support (patients 2, 6, and 13 had BiVAD Levitornics® and patient 18 had ECMO)
<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Pre-Implant Haemodynamics</th>
<th>Pre-Implant Echocardiography</th>
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Table 2.3: Pre-implantation haemodynamics and echocardiographic data
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<th>Urea (2.3-6.7 mmol/L)</th>
<th>Creatinine (60-130 μmol/L)</th>
<th>Bilirubin (0-20 μmol/L)</th>
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<th>ALT(5-65 U/L)</th>
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<td>16</td>
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<td>277</td>
<td>54</td>
<td>80</td>
<td>220</td>
<td>360</td>
</tr>
<tr>
<td>17</td>
<td>8.6</td>
<td>105</td>
<td>29</td>
<td>57</td>
<td>140</td>
<td>&gt;1352</td>
</tr>
<tr>
<td>18</td>
<td>13</td>
<td>248</td>
<td>60</td>
<td>57</td>
<td>1484</td>
<td>&gt;919</td>
</tr>
<tr>
<td>19</td>
<td>7.3</td>
<td>53</td>
<td>85</td>
<td>83</td>
<td>1258</td>
<td>214</td>
</tr>
<tr>
<td>20</td>
<td>13.5</td>
<td>139</td>
<td>8</td>
<td>64</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>21</td>
<td>13.6</td>
<td>138</td>
<td>39</td>
<td>84</td>
<td>32</td>
<td>&gt;919</td>
</tr>
<tr>
<td>22</td>
<td>9.2</td>
<td>145</td>
<td>60</td>
<td>87</td>
<td>101</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>5.5</td>
<td>84</td>
<td>18</td>
<td>91</td>
<td>17</td>
<td>112</td>
</tr>
</tbody>
</table>

**Table 2.3:** Pre-implant haemodynamics, echocardiographic and biochemical profile of individual patients.
Table 2.4: Summary of pre-implantation demographics of the studied population (n=23)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male : female)</td>
<td>21 : 2</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>21non-ischaemic idiopathic DCM, 2 familial</td>
</tr>
<tr>
<td>Age at implantation (years)</td>
<td>35.24 ± 14.27 (16.58 – 58.82)</td>
</tr>
<tr>
<td>Duration of Heart Failure (months)</td>
<td>31.29 ± 38.03 (1.5 – 132; median 12 months)</td>
</tr>
<tr>
<td>Symptoms duration (months)</td>
<td>5.07 ± 4.17 (1 – 14)</td>
</tr>
<tr>
<td>INTERMACS Classification</td>
<td>1.65 ± 0.49 (1 – 2)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.48 ± 11.41 (44.5 – 95)</td>
</tr>
<tr>
<td>Height (cms)</td>
<td>173.54 ± 8.51 (153 – 192)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.67 ± 3.00 (19.00 – 29.32)</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.85 ± 0.19 (1.38 – 2.18)</td>
</tr>
<tr>
<td>Number of inotropes</td>
<td>2.09 ± 0.85 (1 – 4)</td>
</tr>
<tr>
<td>Haemodynamic Parameters</td>
<td></td>
</tr>
<tr>
<td>Systolic PA pressure (mmHg)</td>
<td>47.61 ± 11.17 (31 – 72)</td>
</tr>
<tr>
<td>Diastole PA pressure (mmHg)</td>
<td>27.00 ± 6.32 (17 – 37)</td>
</tr>
<tr>
<td>Mean PA pressure (mmHg)</td>
<td>35.87 ± 6.56 (27 – 52)</td>
</tr>
<tr>
<td>PC Wedge Pressure (mmHg)</td>
<td>29.88 ± 6.42 (22 – 48)</td>
</tr>
<tr>
<td>Cardiac Output (l/min)</td>
<td>2.69 ± 1.01 (1.55 – 5.4)</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m²)</td>
<td>1.46 ± 0.57 (0.40 – 2.9)</td>
</tr>
<tr>
<td>PA saturation (%)</td>
<td>43.00 ± 11.36 (25 – 61.6)</td>
</tr>
<tr>
<td>Echocardiographic Parameters</td>
<td></td>
</tr>
<tr>
<td>End-diastolic diameter (mm)</td>
<td>72.33 ± 8.43 (62 – 91)</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>66.76 ± 7.66 (54 – 82)</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>7.61 ± 3.79 (2.47 – 19.74)</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>20.73 ± 8.96 (7.23 – 48.29)</td>
</tr>
</tbody>
</table>
Table 2.4 (cont.)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemical Parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>12.77 ± 2.22 (8.1 – 16)</td>
</tr>
<tr>
<td>White cell count</td>
<td>9.97 ± 3.58 (5.1 – 19.2)</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>12.01 ± 6.12 (4.6 – 30.3)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>158.61 ± 82.93 (53 – 341)</td>
</tr>
<tr>
<td>Bilirubin (μmol/L)</td>
<td>46.70 ± 24.98 (8 – 99)</td>
</tr>
<tr>
<td>Alkaline Phosphotase (U/L)</td>
<td>83.48 ± 45.21 (36 – 265)</td>
</tr>
<tr>
<td>Alanine Transaminase (U/L)</td>
<td>401.74 ± 590.71 (13 – 2180)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>31.48 ± 5.66 (23 – 45)</td>
</tr>
<tr>
<td>BNP (pmol/L)</td>
<td>338.22 ± 373.20 (16 – 1352)</td>
</tr>
<tr>
<td><strong>Number requiring pre-implant support</strong></td>
<td>4 patients</td>
</tr>
<tr>
<td><strong>Duration of pre-implant support (days)</strong></td>
<td>40.75 ± 30.52 (6 – 74)</td>
</tr>
<tr>
<td><strong>Type of support</strong></td>
<td>3 BiVAD Levitronix®, 1 ECMO</td>
</tr>
<tr>
<td><strong>Number requiring pre-implant ventilation</strong></td>
<td>2 patients</td>
</tr>
<tr>
<td><strong>Number requiring pre-implant haemofilter</strong></td>
<td>2 patients</td>
</tr>
<tr>
<td><strong>Number requiring pre-implant IABP</strong></td>
<td>5 patients</td>
</tr>
</tbody>
</table>

**Table 2.4:** Summary of pre-implantation demographics. Data are presented as means ± S.D. Minimum-maximum, where relevant, is presented in brackets.
2.1.3 **Histological analysis of included patients**

Histological evaluation of the LV cores obtained from the 23 studied patients at time of implantation showed interstitial and replacement fibrosis with myocyte hypertrophy, nuclear enlargement and occasional vacuolated myocytes, compatible with dilated cardiomyopathy. By conventional light microscopy there was no lymphocytic myocarditis, but occasional foci of mixed chronic inflammation surrounding damaged myocytes were noted, suggesting inotrope-related myocardial damage.

2.1.4 **Demographics and clinical characteristics of excluded patients**

As depicted in figure 2.1, 13 patients were excluded as they did not fulfil the inclusion criteria (table 2.1). Pre-implantation demographics, clinical parameters and reasons for exclusion for each individual patient are included in table 2.5.
Table 2.5: Excluded patients (n=13)

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Diagnosis</th>
<th>Gender</th>
<th>Implant Date</th>
<th>Implant Age (yrs)</th>
<th>Global EF (%)</th>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>IHD</td>
<td>M</td>
<td>20/07/2006</td>
<td>60.48</td>
<td>42</td>
<td>Ischaemic Origin</td>
</tr>
<tr>
<td>E2</td>
<td>HOCM</td>
<td>M</td>
<td>28/08/2006</td>
<td>41.07</td>
<td>12</td>
<td>HOCM</td>
</tr>
<tr>
<td>E3</td>
<td>PPM DCM</td>
<td>F</td>
<td>16/10/2006</td>
<td>35.09</td>
<td>28</td>
<td>Developed chest pain every time the speed of the HM II was reduced to assess the underlying myocardial function</td>
</tr>
<tr>
<td>E4</td>
<td>IHD</td>
<td>F</td>
<td>12/02/2007</td>
<td>45.43</td>
<td>30</td>
<td>Ischaemic Origin</td>
</tr>
<tr>
<td>E5</td>
<td>DCM</td>
<td>M</td>
<td>26/02/2007</td>
<td>57.46</td>
<td>10</td>
<td>Apical VSDs</td>
</tr>
<tr>
<td>E6</td>
<td>DCM</td>
<td>M</td>
<td>03/03/2007</td>
<td>27.51</td>
<td>10</td>
<td>Died 23 days after device implantation</td>
</tr>
<tr>
<td>E7</td>
<td>DCM</td>
<td>M</td>
<td>26/06/2007</td>
<td>47.20</td>
<td>-</td>
<td>Died 9 days after device implantation</td>
</tr>
<tr>
<td>E8</td>
<td>Becker’s MD</td>
<td>M</td>
<td>10/07/2007</td>
<td>18.67</td>
<td>-</td>
<td>Becker’s muscular dystrophy</td>
</tr>
<tr>
<td>E9</td>
<td>DCM</td>
<td>M</td>
<td>11/07/2007</td>
<td>21.87</td>
<td>24</td>
<td>Severe MR</td>
</tr>
<tr>
<td>E10</td>
<td>IHD</td>
<td>M</td>
<td>13/10/2007</td>
<td>56.04</td>
<td>25</td>
<td>Ischaemic Origin</td>
</tr>
<tr>
<td>E11</td>
<td>DCM</td>
<td>F</td>
<td>29/12/2007</td>
<td>28.85</td>
<td>15</td>
<td>Severe MR and prolonged ITU stay prevented from recovery assessment</td>
</tr>
<tr>
<td>E12</td>
<td>PPM DCM</td>
<td>F</td>
<td>30/09/2008</td>
<td>41.94</td>
<td>20</td>
<td>Poor clinic attendance and compliance with medication</td>
</tr>
<tr>
<td>E13</td>
<td>IHD</td>
<td>F</td>
<td>09/02/2009</td>
<td>32.08</td>
<td>-</td>
<td>Ischaemic Origin</td>
</tr>
</tbody>
</table>

Table 2.5: Basic demographics of the 13 excluded patients and the reasons for their exclusion from the study.
2.1.5 Pharmacological regimen

Following LVAD implantation, patients were started on Phase I medication of the Harefield Bridge-to-Recovery Protocol to induce reverse remodelling (Birks EJ et al., 2006; Yacoub MH et al., 2001a) immediately after weaning the patients from inotropic therapy with adequate end-organ recovery. As described in chapter 1, the regimen consisted of:

i) Cardiac digitalis (digoxin) – titrated up to 125 μg od;

ii) Non-selective β-blocker (carvedilol) – titrated up to 25 mg bd;

iii) Angiotensin converting enzyme inhibitor (lisinopril) – titrated up to 40 mg od; and

iv) Aldosterone antagonist (spironolactone) – titrated up to 25 mg od.

The second stage of pharmacologic therapy was commenced after maximal regression in the left ventricular end-diastolic diameter had been achieved while the left ventricular assist device was in place. When constant left ventricular end diastolic diameter (<60 mm) and ejection fraction (>50%) had been maintained for at least 2 weeks, according to echocardiographic assessment, carvedilol was replaced by the selective β₁ blocker, bisoprolol. Following maximal bisoprolol up-titration, as per patient tolerance, clenbuterol (β₂ agonist) was started at 40 μg bd and was up-titrated to a maximum dose of 700 μg tds to induce physiological hypertrophy (Birks EJ et al., 2006; Hon J et al., 2001; Hon J & Yacoub M, 2003; Petrou M et al., 1995; Petrou M et al., 1999; Soppa G et al., 2004; Tansley P et al., 2004; Wong K et al., 1998; Yacoub M et al., 2001). Clenbuterol dose was adjusted to maintain a resting heart rate below 100 bpm.
2.2 Post-implantation Clinical Outcome

2.2.1 Post implant RVAD support

Following the implantation of HM II LVAD, five patients had acute right ventricular failure requiring immediate support using RVAD for 25 ± 9.6 days (range 16-36 days). One patient (patient 13) who required 58 days of preoperative support with a BIVAD Levitronix® due to end-organ failure prior to his HM II implantation developed a dense left hemiparesis following his HM II upgrade and BIVAD removal. He recovered well from this event and managed recovery assessments with no further complications.

2.2.2 Recovered and non-recovered patients

By the 31st of October 2009 (the follow-up cut-off date – total study duration was three years and ten months), 15 patients had recovered and fulfilled the Harefield Bridge-to-Recovery explantation criteria whilst the remaining eight did not show any signs of myocardial recovery and were either transplanted or transplant listed (sections 2.3.2.1 and 2.3.2.2 include details of the recovered and the non-recovered patients). Table 2.6 represents a comparison of the pre-implantation clinical parameters between the recovered and the non-recovered patients. The total duration of HF was significantly higher in the non-recovered patients as compared to the recovered patients. In addition, ventricular dimensions were significantly larger in the non-recovered as compared to the recovered patients. Haemodynamic parameters were similar between the two groups.
Table 2.6: Comparison of the pre-implantation clinical parameters between the recovered (n=15) and the non-recovered (n=8) patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recovered Patients (n=15)</th>
<th>Non-Recovered Patients (n=8)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF Duration (months)</td>
<td>15.03 ± 24.81</td>
<td>61.75 ± 41.08</td>
<td>0.002</td>
</tr>
<tr>
<td>Implant Age (yers)</td>
<td>32.30 ± 13.85</td>
<td>40.73 ± 14.26</td>
<td>0.149</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.17 ± 3.20</td>
<td>24.61 ± 2.50</td>
<td>0.357</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.84 ± 0.21</td>
<td>1.88 ± 0.11</td>
<td>0.548</td>
</tr>
<tr>
<td>Systolic PA pressure (mmHg)</td>
<td>45.13 ± 8.20</td>
<td>50.43 ± 13.96</td>
<td>0.536</td>
</tr>
<tr>
<td>Diastole PA pressure(mmHg)</td>
<td>24.38 ± 6.25</td>
<td>30.00 ± 5.29</td>
<td>0.072</td>
</tr>
<tr>
<td>Mean PA pressure (mmHg)</td>
<td>33.38 ± 4.14</td>
<td>38.71 ± 7.91</td>
<td>0.121</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>27.50 ± 4.09</td>
<td>33.29 ± 7.87</td>
<td>0.070</td>
</tr>
<tr>
<td>Cardiac Output (l/min)</td>
<td>2.76 ± 1.17</td>
<td>2.54 ± 0.61</td>
<td>1.000</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m²)</td>
<td>1.54 ± 0.64</td>
<td>1.30 ± 0.39</td>
<td>0.525</td>
</tr>
<tr>
<td>PA saturation (%)</td>
<td>39.43 ± 12.29</td>
<td>47.28 ± 9.59</td>
<td>0.247</td>
</tr>
<tr>
<td>End-diastolic diameter (mm)</td>
<td>69.47 ± 7.38</td>
<td>79.50 ± 6.77</td>
<td>0.011</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>64.00 ± 6.49</td>
<td>73.67 ± 6.06</td>
<td>0.006</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>7.74 ± 4.20</td>
<td>7.29 ± 2.79</td>
<td>0.677</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>21.03 ± 10.04</td>
<td>20.11 ± 6.71</td>
<td>0.680</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>10.08 ± 4.45</td>
<td>16.09 ± 7.06</td>
<td>0.065</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>136.60 ± 69.07</td>
<td>201.78 ± 89.38</td>
<td>0.101</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>45.93 ± 29.56</td>
<td>45.56 ± 15.57</td>
<td>0.728</td>
</tr>
<tr>
<td>Alkaline Phosphotase (U/L)</td>
<td>85.60 ± 53.01</td>
<td>76.33 ± 27.75</td>
<td>0.925</td>
</tr>
<tr>
<td>Alanine Transaminase (U/L)</td>
<td>577.20 ± 671.55</td>
<td>90.78 ± 85.62</td>
<td>0.149</td>
</tr>
<tr>
<td>Albumin(g/dL)</td>
<td>31.67 ± 5.65</td>
<td>30.56 ± 5.90</td>
<td>0.925</td>
</tr>
<tr>
<td>BNP (pmol/L)</td>
<td>241.10 ± 259.31</td>
<td>459.50 ± 470.74</td>
<td>0.460</td>
</tr>
</tbody>
</table>

Table 2.6: Direct comparison of pre-implant demographics, clinical parameters and biochemical profile between the recovered and the non-recovered patients. Analysis performed using unpaired t-test and Mann-Whitney U test for normally distributed and non-parametric variables, respectively. Means ± SD.
Linear regression analysis revealed no correlation between pre-implant heart failure duration and myocardial recovery ($r=0.03; p=0.765$) and pre-implant LV dimension and myocardial recovery (LVEDD and LVESD). The total support duration in the recovered patients was $270.3 \pm 98.8$ days (range 138 – 474 days, median 227 days).

2.2.2.1 Recovered patients

These were the recovered patients: 1, 2, 4, 7, 8, 10, 11, 12, 13, 15, 18, 19, 20, 22, 23. All recovered patients were started on clenbuterol as the second phase therapy after achieving sustained regression in LVEDD (< 60 mm) for at least two weeks (the remaining criterion for commencing clenbuterol was discussed on page 118). Table 2.7 represents the explantation criteria.

Table 2.7: Explantation Criteria

<table>
<thead>
<tr>
<th>Parameter with the speed of HM II reduced to 6000 rpm for 15 minutes *</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end-diastolic diameter (mm)</td>
<td>&lt; 60</td>
</tr>
<tr>
<td>LV end-systolic diameter (mm)</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>&gt; 45</td>
</tr>
<tr>
<td>Maximal VO$_2$ (mls/min/kg)</td>
<td>&gt; 16</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mmHg)</td>
<td>&lt; 12</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m$^2$)</td>
<td>&gt; 2.8</td>
</tr>
</tbody>
</table>

Table 2.7: Explantation criteria as been previously described (Birks EJ et al., 2006).

* Clinical and bench-side experiments revealed that by reducing the speed of the HM II LVAD to 6000 rpm the underlying LV is unmasked sufficiently, enough to allow the assessment of the native LV function (see chapter 3).

A comparison of the pre-implantation and pre-explantation haemodynamic and echocardiographic parameters in the recovered group are presented in table 2.8.
Table 2.8: Comparison between pre-implantation and explantation parameters in the recovered group (n=15).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-implantation</th>
<th>Pre-explantation*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic PA pressure (mmHg)</td>
<td>45.13 ± 8.20</td>
<td>20.00 ± 4.66</td>
<td>0.020</td>
</tr>
<tr>
<td>Diastole PA pressure (mmHg)</td>
<td>24.38 ± 6.26</td>
<td>6.14 ± 2.80</td>
<td>0.040</td>
</tr>
<tr>
<td>Mean PA pressure (mmHg)</td>
<td>33.38 ± 4.14</td>
<td>11.36 ± 3.27</td>
<td>0.020</td>
</tr>
<tr>
<td>PC Wedge Pressure (mmHg)</td>
<td>27.50 ± 4.09</td>
<td>5.71 ± 4.29</td>
<td>0.008</td>
</tr>
<tr>
<td>LV End Diastolic Pressure (mmHg)</td>
<td></td>
<td>6.90 ± 6.39</td>
<td>N/A</td>
</tr>
<tr>
<td>Cardiac Output (l/min)</td>
<td>2.76 ± 1.17</td>
<td>6.33 ± 1.30</td>
<td>0.008</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m²)</td>
<td>1.54 ± 0.64</td>
<td>3.58 ± 0.65</td>
<td>0.008</td>
</tr>
<tr>
<td>PA saturation (%)</td>
<td>39.43 ± 12.29</td>
<td>68.66 ± 9.08</td>
<td>0.08</td>
</tr>
<tr>
<td>End-diastolic diameter (mm)</td>
<td>69.47 ± 7.38</td>
<td>50.07 ± 6.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>64.00 ± 6.49</td>
<td>33.53 ± 5.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>7.74 ± 4.20</td>
<td>33.28 ± 5.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>21.03 ± 10.04</td>
<td>69.81 ± 6.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximal VO₂ (mls/kg/min)</td>
<td></td>
<td>22.56 ± 4.80</td>
<td>N/A</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>10.08 ± 4.45</td>
<td>7.22 ± 2.82</td>
<td>0.311</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>136.58 ± 69.07</td>
<td>92.00 ± 13.22</td>
<td>0.03</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>45.93 ± 29.56</td>
<td>11.85 ± 3.73</td>
<td>0.001</td>
</tr>
<tr>
<td>Alkaline Phosphotase (U/L)</td>
<td>85.60 ± 29.56</td>
<td>73.46 ± 20.26</td>
<td>0.151</td>
</tr>
<tr>
<td>Alanine Transaminase (U/L)</td>
<td>577.20 ± 671.55</td>
<td>32.15 ± 23.96</td>
<td>0.010</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>31.67 ± 5.65</td>
<td>43.38 ± 16.40</td>
<td>0.006</td>
</tr>
<tr>
<td>BNP (pmol/L)</td>
<td>241.20 ± 259.22</td>
<td>8.52 ± 5.13</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2.8: Direct comparison between pre-implant and pre-explant parameters. Analysis performed using paired t-test for normally distributed variables and Wilcoxon signed-rank test for non-parametric variables. Data are presented as means ± SD. * Pre-explantation assessments were performed whilst on low-speed of 6000 rpm for 15 minutes.
2.2.2.1.i  
**Device explantation**

As of the 31st of October 2009, out of the 15 recovered patients, 14 were explanted and one patient (patient 20) was confirmed recovered as demonstrated by the echocardiographic and maximal VO\textsubscript{2} criteria and was waiting to be discussed in the MDT for device explantation.

8 of the 14 (57.1\%) explanted patients (patients 1, 10, 11, 12, 18, 19, 22, and 23) were explanted using the mini-invasive approach, previously described by Professor Yacoub (Tansley P & Yacoub M, 2002). Three patients had planned full redo-sternotomy (patients 2, 8, and 15) for the following reasons:

i) patient 2 had pre-implant BiVAD Levitronix\textsuperscript{®} and thought he may need an RVAD following explantation,

ii) the presence of thrombus in the ascending aorta of patient 8,

iii) patient 15 had parts of the upper lobe of the right lung in front of the outflow cannula as assessed by pre-implantation reconstructive CT scan.

One patient (patient 4) started with the limited surgical approach but developed RV failure once the LVAD was discontinued and hence the procedure was converted into median sternotomy to implant post explant RVAD.

The remaining two patients had limited median and lower sternotomies as precautionary measure. Patient 13 required a BiVAD Levitronix\textsuperscript{®} pre-upgrade to HM II LVAD and patient 7 had recurrent drive line infections which were resistant to antibiotics despite normalisation in his LV function.

2.2.2.1.ii  
**Predicors of myocardial recovery**

Receiver operating characteristics analysis was used to determine possible pre-implant predictors of subsequent recovery (table 2.9). Duration of HF, end diastolic and end-systolic diameters were all identified. HF duration of 12 months or less had 100\% sensitivity and 73.3\% specificity. Both pre-implant end-diastolic and end-systolic diameters of 77 mm or less and 70 mm or less, respectively, had sensitivities of 83.3\% and specificites of 86.7\%. Table 2.10 is a logistic regression
analysis representing the degree of the correlation between all pre-implant parameters and myocardial recovery.

Table 2.9: Predictors of myocardial recovery

<table>
<thead>
<tr>
<th>Pre-implant Variable</th>
<th>Area Under Curve (AUC)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF duration</td>
<td>0.833</td>
<td>100</td>
<td>73.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Pre-implant age</td>
<td>0.692</td>
<td>-</td>
<td>-</td>
<td>0.138</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.625</td>
<td>-</td>
<td>-</td>
<td>0.333</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>0.579</td>
<td>-</td>
<td>-</td>
<td>0.540</td>
</tr>
<tr>
<td>Systolic PA pressure (mmHg)</td>
<td>0.607</td>
<td>-</td>
<td>-</td>
<td>0.487</td>
</tr>
<tr>
<td>Diastole PA pressure (mmHg)</td>
<td>0.786</td>
<td>-</td>
<td>-</td>
<td>0.104</td>
</tr>
<tr>
<td>Mean PA pressure (mmHg)</td>
<td>0.750</td>
<td>-</td>
<td>-</td>
<td>0.105</td>
</tr>
<tr>
<td>PC Wedge Pressure (mmHg)</td>
<td>0.764</td>
<td>-</td>
<td>-</td>
<td>0.098</td>
</tr>
<tr>
<td>Cardiac Output (l/min)</td>
<td>0.509</td>
<td>-</td>
<td>-</td>
<td>0.955</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m²)</td>
<td>0.402</td>
<td>-</td>
<td>-</td>
<td>0.514</td>
</tr>
<tr>
<td>PA saturation (%)</td>
<td>0.733</td>
<td>-</td>
<td>-</td>
<td>0.201</td>
</tr>
<tr>
<td>End-diastolic diameter (mm)</td>
<td>0.856</td>
<td>83.3</td>
<td>86.7</td>
<td>0.013</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>0.872</td>
<td>83.3</td>
<td>86.7</td>
<td>0.009</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>0.567</td>
<td>-</td>
<td>-</td>
<td>0.640</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>0.562</td>
<td>-</td>
<td>-</td>
<td>0.647</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>0.742</td>
<td>-</td>
<td>-</td>
<td>0.208</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>0.717</td>
<td>-</td>
<td>-</td>
<td>0.093</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>0.550</td>
<td>-</td>
<td>-</td>
<td>0.699</td>
</tr>
<tr>
<td>Alkaline Phosphotase (U/L)</td>
<td>0.480</td>
<td>-</td>
<td>-</td>
<td>0.923</td>
</tr>
<tr>
<td>Alanine Transaminase (U/L)</td>
<td>0.308</td>
<td>-</td>
<td>-</td>
<td>0.138</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>0.513</td>
<td>-</td>
<td>-</td>
<td>0.923</td>
</tr>
<tr>
<td>BNP (pmol/L)</td>
<td>0.606</td>
<td>-</td>
<td>-</td>
<td>0.450</td>
</tr>
</tbody>
</table>

Table 2.9: ROC analysis of pre-implant variables
Table 2.10: Logistic regression analysis to correlated pre-implant parameters with myocardial recovery

<table>
<thead>
<tr>
<th>Pre-implant Variable</th>
<th>R-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF duration ≤ 12 months</td>
<td>0.699</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-implant age</td>
<td>0.288</td>
<td>0.183</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>0.232</td>
<td>0.285</td>
</tr>
<tr>
<td>BSA (m(^2))</td>
<td>0.114</td>
<td>0.598</td>
</tr>
<tr>
<td>Systolic PA pressure (mmHg)</td>
<td>0.244</td>
<td>0.378</td>
</tr>
<tr>
<td>Diastole PA pressure (mmHg)</td>
<td>0.459</td>
<td>0.085</td>
</tr>
<tr>
<td>Mean PA pressure (mmHg)</td>
<td>0.342</td>
<td>0.119</td>
</tr>
<tr>
<td>PC Wedge Pressure (mmHg)</td>
<td>0.457</td>
<td>0.065</td>
</tr>
<tr>
<td>Cardiac Output (l/min)</td>
<td>0.105</td>
<td>0.703</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m(^2))</td>
<td>0.214</td>
<td>0.408</td>
</tr>
<tr>
<td>PA saturation (%)</td>
<td>0.367</td>
<td>0.276</td>
</tr>
<tr>
<td>End-diastolic diameter ≤ 77 mm</td>
<td>0.603</td>
<td>0.004</td>
</tr>
<tr>
<td>End-systolic diameter ≤ 70 mm</td>
<td>0.671</td>
<td>0.001</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>0.055</td>
<td>0.812</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>0.045</td>
<td>0.830</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>0.443</td>
<td>0.084</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>0.371</td>
<td>0.081</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>0.045</td>
<td>0.846</td>
</tr>
<tr>
<td>Alkaline Phosphotase (U/L)</td>
<td>0.063</td>
<td>0.766</td>
</tr>
<tr>
<td>Alanine Transaminase (U/L)</td>
<td>0.416</td>
<td>0.082</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>0.045</td>
<td>0.833</td>
</tr>
<tr>
<td>BNP (pmol/L)</td>
<td>0.300</td>
<td>0.228</td>
</tr>
</tbody>
</table>

Table 2.10: Logistic regression analysis of the identified parameters in table 2.9 showed that HF duration ≤ 12 months, EDD ≤ 77 mm, and ESD ≤ 70 mm to correlate with recovery
Of the 14 explanted patients, two died at 14 and 26 days post-explantation from intra-cerebral haemorrhage and cerebral infarcts, respectively. Patient 8 was 21yrs with familial DCM and had signs of good recovery (pre-explant after 15 minutes of 6000 rpm (see chapter 3 for low speed testing protocol) his EF was 61%, LVEDD was 41 mm, LVESD was 30 mm, mVO$_2$ was 27.4 mls/min/kg, CO was 6.4 L/min, CI was 4 L/min/m$^2$, PCWP was 6 mmHg, and the PA saturations was 74.5%. He was explanted after 260 days of support. At explantation, transoesophageal echocardiogram revealed a new thrombus in the ascending aorta around the coronary sinuses. Subsequently the aortic root was explored with the HM II LVAD turned up to 10,000 rpm in the interim period in an attempt to stop the valve opening. However, by the time the root was explored there was no thrombus remaining. It took several attempts to wean him from cardiopulmonary bypass and a Levitronix® short-term LVAD had to be inserted. He then had a period of profound vasoplegia requiring norepinephrine, vasopressin and haemofiltration. By the sixth post operative day he was weaned from all inotropes, extubated and off haemofiltration. However, he then became septic which started resolving when he had a massive intra-cerebral haemorrhage and died on post operative day 14.

The second patient, patient 7, had had reasonable recovery (pre-explant at 6000 rpm, his mVO$_2$ was 21.5 mls/min/kg, CO was 7.06 L/min, CI was 3.4 L/min/m$^2$, PCWP was 2.0 mmHg, PA saturation was 70%, EF was 59%, LVEDD was 58 mm, and LVESD was 43 mm) but had recurrent drive line infection (Enterobacter cloacae and Acinetobacter baumanii). He was explanted after 474 days of support once the driveline infections were considered under control. Following explantation, he was extubated in the operating room and initially did extremely well. However on day 3 he was sitting out of bed when he became very sweaty, tachycardic and had a VF arrest. He required CPR, re-intubation and ECMO insertion (post arrest EF was 5%). Unfortunately he made a poor neurological recovery, decerebrated and CT suggested ischaemic change. ECMO was withdrawn and he died on day 26 post-explantation.
A further patient (patient 4) required 7 days of short-term RVAD support following explantation due to intra-operative air passing down the right coronary artery, but recovered well. She remains extremely well and active 690 days following explantation with LVEDD of 48 mm, LVESD of 29 mm, EF of 64.6%, and mVO$_2$ of 21.2 mls/min/kg.

The remaining 11 explanted patients remain in NYHA class I 371.75 ± 342.85 days post device explantation (range 24 – 1123 days, median 256 days) with LVEDD 61.18 ± 8.66 mm, LVESD 45.50 ± 11.84 mm, FS 25.03 ± 7.97%, EF 56.52 ± 15.14%, and mVO$_2$ 22.97 ± 5.20 mls/min/kg.
2.2.2.2 Non-recovered patients

Pre-implantation parameters of the eight non-recovered patients were included in table 2.6. They were transplant listed following 395.50 ± 139.33 days of LVAD support (range 215 – 607 days, median 354 days) as they had not met the explantation criteria highlighted in table 2.7.

Main reasons for not recovering were chronic drive line infection in two patients (patients 5 and 9) and resistance to recovery with no signs of myocardial recovery on echocardiography despite maximal phase I medication in five patients (3, 6, 16, 17 and 21). Patient 14 showed some signs of myocardial recovery after 8 months of support; however, he developed chronic drive line infection which has resulted in a significant reduction in his LV function / performance. None of the non-recovered patients received phase two therapy - clenbuterol. Table 2.11 represents a direct comparison between pre-implantation and pre-transplant listing echocardiographic parameters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-implantation</th>
<th>Pre-transplant listing</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-diastolic diameter (mm)</td>
<td>79.50 ± 6.77</td>
<td>71.88 ± 10.45</td>
<td>0.147</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>73.67 ± 6.06</td>
<td>64.38 ± 12.61</td>
<td>0.124</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>7.29 ± 2.79</td>
<td>10.80 ± 6.94</td>
<td>0.268</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>20.11 ± 6.71</td>
<td>27.91 ± 16.35</td>
<td>0.261</td>
</tr>
</tbody>
</table>

Table 2.11: Comparison between pre-implantation and transplant listing echocardiographic parameters in the non-recovered group (n=8).

Table 2.11: Echocardiographic comparison between pre-implant and pre-transplant. Analysis performed using paired t-test. Data are presented as means ± SD. Two of the eight patients were transplanted. Patient 5 was transplanted 131 days after listing with total LVAD support duration of 607 days. Patient 9 was transplanted 65 days post listing with total LVAD support duration of 298 days. Both patients died of primary graft failure 38 days and 82 days post transplantation, respectively. The other six patients remain on the transplant waiting list as of the 31st of October 2009. * Pre-transplant listing assessment was performed whilst on low-speed of 6000 rpm for 15 minutes.
2.3 Discussion

DCM is one of the commonest cardiomyopathies. There is a 3:1 male to female ratio and most presentations occur between 3\textsuperscript{rd} - 5\textsuperscript{th} decades with high associated mortality (Dec & Fuster, 1994). In the 23 studied patients the average age of presentation was 35 years (range 16.5 - 59) with male to female ratio of 10.5:1. Aetiological heterogeneity is likely to include direct myocardial toxicity from factors such as alcohol, drugs or heavy metals, viral infection, altered immunity, familial factors, and peri-partum states (Davies et al., 1995; Why et al., 1994; Schonian et al., 1993; Kandolf & Hofschneider, 1989; Caforio et al., 1994). In our studied patients, DCM had non-ischaemic origin, no evidence of myocarditis and none of the patients had post-partum DCM.

The most possible cause of idiopathic DCM in our patients was viral infection. Viral infection in animal models can cause acute myocarditis with a high mortality. After a long latent period in survivors, HF may occur in which myocardial changes consistent with DCM develop (Schnitt et al., 1993). This has been found with Coxsackie infection in mice and Papovavirus in dogs. Similarly in humans, some studies have shown that long-term LV dysfunction can follow viral myocarditis and that some develop DCM (Levi et al., 1988; Matoba et al., 1990; Tazelaar & Billingham, 1987). However, most have no known previous acute myocarditis. This may be true in our patients such that histological evaluation of LV core showed evidence of inflammation with no lymphocytic infiltration which is hallmark in the diagnosis of myocarditis.

The mechanisms by which viral infection may induce LV dysfunction remain unclear. Genetic studies have shown that less than 50% of DCM patients are positive for enteroviruses with viral RNA present in 25% (Kandolf et al., 1987). Proposed mechanisms for the role of viral infection in DCM pathogenesis include direct viral cytotoxicity, immunological involvement and viral RNA persistence (Martino et al., 1994). Enteroviral protease 2A has been shown to cleave dystrophin thus impairing structure and function of the myocardial cytoskeleton, acting as possible mechanism of direct coxsackie myocardial cytotoxicity (Badorff et al., 1999).
Two of the studied patients (patients 8 and 12) had familial DCM and both had their HM II LVAD explanted for myocardial recovery. Familial inheritance has been identified in 20-30%, with a prevalent autosomal dominant trait and variable penetrance (Mestroni & Giacca, 1997). Underlying genetic defects require further investigation along with the molecular basis of the myocardial dysfunction. Genes possibly involved might affect immune regulation or be responsible for normal heart function. X-linked DCM and rare recessive forms also exist.

**Pre-implant support with Levitronix® short-term device**

The use of Levitronix® VAD, a short-term low cost device, has been described as a bridge-to-decision in extremely sick patients who have contraindications to the implantation of a long-term VAD or requiring urgent transplantation (Bhama JK et al., 2009; DeRobertis F et al., 2008). The use of this short-term device has proven to be very effective in stabilising the haemodynamic status and improve the end-organ function prior to deciding if a more expensive device or transplant should be used. Four of our patients required pre-implantation short-term support for 40.75 ± 30.52 days. One of the patients (patient 2) was intubated and the use of short-term support allowed us to assess his neurological status prior to switching to long-term support. The remaining three (patients 6, 13, and 18) were not responsive to inotropic therapy and the decision to support with short-term device was based on the fact that they had severely deranged renal function and a period of stabilisation was required prior to commitment with longer-term support devices.

**DCM and histological features**

In the studied group, histological evaluation of the LV cores acquired at implantation showed interstitial fibrosis, with myocyte hypertrophy and nuclear enlargement. DCM is characterised by abnormal morphological features such as increased heart mass, weight and LV cavity diameter, with normal or reduced LV wall thickness. There is impaired systolic contraction, reduced EF and increased end-systolic and end-diastolic volumes (Richardson et al., 1996). Histological diagnosis of DCM can be difficult due to variable myocyte changes and interstitial fibrosis. The histological pattern of DCM may be described as a complex of non-
specific features, common to many end-stage cardiac conditions. All myocardial components are involved comprising interstitium, small vessels and endocardium but myocytes are the most profoundly affected (Mosseri et al., 1986; Tanganelli et al., 1990). Hypertrophic myocytes often contain irregularly shaped enlarged nuclei. Attenuated myocytes are also present and myofibrillar loss is typical. Myocytes often appear abnormally vacuolated and variable myocyte death may be present. Coexistence of large hypertrophied and small, attenuated cells, therefore produces a characteristically variable picture. Increased intramyocardial fibrillar collagen, especially type I:III ratio is typical (Marijanowski et al., 1995). Increased fine and coarse interstitial fibrosis is common, but focal replacement sclerosis and apical endocardial fibrosis may also be present. About one-third exhibit an increase in inflammatory cells (Davies et al., 1995) which infiltrate the myocardium typically adjacent to areas of replacement fibrosis. Much controversy has arisen regarding their quantification and utility to distinguish DCM from myocarditis (Aretz et al., 1987).

**RVAD support following implantation**

Following implantation 5 patients (21.7%; patients 3, 10, 15, 17, and 21) needed a short term RVAD Levitronix® due to acute right ventricular failure. Interestingly, Maeder et al reported that RV dysfunction is an acute feature and does not worsen during the intermediate term (Maeder MT et al., 2009). The number of RVADs needed post LVAD implantation was consistent with previous studies where 11-30% develop right-sided HF post-LVAD implantation (Goldstein DJ et al., 1998; Frazier OH et al., 1992; Farrar DJ et al., 1985; Pavie A & Leger P, 1996; Van Meter CH, 2001; Fukamachi K et al., 1999). The causes for right-side circulatory failure are multifactorial and are considered to be related to anatomic, intra-operative and perioperative factors. In many instances, right-sided HF becomes clinically evident only after LVAD support has been initiated. It is difficult to predict which patients will develop RV failure after LVAD implantation, and those who do have a poor prognosis (Morgan JA et al., 2004; Deng MC et al., 2005; Kavarana MN et al., 2002; Dang NC et al., 2006). In this studied series none of those requiring an RVAD post HM II LVAD implantation had pre-operative features of right-side HF.
Despite the frequency and significance of RV failure in LVAD recipients, relatively few studies have identified predictors of post-LVAD RV failure. More concerning is the lack of consensus among these. For example, Dang et al reported that elevated central venous pressure (CVP) predicts post-LVAD RV failure, but no study has corroborated this finding (Dang NC et al., 2006). Similarly, Kormos et al identified pulmonary edema, fever without infection and pre-operative mental impairment as predictors of RVAD use, but no subsequent study has provided confirmation (Kormos RL et al., 1996). More recently, Fitzpatrick III et al have devised a score that predicts which LVAD patient will require an RVAD support (Fitzpatrick III JR et al., 2008). Based on analysing data from 266 LVAD recipients, the investigators identified the following six criteria as risk factors: CI $\leq 2.2$ l/min/m$^2$, creatinine $\geq 1.9$ mg/dl, right ventricular stroke work index $\leq 0.25$ mmHg.litre/m$^2$, severe pre-VAD RV dysfunction, previous cardiac surgery, systolic blood pressure $\leq 96$ mmHg. For a high risk criterion the group assigned a score of 1 and for low risk criterion they assigned a score of 0 (e.g. for creatinine $\geq 1.9$ mg/dl a score of 1 was assigned and for creatinine $< 1.9$ mg/dl a score of 0 was assigned). The scores were then entered into the final risk score equation:

$$18(\text{CI}) + 18(\text{right ventricle stroke work index}) + 17(\text{creatinine}) + 16(\text{previous cardiac surgery}) + 16(\text{pre-implant RV dysfunction}) + 13(\text{systolic blood pressure})$$

Maximum possible score is 98 and a score of $\geq 50$ predicted the need of an RVAD with sensitivity and specificity of 83% and 80%, respectively. This score could have not been applied in our group since all 23 patients were on at least two inotropes and those with measurable central haemodynamics had a CI of less than 2.2 (apart from patient 1 who was on IABP and 4 inotropes, had a CI of 2.9). Fitzpatrick III et al did not take inotropic support into consideration (Fitzpatrick III JR et al., 2008). Therefore, the systolic blood pressure would have been an inaccurate representation. In matter of fact the three patients who required pre-HM II BiVAD Levitronix® did not need an RVAD post upgrade with HM II LVAD. To conclude, right-sided HF stems from changes in right ventricular geometry and flow/pressure dynamics as a direct result of LV unloading (Dang
Regardless of the cause, unless properly corrected, right-side HF results in considerable disturbances in volume and pressure distribution across both systemic and pulmonary circulation. These effects have untoward consequences on other organ systems and confer significant morbidity and mortality.

**Use of ICD post implantation**

Andersen et al demonstrated that 12 out of 23 patients implanted with HM II LVAD developed sustained VT or VF with 8 requiring ICD or external defibrillator shock (Andersen M et al., 2009). In our studied patients none had developed VT or VF. The main reason is that we ensured that LV was not sucked down by adjusting the speed using echocardiography to ensure no septal shift. Also digoxin was started as early as possible once inotropes have been stopped.

**Pharmacological therapy**

Phase I pharmacological therapy was commenced as soon as inotropic support was stopped post device implantation. The benefits of using β-blockers, ACE-I, AngII antagonists, aldosterone antagonist and digitalis have been discussed in chapter 1. In most cases lisinopril was commenced as the first oral medication based on the facts that ACE-I was the first agent to demonstrate a mortality benefit in HF patients. Further, ACE-I tend to induce vasodilatation which improves the neurohormonal profile and hence allows a certain degree of stabilisation prior to commencing β-blocker therapy (Hall SA et al., 1995).

Switching between different β-blocker therapies has previously shown to be safe and tolerable providing the switch is managed carefully (Abraham WT, 2003). In our studied patients, carvedilol was switched to bisoprolol to spare β2 receptors for clenbuterol over an average of two weeks. The initial dose of bisoprolol is 1.25 mg once daily which is increased to 10 mg once daily. The use of clenbuterol as a pharmacological agent to induce LV “physiological” hypertrophy was described in chapter 1.
**Clinical outcome**

As discussed earlier, the hallmark of recovery is to assess the native LV function by minimising the input from the device. Previously described weaning protocols have not assessed the true myocardial response to device cessation as the device’s contribution into the circulation remained significant (Dandel M et al., 2005; Maybaum S et al., 2003; Slaughter M et al., 2001). In our previous recovery series, the pulsatile HM I LVAD was switched off for 15 minutes or more and patients were exercised using a 6MW test with continuous monitoring of several clinical, haemodynamic and echocardiographic parameters (Birks EJ et al., 2006). This approach provided a more robust method to assess the true underlying function and inotropic reserve (George RS et al., 2006; George RS et al., 2007b; George RS et al., 2007a).

In this series the speed of the HM II LVAD was reduced to 6000 rpm. The choice of this speed was based on laboratory and clinical experiments (see chapter 3). The recovery rate with the continuous flow HM II LVAD and drug combination therapy was 65.2% (15 out of 23). This was close to our previous series where the rate of recovery was 73.3% (11 out 15) in patients treated with the pulsatile HM I LVAD and the same pharmacological regimen (Birks EJ et al., 2006). Following explantation 12 patients remain alive with excellent myocardial function and exercise tolerance.

Receiver operating characteristics analysis revealed that short HF duration, small end-diastolic and end-systolic diameters are favourables of subsequent recovery. Plotting those variables identified HF duration of 12 months or less, end-diastolic diameter of 77 mm or less, and end-systolic diameter of 70 mm or less, as possible predictors of recovery. Combining these results with logisitc regression analysis determined a correlation between those cut-off points and recovery. To our knowledge this the first report that had identified potential predictors of recovery at the time of implantation. However, these data need to be interpreted cautiously as 4 out of the 15 recovered patients (26.7%) had HF duration longer than 12 months.
Device explantation

Explantation using the mini-invasive approach was previously described by Professor Yacoub in explanting the pulsatile HM I LVAD (Tansley P & Yacoub M, 2002). This approach involves exposing the apex of the heart through an anterolateral mini-thoracotomy. Two other separate small incisions are made; an epigastric incision to expose the device and a limited anterior thoracotomy through the second intercostal space to expose the aortic anastomosis of the outflow cannula (figure 2.2).

Figure 2.2: Mini-invasive explantation technique

Fig 2.2: Sites of the mini-invasive incisions with arteriovenous groin cannulation. From (Tansley P & Yacoub M, 2002). Incision (i) an anterolateral mini thoracotomy to expose the apex of the heart and disconnect the inflow cannula, incision (ii) is performed to expose the device and the power cable, and incision (iii), a limited anterior thoracotomy incision through the second intercostal space to disconnect the outflow graft from the ascending aorta.
To verify the exact position of the inflow and the outflow cannulae, a planned reconstructive CT scan was performed prior to device explantation which was discussed in the transplant / VAD MDT (figure 2.3).

**Figure 2.3: A pre-explant reconstructive CT scan**

![CT scan image showing the inflow cannula in the LV apex and the outflow graft anastomosed to the ascending aorta behind the sternum.](image)

**Fig 2.3:** A pre-explant reconstructive CT scan showing the inflow cannula in the LV apex and the outflow graft anastomosed to the ascending aorta behind the sternum. In this patient median sternotomy was challenging due to the position of the outflow graft.

In 2008, we have described a similar approach in explanting the HM II LVAD (Haj-Yahia S et al., 2008). This surgical approach limited post-operative complications and the necessity of use of blood products. In a retrospective review of 23 patients explanted using limited surgical approach, we have demonstrated that the median post-operative duration of ventilation, intensive care unit length of stay and hospital stay were 1.5, 4, and 23 days, respectively and the median postoperative blood loss was 263ml. The median numbers of blood products given within the first 24 hours postoperatively were 2, 3, 1 and 0 units for cross matched blood, fresh frozen plasma, platelets and cryoprecipitate,
respectively. Two (8.7%) patients had wound infection with a cumulative postoperative survival rate of 87% (Haj-Yahia S et al., 2009).

Finally, none of our studied patient had had device related malfunction / failure despite maximal support duration of 989 days in a non-recovered group patient. This is considered superior to the first generation devices which have been associated with life-threatening device failure incidences (Birks EJ et al., 2004a). The most common types of pulsatile device related malfunction are: inflow valve regurgitation, kinking in the outflow graft, embolisation from the LVAD pumping chamber, acute motor cessation requiring support with pneumatic driver, ruptured pump diaphragm, fractured power cable, and pneumoperitoneum resulting from a leak at the junction of the pneumatic driveline (Birks EJ et al., 2004a; Horton SC et al., 2004; Martin J et al., 2006). Apart from possible power cable fracture, none of the other complications is likely to have occurred in the HM II LVAD as the device consists of one moving component only (figure 2.4) and hence the risk of malfunction is dramatically improved.

**Figure 2.4: The interior structure of the HM II LVAD**

![Image of the interior structure of the HM II LVAD](image)

**Fig 2.4:** A schematic diagram of the interior structure of the HM II LVAD showing one moving component only.

The use of the third generation devices will minimise the risk of device malfunction even further as they will work on bearing-less principle. Further, when the technology of Transcutaneous Energy Transfer System is introduced, drive line related infections will be eradicated.
2.4 Study Plan

Due to different clinical reasons not every patient was able to contribute in every component of the thesis. Reasons for exclusion from each study are included in the beginning of the methods section in each chapter. Figure 2.5 is a consort diagram representing a general overview of the flow of the 23 patients throughout the thesis and table 2.12 is a detailed representation of their flow.

Figure 2.5: Consort diagram representing the number of patients included in each component of the thesis
Table 2.12: Flow of individual patients throughout the thesis

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Recovery Status</th>
<th>Chapter 3 (Low Speed Assessment)</th>
<th>Chapter 4 (Echocardiograms, Contractile Reserve &amp; 6-Minute Walk Test)</th>
<th>Chapter 5 (MIBG)</th>
<th>Chapter 6 (Immunohistochemistry)</th>
<th>Chapter 7 (Catecholamine Levels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Recovered</td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>2</td>
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<td>✓</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>3</td>
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<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>4</td>
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<td>✓</td>
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<td>✓</td>
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<td>×</td>
</tr>
<tr>
<td>6</td>
<td>Non-recovered</td>
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<td>✓</td>
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<td>×</td>
</tr>
<tr>
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<td>✓</td>
</tr>
<tr>
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<tr>
<td>19</td>
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<td>×</td>
</tr>
<tr>
<td>20</td>
<td>Recovered</td>
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<td>×</td>
<td>✓</td>
<td>×</td>
</tr>
<tr>
<td>21</td>
<td>Non-recovered</td>
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<td>✓</td>
<td>✓</td>
<td>×</td>
<td>✓</td>
</tr>
<tr>
<td>22</td>
<td>Recovered</td>
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<td>✓</td>
<td>✓</td>
<td>×</td>
<td>✓</td>
</tr>
<tr>
<td>23</td>
<td>Recovered</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Table 2.12: The flow of individual patients throughout the different components of the thesis. Tick (✓) implies patients involvement in different studies. Cross (×) indicates that the patient did not contribute in different components of the thesis (reasons for exclusion are included in each chapter).
CHAPTER 3 - Low Speed “Off-Pump” Testing
3.1 Background

Recovery of myocardial function can occur following LVAD support (Farrar DJ et al., 1990; Frazier OH & Myers TJ, 1999; Hetzer R et al., 2001; Entwistle JW 3rd, 2003; Birks EJ et al., 2006). This myocardial recovery can be sufficient to allow device removal. However, monitoring recovery prior to device explantation remains an issue such that assessment of the degree of myocardial recovery depends critically on a safe, accurate and reproducible method of monitoring the native LV function during the period of LVAD support.

Some investigators have relied on examining patients while the device is on, after reducing the speed of the device and hence reducing its power, or after momentary discontinuation of the device (Hetzer R et al., 2001; Maybaum S et al., 2003; Slaughter M et al., 2001; Myers T et al., 2006). Each of these techniques, however, provided an indication of the native myocardial function rather than a true assessment of the underlying function.

We have previously described our experience with recovery assessment in patients with pulsatile HM I XVE LVAD (Thoratec Corp., Pleasanton, California, USA) where acute device cessation “off-pump” after heparinisation was safe and efficient to assess the underlying function and the inotropic / contractile reserve of the native myocardium (George RS et al., 2007b; George RS et al., 2006; George RS et al., 2007a). In these studies, HM I XVE LVAD was switched off completely in 22 patients for 15 minutes with intermittent hand-pumping to avoid blood stagnation within the device, followed by a 6MW exercise with continuous monitoring of several clinical, peripheral haemodynamic and echocardiographic parameters. This approach provided a robust method to assess the true underlying function and the inotropic reserve and was implemented in the explantation guidelines for myocardial recovery (Birks EJ et al., 2006).

Unlike the pulsatile flow LVADs, continuous-flow LVADs do not have a one-way valve and therefore switching-off of the continuous flow devices would result in blood regurgitating in the backward direction “reverse flow” into the left ventricle causing full loading of the LV. As could be expected backward flow will
happen in diastole, analogous to aortic regurgitation, and its volume will be maximal when the device is switched off. Having blood regurgitating in the backward direction will result in an inaccurate assessment. Therefore, it is important to examine the native myocardium at a speed where there is no backward flow through the device into the LV and where the forward flow contribution from the device into the circulation is kept to minimal. This approach would provide an “off-pump” testing equivalent to that used in patients with HM I XVE LVAD (George RS et al., 2007a).

In early local pilot examinations, we have used 6000 rpm as the lowest speed to assess the native myocardial function in patients with HM II LVAD. This speed was determined by the Thoratec engineers based on estimation that at 6000 rpm the forward flow generated by the HM II LVAD would counteract the reverse flow occurring in diastole. However, we did not have concrete clinical or experimental data to illustrate whether the speed of 6000 rpm would be sufficient to assess the native myocardial function and the effects of speed reduction on forward and retrograde flows.

The aims of the present study were therefore to: 1) identify the optimal speed at which the native myocardium could be assessed; 2) determine whether 6000 rpm is sufficiently low to assess the native myocardial function; and 3) establish whether it is possible for blood to regurgitate in the backward direction into the LV whilst the rotor is running.

This component of the thesis consists of three parts; part one is a preliminary multi-level statistical modelling illustrating the lack of validity of examining the native LV function whilst the device is on full support and the importance of reducing the speed of the HM II LVAD to assess the underlying LV function. Part two is a bench work experimental study that tests the effects of different speeds on flow in a mock circuit. Part three is a clinical study investigating the effects of different speeds of the HM II LVAD on LV dimensions and function in a subset of the population.
3.2 Part One – Multi-Level Modelling

3.2.1 METHODS

3.2.1.1 Echocardiographic Assessments of the LV Size and Function

All 23 HM II LVAD patients were included in this analysis. Pre-implantation demographics are included in chapter 2.

Echocardiographic parameters were measured sequentially in each patient every 4 to 6 weeks at four different conditions:

i. Condition A - device running at baseline speed;

ii. Condition B - at 5 minutes of 6000 rpm (5Mins);

iii. Condition C - at 15 minutes of 6000 rpm (15Mins); and

iv. Condition D - after a 6-MW test whilst on 6000 rpm.

All measurements were performed in the M-Mode. Left ventricular end diastolic diameter (LVEDD) and Left ventricular end systolic diameter (LVESD) were obtained from the parasternal long axis of the LV at the level of chordal-mitra valve junction. Fractional shortening (FS) was calculated as the percentage change in the LV cavity dimension with systole whilst ejection fraction (EF) was calculated using the cubed function formula of the ventricular dimensions. Further details of the assessments are included in chapter 4.

3.2.1.2 Statistical analysis

To illustrate the necessity of speed reduction in the assessment of the underlying LV function a multilevel modelling over a 1-year of LVAD support was performed using MLwiN version 2.14 for windows (Centre for Multilevel Modelling, university of Bristol, United Kingdom). This model looks at the changes in the trends of the examined parameters over a one year period by averaging the response of each parameter at each condition for all the patients. It is assumed that for each patient there is a linear relationship between the mean of each of the M-Mode measured parameters taken at each of the four different
conditions and the duration of support (a 1-year period). The mean intercept of this relationship represents the value of the measured parameter at time 0 whilst the mean slope refers to the change in the measured parameter over a period of time such that a positive slope indicates an increase in the value and a negative slope indicates a reduction in the value. For model details, refer to Model 1 in Appendix B.

3.2.2 RESULTS

Throughout the LVAD support period, the echocardiographic assessment was performed on 170 occasions (mean of 7.4 tests per patient, range 4-14 tests). The model extrapolated its values from these measurements where the effects of two different speeds (baseline speed and 6000 rpm) on the echocardiographic parameters were tested. Figure 3.1 illustrates the estimates of change in each of the four echocardiographic measured parameters over the 1 year period.

Figure 3.1: Multi-level time model analysis

**Fig 3.1:** Multi-level time model analysis of echocardiographic parameters measured at baseline speed, after speed reduction for 5 and 15 minutes, and after 6MW exercise test.
Multi-level time modelling revealed that all four parameters (LVEDD, LVESD, FS and EF) measured whilst the HM II LVAD running at full speed (range 8600 – 9800 rpm) would not change during the support period (black line on the four graphs). In table 3.1, the model revealed that over a 1-year period the slope of LVEDD increased by 1.638 mm (p=0.634), the slope of LVESD decreased by 0.178 mm (p=0.960), the slope of FS increased by 0.026% (p=0.990) and the slope of EF decreased by 0.565% (p=0.843). These changes were minute, non-significant, and unpredictable despite longer support duration and the changes in medication as per the Harefield Bridge to Recovery Protocol. The lack of alteration in these measurements whilst the device is running on full speed suggests that contribution from the HM II LVAD persists throughout the support period masking the native LV function and preventing real assessment of the underlying LV function.

By applying the model after reducing the speed of the HM II LVAD to 6000 rpm, all four parameters changed over the 1-year modelling period. Such that the slopes of both FS and EF measured after 5 and 15 minutes of low speed and after the 6MW test have increased significantly. The slope of LVESD also decreased significantly at 5 and 15 minutes of low speed and after the 6MW exercise test. The slope of the LVEDD followed the same trend as that of the LVESD but the reduction was not as significant (table 3.1).

**Table 3.1: Multilevel analysis and slope values**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>5-minutes</th>
<th>15-minutes</th>
<th>Post 6MW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>p-value</td>
<td>Slope</td>
<td>p-value</td>
</tr>
<tr>
<td>LVEDD</td>
<td>1.638</td>
<td>0.634</td>
<td>-1.25</td>
<td>0.540</td>
</tr>
<tr>
<td>LVESD</td>
<td>-0.178</td>
<td>0.960</td>
<td>-6.557</td>
<td>0.008</td>
</tr>
<tr>
<td>FS</td>
<td>0.026</td>
<td>0.990</td>
<td>11.186</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EF</td>
<td>-0.565</td>
<td>0.843</td>
<td>17.513</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 3.1:** Values of slopes of LVEDD, LVESD, FS and EF modelled over one-year.
These modelling results illustrate the necessity to reduce the speed of the HM II LVAD to unmask the native LV function. In addition, the speed should be low enough to ensure that the contribution from the device into the cardiac output is kept to minimum and that LV loading, which occurs after speed reduction as evidenced by the increase in the intercept values in both LVESD and LVEDD (figure 3.1), is a physiological response rather than secondary to retrograde filling.

Part two of this study looks at the effects of incremental speed reduction in a mock circuit.
3.3 Part Two – Mock Circulation

3.3.1 METHODS

3.3.1.1 Equipments, System Setup and Data Acquisition

The aim is to set up a model of the LVAD circulatory system. The first stage includes modelling of a patient heart. The Abiomed BVS5000 Extracorporeal Pump (Massachusetts, USA) was chosen as a heart model since it is easy to modify and allows for the addition of a HM II VAD.

Figure 3.2 is a picture of the mock circuit and figure 3.3 is a schematic diagram of the different components of the mock circuit.

Figure 3.2: The mock circuit

Fig 3.2: A photograph of the mock circuit with a HM II VAD attached to the side in parallel to the Abiomed BVS5000 device. The text below and the schematic diagram in figure 3.3 represent detailed description of the different circuit’s components.

† The circuit was set up in Professor Kim Parker’s laboratory, Department of Biomedical Engineering at Imperial College, London.
**Figure 3.3:** The Abiomed BVS5000 extracorporeal system and the HM II VAD

**Fig 3.3:** A schematic diagram of the experimental setup of the circuit excluding the extracorporeal pump drive system and the HM II control system. A, head tank reservoir; B, Abiomed BVS5000 extracorporeal pump; C, extra cylindrical chamber after the ventricle and before the aorta; D, HM II VAD attached to the extra cylindrical chamber (C); E, ruler marked Windkessel system; F, gate clamp providing adjustable resistance to fluid flow; G, ventricular pressure transducer; H, aortic pressure transducer; I, flow probe; J, inlet opening for the pneumatic system to drive the Abiomed device; K, blunt syringe placed in the outflow graft; L, blunt needle placed in the inflow graft; M, direction of fluid flowing from the head tank into the Abiomed BVS5000 system.
**Circuit components:** The circuit consists of three main components, the head reservoir, the Abiomed pump and a Windkessel system. The head reservoir tank (A) represents the pre-load volume and is connected to the Abiomed pump which itself is divided into two chambers by one way valves just as the heart. The first chamber of the device (B) has a valve acting as the mitral valve, whereas the second chamber has an aortic valve between the ventricular chamber and the output to the rest of the system. The ventricular chamber has been extended by adding a cylindrical chamber (C) after the ventricle and before the aortic valve. Chamber (C) has an opening which allowed the installation of the inflow cannula of a HM II VAD (D). The outflow cannula of the HM II VAD is connected to the system using a Y junction located before the Windkessel system (E).

The third component of this circuit is a ruler marked Windkessel system (E). This is a cylinder holding fluid to levels which determine the base value of the aortic pressure (afterload). Using an air valve on the top of the cylinder the fluid level can be adjusted. The idea of the system is to provide a steady flow to the systemic part of the circulation, downstream from the pump. When fluid is pumped into the cylinder during systole the fluid level rises and pressurises the trapped air, during diastole the trapped air pressure forces fluid at a constant rate to the systemic system. After the Windkessel system there is a gate clamp (F) which provides a fully adjustable resistance to fluid flow, just as the body systemic arterioles would provide to blood. Increasing the resistance by closing the gate clamp creates a higher aortic “after-load” pressure. Standard one inch diameter PVC tubing is used throughout the setup.

**Pressure measurements:** There are two pressure transducers (Gaeltec Transducer Interface S13, Isle of Skye, Scotland) that measure the ventricular and aortic pressures. The first pressure transducer, ventricular pressure transducer (G), is placed in the extended ventricle chamber (C) of the Abiomed pump and provides a continuous trace of the left ventricular pressure. The second pressure transducer, the aortic pressure transducer (H), is placed before the Windkessel system and after the Y junction and provides a continuous trace of the aortic pressure. Prior to data acquisition the two probes were calibrated using a standard manometer.
**Flow measurements:** An ultrasonic flow probe (I) is located around the aorta before the Windkessel system and after the Y junction to measure the flow rate. The rate of the fluid flowing after the Y junction (litres/minute) is the sum of the fluid driven by the Abiomed system and the HM II VAD. The probe was immersed in a tank of water after ensuring no air bubbles were trapped in the probe’s two side arms.

**Control of the Abiomed System:** The extracorporeal Abiomed pump is driven by the Thoratec HeartMate Implantable Pneumatic Left Ventricle Assist System Drive Console (TCI, Thermo Cardio System Inc., USA). The system driver uses a pneumatic system to drive the Abiomed pump and is connected to the Abiomed system in the ventricular chamber at opening J in figure 3.3. By changing the amount of air that is being forced by the driver into the system it would be possible to alter the pumping strength of the ventricle. A 50 mls syringe was used on the PVC tube feeding the extracorporeal pump to control pumping strength by either driving air into- or withdrawing air from- the extracorporeal circuit.

**Solution preparation:** To account for the viscosity of blood, the circulating solution was made up with glycerol. Depending on the temperature, the kinematic viscosity of blood is 3.6 – 4.0 centistokes. Using a cannon-fenske routine viscometer (Poulten Selfe & Lee, Ltd), 99% glycerol (Sigma, UK) was diluted to 40% at 23°C (room temperature) to obtain a viscosity of 3.8 centistokes. Since the system’s capacity is 6 litres, the system required 2.4 litres of 99% glycerol (Sigma, UK) and 3.6 litres of water. The viscosity of the circulating solution was repeated at the end of the day to ensure no alteration in the viscosity had occurred throughout the experiment. Refer to Appendix C for the viscosity calculations.

**Dye simulation:** 18G blunt needles (K and L) were inserted into the outflow and the inflow grafts of the HM II VAD before the HM II VAD itself and before the Y junction, respectively. Each needle was connected to a 50 mls syringe filled with 10 mls of blue ink and 40 mls of the diluted glycerol solution (20% blue ink concentration) to simulate the dye injected during cardiac catheterisation. The syringe was driven at a constant pressure using an IVAC P7000 syringe pump (Cardinal Health, Switzerland). This technique allowed visualisation and
qualitative analysis of the flow pattern within the inflow and outflow grafts of the HM II VAD.

The HM II VAD was controlled by a modified speed controller especially manufactured by Thoratec Corporation Inc (software version 3.11) which can control the speed of the device between 4000 rpm and 14000 rpm compared to the standard controller which drops the speed of the device to a minimum of 6000 rpm. The speed was adjusted using a standard HM II monitor (figure 3.4).

Figure 3.4: HM II monitor with low speed controller

Fig 3.4: A touch digital monitor connected to the low speed controller to control the speed of the HM II VAD.
3.3.1.2 Experimental Protocols

In most clinical applications the baseline speed was set to ensure no septal shift and that the aortic valve remains closed i.e. blood will flow into the HM II’s inflow cannula and then into the ascending aorta bypassing the aortic valve. This ensures maximal unloading of the LV. In the studied population the speed of the HM II LVAD ranged from 8600 rpm to 9800 rpm.

In the mock circuit the effects of 7 different speeds (range 4000 – 10,000 rpm) were tested in four different experimental ventricular conditions. In the beginning of each experiment the systolic ventricular pressure of the Abiomed device was set to simulate four different ventricular conditions:

i) Abiomed system off (0 mmHg – experiment 1) to understand the independent effects of the HM II VAD on the circulation;

ii) mild ventricle (40 mmHg – experiment 2);

iii) moderate ventricle (80mmHg – experiment 3);

iv) strong ventricle (120 mmHg – experiment 4).

Experiments 2, 3 and 4 were performed whilst the Abiomed system running at 60 bpm. Table 3.2 shows the matrix of the runs performed at various conditions of the ventricle and the different speeds of the HM II VAD.

**Table 3.2: Experimental Matrix**

<table>
<thead>
<tr>
<th>Peak Ventricular Condition</th>
<th>Speed of HM II VAD (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mmHg</td>
<td>10000 9000 8000 7000 6000 5000 4000</td>
</tr>
<tr>
<td>40 mmHg</td>
<td>10000 9000 8000 7000 6000 5000 4000</td>
</tr>
<tr>
<td>80 mmHg</td>
<td>10000 9000 8000 7000 6000 5000 4000</td>
</tr>
<tr>
<td>120 mmHg</td>
<td>10000 9000 8000 7000 6000 5000 4000</td>
</tr>
</tbody>
</table>

Table 3.2: The experimental matrix of the 28 different runs performed at 4 different ventricular strengths and 7 different HM II VAD speeds at a constant Abiomed rate of 60 bpm.

‡ 60 bpm was chosen instead of 80 bpm to allow for slower flow and hence better visualisation and recording of the flow pattern.
As stated above the ventricular pressure was controlled by the pneumatic system that drives the Abiomed system (label J in figure 3.3). The ventricular strength of the Abiomed system was set by altering the amount of air driven into it whilst the HM II VAD is switched off and the inflow cannula is clamped. This ensures accurate setting of the ventricular pressure without being affected by the suctioning effects of the rotor of the HM II VAD. After setting up the LV pressure at the desired value, the clamp was released and the HM II VAD was started at 10000 rpm. The first recording was taken at 10000 rpm and then at the 6 other lower speeds after allowing the system to settle for 5 minutes after each incremental reduction. During each run, the quoted ventricular and aortic pressures are the peak pressures.

To visualise and record the flow pattern and the direction of flow, 2 mls of 20% blue ink were purged into the outflow and inflow grafts through the blunt needles (structures K and L in figure 3.3) in each of the 28 runs using the syringe driver. The pattern and direction of flow were recorded visually using a 30 frame per second Sony HD movie camera (model DSC-T500, 5x optical zoom) in the macro mode. The camera lens was placed 5 centimetres away from the outflow graft focusing on the blunt needle’s point of insertion in the centre of the frame. Each recording started 2 seconds prior to purging, continued throughout the ink injection, and for 5 seconds after purging. Recording was repeated by repositioning the camera at the inflow cannula to determine if any of the ink “dye” would regurgitate back into the LV.

### 3.3.1.2 Data Acquisition and Analysis

The two pressure probes and the flow probe were connected to a transducer amplifier (PM – 1000, CWE, Inc., USA, figure 3.5). Pressure and flow signals were recorded at 2000 Hz (i.e. 2000 data points per second) and entered to a Notocord Systems Software (France). Data was imported onto Matlab Statistics Software version 7.5 (MathWorks, UK) where Savitzky-Golay filter § was applied

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§ The Savitzky-Golay electronic filter performs a local polynomial regression on a trace to determine the smoothed value for each point. It eliminates noise and preserves features of the distribution such as relative maxima, minima and width.
See Appendix D for the computer script written to plot 2000 filtered data points per second in single graphs.

**Figure 3.5: Pressure and flow signal amplifier**

![Image of four channel signal amplifier](image)

**Fig 3.5:** Four channel signal amplifier that amplifies ventricular pressure, aortic pressure and flow signals. This amplifier is connected to the Notocord Systems Software.

**Figure 3.6: An example of Savitzky-Golay electronic filter being applied to a ventricular pressure trace**

![Graphs showing ventricular pressure trace with and without Savitzky-Golay filter](image)

**Fig 3.6:** Savitzky-Golay electronic filter being applied to an example of ventricular trace. The filter smoothes the curves by eliminating the noise and preserves the main features of the distribution i.e. relative maxima, minima and width.

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3.3.2 RESULTS

The first step was to test the setup of the mock circuit without the HM II VAD being connected to compare the pressure traces produced by the model with normal heart traces. Figure 3.7A shows a screen capture of Notocord and the traces obtained from the test-run. There is marked similarity with figure 3.7B, normal heart traces. The ventricular pressure and the aortic pressure traces were reproducible with every beat at heart rate of 60 bpm.

**Figure 3.7: Comparison between acquired pressure and flow traces from Notocord System and the traces from a normal heart**

![Figure 3.7: Comparison between acquired pressure and flow traces from Notocord System and the traces from a normal heart](image)

**Fig 3.7:** A) a screen capture of Notocord System illustrating the pressure and the flow traces. B) LV and Aortic pressure traces in normal LV. It is worth noting the noise in the traces acquired from the Notocord screen. The noise was eliminated using the Savitzky-Golay filter as described above.

The next step was to test the effectiveness of the air syringe system to adjust the ventricular pressure of the Abiomed system. As mentioned above, peak ventricular pressure values of 120 mmHg, 80 mmHg and 40 mmHg were obtained by either removing air from- or driving air into- the drive tube that is connected to the Abiomed Extracorporeal pump whilst the HM II VAD was switched off and the inflow cannula was clamped. The aortic pressure trace was initially altered by
adjusting the Windkessel fluid level and changing the systemic circulatory resistance using the gate clamp then it left to self adjust once the HM II VAD was switched on and the clamp was removed.

**Experiment 1: Abiomed System Switched off – 0 mmHg**

Figure 3.8 represents the traces obtained whilst the Abiomed system was switched off and the HM II VAD running at seven different speeds. In this experiment, air was fully drawn from the Abiomed system leaving the ventricle with no pumping strength and the aortic valve closed i.e. simulating a dead ventricle such that blood was flowing from the head reservoir through the HM II VAD into the Windkessel system bypassing the closed aortic valve. At 10000 rpm the ventricular pressure was a flat line and subzero due to the negative pressure being generated as a result of the suctioning effect of the HMII VAD. The aortic pressure was 50mmHg. This was generated by the forward flow driven from the HM II VAD. The flow measured using the flow transducer was approximately 6 l/min. As the speed of the HM II VAD decreased by increments of 1000 rpm, both flow and aortic pressure decreased steadily reflecting reduced contribution from the HM II VAD. Inversely, the ventricular pressure increased steadily to reach almost 0 mmHg. The inverse relationship between the ventricular pressure and the speed of the HM II VAD is due to the decreasing negative pressure generated from the suctioning power of the HM II VAD.

Video captures (figure 3.9) of ink flowing within the outflow graft at 10,000 rpm revealed a turbulent, complex and a non-pulsatile flow pattern that is being driven only in the forward direction i.e. towards the Windkessel system. As the speed decreased the ink pattern became less turbulent but remained complex with no evidence of pulsatility and continued to be driven in the forward direction. In addition, the ink flow became stagnant within the outflow cannula and took longer time to clear. At no point the ink has reached the ventricle in the retrograde direction.
Figure 3.8: Pressure and flow traces with a switched off Abiomed system

Fig 3.8: The pressure and flow traces recorded with the HM II VAD running at 7 different speeds whilst the Abiomed is effectively “switched off”. Red trace represents aortic pressure; blue trace represents ventricular pressure; and black trace represents flow in l/min. The ventricular pressure is subzero at 10,000 rpm due to the suctioning effect of the HM II VAD whilst the Abiomed is “switched off”. The aortic pressure was 50 mmHg at 10,000 rpm and represents the pressure generated from the HM II VAD. As the speed decreased, the ventricular pressure increased as a consequence of the reduction in the suctioning power of the HM II VAD, and the aortic pressure decreased as a consequence of reduction in flow being generated by the HM II VAD. It is important to note that the aortic valve remained closed throughout the 7 runs due to the absence of ventricular contractility.
Figure 3.9: Ink flow pattern within the outflow graft in experiment 1 with the Abiomed system switched off

Fig 3.9: The ink flow pattern was turbulent, complex, and non-pulsatile at higher speeds (10,000-7000 rpm). As the speed of the HM II VAD decreased below 7000 rpm, the pattern became less turbulent and more central but remained complex with no evidence of pulsatility. In addition, the force driving the ink towards the Windkessel System was lower such that it had the tendency to stagnate within the outflow graft. Arrow represents flow direction.
**Experiment 2: Poor ventricle**

In this experiment the initial ventricular systolic pressure was set at 40 mmHg. Figure 3.10 represents the pressure and flow traces obtained with the HM II VAD running at 7 different speeds. At 10,000 rpm the aortic valve remained closed, the ventricular diastolic pressure was below zero, and the aortic diastolic pressure was approximately 50 mmHg. The systolic aortic pressure was consistent with ventricular pulsatility such that the rise in the aortic pressure was due to the pulsatility being generated from the Abiomed’s ventricular system. At 7000 rpm as the ventricular pressure exceeded the aortic pressure the aortic valve started opening and continued to open in systole at 6000 rpm, 5000 rpm and 4000 rpm.

The flow pattern of the injected ink at 10,000 rpm continued to be complex and turbulent (figure 3.11). As the speed of the HM II decreased there was evidence of pulsatility in the flow pattern of the injected ink into the outflow graft consistent with systole and diastole. At 6000 rpm, the ink injected in the outflow graft formed a compact pattern “cloud-like” that moved forward in systole with a minute non-significant backward movement in early diastole. However, at a certain point the pressure generated by the turbine nature of the rotor of the HM II VAD was high enough to stop the ink flowing further backward even at aortic pressure higher than the ventricular pressure and pushed the ink back into the forward direction. This imaginary point was referred to as a point of stagnation (figure 3.12). The amount of backward movement did not change with further speed reduction and again at no point the flow bypassed the rotor of the HM II in the reverse direction to reach the ventricle.

Visualisation of flow in the inflow cannula identified flow moving forward towards the tip of the inflow cannula in systole. In early diastole there was a minute non-significant backward displacement of ink in the inflow cannula that did not change in quantity as the speed decreased to 4000 rpm. At 10,000 rpm the systolic flow was 6.5 l/min and the diastolic flow was 5 l/min giving a difference in flow rate between systole and diastole (ΔQ) of 1.5 l/min. However, as the speed of the HM II VAD decreased, ΔQ increased to 3 l/min at 4000 rpm suggesting increased contribution from the Abiomed system and reduced contribution from the HM II VAD.
Figure 3.10: Pressure and flow traces with the ventricular pressure set at 40mmHg

Fig 3.10: The pressure and flow traces recorded with the HM II VAD running at 7 different speeds with the initial ventricular pressure set at 40mmHg. Red trace represents aortic pressure; blue trace represents ventricular pressure; and black trace represents flow in l/min. Similar to the “switched off” setting (figure 3.8), at 10,000 rpm the aortic pressure was approximately 50mmHg and was exceeding the ventricular pressure. The aortic valve started opening at 7000 rpm when the peak ventricular pressure exceeded the aortic pressure. As the speed decreased the ventricular pressure continued to rise and the aortic pressure continued to decrease due to the reduced flow being generated by the HM II VAD. Also the flow generated from the pulsatility of the Abiomed system and the HM II decreased as the speed of HM II decreased.
Figure 3.11: Ink flow pattern within the outflow graft in experiment 2

The ink flow pattern in experiment 2 became more turbulent and complex. Evidence of pulsatility started appearing as the speed of the HM II VAD was reduced to be consistent with systole and diastole. At 6000 rpm there was a “cloud-like” pattern. Ink never flowed back into the LV. Arrow represents flow direction.
**Figure 3.12:** A schematic diagram illustrating the flow of the ink in the outflow graft

**Fig 3.12:** As the speed of the device is reduced the flow becomes steadier and less turbulent forming a compact pattern “cloud-like”. There is displacement of ink in the backward direction towards the tip of the outflow cannula in early diastole. However, at point X (point of stagnation), the ink stops travelling backwards and starts moving along the peripheries.
Experiments 3 and 4: Moderate and strong ventricle

In these two experiments (figures 3.13 and 3.14), the initial ventricular systolic pressure was set at 80 mmHg and 120 mmHg, respectively. In both experiments the aortic valve was opening at 10000 rpm. As the speed of the HM II VAD decreased, the ventricular diastolic pressure increased and the aortic pressure decreased steadily. Furthermore, throughout the two experiments the aortic systolic pressure was related to the ventricular systolic pressure.

Similar to experiment 2, the flow pattern at 10,000 rpm continued to be complex and turbulent and at 6000 rpm the ink injected in the outflow graft formed a well defined “cloud-like” pattern that moved forward in systole with more exaggerated but non-significant backward displacement in early diastole (figures 3.15 and 3.16). The ink stopped flowing in the backward direction at a certain point and it seemed that the stagnation point moved closer towards the HM II VAD. Again at no point ink travelled back into the ventricular chamber. Visualisation of the flow in the inflow cannula identified ink moving forward towards the tip of the inflow cannula in systole. In early diastole there was a backward displacement of ink in the inflow cannula that did not change in quantity as the speed decreased to 4000 rpm in either experiment.

In both experiments, the peak flow remained almost constant whereas the baseline flow continued to decrease as the speed of the device decreased resulting in an increase in ΔQ. The rise in ΔQ was a response to both reduction in the speed of the HM II VAD and increased pulsatility (pulse pressure) of the Abiomed system. Figure 3.17 represents the changes in the ΔQ with different speeds of HM II at three different LV functions: poor, moderate and strong.
Figure 3.13: Pressure and flow traces with the ventricular pressure set at 80mmHg

The pressure and flow traces recorded with the HM II VAD running at 7 different speeds with the initial ventricular pressure set at 80mmHg. Red trace represents aortic pressure; blue trace represents ventricular pressure; and black trace represents flow in l/min. Throughout the seven runs the aortic valve was opening and the flow at 10000 rpm reached a peak of 9 l/min. As the speed decreased the pressure difference decreased and the aortic pressure decreased steadily. Also the base flow decreased in diastole as the speed was reduced whilst the peak flow remained at a range of 9-10 l/min.
Figure 3.14: Pressure and flow traces with the ventricular pressure set at 120mmHg

Fig 3.14: The pressure and flow traces recorded with the HM II VAD running at 7 different speeds with the initial ventricular pressure set at 120mmHg. Red trace represents aortic pressure; blue trace represents ventricular pressure; and black trace represents flow in l/min. Throughout the seven runs the aortic valve was opening and the flow at 10000 rpm reached a peak of 12 l/min. As the speed decreased, the pressure difference decreased, the aortic pressure decreased, the base flow decreased in diastole, and the peak flow increased to reach 15 l/min.
Figure 3.15: The ink flow pattern in experiment 3 became more turbulent and complex. At 10,000 rpm the pattern was very complex and more diluted such that the transient time of the ink was shorter as compared to experiment 2. Evidence of pulsatility was apparent at lower speeds of the HM II VAD and the pattern became cloudier. Ink never flowed back into the LV. Arrow represents flow direction.
Figure 3.16: Ink flow pattern within the outflow graft in experiment 4

Fig 3.16: Similar to experiment 3, the ink flow pattern in experiment 4 was more turbulent and complex. Evidence of pulsatility was apparent at lower speeds of the HM II VAD and the pattern became cloudier. Ink never flowed back into the LV. Arrow represents flow direction.
Figure 3.17: Changes in ΔQ at the seven different HM II speeds and three different ventricular contractility functions (mild, moderate, strong).

In figure 3.17, the ΔQ increased steadily with moderate and strong ventricular contractility as the speed of the HM II decreased from 10000 rpm to 4000 rpm. Interestingly when the initial setting of the ventricular pressure was set to 40 mmHg (poor ventricle), the ΔQ decreased initially as the speed of the HM II decreased. This reduction only occurred whilst the aortic valve remained closed. When the aortic valve started opening at 7000 rpm the ΔQ started to increase. From the above it could be assumed that the ΔQ is an experimental representation of the pulse pressure.
3.4 Part Three – Clinical Study

The aim of this component of the study is to clinically investigate the effects of different HM II LVAD speeds on the myocardial function and structure and to determine if 6000 rpm is an appropriate speed to examine the native myocardial function safely and effectively.

3.4.1 METHODS

3.4.1.1 Study Population

Flow across the HM II LVAD was studied in 13 male patients out of the 23 patients on 33 occasions. Reasons for excluding 10 patients are included in table 3.3. Demographics and pre-implantation data for the 13 studied patients are presented in table 3.4. The study was performed using the low speed controller provided by Thoratec Inc in December 2007. This study was completed in March 2009.

Table 3.3: Patients excluded from low speed study (n=10)

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 4</td>
<td>Patients were explanted prior to obtaining the low speed controller</td>
</tr>
<tr>
<td>8, 9, 15, 18, 19, 20, 22</td>
<td>Low speed device Doppler could not be acquired due to cannula position</td>
</tr>
</tbody>
</table>

Table 3.3: Reasons for excluding 10 patients from this analysis.
Table 3.4: Demographics of the studied patients (n=13)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at HM II implantation (years)</td>
<td>35.46 ± 12.86</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.89 ± 2.13</td>
</tr>
<tr>
<td>HF duration (months)</td>
<td>40.35 ± 39.65</td>
</tr>
<tr>
<td>Average number of pre-implant inotropes per patient</td>
<td>2.00 ± 0.71</td>
</tr>
<tr>
<td>Pre-implantation Echo</td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>76.00 ± 9.06</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>70.60 ± 7.51</td>
</tr>
<tr>
<td>FS (%)</td>
<td>6.89 ± 2.42</td>
</tr>
<tr>
<td>EF (%)</td>
<td>19.14 ± 7.34</td>
</tr>
<tr>
<td>Pre-implantation Haemodynamics</td>
<td></td>
</tr>
<tr>
<td>Mean Pulmonary Artery Pressure (mmHg)</td>
<td>35.70 ± 5.64</td>
</tr>
<tr>
<td>Mean PCWP (mmHg)</td>
<td>30.10 ± 4.28</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>2.56 ± 0.87</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>1.31 ± 0.42</td>
</tr>
</tbody>
</table>

Table 3.4: Pre-implantation demographics of the 13 studied patients.

After ensuring an INR of 2 or above, device flow pattern, LV echocardiographic parameters, and peripheral haemodynamics were measured on each of the following three speeds:

i) the baseline speed of the HM II LVAD (range 8800-9400 rpm),

ii) after 15 minutes of 6000 rpm, and

iii) after 5 minutes of reducing the speed to either 5000 rpm or 4000 rpm.

Due to time constraint in performing the echocardiographic examination, each patient was examined at either 5000 rpm or 4000 rpm on each occasion. Hence two groups were generated; Group A (18 occasions at 5000 rpm) and Group B (15 occasions at 4000 rpm).
3.4.1.2 Echocardiography

Regular echocardiographic examinations were performed with an advanced cardiac ultrasound machine using harmonic imaging as standard. Examinations were performed on a Philips iE33 machine (Philips Electronics, Eindhoven, the Netherlands). Images were obtained by a senior echocardiographer (Mrs. Carole Webb) from standard parasternal, apical, subcostal and suprasternal views. Occasionally “off-axis” images were obtained when the LVAD apical inflow cannula provided acoustic interference. LVAD flow pattern and direction were assessed by positioning a pulsed wave Doppler (PW) sample volume at the inflow cannula in the best possible window which revealed two spectra occurring with every heart beat; a positive spectrum occurring in systole representing forward flow i.e. blood flowing from the ventricle into the inflow cannula of the HM II LVAD (figure 3.18). The second is a negative spectrum occurring in diastole immediately following the positive spectrum. Images were digitally acquired and read off line.
Figure 3.18: Doppler Examination of flow through the inflow cannula

**Fig 3.18:** An illustration of how the forward flow was quantified using the pulsed wave Doppler. A) Peak velocity in forward direction ($V_{\text{max}}^f$) was measured by measuring the distance between the baseline and the peak of the positive velocity spectrum. Forward velocity time integral ($\text{VTI}_f$) is an integration of the area under the Doppler curve representing the distance that blood travelled into the inflow cannula. $Z$, is the negative spectrum observed immediately after each positive spectrum. Figures 3.14B-D are representative examples of the changes in the device forward flow measured spectrum in a patient who had the speed of the device reduced from 9000 rpm (B) to 6000 rpm for 15 minutes (C) then to 4000 rpm for 5 minutes (D). The $V_{\text{max}}^f$ (thin yellow arrow) becomes less prominent as the LVAD speed is reduced.
3.4.1.3 Device Forward Flow

Peak velocity in forward direction (V\text{max}_f) was measured by measuring the distance between the baseline and the peak of positive velocity spectrum on Doppler images. The changing velocity over the flow period in the forward direction was determined by tracing (integrating) the area under the Doppler curve from the leading edges of the positive velocity spectrum to obtain forward velocity time integrals (VTI\text{f}). In other words, VTI\text{f} represents the distance that the blood travelled into the inflow cannula with each heart beat. Since the inflow cannula is a rigid circular tube with a constant cross sectional area (CSA), the flow through the inflow cannula would have a uniform velocity. Using the volumetric flow equation, the blood volume that flowed into the inflow cannula was calculated as the product of the forward VTI\text{f} and the CSA of the inflow cannula: BV = CSA \times VTI\text{f}, where, BV, blood volume (cm\(^3\)); VTI\text{f}, velocity time integral (cms); CSA, cross-sectional area of the inflow cannula (cm\(^2\)).

3.4.1.4 Ventricular Dimensions and Function

Both LVEDD and LVESD were obtained from the parasternal long axis of the LV at the level of the chordal-mitral valve junction. Fractional shortening (FS) was calculated as the percentage of change in the LV cavity dimension with systole:

$$FS = \frac{LVEDD - LVESD}{LVEDD} \times 100\%.$$ 

Ejection fraction (EF) was calculated using the cubed function of the ventricular dimension formula:

$$EF = \frac{LVEDD^3 - LVESD^3}{LVEDD^3} \times 100\%.$$ 

\*\* CSA was determined as 2.5447 cm\(^2\) based on an internal diameter of the inflow cannula of 1.8 cm
### 3.4.1.5 Peripheral Haemodynamic Measurements

Haemodynamic measurements were performed using an automatic blood pressure monitor, Datascope Accutorr Plus and included systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR). Pulse pressure (PP) was calculated as the difference between SBP and DBP. Mean arterial blood pressure (MAP) was calculated by adding one third of the PP to the DBP. During low speed duration patients were closely observed clinically for any untoward symptoms such as dizziness, sweating, palpitations, or dyspnoea.

### 3.4.1.6 Data Collection and Statistics

All data were collected prospectively and are represented as mean ± stdev. Data analysis was done using SPSS version 16.0 for windows (Lead Technologies). For normally distributed samples paired $t$-test and unpaired $t$-test were used. Wilcoxon signed-rank test and Mann-Whitney U tests were used to compare non-parametric variables between 2 and 3 groups, respectively. A p-value $< 0.05$ was considered to be statistically significant.
3.4.2 RESULTS

3.4.2.1 Safety

No adverse incidents were noted on any of the 33 occasions when the speed was reduced from baseline to 6000 rpm. Further speed reduction to either 5000 rpm or 4000 rpm was also tolerated by all patients with no evidence of dizziness, palpitations, or dyspnoea. The longest period of time at less than baseline flow was 27 minutes. Patients were followed up for 72 hours and none reported any thrombotic related complications.

3.4.2.2 Group Comparison

There were no significant differences in the baseline haemodynamic, echocardiographic and PW Doppler measured parameters between Groups A and B when the HM II LVAD was at either baseline speed or at 6000 rpm (table 3.5). Also, none of the patients had significant mitral regurgitation on echocardiography and the aortic valve was closed whilst the device was running at baseline speed.
Table 3.5: Inter-group comparison.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline Speed</th>
<th>Speed 6000 rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>92.71 ± 10.52</td>
<td>88.06 ± 8.14</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>61.12 ± 10.84</td>
<td>58.94 ± 6.51</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>71.65 ± 8.73</td>
<td>68.65 ± 5.63</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>86.89 ± 14.03</td>
<td>88.44 ± 16.13</td>
</tr>
<tr>
<td>EDD (mm)</td>
<td>53.16 ± 11.32</td>
<td>49.93 ± 14.79</td>
</tr>
<tr>
<td>ESD (mm)</td>
<td>40.47 ± 16.17</td>
<td>40.47 ± 16.17</td>
</tr>
<tr>
<td>FS (%)</td>
<td>20.90 ± 8.52</td>
<td>20.75 ± 10.42</td>
</tr>
<tr>
<td>EF (%)</td>
<td>48.87 ± 16.31</td>
<td>47.84 ± 19.31</td>
</tr>
<tr>
<td>Vmaxf(cm/sec)</td>
<td>69.21 ± 28.36</td>
<td>76.01 ± 38.54</td>
</tr>
<tr>
<td>VTI(cm)</td>
<td>22.49 ± 11.73</td>
<td>25.11 ± 12.35</td>
</tr>
<tr>
<td>BV (cm³)</td>
<td>57.23 ± 29.85</td>
<td>63.91 ± 31.43</td>
</tr>
</tbody>
</table>

Table 3.5: Comparison of the baseline and 6000 rpm measured peripheral haemodynamics, echocardiographic and Doppler parameters between the two groups.
3.4.2.3 Device Forward Flow

In group A, speed reduction from an average baseline speed of 9055 ± 165 rpm (range 8800 – 9400 rpm) to 6000 rpm resulted in a significant reduction in both $V_{\text{max}}$ and $VTI_f$ from $69.21 ± 28.36$ cm/sec to $47.33 ± 11.33$ cm/sec ($p=0.006$) and from $22.49 ± 11.73$ cms to $12.15 ± 4.16$ cms ($p=0.001$), respectively (figure 3.19). Although speed reduction to 5000 rpm resulted in a significant decrease in both $V_{\text{max}}$ and $VTI_f$ as compared to the baseline speed measurements ($V_{\text{max}}$: $69.21 ± 28.36$ cm/sec versus $43.54 ± 10.30$ cm/sec, $p=0.001$; $VTI_f$: $22.49 ± 11.73$ cms versus $10.94 ± 2.65$ cms, $p<0.001$), both $V_{\text{max}}$ and $VTI_f$ measured at 5000 rpm were not significantly lower when compared to measurements acquired at 6000 rpm ($V_{\text{max}}$: $47.33 ± 11.33$ cm/sec versus $43.54 ± 10.30$ cm/sec, $p=0.302$; $VTI_f$: $12.15 ± 4.16$ cms versus $10.94 ± 2.65$ cms, $p=0.306$, respectively).

Similar to group A, speed reduction in group B from an average baseline speed of $9160 ± 260$ rpm (range 8800 – 9800 rpm) to 6000 rpm resulted in a significant decrease in both $V_{\text{max}}$ and $VTI_f$ from $76.01 ± 38.54$ cm/sec to $51.67 ± 21.23$ cm/sec ($p=0.044$) and from $25.11 ± 12.35$ cms to $13.29 ± 7.04$ cms ($p=0.004$), respectively (figure 3.20). $V_{\text{max}}$ and $VTI_f$ measured at speed 4000 rpm were also significantly lower as compared to the baseline speed measurements ($V_{\text{max}}$: $76.01 ± 38.54$ cm/sec versus $38.44 ± 16.81$ cm/sec, $p=0.004$; $VTI_f$: $25.11 ± 12.35$ cms versus $9.93 ± 5.60$ cms, $p<0.001$) but not significantly lower when compared to measurements obtained at 6000 rpm although there was a lower trend ($V_{\text{max}}$: $51.67 ± 21.23$ cm/sec versus $39.43 ± 16.72$ cm/sec, $p=0.07$; $VTI_f$: $13.29 ± 7.04$ cms versus $9.93 ± 5.60$ cms, $p=0.165$, respectively).

Figure 3.21 illustrates the effects of speed reduction on blood volume flowing into the inflow cannula. Following the same pattern as the $VTI_f$, the BV that travelled into the inflow cannula decreased as the speed decreased from baseline to 6000 rpm by more than 40% in both groups (Group A: $57.23 ± 29.88$ cm$^3$ versus $30.92 ± 10.58$ cm$^3$, $p=0.002$; Group B: $63.99 ± 31.43$ cm$^3$ versus $32.24 ± 14.21$ cm$^3$, $p=0.004$). Further speed reduction in both groups resulted in a non-significant decrease in the forward BV travelling into the inflow cannula (Group A: $30.92 ± 10.58$ cm$^3$ versus $27.84 ± 6.74$ cm$^3$, $p=0.306$; Group B: $32.24 ± 14.21$ cm$^3$ versus $25.27 ± 14.24$ cm$^3$, $p=0.165$).
Figure 3.19: Effect of speed reduction on the HM II forward flow in Group A

(i)

Fig 3.19: Changes in device forward flow measurements in group A as the speed was reduced from baseline to 5000 rpm. Figures 3.19 (i) and 3.19 (ii) illustrate the reduction in both $V_{\text{max}}$ and $V_{T.I.}$, respectively. Speed reduction to 6000 rpm resulted in a significant decrease in both parameters. Further speed reduction to 5000 rpm did not cause a significant decrease in either measurement when compared to 6000 rpm. * $p=0.006$, ** $p=0.001$, *** $p<0.001$. 
Figure 3.20: Effect of speed reduction on the HM II forward flow in Group B

(i)

Fig 3.20: Changes in device forward flow measurements in group B as the speed was reduced from baseline to 4000 rpm. Figures 3.20 (i) and 3.20 (ii) illustrate the reduction in both Vmax_f and VTI_f, respectively. Speed reduction to 6000 rpm resulted in a significant decrease in both parameters. Further speed reduction to 4000 rpm did not cause a significant decrease in either measurement when compared to 6000 rpm. * p=0.04, ** p=0.004, *** p<0.001.
Figure 3.21: Effects of speed reduction on the blood volume being driven into the HM II LVAD

Fig 3.21: Speed reduction resulted in a significant drop in the BV being driven into the HM II LVAD in both group A (figure 3.21 (A)) and group B (figure 3.21 (B)). There was no significant difference in the BV that entered the LV inflow cannula between 6000 rpm and the lower speeds in either group. * p=0.002, ** p=0.004.
After combining the two groups’ 6000 rpm measured parameters, an inter-group analysis of \( \text{Vmax}_f \), \( \text{VTI}_f \) and \( \text{BV} \) between 6000 rpm, 5000 rpm and 4000 rpm revealed that they were not significantly lower when measured at 5000 rpm and 4000 rpm as compared to 6000 rpm (\( \text{Vmax}_f \): 49.30 ± 16.44 cm/sec versus 43.54 ± 10.30 cm/sec versus 38.44 ± 16.81 cm/sec, \( p=0.082 \); \( \text{VTI}_f \): 12.67 ± 5.58 cms versus 10.94 ± 2.65 cms versus 9.93 ± 5.60 cms, \( p=0.074 \); \( \text{BV} \): 32.24 ± 14.21 cm\(^3\) versus 27.84 ± 6.74 cm\(^3\) versus 25.27 ± 14.24 cm\(^3\), \( p=0.093 \)).

As shown in figure 3.18, there was a negative spectrum below the baseline on the PW Doppler assessment. This negative spectrum only occurred in diastole immediately after the positive spectrum. From the mock circuit, visualisation of the ink injected at the inflow cannula (similar position to the sample volume positioned whilst performing echocardiography) revealed a minute and a non-significant retrograde flow in the inflow cannula which could not have been quantified. Based on this observation the negative spectrum has been not calculated. See discussion for further explanation for the possible reasons for the existence of the negative spectrum.
3.4.2.4  Left Ventricular Structure and Function

The reduction in the speed of the HM II LVAD did not have a significant effect on either the LV dimensions or function (table 3.6).

Table 3.6: Changes in LV Structure and Function

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline Speed</th>
<th>6000 rpm</th>
<th>5000 rpm</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>53.16 ± 11.32</td>
<td>56.53 ± 8.45</td>
<td>56.21 ± 7.11</td>
<td>0.581</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>42.74 ± 12.40</td>
<td>44.37 ± 9.80</td>
<td>42.47 ± 9.43</td>
<td>0.832</td>
</tr>
<tr>
<td>FS (%)</td>
<td>20.90 ± 16.31</td>
<td>22.15 ± 8.23</td>
<td>25.18 ± 8.57</td>
<td>0.281</td>
</tr>
<tr>
<td>EF (%)</td>
<td>48.87 ± 16.31</td>
<td>51.33 ± 15.17</td>
<td>56.54 ± 14.91</td>
<td>0.281</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>49.93 ± 14.79</td>
<td>56.40 ± 12.26</td>
<td>57.40 ± 12.59</td>
<td>0.295</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>40.47 ± 16.17</td>
<td>44.47 ± 16.04</td>
<td>45.07 ± 15.21</td>
<td>0.613</td>
</tr>
<tr>
<td>FS (%)</td>
<td>20.75 ± 10.42</td>
<td>22.96 ±10.89</td>
<td>22.97 ± 9.36</td>
<td>0.636</td>
</tr>
<tr>
<td>EF (%)</td>
<td>47.84 ± 19.31</td>
<td>51.71 ± 20.40</td>
<td>52.36 ± 17.93</td>
<td>0.636</td>
</tr>
</tbody>
</table>

Table 3.6: Changes in the echocardiographic parameters following incremental speed reduction in both groups. There was no significant change in LVEDD, LVESD, FS or EF as the speed of the device was reduced in either group.
### 3.4.2.5 Peripheral Haemodynamics

Table 3.7 illustrates the changes noted in the peripheral haemodynamics parameters following incremental speed reduction. There was no significant change in SBP in either group as the speed of the device was reduced. However, both DBP and MAP decreased significantly as the baseline speed decreased to 6000 rpm in both groups. Further device speed reduction did not have a significant effect on either DBP or MAP. The reduction in the DBP resulted in an increase in PP, as would be expected, but not significantly.

**Table 3.7: Blood pressure response to speed reduction**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline Speed</th>
<th>6000 rpm</th>
<th>5000 rpm</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>92.71 ± 10.52</td>
<td>86.06 ± 9.38</td>
<td>86.33 ± 10.74</td>
<td>0.125</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>61.12 ± 10.84 *</td>
<td>52.17 ± 6.14 *</td>
<td>49.22 ± 5.77</td>
<td>0.004</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>31.59 ± 13.25</td>
<td>33.89 ± 7.50</td>
<td>37.11 ± 9.14</td>
<td>0.115</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>71.65 ± 8.73 *</td>
<td>63.46 ± 6.48 *</td>
<td>61.59 ± 6.54</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>88.06 ± 8.14</td>
<td>85.94 ± 12.11</td>
<td>85.75 ± 11.33</td>
<td>0.908</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>58.94 ± 6.51 *</td>
<td>53.56 ± 7.85 *</td>
<td>47.94 ± 7.82</td>
<td>0.001</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>29.13 ± 9.15</td>
<td>32.38 ± 8.7</td>
<td>37.81 ± 8.36</td>
<td>0.106</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>68.65 ± 5.63 *</td>
<td>64.35 ± 8.55 *</td>
<td>60.54 ± 8.25</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Table 3.7: Changes in blood pressure parameters following incremental speed reduction in both groups. There was no significant change in SBP as the speed of the device was reduced. However, the DBP decreased significantly as the baseline speed decreased to 6000 RPM in both groups. Further reduction to 5000 RPM in Group A and 4000 RPM in Group B did not have a significant effect on the DBP. MAP followed the same trend as DBP.
Furthermore, both groups exhibited an initial non-significant decline in the HR trend as the speed decreased from baseline to 6000 rpm; Group A, 86.89 ± 14.03 bpm versus 83.22 ± 18.68 bpm, p=0.205; Group B, 88.44 ± 16.1 bpm versus 85.63 ± 14.92 bpm, p=0.112. Further speed reduction in either group did not result in significant additional change in the HR when compared to 6000 rpm (Group A: 83.22 ± 18.68 bpm versus 84.39 ± 18.24 bpm, p=0.731; Group B: 85.63 ± 14.92 bpm versus 85.88 ± 15.32 bpm, p=0.734).
3.5 Discussion

This chapter sets the scene on how to assess the native myocardial function in patients with the continuous flow HM II LVAD. Unlike pulsatile flow LVADs, continuous flow devices provide less pulsatility and generate negative pressure in the inflow cannula as a result of the suctioning effect of the rotary pump affecting the blood rheology (DiGiorgi PL et al., 2004). Despite this difference in the characteristics of the device, continuous flow devices provide an equivalent degree of haemodynamic support, LV unloading, end-organ perfusion, and exercise capacity to pulsatile LVADs (Feller ED et al., 2007; Garcia S et al., 2008; Haft J et al., 2007; Radovancevic B et al., 2007; Yu JJ et al., 2008).

It is well recognised that successful assessment of the underlying myocardial function in patients with LVAD can only occur after minimising the contribution from the device into the circulation and after ensuring that LV loading is a physiological response to the reduction of the LVAD contribution. Practically, this occurs when the mechanical support is either reduced or effectively discontinued.

We have previously reported that acute cessation of the pulsatile HeartMate I LVAD under heparin cover, is a safe and an efficient method in assessing the underlying function of the native myocardium (George RS et al., 2007b). It is well recognised that cessation of LVADs will result in blood to regurgitate from the ascending aorta into the outflow graft in diastole (i.e. reverse direction) towards the LVAD and subsequently into the LV. The regurgitant volume will be maximal when the device is switched off. In patients supported with pulsatile devices such as the HeartMate I LVAD the regurgitant volume is of insignificance due to the presence of a one-way valve at the inflow cannula obstructing it from reaching the LV and hence retrograde loading of the LV does not occur. Therefore, it can be concluded that LV loading when the pulsatile device is switched off is a physiological response to device cessation which reveals the true function of the underlying myocardium rather than being a consequence of retrograde filling.
This is not true for continuous flow devices such as the HM II LVADs due to the continuous rotary nature of the device and the absence of the one-way valve system. In part one of this tri-component study, a multi-level time model analysis revealed that measurements taken whilst the LVAD running at baseline speed did not change over a 1-year period suggesting that the contribution from the HM II LVAD continued to mask the native LV function preventing real assessment of the underlying LV function. Measurements taken at 5 and 15 minutes of low speed and after the 6MW, on the other hand, showed a significant alteration over the 1-year modelling period. Hence it could be assumed that reducing the input from the HM II LVAD into the circulation by decreasing the speed of the device would allow the LV to partially reload and hence the underlying true function is unmasked. However, reducing the speed of continuous flow devices will lead to less power being generated and less suctioning power resulting in blood flowing back into the outflow graft towards the HM II rotor device in diastole. It can be anticipated that when the device is completely switched off the regurgitant volume will bypass the rotor placed in the housing chamber (figure 2.3) to load the LV i.e. retrograde loading secondary to device cessation.

Should the regurgitant volume reach the LV, assessment of the native myocardial function will be unreliable. Myers et al, have identified a direct relationship between the value of the regurgitant flow measured at the LV outflow tract and the tolerability of device cessation (Myers T et al., 2006). In their study, those who did not tolerate the discontinuation of Jarvik 2000 (another type of continuous flow LVAD) had a bigger regurgitant flow compared to those who did (Myers T et al., 2006). Myers’s approach to assess the regurgitant flow through the LV outflow tract does not solely reflect the reverse flow through the device as it depends on the function of the aortic valve. Slaughter et al, have used the HM II flow estimator to assess the VAD flow (Slaughter MS et al., 2009a). They significantly correlated the readings obtained from the estimator to the intraoperative evaluation of VAD flows using high-fidelity probe placed in the outflow tract (p=0.0001), although the degree of correlation was moderate (r=0.56). They also concluded that data obtained from the estimator should be used to provide directional information for trend purposes only. Therefore, it was important in our patient population to identify a speed where the underlying
myocardial function could be safely and effectively assessed i.e. a speed where blood does not regurgitate back into the LV and that the forward flow generated by the HM II VAD does not interfere with the assessment.

In the mock circuit, part two, a relationship between the device’s speed, the ventricular pressure, the aortic pressure and the flows throughout the circuit, at three different ventricular strengths (poor, moderate, and strong) was identified. At each of the three different ventricular conditions, as the speed of the HM II decreased both aortic pressure and baseline flow decreased steadily whereas the diastolic ventricular pressure and the difference in flow between systole and diastole (ΔQ) increased. These changes could all be related to the reduction in the speed of the HM II VAD in an experimental environment such that:

1) The reduction in the aortic pressure and the baseline flow were due to the reduction in the power being generated by the HM II that drives flow towards the Windkessel system,

2) The rise in the diastolic ventricular pressure was due to the reduction in the negative pressure and the suctioning power generated by the rotary effect of the HM II VAD,

3) The rise in ΔQ was a response to the increase in the pulse pressure being generated in the system due to increased contribution from the Abiomed 5000 pulsatile system and was only apparent when the aortic valve was opening (figure 3.17).

Unfortunately, the video captures of the ink injected in either the outflow or inflow grafts during ventricular contractility were qualitative and could have not been quantified. Nevertheless they provided a clear indication of the complex nature of the pattern of the flow being generated from the HM II VAD and its response to pre-load and after-load. In experiments 2, 3 and 4 where the ventricular contractility was poor, moderate and strong, respectively, the flow pattern at 10000 rpm was complex and turbulent. As the speed of the HM II VAD decreased to 6000 rpm the flow became less turbulent and formed a well defined “cloud-like” pattern that moved forward in systole with minimal but non-significant backward movement in early diastole. The ink stopped flowing in the backward direction at a certain point (referred to as stagnation point i.e. end of
diastole – figure 3.12) and moved forward in systole. At no point the injected ink travelled back into the ventricular chamber of the Abiomed system even when the aortic pressure exceeded the ventricular pressure.

In part three, assessment of flows within the HM II LVAD and the impact of speed alteration on the flow was performed by placing a Pulsed Wave Doppler sample volume at the tip of the inflow cannula. This approach has been previously described by Chumnanvej et al (Chumnanvej S et al., 2007). Using this approach it became clearer that flow across the HM II LVAD consists of two components; a positive spectrum that occurs in systole representing forward flow and a negative spectrum occurring in diastole.

In this part of the study it was shown that speed reduction of the HM II LVAD to 6000 rpm and subsequently lower to either 5000 rpm or 4000 rpm was safe in the clinical setting. Speed reduction was tolerated on all 33 occasions in the 13 studied patients with no observable side effects. Participants were divided into two groups determined by the lowest speed of the device, either 5000 rpm (Group A) or 4000 rpm (Group B). There was no difference in the baseline speed between the two groups (9060 ± 160 rpm versus 9140 ± 240 rpm, median 9000 rpm in each group). The baseline speed was set to ensure no septal shift and that the aortic valve remained closed to ensure maximal LV unloading whilst on LVAD support. Others have used a HM II pulsatility index of 4 or more to provide maximal power output as a guide to setting the baseline speed (Garcia S et al., 2008). Although it would have been desirable, simultaneous assessment of device flow at 5000 rpm and 4000 rpm in every patient was not feasible due to safety concerns over prolonged periods of low flow.

The volume of blood that travelled into the inflow cannula with each cardiac cycle is generated from the suction effect of the HM II LVAD and from LV contractions. This volume could not have been measured directly and hence it had to be calculated as a product of the VTI\textsubscript{f} and the CSA of the inflow cannula, a constant value. Although the calculation assumes that the sample volume remained in a constant position throughout the period of the flow to obtain an accurate VTI\textsubscript{f}, it could be assumed that the calculated volume reflects a true representation of the BV that flows into the inflow cannula.
In both groups speed reduction from the baseline speed to 6000 rpm for 15 minutes resulted in a significant decrease in the forward flow across the inflow cannula by more than 25%. The calculated BV that flowed into the inflow cannula also decreased significantly. This reduction unmasked the native LV function and resulted in the redirection of the blood flow from the LV into the LV outflow tract through the opening aortic valve. Speed reduction to less than 6000 rpm resulted in a further non-significant decrease in the forward flow velocity, the forward velocity time integral, and the blood volume suggesting that more blood has been redirected from the LV into the LV outflow tract and that speed reduction to levels below 6000 rpm is unnecessary to assess the myocardial function.

From figures 3.18 B-D it could be noted that there was an identifiable negative spectrum below the baseline in diastole at the baseline speed and at lower speeds immediately after the positive spectrum (label Z). This may imply that the blood that had flowed into the outflow graft had bypassed the running rotor within the housing chamber and was driven into the LV. This may be true at lower speeds where the aortic valve is opening and when the power generated by the rotor is not high to overcome the pressure difference between the inflow and the outflow ends. However at baseline speeds when the aortic valve does not open and the power generated from the rotor is so high that it overcomes the pressure difference between the inflow and the outflow ends, reverse flow as far as the LV is unlikely. Therefore, the negative spectrum seen at the baseline speeds was thought to be an artefact.

The appearance of the negative spectrum at lower speeds could be explained differently. From the mock circuit experiments the amount of ink that travelled in the backward direction in the outflow graft was under the effects of the speed of the device and the pressure gradient between the aorta and the ventricular chamber. When the speed of the HM II VAD was reduced some ink travelled in the backward direction until it was stopped at the point of stagnation †† (figure 3.12) before reaching the rotary pump of the HM II VAD and never reached the ventricular chamber. Further, visualisation of the flow of the ink injected in the inflow cannula confirmed that the ink moved only forward towards the tip of the

††The point of stagnation is an imaginary point and does not physically stop the flow (figure 3.12)
inflow cannula in systole. In early diastole there was a minute backward displacement of ink in the inflow cannula that did not change in quantity with further speed reduction. This retrograde displacement of flow in the inflow cannula at lower speeds could possibly explain the negative spectrum seen in the echocardiographic PW Doppler assessment of the flow across the inflow cannula which results from the recoil feature of the elastic component of the inflow cannula in diastole secondary to the negative pressure being generated by the LV in diastole. Another explanation for the appearance of the negative spectrum is that it is a representation of a propagated pulse / pressure generated from the blood entering the outflow graft in diastole which itself never reaches the LV.

Left ventricular dimensions and LV function (FS and EF) changed significantly as the speed decreased from baseline to 6000 rpm with no further significant changes at either 5000 rpm or 4000 rpm. This again confirms that sub-6000 rpm speeds are not needed to assess the underlying LV function.

Both diastolic and mean arterial blood pressures decreased significantly in both groups following speed reduction to 6000 rpm. Further speed reduction caused a non-significant decrease in both. There was also a non-significant change in the SBP readings. As the speed decreased from baseline to 6000 rpm and subsequently to lower speed, the PP increased which could be a response to the drop in the DBP. The rise in PP was not significant and its inverse relationship with DBP confirms that the necessity to minimise the suctioning power of the HM II LVAD in order to assess the native LV function. Again there were no significant differences between 6000 rpm and lower speeds.

Limitations

Apart from visualising the ink pattern in the outflow cannula in the mock circuit, there was no other means of quantifying the volume / extent of the regurgitant volume that travelled in the backward direction. Another limitation of the mock circuit was its static characteristic. A third limitation was the position of the HM II VAD which was connected in parallel to the Abiomed system rather than in series (in LVAD patients the HM II is connected at the apex of the LV i.e. in series).
With regards to the clinical component of this study, parameters measured at speeds of 6000 rpm, 5000 rpm and 4000 rpm were not assessed serially in each patient and that the population had to be divided into two groups. A fifth limitation was the inability to differentiate between the blood flow that is being driven into the inflow cannula by the rotary action of the device and the volume that is being generated by LV contractility. It was also not possible to assess the LV outflow tract stroke volume as the inflow cannula position prevented correct alignment of the PW Doppler sample volume on the LV outflow tract.

**Conclusion**

The first component of this study was based on multi-level modelling analysis to show that speed reduction is necessary in patients with HM II LVAD to enable the assessment of the underlying LV function. The choice of the lowest possible speed to assess the native myocardium, where there will be minimum contribution from the device into the circulation, had never been clarified to-date. A bench work study revealed a direct relationship between the device’s speed, pressure and flow. No significant difference in the flow pattern was noted between 6000 rpm, 5000 rpm, and 4000 rpm and although a minute amount of blood travelled in the backward direction in diastole, it never reached the LV; suggesting that ventricular loading following speed reduction is a physiological response rather than retrograde filling from the blood entering through the inflow cannula. In the clinical setting, direct comparison of echocardiographic, haemodynamic, and device flow related parameters between speeds of 6000 rpm, 5000 rpm and 4000 rpm revealed no significant difference. This suggests that reducing the speed to 6000 rpm is adequate to safely and effectively assess the native myocardial function as will be demonstrated in chapter 4.
CHAPTER 4 - Effects of LVAD and Drug Combination Therapy on Left Ventricular Structure, Function and Contractile Reserve
4.1 Background

In 1996, Dr Frazier reported that the LV structure and function change with LVAD support (Frazier OH et al., 1996). His observation was confirmed when several groups studied the left ventricular’s response to LVAD support and identified sufficient degree of myocardial recovery for device explantation (Frazier OH et al., 1996; Levin HR et al., 1996; Loebe M et al., 1997; Müller J et al., 1997; Mancini DM et al., 1998; Frazier OH & Myers TJ, 1999; Farrar DJ et al., 2002; Dandel M et al., 2005). The explantation rate, however, was small and ranged from 5-24%. In 2006, Birks et al prospectively studied 15 non-ischaemic DCM patients receiving pulsatile HM I LVAD and drug combination therapy, the Harefield Bridge-to-Recovery Protocol (Birks EJ et al., 2006). This strategy resulted in sufficient myocardial recovery that allowed LVAD explantation in 73% of the patients. Detailed analyses of the changes in both LV structure and function were later reported by George et al (George RS et al., 2006; George RS et al., 2007a; George RS et al., 2007b). In these studies, it was determined that recovery assessment is a continuous process and that cessation of the pulsatile HM I LVAD is essential to assess the native myocardium.

The Harefield Bridge-to-Recovery protocol uses peak oxygen consumption (VO₂) assessment as one of the explantation criteria (Chapter 2, table 2.2), however, this test requires specific equipment, personnel, and exercise to exhaustion which does not reflect daily activities. The 6-minute walk (6MW) exercise test, although considered a submaximal test, has been shown to correspond to the demands of day-to-day activities and the changes in the LV function which would reflect the contractile reserve (CR) needed in normal day-to-day activities (Bittner V, 1997; Bittner V, 2003).

CR, also known as inotropic reserve, is an old concept brought together from observations made by many investigators who studied the performance of the LV in the failing and the non-failing hearts. In general, CR describes the changes in myocardial contractility in response to a variety of physiologic or pharmacologic stress agents including the sympathetic nervous system (SNS). Over the years
myocardial CR has been shown to provide important prognostic information in both ischaemic and non-ischaemic DCM patients (Pratali L et al., 2007).

Different investigators have used different means in an attempt to provide an objective assessment of CR. These included haemodynamic measurements during cardiac catheterisation and EF assessment by echocardiography performed during either exercise or stress echocardiography (Borow K et al., 1985). In 1997, Nagaoka et al, reported that the change in LV EF following exercise is the most powerful indicator of CR and a strong predictor of survival in DCM patients (Nagaoka H et al., 1997).

The aim of the present is to primarily determine the effects of continuous flow HM II LVAD and drug combination therapy on the LV size (dimensions), function and CR. The second aim is to correlate the changes in the LV function with myocardial recovery and attempt to determine echocardiographic and peripheral haemodynamic predictors of myocardial recovery. Thirdly, to evaluate and compare the differences in the LV response to two different types of LVADs; pulsatile HM I LVAD and continuous flow HM II LVAD.
4.2 Part One – Impact of HM II LVAD on Left Ventricular Size, Function and Contractile Reserve

4.2.1 METHODS

4.2.1.1 Patient population

All 23 patients were included in this study. As mentioned in chapter 2 all were in end-stage HF and on inotropic support prior to device implantation (table 2.1). The Harefield Bridge to Recovery protocol was previously discussed in chapters 1 and 2. In summary anti-failure medication was commenced immediately after weaning the patients from inotropic therapy with adequate end-organ recovery. Assessment of the native LV function was started once patients have started mobilisation. In those who required RVAD support, assessment of the native LV function was started following RVAD explantation and patient mobilisation.

4.2.1.2 Speed reduction protocol

After ensuring an INR of 2.0 and above assessment of the native myocardial function in patients with HM II LVAD was performed after reducing the speed of the device to 6000 rpm, a tested speed (Chapter 3 - experimental and clinical analysis) where forward flow contribution from the device is minimal and that LV loading is a physiological response to speed reduction rather than a response to retrograde filling i.e. off-pump testing equivalent. Assessment of the native LV function was implemented on an out of hour basis in the outpatient setting at Harefield Hospital.

4.2.1.3 Peripheral haemodynamic measurements

Peripheral haemodynamic parameters were measured sequentially in each patient using an automatic blood pressure monitor, Datascope Accutrorr Plus, Datascope, USA. Measurements SBP, DBP, HR, and were performed at six conditions in each patient on every assessment:
i) device running at baseline speed;
ii) immediately after speed reduction to 6000 rpm (0Min);
iii) at 5 minutes of 6000 rpm (5Mins);
iv) at 10 minutes of 6000 rpm (10Mins);
v) at 15 minutes of 6000 rpm (15Mins); and
vi) after a 6MW whilst on 6000 rpm (see section 4.2.1.5 for the protocol).

PP was calculated as the difference between the SBP and DBP whereas the MAP was calculated by adding one third of the PP to the DBP.

4.2.1.4 Echocardiographic assessment of the LV size and function

Echocardiographic parameters were also measured sequentially in each patient throughout the study at four different conditions:

i) device running at baseline speed;
ii) at 5 minutes of 6000 rpm (5Mins);
iii) at 15 minutes of 6000 rpm (15Mins); and
iv) after a 6MW whilst on 6000 rpm (see section 4.2.1.5 for the protocol).

All echocardiographic assessments were performed by one senior echocardiographer (Mrs Carole Webb) at Harefield using either the iE33 machine (Philips, The Netherlands), or the Vivid 7 (GE Healthcare, USA). All measurements were performed in the M-Mode using a transducer with harmonic imaging. To eliminate bias the radiographer was blinded to the recovery status of each patient.

LVEDD and LVESD were obtained from the parasternal long axis of the LV at the level of chordal-mitral valve junction. The systolic diameter was measured at the onset of the QRS complex using the leading edge method i.e. from the leading edge of the LV septal endocardial tissue to the leading edge of the posterior wall endocardial tissue. The diastolic diameter was also measured by the leading edge method at either the peak of posterior wall motion or the nadir of septal motion.
FS was calculated as the percentage change in the LV cavity dimensions with systole (equation 4.1):

\[ FS = \frac{(LVEDD - LVESD)}{LVEDD} \times 100\%. \]  

(4.1)

Although it would have been desirable, but due to time constraint (each assessment takes at least 1 hour) and the altered internal architecture of the LV in patients with LVAD, Simpson’s Biplane EF could not have been acquired on every assessment. Therefore, to ensure standardisation in determining the LV function, EF was calculated using the cubed function formula of the ventricular dimensions (equation 4.2):

\[ EF = \frac{(LVEDD^3 - LVESD^3)}{LVEDD^3} \times 100\%. \]  

(4.2)

4.2.1.5 Six-Minute walk exercise test to assess myocardial contractile reserve

The absolute change in EF following dobutamine infusion has been commonly used to describe the contractile ability of the LV in response to pharmacological stress (dobutamine infusion). An absolute rise in the EF by 5% during dobutamine infusion as compared to resting was considered to be a positive indicator for the presence of CR with strong correlation to both prognosis and to different therapeutic approaches. In this study, dobutamine stress echocardiography (DSE) was not utilised as means for assessing CR as the presence of the LVAD in situ would distort the internal structure of the LV, hence rendering the pattern of LV wall motion and segmental viability obtained from DSE unreliable. Furthermore, assessment of the extent of myocardial recovery is a continuous and rigorous process that needed to be performed on regular basis and the use of DSE on 4-6 weekly basis would have been unethical, unacceptable by the patient, and would have required a consultant cardiologist performing the examination.

The 6MW test on the other hand had proven to be a reproducible and an inexpensive technique which can be performed serially in each patient in the outpatient clinic setting and was accepted by all patients. **Similar to DSE technique,**
we have used an absolute increase in FS and EF following the 6MW by at least 5% as cut-off points to indicate preservation of CR.

If the speed of 6000 rpm, although recognized that may not be optimal to assess the native LV function, was tolerated for 15 minutes, the patient performed a 6MW exercise test on a flat, zero-gradient surface (75 meters long) with the speed of the HM II LVAD running at 6000 rpm. Upon completion of the 6MW, repeat echocardiographic and haemodynamic measurements were acquired to determine the LV’s response to exercise. *None of the patients performed the 6MW test during their first “low-speed” assessment such that the first low speed assessment test was confined to a 15 minutes of low speed testing only.* This approach was taken for safety reasons and to make sure patients could tolerate a period of 15 minutes of low speed before the 6MW test and that they are more rehabilitated in order to complete the 6MW exercise test. CR was defined as the percentage difference between FS and EF obtained after the 6MW with the HM II LVAD running at 6000 rpm and after 15 minutes of 6000 rpm. The distance walked was also calculated.

### 4.2.1.6 Data collection and statistics

All data were collected prospectively. Data analysis was done using SPSS version 16.0 for windows (Lead Technologies). All data was normally distributed. Parametric *t*-test was used for sequential variable analysis and unpaired *t*-test was used for inter-group analysis (recovery versus non-recovery). Receiver Operating Characteristic (ROC) analysis was used to determine echocardiographic and haemodynamic predictive factors for myocardial recovery. Linear correlation analysis was used to determine the correlation between CR measured indices and myocardial recovery. Percentage changes between subsequent measurements were calculated as:

\[
\text{Percentage change} = \frac{\text{Second Measurement} - \text{First Measurement}}{\text{First Measurement}} \times 100
\]
Time model analysis was performed to determine the trends in the echocardiographic and haemodynamic parameters over a 1-year period and the differences in the trends between the recovered and the non-recovered patients. This model assumes that for each patient there is a linear relationship between the mean of each measured parameter and a 1-year period of support. The mean intercept of this relationship represents the value of the measured parameter at time 0. The mean slope of the relationship refers to the change in the measured parameter over a period of time such that a positive slope indicates an increase in the value and a negative slope indicates a reduction in the value. Refer to Model 2 in Appendix B for model details.

Unless stated otherwise, all measurements are presented as means ± stdev. A p-value < 0.05 was considered to be statistically significant.
4.2.2 RESULTS

4.2.2.1 Feasibility and tolerability of testing

The first low-speed assessment was performed within 37 days (range 20-76 days) of device implantation. The test was repeated thereafter every 4 to 6 weeks. In total, the speed of the device was reduced to 6000 rpm on 170 occasions (mean of 7.4 tests per patient, range 4-14 tests with median of 7 tests).

Running the HM II LVAD at 6000 rpm for 15 minutes was tolerated on all 170 assessments. None of the patients has developed any signs of overt HF. As stated above, none of the patients undertook the 6MW test on the first low-speed assessment leaving the total number of occasions where the 6MW test could have been performed to 147. The 6MW, however, was only tolerated on 144 out of the 147 potential occasions (98%). On three occasions, patient number 6 has developed shortness of breath and the test was interrupted and the speed was re-adjusted to his baseline speed (9000 rpm). All patients were followed up for 72 hours and none had experienced short-term or long-term adverse events especially thromboembolic related complications.

4.2.2.2 Effects of low speed on peripheral haemodynamic parameters

Figure 4.1A illustrates the mean instantaneous effects of speed reduction on the SBP, DBP, PP, and MAP during the 170 low-speed assessments. Immediate speed reduction from the baseline speed (range 8600 rpm – 9800 rpm) to 6000 rpm has resulted in a significant decrease in the SBP from 87.64 ± 6.36 mmHg to 83.56 ± 6.88 mmHg (p=0.005), DBP from 58.06 ± 5.72 mmHg to 50.15 ± 4.04 mmHg (p<0.001), and MAP from 68.17 ± 5.32 mmHg to 61.41 ± 4.04 mmHg (p<0.001). The PP increased significantly by 12.61% immediately after speed reduction (29.64 ± 5.52 mmHg versus 33.38 ± 6.87 mmHg, p=0.002). At 5, 10 and 15 minutes of 6000 rpm there were no significant changes in any of the blood pressure measured parameters compared to those taken immediately after speed reduction i.e. 0Min.
Figure 4.1A: Blood pressure response to speed reduction

**Fig 4.1A:** There was a significant reduction in SBP, DBP and MAP measurements and a significant increase in PP immediately after speed reduction from the baseline speed to 6000 rpm. All parameters were stabilized thereafter as the speed of the HM II LVAD continued to run at 6000 rpm. * p=0.005, ** p<0.001, † p=0.002.
No significant change in the HR was noted as the speed of the HM II LVAD decreased from baseline to 6000 rpm (83.35 ± 10.40 versus 83.96 ± 11.68, p=0.404). There was also no significant difference in the HR at 5, 10 or 15 minutes of low speed (figure 4.1B).

Following the 6MW exercise test, there was a significant increase in all peripheral haemodynamic parameters as compared to those acquired at 15 minutes (table 4.1).

### Table 4.1: Peripheral haemodynamic response to the 6MW exercise test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>15Mins of 6000 rpm</th>
<th>Post 6MW</th>
<th>Change (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>85.23 ± 9.00</td>
<td>92.76 ± 10.67</td>
<td>8.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>51.68 ± 5.34</td>
<td>55.04 ± 7.12</td>
<td>6.50</td>
<td>0.011</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>63.07 ± 5.80</td>
<td>67.69 ± 7.58</td>
<td>7.32</td>
<td>0.001</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>33.50 ± 6.78</td>
<td>37.74 ± 8.87</td>
<td>12.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>82.39 ± 11.03</td>
<td>98.80 ± 12.64</td>
<td>19.91</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4.1: Following the 6MW all peripheral haemodynamic parameters increased significantly as compared to the measurements acquired at 15 minutes of low speed test.
Immediate speed reduction did not have a significant effect on the HR which remained stable with minimal change over the 15 minutes of 6000 rpm.
4.2.2.3  Effects of low speed on echocardiographic parameters

Following 5 minutes of low speed, echocardiographic assessment revealed a significant increase in both LVEDD and LVESD as compared to the baseline measurements (57.43 ± 11.45 mm versus 51.60 ± 12.26 mm, p<0.001; and 45.21 ± 13.89 mm versus 41.19 ± 14.23 mm, p<0.001, respectively). Continuous support at 6000 rpm for 15 minutes did not have a significant effect on either dimension (figure 4.2A) although they remained significantly higher as compared to the baseline measurements (LVEDD: 57.24 ± 12.25 mm versus 51.60 ± 13.10 mm, p<0.001; LVESD: 45.35 ± 14.33 mm versus 41.19 ± 14.23 mm, p<0.001).

Unlike the changes seen in the ventricular dimensions, speed reduction from baseline speed to 6000 rpm did not have a significant impact on either FS or EF at either 5 or 15 minutes (figure 4.2B). Furthermore, there was no significant difference between 5 and 15 minutes of low speed (FS: 22.70 ± 8.55% versus 22.80 ± 9.04%, p=0.785; EF: 51.46 ± 16.40% versus 51.53 ± 17.30%, p=0.905).

Following the 6MW exercise test, the LVESD decreased significantly by 4.19% as compared to 15 minutes low speed (43.45 ± 15.11 mm versus 45.35 ± 14.33 mm, p=0.001) whereas the change in LVEDD was very small (0.04%) and not significant (57.60 ± 11.49 mm versus 57.58 ±11.62 mm, p=0.970). Also, both FS and EF increased significantly when compared to 15 minutes of low speed (26.29 ± 11.23% versus 22.80 ± 9.04%, p<0.001; and 56.79 ± 20.17% versus 51.53 ± 17.30 %, p<0.001, respectively). The absolute increases in both parameters were equivalent to 3.49± 3.70% and 5.25 ± 5.30%, respectively. These changes corresponded to 15.31% and a 10.20% percentage rise after the 6MW exercise test, respectively (figure 4.2C).
Figure 4.2A: Changes in LVEDD and LVESD following speed reduction. At 5 minutes, both LVEDD and LVESD increased significantly with no further changes at 15 minutes although the measurements taken at 15 minutes remained significantly higher than the baseline measurements. * p<0.001
**Fig 4.2B:** Changes in FS and EF following speed reduction. There were no significant change at either 5 or 15 minutes.
Figure 4.2C: Response of LV function to 6MW

Fig 4.2C: The percentage increase in both FS and EF following the 6MW was 15.31% and 10.20%, respectively. * p<0.01
4.2.2.4 Recovered versus Non-Recovered

As been described in chapter 2, 15 patients had recovered (Recovered (Rec) Group) whereas 8 did not recover (Non-Recovered (NRec) Group). A comparison of the pre-implantation demographics and clinical data between the two groups is included in table 4.2. There were no differences in the pre-implantation clinical parameters between the two subpopulations.

Table 4.3 compares the haemodynamic and the echocardiographic parameters measured between the two groups with the HM II LVAD running at baseline speed and the amount of the phase I therapy at the first assessment. The average baseline speed was 9080 ± 250 rpm in the Rec group (median 9200 rpm, range 8600 – 9400 rpm) as compared to 9310 ± 280 rpm in the NRec group (median 9400 rpm, range 8800 – 9800 rpm). There was no statistical difference between the two average speeds (p=0.110). Furthermore, there were no significant differences in: (i) the time points when the first low speed assessment was performed, (ii) the drug regimen, and (iii) the peripheral haemodynamic parameters. LVESD, FS and EF were significantly better in the Rec group at the baseline speed on the first assessment as compared to the NRec group. The LVEDD was also smaller in the Rec group as compared to the NRec group but did not reach the level of significance. There was no correlation between the baseline speed of the device and the acquired haemodynamic and echocardiographic parameters ($R^2 = 0.29$, p=0.310) suggesting that the initial echocardiographic and haemodynamic differences between the two groups were not secondary to speed differences. There was also no correlation between the echocardiographic parameters measured at pre-implantation and at the first assessment and myocardial recovery.

The following three subsections (4.2.2.4.i – 4.2.2.4.iii) evaluate the different responses to speed reduction and the 6MW exercise test between the Rec and the NRec groups.
Table 4.2: Differences in the pre-implantation demographics, haemodynamics and echocardiographic parameters between the recovered and the non-recovered patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recovered Patients (n=15)</th>
<th>Non-Recovered Patients (n=8)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF Duration (months)</td>
<td>15.03 ± 24.81</td>
<td>61.75 ± 41.08</td>
<td>0.002</td>
</tr>
<tr>
<td>Implant Age (yrs)</td>
<td>32.30 ± 13.85</td>
<td>40.73 ± 14.26</td>
<td>0.149</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.17 ± 3.20</td>
<td>24.61 ± 2.50</td>
<td>0.357</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.84 ± 0.21</td>
<td>1.88 ± 0.11</td>
<td>0.548</td>
</tr>
<tr>
<td>Systolic PA pressure (mmHg)</td>
<td>45.13 ± 8.20</td>
<td>50.43 ± 13.96</td>
<td>0.536</td>
</tr>
<tr>
<td>Diastole PA pressure (mmHg)</td>
<td>24.38 ± 6.25</td>
<td>30.00 ± 5.29</td>
<td>0.072</td>
</tr>
<tr>
<td>Mean PA pressure (mmHg)</td>
<td>33.38 ± 4.14</td>
<td>38.71 ± 7.91</td>
<td>0.121</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>27.50 ± 4.09</td>
<td>33.29 ± 7.87</td>
<td>0.070</td>
</tr>
<tr>
<td>Cardiac Output (l/min)</td>
<td>2.76 ± 1.17</td>
<td>2.54 ± 0.61</td>
<td>1.000</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m²)</td>
<td>1.54 ± 0.64</td>
<td>1.30 ± 0.39</td>
<td>0.525</td>
</tr>
<tr>
<td>PA saturation (%)</td>
<td>39.43 ± 12.29</td>
<td>47.28 ± 9.59</td>
<td>0.247</td>
</tr>
<tr>
<td>End-diastolic diameter (mm)</td>
<td>69.47 ± 7.38</td>
<td>79.50 ± 6.77</td>
<td>0.011</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>64.00 ± 6.49</td>
<td>73.67 ± 6.06</td>
<td>0.006</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>7.74 ± 4.20</td>
<td>7.29 ± 2.79</td>
<td>0.677</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>21.03 ± 10.04</td>
<td>20.11 ± 6.71</td>
<td>0.680</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>10.08 ± 4.45</td>
<td>16.09 ± 7.06</td>
<td>0.065</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>136.60 ± 69.07</td>
<td>201.78 ± 89.38</td>
<td>0.101</td>
</tr>
<tr>
<td>Bilirubin (μmol/L)</td>
<td>45.93 ± 29.56</td>
<td>45.56 ± 15.57</td>
<td>0.728</td>
</tr>
<tr>
<td>Alkaline Phosphotase (U/L)</td>
<td>85.60 ± 53.01</td>
<td>76.33 ± 27.75</td>
<td>0.925</td>
</tr>
<tr>
<td>Alanine Transaminase (U/L)</td>
<td>577.20 ± 671.55</td>
<td>90.78 ± 85.62</td>
<td>0.149</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>31.67 ± 5.65</td>
<td>30.56 ± 5.90</td>
<td>0.925</td>
</tr>
<tr>
<td>BNP (pmol/L)</td>
<td>241.10 ± 259.31</td>
<td>459.50 ± 470.74</td>
<td>0.460</td>
</tr>
</tbody>
</table>

Table 4.2: Direct comparison of pre-implant demographics, clinical parameters and biochemical profile between the recovered (n=15) and the non-recovered (n=8) patients.
**Table 4.3:** Differences between the Rec and the NRec groups during the first low speed assessment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rec Group (n=15)</th>
<th>NRec Group (n=8)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Point of first assessment since HMII implantation (days)</td>
<td>37.53 ± 17.74</td>
<td>42.89 ± 20.31</td>
<td>0.438</td>
</tr>
<tr>
<td>Baseline Speed (rpm)</td>
<td>9080 ± 250</td>
<td>9310 ± 280</td>
<td>0.110</td>
</tr>
<tr>
<td>Haemodynamic Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>88.00 ± 7.92</td>
<td>87.57 ± 12.82</td>
<td>0.944</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>60.13 ± 6.53</td>
<td>59.71 ± 13.02</td>
<td>0.596</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>27.87 ± 2.89</td>
<td>27.86 ± 4.76</td>
<td>0.974</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>69.42 ± 5.84</td>
<td>73.14 ± 9.81</td>
<td>0.217</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>92.80 ± 16.50</td>
<td>88.43 ± 16.72</td>
<td>0.549</td>
</tr>
<tr>
<td>Echo Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>49.60 ± 13.39</td>
<td>61.67 ± 11.99</td>
<td>0.070</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>39.27 ± 12.66 *</td>
<td>54.11 ± 12.10 *</td>
<td>0.010</td>
</tr>
<tr>
<td>FS (%)</td>
<td>21.50 ± 7.15 *</td>
<td>12.65 ± 4.64 *</td>
<td>0.005</td>
</tr>
<tr>
<td>EF (%)</td>
<td>50.52 ± 12.60 *</td>
<td>32.85 ± 10.80 *</td>
<td>0.004</td>
</tr>
<tr>
<td>Phase I Medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lisinopril (mg)</td>
<td>20.00 ± 8.90</td>
<td>20.00 ± 16.51</td>
<td>0.755</td>
</tr>
<tr>
<td>Spironolactone (mg)</td>
<td>25.00 ± 0.00</td>
<td>25.00 ± 0.00</td>
<td>1.000</td>
</tr>
<tr>
<td>Digoxin (mcg)</td>
<td>119.32 ± 18.84</td>
<td>125.00 ± 0.00</td>
<td>0.425</td>
</tr>
<tr>
<td>Carvedilol (mg)</td>
<td>15.40 ± 11.79</td>
<td>17.71 ± 16.50</td>
<td>0.932</td>
</tr>
</tbody>
</table>

Table 4.3: The echocardiographic and haemodynamic parameters obtained at the first assessment with the HM II LVAD running at baseline speed. LVESD and LV function were significantly better in the Rec group as compared to the NRec group. There were no significant differences in the time point at which the first assessment was performed, the baseline speed, haemodynamic parameters, or the amount of phase I medication.
4.2.2.4.i  Haemodynamic responses to immediate speed reduction and the 6MW test

a) Response to immediate speed reduction

In the Rec group immediate speed reduction to 6000 RPM resulted in a drop in SBP, DBP, and MAP by 2.74%, 13.88%, and 9.01%, respectively, and in an increase in the PP by 18.92% (figure 4.3A). The reductions in both DBP and MAP and the increase in PP were statistically significant (DBP: 48.75 ± 3.50 mmHg versus 56.61 ± 5.41 mmHg, p<0.001; MAP: 60.34 ± 3.70 mmHg versus 66.32 ± 4.67 mmHg, p<0.001; PP: 34.63 ± 6.23 mmHg versus 29.12 ± 5.94 mmHg, p<0.001) whereas the reduction in SBP was not significant (83.38 ± 6.51 versus 85.73 ± 5.53, p=0.116). There was no further significant change in any of the four parameters as the HM II LVAD continued to run at 6000 rpm.

In the NRec group (figure 4.3B) immediate speed reduction also resulted in a significant decrease in SBP, DBP, and MAP (SBP: 83.85 ± 7.87 mmHg versus 90.82 ± 6.68 mmHg (-7.67%), p=0.020; DBP: 52.47 ± 3.99 mmHg versus 60.47 ± 5.69 mmHg (-13.23%), p=0.001; and MAP: 63.19 ± 4.17 mmHg versus 71.27 ± 5.09 mmHg (-11.34%), p=0.001) and a fractional non-significant increase in the PP (31.29 ± 7.74 versus 30.51 ± 4.95 mmHg (2.57%), p=0.713).

The drop in the SBP was more significant in the NRec group as compared to the Rec group (7.67% versus 2.74%, p=0.04). There were no significant differences in the percentage reductions in either DBP or MAP between the two groups (13.88% versus 13.23%, p=0.186; and 9.01% versus 11.34%, p=0.186, respectively). The percentage change in PP at 0Min was significantly higher in the Rec group as compared to the NRec group (18.92% versus 2.57%, p=0.04).

There was no significant change in the HR in either group following immediate speed reduction with no further changes at 5, 10 or 15 minutes (figure 4.3C).
Figure 4.3A: Response of blood pressure to speed reduction in the Rec group

Fig 4.3A: Immediate speed reduction resulted in a significant decrease in both DBP and MAP and a significant increase in PP in the Rec group. The reduction in SBP was not significant. * p<0.001.
Figure 4.3B: Response of blood pressure to speed reduction in the NRec group

**Fig 4.3B:** Immediate speed reduction resulted in a significant decrease in SBP, DBP and MAP. The reduction in SBP was more profound in the NRec group as compared to the Rec group (7.67% versus 2.74%, p=0.040). * p=0.02, ** p=0.001.
Figure 4.3C: Response of heart rate to speed reduction in both Rec and NRec groups

Fig 4.3C: Speed reduction did not have a significant effect on the HR in either group.
b) Response to the 6MW exercise test

In the Rec group, the 6MW exercise test resulted in a significant increase in SBP, DBP, PP, MAP, and HR as compared to the measurements acquired at 15 minutes of low speed (SBP: 94.56 ± 10.46 mmHg versus 85.13 ± 8.75 mmHg, p<0.001; DBP: 53.90 ± 6.97 mmHg versus 50.45 ± 4.81 mmHg, p=0.019; PP: 40.66 ± 7.13 mmHg versus 34.68 ± 6.58 mmHg, p<0.001; MAP: 67.55 ± 7.64 mmHg versus 62.08 ± 5.57 mmHg, p=0.001; HR: 100.48 ± 14.91 bpm versus 83.19 ± 11.43 bpm, p<0.001, Figure 4.4A). The changes in SBP, DBP, PP, MAP, and HR following the 6MW exercise test represented an increase by 11.07%, 6.83%, 17.23%, 8.82%, and 20.79%, respectively.

In the NRec group, the 6MW did not result in a similar significant increase in any of the SBP, DBP, PP, and MAP parameters (SBP: 89.75 ± 10.61 mmHg versus 85.38 ± 9.93 mmHg, p=0.117; DBP: 56.94 ± 7.37 mmHg versus 53.72 ± 5.84 mmHg, p=0.240; PP: 32.86 ± 6.80 versus 31.52 ± 7.05, p=0.333; MAP: 67.93 ±7.92 mmHg versus 64.74 ± 6.11 mmHg, p=0.242, Figure 4.4B). The HR, however, increased significantly by 18.41% following the 6MW (95.99 ± 7.50 bpm versus 81.07 ± 10.87 bpm, p<0.001).

Figure 4.4C represents the differences in the percentage change in the peripheral haemodynamic parameters following the 6MW exercise test between the two groups such that the percentage change in SBP, DBP, MAP and HR were higher in the Rec group as compared to the NRec group but not significantly (11.07% versus 5.12%, p=0.155; 6.83% versus 5.98%, p=0.723; 8.82% versus 4.92%, p=0.379; and 20.79% versus 18.41%, p=0.904, respectively). The percentage change in the PP, however, was significantly higher in the Rec group as compared to the NRec group (17.23% versus 4.27%, p=0.028).
Figure 4.4A: Response of peripheral haemodynamics to the 6MW exercise test in the Rec group

**Fig 4.4A:** There was a significant increase in SBP, DBP, PP, MAP, and HR following the 6MW in the Rec group. * p<0.001, ** p=0.019, † p=0.001
Figure 4.4B: Response of peripheral haemodynamics to the 6MW exercise test in the NRec group

**Fig 4.4B:** There was no significant change in SBP, DBP, PP, or MAP following the 6MW. Similar to the Rec Group, the HR increased significantly after the 6MW. * p<0.001
Figure 4.4C: Comparison in the percentage change in the haemodynamic parameters following the 6MW exercise test

Fig 4.4C: Following the 6MW, the percentage change in SBP, DBP, PP, MAP and HR was higher in the Rec subpopulation compared to the NRec subpopulation. Data presented as mean percentage change. * p=0.028
4.2.2.4.ii  

**Echocardiographic responses to speed reduction and the 6MW exercise test**

a) **Response to 5Mins of low-speed**

In the two groups, the changes seen in the LV dimensions over 15 minutes of low speed followed the same trend as the entire 23 studied patients (section 4.2.2.3 and figure 4.2A). In each group, both LVEDD and LVESD were significantly higher at 5 minutes and 15 minutes as compared to the baseline measurements. Intra-group analysis revealed no difference between the 5 and 15 minutes measurements in either group (figure 4.5A). However, inter-group analysis revealed that both LVEDD and LVESD at 15 minutes were significantly lower in the Rec group as compared to the NRec group (LVEDD: 51.24 ± 6.67 mm versus 68.13 ± 10.48, p<0.001; and LVESD: 36.81 ± 6.20 mm versus 59.59 ± 12.58 mm, p<0.001, both parameters are denoted by Ω on figure 4.5A).

Speed reduction did not have a significant effect on the LV function in either group (figure 4.5B). At 15 minutes both FS and EF were significantly higher in the Rec group as compared to the NRec group (FS: 28.61 ± 4.13% versus 13.11 ± 5.98%, p<0.001; and EF: 62.55 ± 6.24% versus 33.17 ± 13.63%, p<0.001).
Figure 4.5A: Effects of speed reduction on LV dimensions in the Rec and the NRec groups.

**Fig 4.5A:** After 5 minutes of speed reduction both LVEDD and LVESD increased significantly in both groups and remained significantly higher at 15 minutes of low speed. There were no significant differences in the LV dimensions between 5 minutes and 15 minutes of low speed. Both LVEDD and LVESD were significantly lower in the Rec group versus the NRec group. * p<0.001, Ω p<0.001, ** p=0.001, *** p=0.005.
Figure 4.5B: Effects of speed reduction on LV function in the Rec and the NRec groups

Fig 4.5B: There were no significant changes in either FS or EF in either group after reducing the speed of the HM II LVAD for 5 minutes. There was no significant change in the function between 5 minutes and 15 minutes of low speed. At 15 minutes both FS and EF were significantly higher in the Rec group as compared to the NRec group. * p<0.001.
b) Response to the 6MW exercise test

Following the 6MW, the LVESD in the Rec group decreased significantly as compared to the 15 minutes low-speed (34.42 ± 6.20 mm versus 36.81 ± 6.20 mm, p=0.003). There was no significant change in the LVEDD (51.83 ± 7.20 mm versus 51.24 ± 6.67 mm, p=0.463). Both FS and EF increased significantly by 5.29 ± 3.39% and 7.73 ± 4.37%, respectively. The percentage increase was equivalent to **18.50%** increase in FS following the 6MW exercise test and a **12.35%** increase in EF following the 6MW exercise test (FS: 33.91 ± 4.46% versus 28.61 ± 4.13%, p<0.001; and EF: 70.27 ± 5.99% versus 62.55 ± 6.24 %, p<0.001, figure 4.6).

Figure 4.7 illustrates the LV response to 6MW exercise test in the NRec group. There was no significant change in either LVEDD or LVESD as compared to the 15 minutes low speed (67.21 ± 11.08 mm versus 68.13 ± 10.48 mm, p=0.177, and 58.51 ± 13.46 mm versus 59.59 ± 12.58 mm, p=0.212, respectively). Both FS and EF increased but not significantly by an absolute value of 0.48 ± 1.71% (13.59 ± 6.14% versus 13.11 ± 5.98%, p=0.419) and 1.13 ± 4.20% (34.31 ± 14.05% versus 33.17 ± 13.63%, p=0.442), respectively. These changes were equivalent to a percentage increase of 3.70% in FS and a percentage increase in the EF by 3.42% following the 6MW.

The percentage change in both FS and EF following the 6MW exercise test was significantly higher in the Rec group as compared to the NRec group (18.50% versus 3.70%, p=0.012, and 12.35% versus 3.42%, p=0.012, figure 4.8). The differences in these changes suggest that the CR in the recovered patients is better than those who did not recover reflecting the poorer LV response to exercise in the non-recovered patients.

**4.2.2.4.iii Differences in the 6MW distance between the Rec and the NRec groups**

In total all 23 patients managed 571.96 ± 101.05 meters. The recovered group managed 604.07 ± 94.02 meters as compared to 518.45 ± 93.46 meters (p=0.048).
Figure 4.6: Response of LV structure and function to the 6MW exercise test in the Rec group

Fig 4.6: There was a significant increase in FS and EF following the 6MW there was an absolute increase in both FS and EF by 5.30% and 7.72%, respectively, corresponding to a percentage change of 18.50% and 12.35%, respectively. ** p=0.003, * p<0.001.
Figure 4.7: Response of LV structure and function to the 6MW exercise test in the NRec group

**Fig 4.7:** There was no significant change in ESD and EDD following 6MW. Unlike the recovered group the absolute increase in both FS and EF corresponded to 0.49% and 1.14%, respectively. These changes represent a percentage change of 3.70% and 3.42%, respectively.
Figure 4.8: Percentage change in the echocardiographic parameters following the 6MW exercise test– Rec versus NRec groups

Fig 4.8: Following the 6MW, the percentage change in FS and EF were significantly higher in the Rec group as compared to the NRec group. Data presented as means of percentage change. * p=0.012
4.2.2.4.iv Difference in the left ventricular structural and functional trends between the Rec and the NRec groups over a 1-year period – time modelling

A multi-level time modelling was performed to determine the differences in the LV structural and functional trends between the Rec and the NRec groups over a 1 year period of support (please refer to Model 2 in Appendix B for details of the analysis).

The model revealed that at 15 minutes of low speed (figure 4.9A) and after the 6MW exercise (figure 4.9B) the mean intercepts of all echocardiographic measured parameters are significantly better in the Rec group i.e. the mean value at time 0 is significantly better in the Rec group. This was also shown in table 4.3 where the Rec group had significantly better echocardiographic parameters as compared to the NRec patients at the first low-speed assessment.

Furthermore, the mean slopes were significantly better in the Rec group suggesting that the mean changes over the 1-year modelling period is significantly better in the Rec group. These results conclude that the echocardiographic differences seen after 15 minutes of low speed and after the 6MW exercise test between the Rec and the NRec groups are not just differences in average measurements but also differences in the trends over a period of time with better outcome in the recovered patients.
Figure 4.9A: Differences in the echocardiographic trends between the Rec and the NRec groups at 15 minutes of low speed

**LVEDD**
- p<0.001
- p-value of slope = 0.017

**LVESD**
- p<0.001
- p-value of slope = 0.002

**FS**
- p-value of slope < 0.001
- p<0.001

**EF**
- p<0.001
- p-value of slope = 0.002

**Fig 4.9A:** Differences in the echocardiographic trends between the Rec (—) and the NRec (—) groups at 15 minutes of low speed. The intercept was significantly better in the recovered group compared to the non-recovered group. The mean slope was also significantly better in the Rec group.
Figure 4.9B: Differences in the echocardiographic trends between the Rec and the NRec groups after the 6MW exercise test

**LVEDD**
- Intercept was significantly better in the recovered group compared to the non-recovered group.
- Mean slope was also significantly better in the Rec group.

**LVESD**
- Intercept was significantly better in the recovered group compared to the non-recovered group.
- Mean slope was also significantly better in the Rec group.

**FS**
- Intercept was significantly better in the recovered group compared to the non-recovered group.
- Mean slope was also significantly better in the Rec group.

**EF**
- Intercept was significantly better in the recovered group compared to the non-recovered group.
- Mean slope was also significantly better in the Rec group.

**Fig 4.9B**: Differences in the echocardiographic trends between the Rec (—) and the NRec (—) groups after 6MW. The intercept was significantly better in the recovered group compared to the non-recovered group. The mean slope was also significantly better in the Rec group.
4.2.2.5 Echocardiographic and haemodynamic predictors of myocardial recovery

Linear correlation analysis revealed that the strongest correlation existed between echocardiographic parameters measured after the 6MW exercise test and myocardial recovery (R value ranged between 0.731 and 0.8, table 4.4). The correlation between the haemodynamic parameters measured throughout the 6 different conditions and myocardial recovery were very poor with an R value ranging between 0.03 and 0.336 and never reached level of significance.

Logistic regression analysis of the echocardiographic parameters measured after the 6MW exercise test showed that an EF of 56.3% was a strong predictor of myocardial recovery (ROC curve area = 1.0, 100% sensitivity, and 97.5% specificity, p<0.001). The percentage change in EF by 8.3%, which corresponds to an absolute increase by 5% after the 6MW exercise test was a strong predictor of myocardial recovery (ROC curve area = 0.889, sensitivity 73%, and specificity 89% p=0.002).
Table 4.4: Correlation analysis between echocardiographic parameters and myocardial recovery

<table>
<thead>
<tr>
<th>Echocardiographic parameter</th>
<th>LVEDD R</th>
<th>p-value</th>
<th>LVESD R</th>
<th>p-value</th>
<th>FS R</th>
<th>p-value</th>
<th>EF R</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM II LVAD at baseline speed</td>
<td>0.600</td>
<td>&lt;0.001</td>
<td>0.677</td>
<td>&lt;0.001</td>
<td>0.653</td>
<td>&lt;0.001</td>
<td>0.653</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5 Minutes low speed</td>
<td>0.631</td>
<td>&lt;0.001</td>
<td>0.698</td>
<td>&lt;0.001</td>
<td>0.665</td>
<td>&lt;0.001</td>
<td>0.665</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>15 minutes low speed</td>
<td>0.636</td>
<td>&lt;0.001</td>
<td>0.704</td>
<td>&lt;0.001</td>
<td>0.694</td>
<td>&lt;0.001</td>
<td>0.694</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post 6MW</td>
<td>0.789</td>
<td>&lt;0.001</td>
<td>0.731</td>
<td>&lt;0.001</td>
<td>0.800</td>
<td>&lt;0.001</td>
<td>0.800</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4.4: Linear correlation analysis revealed that all echocardiographic parameters were predictive of myocardial recovery, however, measurements acquired following the 6MW exercise test, specifically FS and EF, were the strongest with an R value of 0.8.
4.3 Part Two – Comparison of the LV Response between HM I and HM II LVAD

In this section, the haemodynamic and the LV responses to off-pump testing and the 6MW exercise testing is compared between patients receiving the pulsatile HM I LVAD and the continuous flow HM II LVAD. Differences in device specification and properties have been previously discussed in Chapter 1.

In 2006 and 2007, we have reported the effects of pulsatile HM I LVAD and drug combination therapy on the peripheral haemodynamics, LV structure and function, and CR in non-ischaemic DCM patients (George RS et al., 2006; George RS et al., 2007a). The analysis depended on acute device cessation of the HM I LVAD i.e. “off-pump” testing.

4.3.1 METHODS

4.3.1.1 HM I LVAD patient population

Since December 1999, 39 patients with non-ischaemic DCM were implanted with the pulsatile HM I LVAD. Out of those four had the device implanted on compassionate ground and died in the interim of the peri-operative course, nine had early post-operative deaths, two had their device failed and one received a heart transplant without starting the phase II therapy. The remaining 23 patients remained potential candidates for myocardial recovery and received both phase I and phase II therapy and were assessed for recovery. A comparison in the pre-implantation demographics and clinical characteristics between the 23 HM I LVAD patients and the 23 HM II LVAD patients (the main cohort of the thesis) is presented in table 4.5. The HM I population had a significantly higher BMI and BSA as compared to the HM II population justifying the use of the larger HM I device in the former group. Cardiac output and renal function were significantly worse in the HM II population. The remaining clinical parameters were also worse in the HM II population but did not reach level of significance.

‡‡ The inclusion of the HM I LVAD group in this chapter is for comparative purposes only to determine the responses of the patients to different LVAD types.
### Table 4.5: Pre-implantation demographics and clinical characteristics differences between the HM I and the HM II LVAD patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>HM I (n=23)</th>
<th>HM II (n=23)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male: female)</td>
<td>20 : 3</td>
<td>21 : 2</td>
<td>0.605</td>
</tr>
<tr>
<td>Age at implant (years)</td>
<td>37.09 ± 12.67</td>
<td>35.24 ± 14.27</td>
<td>0.555</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.52 ± 7.39</td>
<td>23.67 ± 3.00</td>
<td>0.005</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.93 ± 0.48</td>
<td>1.85 ± 0.19</td>
<td>0.012</td>
</tr>
<tr>
<td>Heart failure duration (months)</td>
<td>42.85 ± 49.65</td>
<td>31.29 ± 38.03</td>
<td>0.751</td>
</tr>
<tr>
<td>Symptoms duration (months)</td>
<td>7.30 ± 7.36</td>
<td>5.07 ± 4.17</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Haemodynamic Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA Syst (mmHg)</td>
<td>49.28 ± 15.28</td>
<td>47.60 ± 11.17</td>
<td>0.758</td>
</tr>
<tr>
<td>PA Diast (mmHg)</td>
<td>27.56 ± 10.93</td>
<td>27.00 ± 6.32</td>
<td>0.745</td>
</tr>
<tr>
<td>PA Mean (mmHg)</td>
<td>34.33 ± 8.08</td>
<td>35.87 ± 6.56</td>
<td>0.663</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>27.55 ± 8.44</td>
<td>29.88 ± 6.42</td>
<td>0.300</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td><strong>3.55 ± 0.98</strong></td>
<td><strong>2.69 ± 1.01</strong></td>
<td><strong>0.012</strong></td>
</tr>
<tr>
<td>Cardiac index (l/min/m²)</td>
<td>1.74 ± 0.47</td>
<td>1.46 ± 0.57</td>
<td>0.345</td>
</tr>
<tr>
<td>Pulmonary artery saturation (%)</td>
<td>51.17 ± 11.41</td>
<td>43.00 ± 11.36</td>
<td>0.254</td>
</tr>
<tr>
<td><strong>Echocardiographic Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-diastolic diameter (mm)</td>
<td>73.04 ± 12.10</td>
<td>72.33 ± 8.43</td>
<td>0.621</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>66.39 ± 12.16</td>
<td>66.76 ± 7.66</td>
<td>0.888</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>9.51 ± 5.09</td>
<td>7.61 ± 3.79</td>
<td>0.093</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>23.50 ± 9.01</td>
<td>20.73 ± 8.96</td>
<td>0.173</td>
</tr>
<tr>
<td><strong>Biochemical Profile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td><strong>8.77 ± 4.08</strong></td>
<td>12.01 ± 6.12</td>
<td><strong>0.041</strong></td>
</tr>
<tr>
<td>Creatinine (g/dl)</td>
<td><strong>114.63 ± 32.56</strong></td>
<td><strong>158.61 ± 82.93</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>39.29 ± 18.95</td>
<td>46.70 ± 24.98</td>
<td>0.435</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td><strong>172.71 ± 103.52</strong></td>
<td><strong>83.48 ± 45.21</strong></td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Alanine transaminase (IU/L)</td>
<td>163.33 ± 108.02</td>
<td>401.74 ± 590.71</td>
<td>0.909</td>
</tr>
</tbody>
</table>

Table 4.5: Differences in the pre-implantation parameters between the HM I and the HM II LVAD patients
Out of the 23 HM I LVAD patients, 15 had recovered and had their HM I LVAD explanted whereas 8 did not recover and were transplanted. A comparison between the recovered and the non-recovered patients of the HM I LVAD population is presented in table 4.6. Unlike the HM II group (table 4.2), there was no significant differences in the HF duration, LVESD and LVEDD between the recovered and the non-recovered patients.

Table 4.6. Differences in the pre-implantation demographics, haemodynamics and echocardiographic parameters between the recovered and the non-recovered patients in the HM I LVAD population

<table>
<thead>
<tr>
<th>Variable</th>
<th>HM I Rec Group (n=15)</th>
<th>HM I NRec Group (n=8)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implant Age (years)</td>
<td>35.94 ± 12.38</td>
<td>39.25 ± 13.78</td>
<td>0.478</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.57 ± 4.58</td>
<td>24.53 ± 11.08</td>
<td>0.796</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>2.02 ± 0.21</td>
<td>1.76 ± 0.76</td>
<td>0.561</td>
</tr>
<tr>
<td>HF duration</td>
<td>42.08 ± 56.72</td>
<td>44.29 ±36.98</td>
<td>0.632</td>
</tr>
<tr>
<td>Haemodynamic Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA Syst (mmHg)</td>
<td>46.36 ± 17.45</td>
<td>53.86 ± 10.68</td>
<td>0.102</td>
</tr>
<tr>
<td>PA Diast (mmHg)</td>
<td>28.64 ±13.57</td>
<td>25.86 ± 5.15</td>
<td>0.785</td>
</tr>
<tr>
<td>PA Mean (mmHg)</td>
<td>32.00 ± 7.47</td>
<td>38.00 ± 8.14</td>
<td>0.187</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>25.83 ± 7.86</td>
<td>30.13 ± 9.14</td>
<td>0.562</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>3.69 ± 1.15</td>
<td>3.36 ± 0.71</td>
<td>0.364</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>1.81 ± 0.55</td>
<td>1.61 ± 0.29</td>
<td>0.421</td>
</tr>
<tr>
<td>SvO₂ (%)</td>
<td>42.50 ± 13.44</td>
<td>55.50 ± 9.04</td>
<td>0.355</td>
</tr>
<tr>
<td>Echocardiographic Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>73.73 ± 14.45</td>
<td>71.75 ± 6.27</td>
<td>0.583</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>66.67 ± 14.23</td>
<td>65.88 ± 7.72</td>
<td>0.651</td>
</tr>
<tr>
<td>FS (%)</td>
<td>10.07 ± 5.40</td>
<td>8.47 ± 4.61</td>
<td>0.872</td>
</tr>
<tr>
<td>EF (%)</td>
<td>24.56 ± 7.55</td>
<td>21.50 ± 11.58</td>
<td>0.605</td>
</tr>
</tbody>
</table>

Table 4.6: Main demographics, haemodynamics and echocardiographic parameters between the recovered and the non-recovered patients of the HM I LVAD patients.
4.3.1.2 Device cessation “off-pump” protocol

Ten minutes prior to cessation of the HM I LVAD, patients were given 10,000 units of intravenous unfractionated heparin. Pneumatic hand pumping was instituted at a rate of 3 hand pumps every 15 seconds as soon as the device was switched off in order to avoid blood stagnation within the pump. Pneumatic hand pumping was stopped while taking haemodynamic and echocardiographic measurements. These assessments were performed using similar protocol as has been previously described for the HM II LVAD patients (sections 4.2.1.3 and 4.2.1.4). If patients tolerated device cessation for 15 minutes, a 6MW exercise test was undertaken to assess their CR and response to exercise (see section 4.2.1.5 for the 6MW exercise test protocol). Haemodynamic and echocardiographic measurements were repeated after the 6MW exercise test. Similar to the HM II population, HM I LVAD patients missed the 6MW exercise test during their first “off-pump” assessment.

4.3.1.3 Data collection and statistics

Tests on patients implanted prior to February 2005 were performed by Dr. Patrick Tansley and Dr. James Hardy (previous clinical research fellows in the VAD programme at Harefield). Following February 2005, all tests on the HM I LVAD patients were performed prospectively. Data analysis were done using SPSS version 16.0 for windows (Lead Technologies) and presented as means ± stdev. Parametric paired t-test was used for intra-group analysis and unpaired t-test was utilised for the HM I LVAD inter-group analysis (Recovered versus Non-Recovered) and to compare between the HM I and the HM II LVAD patients. Time modelling analysis was performed to compare the differences in the trends in the LV response to the 6MW exercise test between HM I and HM II LVAD patients. A p-value < 0.05 was considered to be statistically significant.
4.3.2 RESULTS

4.3.2.1 Feasibility and tolerability of testing in HM I patients

The first off-pump assessment was performed within 53 days (range 30-82 days) of HM I LVAD implantation as compared to the average 37 days in the HM II population (p=0.007) and was repeated at 4 to 6 weeks interval.

In total, the HM I LVAD was switched off on 226 occasions (mean of 9.8 tests per patient, range 2-18 tests, median 10 tests). Device cessation for 15 minutes was tolerated on 223 occasions. On 3 occasions 1 patient did not tolerate device cessation for 15 minutes and developed mild chest discomfort and the HM I had to be reconnected after 10 minutes. Similar to the HM II population none of the patients undertook the 6MW test on their first off-pump assessment leaving the total possible number of occasions where the 6MW exercise test that could be performed to 203. Out of the 203 potential tests, the 6MW was tolerated on 197 occasions (97%). On those six occasions, 1 patient became very anxious and the HM I LVAD had to be reconnected abandoning the 6MW exercise test. At no point any of the patients has developed signs of overt heart failure during the assessments that necessitated hospital admission. All patients were followed up for 72 hours and none had experienced short term or long term adverse events.

4.3.2.2 Summary of the Effects of Pump Cessation in the HM I Population

A similar analysis to the HM II LVAD patients was conducted on the HM I studied population and presented in tables 4.7 and 4.8, respectively.
Table 4.7: Changes in the haemodynamic and echocardiographic parameters that occur immediately after cessation of the HM I LVAD (n=23)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>On-Pump</th>
<th>Immediately after device cessation</th>
<th>Change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haemodynamic Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>99.49 ± 11.57</td>
<td>92.22 ± 16.13</td>
<td>-7.31</td>
<td>0.006</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>59.95 ± 10.58</td>
<td>58.74 ± 10.58</td>
<td>-2.00</td>
<td>0.222</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>39.55 ± 5.48</td>
<td>33.48 ± 7.94</td>
<td>-15.37</td>
<td>0.007</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>73.12 ± 10.61</td>
<td>69.90 ± 12.14</td>
<td>-4.41</td>
<td>0.020</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>83.40 ± 8.91</td>
<td>90.66 ± 12.88</td>
<td>8.71</td>
<td>0.020</td>
</tr>
<tr>
<td><strong>Echo Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>48.33 ± 8.19</td>
<td>54.62 ± 7.64</td>
<td>13.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>33.40 ± 7.72</td>
<td>40.95 ± 8.91</td>
<td>22.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FS (%)</td>
<td>31.56 ± 6.81</td>
<td>25.68 ± 8.54</td>
<td>-18.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EF (%)</td>
<td>66.26 ± 10.17</td>
<td>56.76 ± 15.30</td>
<td>-14.34</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4.7: A summary of the immediate haemodynamic and echocardiographic response to acute device cessation in the HM I population.

There was a significant reduction in SBP, PP, MAP, FS, and EF, and a significant increase in HR, LVEDD, and LVESD immediately after acute cessation of the HM I LVAD. Although the DBP decreased after device interruption, it did not reach level of significance.

Device cessation for 15 minutes did not have further significant impact on either the haemodynamic or the echocardiographic measurements (table 4.8).
Table 4.8: Sequential effects of pump cessation for 15 minutes on the HM I LVAD patients (n=23)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>0Mins</th>
<th>5Mins</th>
<th>10Mins</th>
<th>15Mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>99.49 ± 11.57</td>
<td>92.22 ± 16.13</td>
<td>92.76 ± 16.95</td>
<td>92.85 ± 18.51</td>
<td>93.53 ± 17.02</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>59.95 ± 10.58</td>
<td>58.74 ± 10.58</td>
<td>59.63 ± 10.69</td>
<td>59.34 ± 9.46</td>
<td>57.60 ± 11.25</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>39.55 ± 5.48</td>
<td>33.48 ± 7.94</td>
<td>33.13 ± 10.95</td>
<td>38.52 ± 13.96</td>
<td>35.94 ± 7.75</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>73.12 ± 10.61</td>
<td>69.90 ± 12.14</td>
<td>70.67 ± 12.05</td>
<td>72.17 ± 11.42</td>
<td>69.58 ± 12.95</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>83.40 ± 8.91</td>
<td>90.66 ± 12.88</td>
<td>89.85 ± 13.72</td>
<td>89.76 ± 13.52</td>
<td>89.32 ± 13.15</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>48.33 ± 8.19</td>
<td>-</td>
<td>54.62 ± 7.64</td>
<td>-</td>
<td>55.32 ± 8.00</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>33.40 ± 7.72</td>
<td>-</td>
<td>40.95 ± 8.91</td>
<td>-</td>
<td>41.59 ± 9.05</td>
</tr>
<tr>
<td>FS (%)</td>
<td>31.56 ± 6.81</td>
<td>-</td>
<td>25.68 ± 8.54</td>
<td>-</td>
<td>25.52 ± 7.64</td>
</tr>
<tr>
<td>EF (%)</td>
<td>66.26 ± 10.17</td>
<td>-</td>
<td>56.76 ± 15.30</td>
<td>-</td>
<td>57.05 ± 13.30</td>
</tr>
</tbody>
</table>

Table 4.8: A summary of the sequential effects of device cessation in the 23 HM I LVAD population.
4.3.2.3 A comparison of the haemodynamic and the echocardiographic responses to off-pump testing and to the 6MW exercise test between the HM I and the HM II LVAD patients

Figure 4.10A illustrates a direct comparison between the percentage change in the haemodynamic and the echocardiographic parameters between the HM I (n=23) and the HM II (n=23) groups. Immediately after interruption of LVAD support the percentage reductions in DBP and MAP were significantly higher in the HM II group as compared to the HM I group (-13.62% versus -2.00%, p<0.001, and -9.92% versus -4.41%, p=0.011, respectively). In the HM II patients there was a significant increase in PP in contrast to a significant decrease in the HM I group (12.62% versus -15.37%, p<0.001). In addition, there were no differences in the percentage change in either HR or SBP although the changes were more pronounced in the HM I group.

After 5 minutes of off-pump testing, although the absolute FS and EF in the HM I LVAD patients had decreased, values remained higher as compared to the HM II group (25.68 ± 8.54% versus 22.70 ± 8.55%, p=0.338, and 56.76 ± 15.30% versus 51.46 ± 6.40%, p=0.297, respectively). Interestingly, the HM II group demonstrated a percentage increase in both parameters whereas the HM I group demonstrated a percentage reduction in both parameters (FS: 2.66% versus -18.64%, p<0.001; and EF: 3.43% versus -14.34%, p<0.001, figure 4.10A). The rise in the absolute value in the FS and EF in the HM II group at 5 minutes following speed reduction (22.70 ± 8.55% versus 22.11 ± 9.53%, p=0.179, and 51.46 ± 16.40% versus 49.75 ± 18.62%, p=0.102, respectively) was unexpected and it could be related to either the suctioning power generated by the rotor pump being is reduced when the speed decreases hence allowing the LV to provide better contractility or the second possibility is that the LV may not be fully unloaded and its function is still influenced by the effect of the HM II LVAD unlike the HM I LVAD patients where the device is completely switched off.
Figure 4.10A: The percentage change in the haemodynamic and the echocardiographic parameters following interruption of LVAD support

Fig 4.10A: A comparison of the haemodynamic and echocardiographic parameters measured after interruption of LVAD support between the HM I and HM II LVAD patients. Percentage decrease in DBP and MAP were significantly higher in the HM II LVAD patients. The HM II group also demonstrated an increase both FS and EF after speed reduction as compared to the HM I patients who showed a reduction. *p<0.001, **p=0.002, †p=0.011.
Left ventricular dimensions showed a trend to increase following interruption of support in both groups. The percentage change in both LVEDD and LVESD were more pronounced in the HM I LVAD patients as compared to the HM II group (13.02% versus 11.31%, p=0.798, and 22.62% versus 9.78%, p<0.001, respectively) although the absolute dimensions in the HM II group remained higher as compared to the absolute dimensions in the HM I LVAD patients (LVEDD: 57.43 ± 11.45 mm versus 54.62 ± 7.64 mm, p=0.573; LVESD: 45.21 ± 13.89 mm versus 40.95 ± 8.91 mm, p=0.489).

There was a significant increase in the SBP, PP and HR following the 6MW exercise test in both groups (figure 4.10B). The percentage change in SBP, DBP, PP, MAP and HR were not significantly different between the two groups (8.84% versus 5.88%, p=0.180; 6.50% versus 1.42%, p=0.120; 12.66 % versus 13.03%, p=0.468; 7.32 % versus 3.42%, p=0.131; and 19.91% versus 15.50%, p=0.287, respectively).

The HM I LVAD patients demonstrated a significant increase in the absolute FS following the 6MW exercise test as compared to the 15 minutes off-pump testing (26.58 ± 8.63% versus 25.52 ± 7.64%, p=0.034, an absolute increase by 1.06 ± 2.26% corresponding to a percentage change of 4.17%) and a slight increase in the absolute EF (58.42 ± 15.06% versus 57.05 ± 13.30%, p=0.136, an absolute increase by 1.37 ± 4.24% corresponding to a percentage change of 2.40%).

As discussed in section 4.2.2.3 and illustrated in figure 4.2C, the HM II LVAD patients exhibited a significant rise in both the absolute FS and the absolute EF following the 6MW exercise test as compared to the 15 minutes off-pump testing such that both FS and EF increased by an absolute value of 3.49 ± 3.70% and 5.36 ± 5.30%, respectively, which correspond to 15.31% and 10.21% percentage change from the 15 minutes of low-speed testing. The percentage change in both FS and EF were significantly higher in the HM II LVAD patients as compared to the HM I LVAD patients (15.31% versus 4.17%, p=0.050, and 10.20% versus 2.40%, p=0.035, respectively) suggesting that the response to exercise was higher and more evident in the HM II LVAD patients.
Figure 4.10B: The percentage changes in the haemodynamic and the echocardiographic parameters after the 6MW exercise test

Fig 4.10B: A comparison of the haemodynamic and echocardiographic parameters measured after the 6MW test between the HM I and HM II LVAD patients. There were no significant differences in the percentage changes in the haemodynamic parameters after the 6MW test as compared to the 15 minutes off-pump. The percentage increase in both FS and EF were significantly higher in the HM II LVAD patients as compared to the HM I LVAD patients. *p<0.050, †p=0.035.

The following section focuses on the recovered patients in each group to assess the effects of different devices on their recovery status and contractile reserve response.
4.3.2.4 Differences in the haemodynamic and the echocardiographic responses between recovered HM I (n=15) and recovered HM II (n=15) LVAD patients

Before determining the differences in the haemodynamic and the echocardiographic responses to off-pump testing and the 6MW exercise test between the two recovered groups, a comparison of the pre-implantation clinical parameters between the two recovered subgroups and their medication was performed and presented in table 4.9.

Table 4.10 represents the differences in the haemodynamic parameters between the two recovered sub-groups at all measurement time points. Apart from the PP and the HR measurements, the HM I recovered group had significantly higher peripheral haemodynamic parameters as compared to the HM II recovered patients.
Table 4.9: Differences in pre-implantation clinical parameters and maximal medication between both recovered groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>HM I Group (n=15)</th>
<th>HM II Group (n=15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at implantation</td>
<td>35.94 ± 12.38</td>
<td>32.30 ± 13.85</td>
<td>0.461</td>
</tr>
<tr>
<td>HF Duration (months)</td>
<td>42.08 ± 56.72</td>
<td>15.04 ± 24.81</td>
<td>0.440</td>
</tr>
<tr>
<td>Pre-implantation Haemodynamic Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA Systole (mmHg)</td>
<td>46.36 ± 17.45</td>
<td>45.13 ± 8.20</td>
<td>0.968</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>25.83 ± 7.86</td>
<td>27.50 ± 4.09</td>
<td>0.582</td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>1.81 ± 0.55</td>
<td>1.54 ± 0.64</td>
<td>0.512</td>
</tr>
<tr>
<td>Pre-implantation Echocardiographic Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>73.73 ± 14.45</td>
<td>69.47 ± 7.38</td>
<td>0.148</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>66.67 ± 14.23</td>
<td>64.00 ± 6.49</td>
<td>0.217</td>
</tr>
<tr>
<td>FS (%)</td>
<td>10.07 ± 5.40</td>
<td>7.74 ± 4.20</td>
<td>0.081</td>
</tr>
<tr>
<td>EF (%)</td>
<td>24.56 ± 7.55</td>
<td>21.03 ± 10.04</td>
<td>0.098</td>
</tr>
<tr>
<td>Maximal Medication</td>
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<td></td>
</tr>
<tr>
<td>Carvedilol (mg)</td>
<td>40 ± 20</td>
<td>38.45 ± 107.50</td>
<td>0.845</td>
</tr>
<tr>
<td>Bisoprolol (mg)</td>
<td>9.50 ± 1.10</td>
<td>10 ± 2.10</td>
<td>0.923</td>
</tr>
<tr>
<td>Lisinopril (mg)</td>
<td>35.00 ± 10.00</td>
<td>35.00 ± 8.00</td>
<td>0.905</td>
</tr>
<tr>
<td>Digoxin (mcg)</td>
<td>125</td>
<td>125</td>
<td>1.000</td>
</tr>
<tr>
<td>Spironolactone (mg)</td>
<td>25</td>
<td>25</td>
<td>1.000</td>
</tr>
<tr>
<td>Clenbuterol (mcg)</td>
<td>1370.00 ± 850</td>
<td>1870.00 ± 470</td>
<td>0.632</td>
</tr>
<tr>
<td>Clenbuterol start since implantation (days)</td>
<td>151.00 ± 96.70</td>
<td>123.80 ± 43.30</td>
<td>0.312</td>
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</table>

Table 4.9: Difference in pre-implantation clinical parameters and maximal medication between both recovered groups. There were no differences in the clinical parameters or the maximal medication taken by each sub-group.
Table 4.10: Differences in the peripheral haemodynamics between the two recovered groups

<table>
<thead>
<tr>
<th>Assessment time-point</th>
<th>Baseline</th>
<th>0Mins</th>
<th>5Mins</th>
<th>10Mins</th>
<th>15Mins</th>
<th>Post 6MW</th>
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<tr>
<td></td>
<td>SBP (mmHg)</td>
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</tr>
<tr>
<td>HM I</td>
<td>102.26 ± 10.18</td>
<td>99.32 ± 14.00</td>
<td>100.59 ± 13.91</td>
<td>105.93 ± 17.57</td>
<td>101.02 ± 15.79</td>
<td>108.28 ± 21.80</td>
</tr>
<tr>
<td>HM II</td>
<td>85.73 ± 5.53</td>
<td>83.38 ± 11.33</td>
<td>84.89 ± 8.12</td>
<td>84.73 ± 8.17</td>
<td>85.13 ± 8.75</td>
<td>94.56 ± 10.46</td>
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<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>DBP (mmHg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HM I</td>
<td>63.09 ± 9.10</td>
<td>62.42 ± 10.31</td>
<td>63.26 ± 8.60</td>
<td>63.61 ± 8.16</td>
<td>62.17 ± 10.29</td>
<td>63.22 ± 13.31</td>
</tr>
<tr>
<td>HM II</td>
<td>56.61 ± 5.41</td>
<td>48.75 ± 3.50</td>
<td>49.87 ± 3.77</td>
<td>49.77 ± 4.55</td>
<td>50.45 ± 4.81</td>
<td>53.90 ± 6.97</td>
</tr>
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<td>p-value</td>
<td>0.002</td>
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<td>&lt;0.001</td>
<td>0.001</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>PP (mmHg)</td>
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<tr>
<td>HM I</td>
<td>39.17 ± 5.89</td>
<td>36.91 ± 5.55</td>
<td>37.33 ± 6.41</td>
<td>42.31 ± 15.62</td>
<td>38.85 ± 7.02</td>
<td>45.06 ± 9.60</td>
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<tr>
<td>HM II</td>
<td>29.12 ± 5.94</td>
<td>34.63 ± 6.23</td>
<td>35.02 ± 7.14</td>
<td>34.96 ± 6.82</td>
<td>34.68 ± 6.57</td>
<td>40.66 ± 7.13</td>
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<td>p-value</td>
<td>&lt;0.001</td>
<td>0.233</td>
<td>0.233</td>
<td>0.100</td>
<td>0.116</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HM I</td>
<td>76.15 ± 9.06</td>
<td>74.72 ± 11.38</td>
<td>75.71 ± 10.23</td>
<td>77.72 ± 9.64</td>
<td>75.12 ± 11.95</td>
<td>78.24 ± 16.00</td>
</tr>
<tr>
<td>HM II</td>
<td>66.32 ± 4.67</td>
<td>60.34 ± 3.70</td>
<td>61.64 ± 4.44</td>
<td>61.53 ± 4.99</td>
<td>62.08 ± 5.57</td>
<td>67.55 ± 7.64</td>
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<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>HR (bpm)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HM I</td>
<td>84.53 ± 7.62</td>
<td>93.40 ± 13.61</td>
<td>92.31 ± 14.68</td>
<td>91.47 ± 14.69</td>
<td>91.78 ± 13.96</td>
<td>105.65 ± 14.25</td>
</tr>
<tr>
<td>HM II</td>
<td>86.41 ± 11.33</td>
<td>85.62 ± 12.61</td>
<td>84.42 ± 11.78</td>
<td>85.01 ± 11.51</td>
<td>83.19 ± 11.43</td>
<td>100.48 ± 14.91</td>
</tr>
<tr>
<td>p-value</td>
<td>1.000</td>
<td>0.116</td>
<td>0.074</td>
<td>0.174</td>
<td>0.074</td>
<td>0.250</td>
</tr>
</tbody>
</table>

Table 4.10: There were significant differences in SBP, DBP, and MAP with the values being significantly higher in the recovered HM I LVAD patients as compared to the recovered HM II LVAD patients reflecting the pulsatility effects of the HM I LVAD.
Whilst on full support the HM I recovered group had significantly higher FS and EF as compared to the HM II recovered group (Table 4.11). There were no significant differences in the echocardiographic parameters measured at either 5 or 15 minutes of “low-speed / off-pump” testing between the two recovered groups. Following the 6MW exercise test there was a significant decrease in LVESD in both groups but more pronounced in the recovered HM II LVAD patients as compared to the recovered HM I LVAD patients.

The rise in both FS and EF in each group following the 6MW exercise test was significantly higher as compared to the 15 minutes of “low-speed / off-pump” testing (HM I: FS, 29.96 ± 7.13 % versus 28.20 ± 6.33%, p=0.009, EF, 64.30 ± 11.18% versus 61.80 ± 10.79%, p=0.016; HM II, FS, 33.91 ± 4.46% versus 28.61 ± 4.13%, p<0.001, EF, 70.27 ± 5.99% versus 62.55 ± 6.24%, p<0.001). Although there were no differences in the absolute FS and EF values acquired following the 6MW exercise test between the two recovered groups, the absolute increase in both FS and EF were higher in the recovered HM II LVAD patients as compared to the recovered HM I LVAD patients (FS: 5.29 ± 3.39% versus 1.76 ± 2.27%, and EF: 7.73 ± 4.37% versus 2.50 ± 3.55%). These values corresponded to a percentage rise in the FS by 18.50% in the recovered HM II LVAD group and 6.26% in the recovered HM I LVAD group (p=0.004, figure 4.11). Also the absolute rise in the EF corresponded to a percentage increase in the EF by 12.35% in the HM II LVAD recovered patients as compared to a percentage rise of 4.04% in the recovered HM I LVAD patients (p=0.007, figure 4.11). These changes suggest that patients who recover on continuous flow LVAD may have enhanced CR as compared to those who recover on pulsatile LVADs despite the similarity in the LV function whilst the device’s input has been interrupted for 15 minutes.
Table 4.11: Differences in the echocardiographic parameters between the two recovered groups

<table>
<thead>
<tr>
<th>Assessment time-point</th>
<th>Baseline</th>
<th>5Mins</th>
<th>15Mins</th>
<th>Post 6MW</th>
<th>p-value between 15Mins and Post 6MW</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LVEDD (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HM I</td>
<td>47.16 ± 9.67</td>
<td>53.85 ± 8.45</td>
<td>54.42 ± 8.67</td>
<td>54.18 ± 8.33</td>
<td>0.618</td>
</tr>
<tr>
<td>HM II</td>
<td>44.92 ± 7.51</td>
<td>51.15 ± 6.37</td>
<td>51.24 ± 6.67</td>
<td>51.83 ± 7.20</td>
<td>0.463</td>
</tr>
<tr>
<td>p-value</td>
<td>0.539</td>
<td>0.486</td>
<td>0.436</td>
<td>0.512</td>
<td></td>
</tr>
<tr>
<td><strong>LVESD (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HM I</td>
<td>31.56 ± 8.25</td>
<td>38.46 ± 8.57</td>
<td>39.41 ± 8.91</td>
<td>38.29 ± 8.69</td>
<td>0.046</td>
</tr>
<tr>
<td>HM II</td>
<td>32.60 ± 6.89</td>
<td>36.93 ± 6.10</td>
<td>36.81 ± 6.20</td>
<td>34.42 ± 6.20</td>
<td>0.003</td>
</tr>
<tr>
<td>p-value</td>
<td>0.805</td>
<td>0.838</td>
<td>0.567</td>
<td>0.325</td>
<td></td>
</tr>
<tr>
<td><strong>FS (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HM I</td>
<td>33.76 ± 5.30</td>
<td>29.29 ± 6.44</td>
<td>28.20 ± 6.33</td>
<td>29.96 ± 7.13</td>
<td>0.009</td>
</tr>
<tr>
<td>HM II</td>
<td>28.17 ± 4.76</td>
<td>28.16 ± 4.06</td>
<td>28.61 ± 4.13</td>
<td>33.91 ± 4.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p-value</td>
<td>0.004</td>
<td>0.595</td>
<td>0.806</td>
<td>0.116</td>
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</tr>
<tr>
<td><strong>EF (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HM I</td>
<td>69.67 ± 7.18</td>
<td>63.47 ± 9.95</td>
<td>61.80 ± 10.79</td>
<td>64.30 ± 11.18</td>
<td>0.016</td>
</tr>
<tr>
<td>HM II</td>
<td>61.36 ± 7.52</td>
<td>61.76 ± 6.47</td>
<td>62.55 ± 6.24</td>
<td>70.27 ± 5.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p-value</td>
<td>0.003</td>
<td>0.486</td>
<td>0.713</td>
<td>0.137</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.11: Differences in the echocardiographic measurements between the recovered HM I (n=15) and the recovered HM II (n=15) LVAD patients. There were no intergroup differences between the two groups. Intra-group analysis, however, revealed that following the 6MW LVESD decreased significantly and both FS and EF increased significantly.
Figure 4.11: Differences in the percentage increase in FS and EF following the 6MW exercise test

Fig 4.11: A comparison in the percentage increases in both FS and EF between the recovered HM I and the recovered HM II LVAD patients. The percentage rise in both parameters was significantly higher in the recovered HM II group as compared to the recovered HM I group.
4.3.2.5 Differences in the 6MW distance between the HM I and the HM II LVAD patients

The total distance walked by the HM I LVAD patients was significantly lower as compared to the HM II LVAD patients (491.03 ± 115.34 meters versus 571.96 ± 101.05 meters, p=0.024).

Similarly, the recovered HM II LVAD patients walked a longer distance walked a longer distance as compared to the recovered HM I LVAD patients (604.07 ± 94.02 versus 535.52 ± 99.26) however, it did not reach the level of significance (p=0.081, figure 4.12).

Figure 4.12: Difference in the distance walked between the recovered HMI and the recovered HM II LVAD patients

![Figure 4.12](image)

Figure 4.12: The total distance walked by the recovered HM II patients was higher than the recovered HM I LVAD patients.
A multi-level time modelling was performed to determine the differences in the LV structural and functional trends between the recovered HM I and the recovered HM II LVAD patients over a 1 year period of support (see model 3 in Appendix B for details of the analysis).

The model revealed that at 15 minutes of off-pump testing the intercepts of the LVEDD, LVESD, FS, and EF were poorer in the recovered HM II LVAD patients as compared to the recovered HM I LVAD patients, but not significantly (figure 4.13A). However, over a 1-year period of support, FS and EF parameters would improve in both recovered groups but more so in the recovered HM II LVAD patients. This illustrated by better improvement in the slope values in the recovered HM II LVAD patients as compared to the recovered HM I LVAD patients over a 1-year period of support (FS: 16.34 versus 2.26, p=0.003; and EF: 25.92 versus 4.46, p=0.005). In contrast, the trend in the LV dimensions in the recovered HM I LVAD patients tend to increase over a 1-year period of support as compared to the trend in the dimensions in the recovered HM II LVAD group (LVEDD: -7.06 versus 5.60, p=0.004; and LVESD: -14.19 versus 2.67, p<0.001).

The model also revealed that following the 6MW exercise test the mean intercepts of both FS and EF were significantly better in the recovered HM II LVAD group as compared to the recovered HM I LVAD group but not statistically significant (figure 4.13B). Furthermore, the mean slopes were better in the recovered HM II LVAD patients as compared to the mean slopes of the recovered HM I LVAD patients but did not reach level of significance (FS: 10.74 versus 3.13, p=0.120; and EF: 13.25 versus 5.11, p=0.240). Therefore, this analysis concludes that the trends in the both FS and EF over a 1-year period of support tend to be better in the recovered HM II LVAD patients. Further, the analysis also suggests that the differences shown in the FS and EF (section 4.3.2.3) between the two recovered groups following the 6MW exercise test are not just differences in averaged measurements but differences in the trends over a period of time with better
outcome in the recovered HM II LVAD patients and that the CR tends to be more preserved in the recovered HM II LVAD patients.

**Figure 4.13A: Differences in the echocardiographic trends between the recovered HM I and HM II LVAD patients at 15 minutes of off-pump testing.**

Figure 4.13A: Differences in the echocardiographic trends between the recovered HM I ( ) and the recovered HM II ( ) LVAD patients at 15 minutes of off-pump testing.
Figure 4.13B: Differences in the echocardiographic trends between the recovered HM I and HM II LVAD patients following the 6MW exercise test.

![Graphs showing echocardiographic trends](image)

Figure 4.13B: Differences in the echocardiographic trends between the recovered HM I (---) and the recovered HM II (- - - -) LVAD patients following the 6MW exercise test.
4.4 Discussion

The main findings of the present study include the following:

- HM II LVAD patients tolerated off-pump testing with no ill-side effects;
- The 6MW exercise test is a safe and a useful tool that corresponds to day-to-day activity and has a role in assessing the native LV function. The test was tolerated by 97-98% of the studied populations;
- Following the 6MW exercise test, the HM II recovered patients exhibited a better haemodynamic and echocardiographic responses as compared to the non-recovered patients which could correspond to improvement in CR. In addition both EF and the percentage change in EF following the 6MW exercise test correlated strongly with myocardial recovery and may predict recovery;
- The changes seen in the echocardiographic parameters are not due to data averaging but are trends that had occurred throughout the support period;
- HM II LVAD patients had a better response to the 6MW exercise test than the HM I LVAD patients with better CR. Further, the recovered HM II patients have a higher response to exercise testing than the corresponding recovered HM I patients;
- The walking distance was significantly higher in the recovered HM II LVAD patients as compared to the non-recovered HM II LVAD patients and also higher (but not significantly) than the recovered HM I LVAD patients.

Assessment of the underlying LV function

As has been previously described, the identification of the extent of myocardial recovery relies heavily on unmasking the underlying myocardial function. In chapter 3, the necessity for reducing the speed of the HM II LVAD was established as an approach to reveal the native LV function. By lowering the baseline speed to 6000 rpm, the contribution from the LVAD into the circulation was found to be minimal with no evidence of retrograde filling. Hence, LV filling following speed reduction was attributed to physiological loading rather than retrograde filling. In part one of this chapter, the clinical, peripheral
haemodynamic and echocardiographic responses in all 23 patients to both speed reduction and exercise were studied.

Reducing the speed of the continuous flow HM II LVAD to 6000 rpm was performed after ensuring an INR of 2.0 and above and was tolerated by all patients on all 170 occasions. None of the patients had developed any signs of overt heart failure such as breathlessness, chest pain or discomfort. All patients were followed up for 72 hours Although none had reported thrombo-embolic or bleeding related complications the exact level of INR prior to speed reduction remains to be confirmed experimentally and clinically to ensure that patients are exposed to the least anticoagulation therapy (Amir O et al., 2005; Frazier OH et al., 2004a; Frazier OH et al., 2007).

Unfortunately, the first assessment was not performed until patients were clinically stable (ESC, 2001), inotrope independent, started their phase I pharmacological therapy, and mobile. The first assessment was performed 4-6 weeks after device implantation and hence immediate (i.e. 24-48 hours) and short-term (i.e. 3-7 days) responses to device implantation and the effects on LV unloading could have not been assessed. In addition, none of the patients was exercised on the first assessment such that the first assessment was only confined to a 15 minutes of low-speed testing. This approach was taken for safety reasons and to ensure that patients could tolerate the 15-minutes of low-speed testing before the 6MW exercise test and that they are more rehabilitated in order to complete the 6MW exercise test. Out of the potential 147 exercise tests, the 6MW was tolerated on 144 occasions (98%). On 3 occasions, one patient developed shortness of breath within two minutes of the walk and the speed of the device had to be re-adjusted to his baseline speed. Previous groups have also reported successful toleration of speed reduction of the axial-flow Jarvik 2000 LVAD (Myers T et al., 2006).

**6MW exercise test to assess CR and response to exercise**

According to the American Thoracic Society guidelines, the 6MW exercise test evaluates the global and integrated responses of all systems involved during exercise, including the pulmonary and cardiovascular systems, systemic
circulation, blood, neuromuscular units, and muscle metabolism (ATS Statement: 2002). In 1985, Guyatt et al were the first to report its use in patients with end-stage HF (Guyatt GH et al., 1985) and since then it has been widely applied in clinical and research setting as a useful prognostic marker and a reliable test for the identification of effective interventions (Roul G et al., 1998; Sharma R & Anker SD, 2001; Bittner V et al., 1993; Rostagno C et al., 2003; Opasich C et al., 2001; Cahalin LP et al., 1996; Schaufelberger M & Swedberg K, 1998; Olsson LG et al., 2005; Bittner V, 1999; Lucas C et al., 1999; Passantino A et al., 2006; Zugck C et al., 1998).

Although, it is considered a submaximal test, we have used it in our LVAD patients as it has been shown to correspond to the demands of day-to-day activities, easier to accomplish by the patient, needs less operational power than other tests such as treadmill exercise, and not expensive (Bittner V et al., 1993; Bittner V, 1997; Bittner V, 2003). In addition to calculating the distance walked this test was utilised in LVAD patients as means of assessing the cardiac response to exercise by repeating peripheral haemodynamic and echocardiographic assessments immediately after the test. The absolute changes in both FS and EF corresponded to the LV CR needed in normal day-to-day activity. Further, the percentage changes have also proven to be additional important indicators / predictors of myocardial recovery.

**Contractile reserve**

By definition, contractile reserve (also known as inotropic reserve) refers to the objective quantification of the LV contractility following either a pharmacological or physiological stress (Gudjonsson T & Rahko PS, 2002a). It is well established that CR is reduced in patients with ischaemic and non-ischaemic DCM (Nagaoka H et al., 1996). Mechanisms that would be responsible for this reduction include (Nagaoka H et al., 1996; Nagaoka H et al., 1996):

1. Alteration in the autonomic nervous system;
2. β-adrenergic pathway abnormalities;
3. Defect in receptor adneylate cyclase coupling;
4. Impaired exercise induced physiologic up-regulation of β-adrenoceptors;
5. Depletion of myocardial norepinephrine (NE).

Studies have shown that CR abnormality had a strong correlation with abnormal cardiac sympathetic innervation (Ohshima S et al., 2005; Kobayashi M et al., 2008) such that abnormal $^{123}$I-MIBG uptake (see Chapter 5 for the $^{123}$I-MIBG uptake in HM II LVAD patients study) was related to impaired myocardial CR in DCM patients ($R=0.64$, $p<0.001$).

Different indices have been used to describe the contractile ability of the LV in response to pharmacological stress (dobutamine infusion). Examples of these indices include wall motion score index, ejection fraction, cardiac power output, end systolic pressure to volume ratio, maximal first derivative of LV pressure ($\text{LV } \frac{dP}{dt_{\text{max}}}$), and functional myocardial segments (Bax JJ et al., 2002; Chaudhry FA et al., 1999; Kobayashi M et al., 2008; Lim P et al., 2007; Otasevic P et al., 2005; Szili-Torok T et al., 2005; Moonen M et al., 2008; Gudjonsson T & Rahko PS, 2002a). The most frequently used, however, is the absolute change in EF following dobutamine infusion (Bax JJ et al., 2002; Chaudhry FA et al., 1999; Kobayashi M et al., 2008; Otasevic P et al., 2005; Ypenburg C et al., 2007) despite its inability to distinguish abnormalities in contractility from alterations in preload or afterload (Neskovic AN & Otasevic P, 2005).

Conventionally, an absolute rise in the EF by 5% during dobutamine infusion as compared to resting was considered to be a positive indicator for the presence of CR with strong correlation to both prognosis and to different therapeutic approaches (Khan N et al., 2003; Nagaoka H et al., 1996; Nagaoka H et al., 1997; Pratali L et al., 2007; Ypenburg C et al., 2007).

In this study, dobutamine stress echocardiography (DSE) was not utilised as means for assessing CR as the presence of the LVAD in situ would distort the internal structure of the LV, hence rendering the pattern of LV wall motion and segmental viability obtained from DSE unreliable. Furthermore, assessment of the extent of myocardial recovery is a continuous and rigorous process that needs to be performed on regular basis and the use of DSE on 4-6 weekly basis would have been unethical, unacceptable by the patient, and would have required a consultant cardiologist performing the examination. The 6MW test on the other hand had
proven to be a reproducible and an inexpensive technique which can be performed serially in each patient in the out-patient clinic setting and was accepted by all patients. Similar to DSE technique, we have used an absolute increase in FS and EF following the 6MW by at least 5% as cut-off points to indicate preservation of CR.

**Effects of the HM II LVAD on peripheral haemodynamics, LV structure and function, and exercise tolerance (contractile reserve)**

In the entire HM II LVAD population immediate speed reduction resulted in a significant decrease in SBP, DBP and MAP, and a significant increase in PP. The rise in PP could be attributed to the profound drop noted in DBP immediately following speed reduction. Following the 6MW exercise test all peripheral haemodynamic parameters increased significantly as compared to measurements taken at 15 minutes of low-speed.

Although, there was a significant increase in both LVEDD and LVESD at 5 minutes following speed reduction, there was no difference in the ventricular FS and EF. Following the 6MW exercise test, there was a significant reduction in the LVESD as compared to the 15-minutes low speed measurements (43.45 ± 15.11 mm versus 45.35 ± 14.33 mm, p=0.001). This drop may reflect the improved degree of unloading after exercise as a result of improved stroke volume. The reduction in the LVESD has also contributed to the absolute increase seen in both FS and EF (3.49 ± 3.70% and 5.25 ± 5.3%, respectively) which corresponded to a percentage change of 15.31 % and 10.20%, respectively (figure 4.2C). Until this point the rise in the EF has just fulfilled the cut-off point of CR but did not differentiate the degree of CR preserved between the recovered and the non-recovered patients.

Comparing the pre-implantation parameters between the recovered (n=15) and the non-recovered (n=8) patients (tables 2.3 and 4.2) revealed no significant differences in pre-implantation haemodynamics, LV function and biochemical profile. HF duration and pre-implantation LV dimensions were significantly better in the recovered patients as compared to the non-recovered patients. Further, a comparison between the two groups at the first assessment revealed no significant
differences in peripheral haemodynamics, the time point at which the first assessment was performed, the baseline speed, and the phase I therapy (table 4.3). Ventricular dimensions and function remained significantly better in the recovered group with no correlation with the speed of the device suggesting that the identified differences were not secondary to speed differences (in matter of fact the non-recovered group had a higher baseline speed as compared to the recovered patients: 9310 ± 280 rpm versus 9080 ± 250 rpm (p=0.110)).

Following the 6MW exercise test, all peripheral haemodynamic measurements improved significantly in the recovered group, as compared to a lone rise in the HR in the non-recovered group. Interestingly the percentage rise in PP in the recovery group was significantly higher as compared to the percentage rise in the non-recovered group (17.23 % versus 4.27%, p=0.0028) which may reflect a higher stroke volume value in the recovered group. Unfortunately, stroke volume could have not been assessed non-invasively using Doppler as it consists of the volume travelling into the LVOT and the LVAD; the former could have not been quantified effectively.

Both FS and EF increased in both groups following the 6MW exercise test. The absolute change, however, was significantly higher in the recovered as compared to the non-recovered group for both FS and EF (FS: 5.29 ± 3.39% versus 0.48 ± 1.71%, p=0.001; and EF: 7.73 ± 4.37% versus 1.13 ± 4.20%, p=0.003). This absolute increase in EF in the recovered group was higher than the cut off point and corresponded to a 12.35% increase in the recovered group as compared to a 3.42% in the non-recovered group. This could be interpreted as a significant improvement in cardiac sympathetic dysfunction since Ohshima et al elegantly correlated cardiac sympathetic dysfunction with abnormal CR in DCM patients (Ohshima S et al., 2005). See chapter 5 for the correlation between CR and MIBG uptake.

To confirm that the changes seen in LV function following the 6MW exercise test were not due to averaging the data over the support period, a multi-level time modelling analysis was performed to determine the response over a 1-year support period. This revealed that the recovered group had a significantly higher slope
value for both FS and EF suggesting that CR preservation is a positive trend which is exhibited highly in the recovered patients as compared to the non-recovered patients. Furthermore, the results also suggest that CR in the recovered patients predominates over the support period and hence it could have a prognostic value as well as being a predictor for recovery.

No reliable morphological parameter has emerged to predict recovery whilst on LVAD support. In patients with the first generation pulsatile HM I LVAD, we have identified that only MAP of 60 mm Hg after the 6MW (p= 0.006, ROC=0.89, sensitivity 87.5%, specificity 83%) and EF of ≥53% after the 6MW (p=0.02, ROC=0.82, sensitivity 93%, specificity 80%) were the strongest predictors of recovery. In this study, linear correlation analysis revealed that all echocardiographic parameters exhibited a correlation with myocardial recovery; however, the strongest correlation was associated with the parameters examined following the 6MW exercise test. ROC analysis revealed that an absolute value for EF of 56.3% or more following the 6MW and a percentage rise in the EF by at least 8.3% (absolute rise by 5%) were independent strong predictors of myocardial recovery. Interestingly, this value corresponded to the cut-off of CR i.e. those who have preserved LV CR have a higher chance to recover. Finally, the recovered group walked a significantly longer walking distance as compared to the non-recovered group.

**Comparison between the effects of HM I and HM II LVAD on peripheral haemodynamics, LV structure and function, and the response to exercise**

In part two of the present study the differences in the LV response between the continuous flow HM II LVAD and the pulsatile flow HM I LVAD were assessed (the HM I LVAD group was independent from the main study group and was utilised only to compare the effects of different devices on peripheral haemodynamics, LV structure and function, and the response to exercise). Previously we have reported the effects of the pulsatile HM I LVAD and drug combination therapy on CR in non-ischaemic DCM patients (George RS et al., 2006;George RS et al., 2007a;George RS et al., 2007b). The number of the recovered patients was similar between the two groups; 15 out of 23 (65.2%). In both groups off-pump testing was considered to be a safe and a reproducible
approach to unmask the native LV function in an outpatient clinic, such that speed reduction was tolerated in all 170 occasions in the HM II LVAD patients as compared to 98.7% of occasions in the HM I population (p=0.932). Similarly, the 6MW exercise test was tolerated on 97% and 98% of occasions in the HM I and the HM II LVAD groups, respectively.

Although the term non-pulsatile device has been considered a misnomer in defining continuous flow devices, in matter of fact it is an accurate term. Unlike the pulsatile devices, continuous flow devices do not generate pulsatile flow and the pulsatility that is being detected in the clinical setting is secondary to the underlying ventricular action which itself increases as the ventricle recovers (Thalmann M et al., 2005). Continuous-flow LVADs have certainly reduced morbidity and mortality due to easier and less traumatic surgical implantation and are associated with much longer durability due to their simpler design with only a single moving part, the internal rotor (Frazier OH et al., 2004a). However it has been felt their non-pulsatile pattern of flow may not be so effective for unloading for myocardial recovery.

In goats, using long-term non-pulsatile left heart bypass, Nishimura et al investigated in detail the mechanical properties of the descending aortic wall relative to structural changes due to continuous flow (Nishimura T et al., 1999b;Nishimura T et al., 1999a). The group showed that the wall was significantly thinner, and the volume ratio of smooth muscle cells was much lower, indicating morphologic, atrophic changes in the aorta. The effects on cerebral metabolism and cerebral blood flow was controversial with some groups suggesting no differences in carotid blood flow and cerebral blood flow autoregulation (Kashiwazaki S, 2000;Hindman B, 1994) whilst others showed significant detrimental effects on blood-brain flow, metabolism and autoregulation (Nishinaka T et al., 2000).

Pulsatile-flow LVADs provide pulsatile blood flow patterns mimicking the normal physiologic blood circulation. Their use, however, has been associated with significant co-morbidity including the need for extensive surgery and frequent device related infections. Another restrictive factor is their limited long-term durability and the increased incidence of device malfunction (Birks EJ et al.,
Despite the several design enhancements of the HM I TCI to the HM I XVE, durability at 2-years post-implantation remains around 5% (Martin J et al., 2006).

The purpose of part two was to assess whether there are any differences in the degree of LV unloading between pulsatile- and continuous-flow LVAD. Previous studies have shown that differences in LV unloading between the two types of devices as assessed by echocardiography were similar following six months or more of support (Drews T et al., 2008; Garcia S et al., 2008; Klotz S et al., 2004; Haft J et al., 2007). Others have shown that pulsatile devices were superior to non-pulsatile devices in myocardial volume unloading and oxygen consumption / supply balance (Yu JJ et al., 2008) and in peripheral vascular reactivity (Amir O et al., 2006).

The immediate peripheral haemodynamic responses to speed reduction in the HM I LVAD population were less significant as compared to the HM II LVAD population. This difference reflects the extent of support withdrawal in the HM II LVAD patients. The 6MW exercise test resulted in an absolute increase in both FS and EF in the HM I LVAD population by 1.06 ± 2.26% and 1.37 ± 4.24%, respectively. This rise was significantly lower as compared to the absolute rise seen in the HM II LVAD population.

A direct comparison between the two recovered groups revealed that both absolute and percentage increase in both FS and EF were significantly higher in the HM II recovered LVAD patients as compared to the HM I recovered LVAD patients suggesting that CR was more abundant in the HM II recovered LVAD patients as compared to the HM I recovered LVAD patients. Also the 6MW exercise test distance was higher in the recovered HM II LVAD patients as compared to the recovered HM I LVAD patients but not significantly.

Finally, the echocardiographic 1-year trends following device interruption and exercise testing were better in the HM II recovered patients. It could be argued that the improvement in unloading in the HM II population with time was related to an increase in the device speed. In all patients, however, the speed was mainly
adjusted within the first week of device implantation under echocardiographic assessment and rarely changed by much after that.

**Limitations**

The sample size of this study was relatively small. It is a non-randomised, observational, prospective, and single-centred study. Another major limitation of the present study was to calculate the EF from the cubed function formula of the ventricular dimensions. It was not possible to use Simpson’s Biplane method at every assessment due to the architectural changes seen in the internal structure of the LV cavity and due to difficulties met in aligning the echo transducer at every assessment to acquire two appropriate planes to measure the EF. Another possible limitation was the use of the 6MW exercise test as a tool to assess CR. Although, the 6MW exercise test reflects day-to-day activity, reproducible at every clinic assessment, and acceptable by all patients it remains submaximal and there are no benchmark studies that have it to measure the CR. Further, the use of DSE would have been time-consuming, unethical, unacceptable by patients, and would have provided us with segmental viability (a non-required parameter in the assessment of the degree of myocardial recovery). A final limitation was the inability to assess the response of the myocardium to exercise sooner post-device implantation as this would have enabled us to correlate the response of the LV function to exercise with the different components of pharmacological therapy during the up-titration phase.

**Conclusion**

Off-pump testing is a safe approach in patients with continuous flow and pulsatile flow LVADs. The 6MW exercise test is also safe and a useful clinical tool that corresponds to day-to-day activity and has a role in assessing the underlying LV function and response to exercise, such that, recovered patients have exhibited better haemodynamic and echocardiographic responses as compared to the non-recovered patients. The significantly enhanced EF following the 6MW could reflect better preservation of CR in recovered patients since it has correlated strongly with myocardial recovery and has proven to be a strong predictor for
myocardial recovery. Interestingly, patients with continuous flow LVADs had a better response to the 6MW exercise test as compared to those with the pulsatile device suggesting that the continuous flow LVADs and drug combination therapy result in better CR preservation and enhancement in the myocardial sympathetic nervous system which is significantly deranged in end-stage HF. The changes observed were not due to data averaging but were continuous trends that have occurred throughout the support period. Finally, recovered patients managed longer walking distances as compared to the non-recovered patients in both HM I and HM II LVAD populations with the recovered HM II patients managing a longer walking distance than the recovered HM I LVAD patients.
Chapter 5 - Mechanical LV unloading results in normalisation of the cardiac NET uptake mechanism
5.1 Background

As been described in chapter 1, patients with HF have increased activity in their sympathetic efferent neuronal activity leading to excessive exposure of the myocardium to norepinephrine (NE). Abundance of NE in the synapse leads to increased sympathetic drive which acts as a method of **compensatory** inotropic and chronotropic mechanisms (Eisenhofer G, 2001) explaining the initial phase of hypertension and tachycardia seen in the early stages of DCM. However, it has been clearly documented that long term exposure of the heart to NE contributes to the progression of CHF with the development of arrhythmias, down regulation of α- and β-AR, myocardial apoptosis and necrosis, tissue hypoxia, increased cardiac oxygen consumption and myocardial oxygen wastage, reduced coronary blood flow, and loss of CR (Fukuoka S et al., 1997; Gudjonsson T & Rahko PS, 2002b; Liang C, 2003; Opie LH, 2002; Inoue H & Zipes DP, 1987; Udelson JE et al., 2002).

In normal individuals 92% of the synaptic NE is removed from the synapse via norepinephrine transporter (NET) located at the presynaptic side. This percentage is reduced to 85% in DCM patients due to a reduction of NET uptake sites (Böhm M et al., 1995). Backs et al demonstrated that reduction of NET binding sites is neither mediated by a decreased NET gene expression nor by a loss of noradrenergic nerve terminals, however, a posttranscriptional downregulation of NET per neuron is postulated (Backs J et al., 2001).

5.1.1 Norepinephrine Transporter

The main role of NET is to limit the action of NE through reuptake into the cytoplasm (Mandela P & Ordway GA, 2006). NET is a Na+/Cl⁻ dependent transporter (Kitayama S & Dohi T, 2003) with 12-hydrophobic transmembrane domains ((Brüss M et al., 1995) - figure 5.1). The electrochemical gradient derived from the inward gradient of Na⁺ drives the intracellular accumulation of NE via NET. The function of NET can be selectively blocked by the Na⁺/Cl⁻ dependent binding of drugs such as desipramine (Liang C, 2007).
Figure 5.1: Topological structure of NET

![Topological structure of NET](image)

Fig 5.1: Topological structure of NET depicting 3 N-glycosylation sites on the second extracellular loop. From (Stober G et al., 1996).

The gene encoding NET (SLC6A2) is located on the long arm of chromosome 16 (locus 16q12.2) and consists of 15 exons spanning 45 kilobases (Stober G et al., 1996; Uhl GR & Johnson PS, 1994; Bauman PA & Blakely RD, 2002).

NET consists of 617 amino acids with both amino- and carboxy- terminals being located intracellularly. The first 5 trans-membrane domains of the amino-terminal have been shown to be involved primarily in general uptake mechanisms and are well conserved in the Na⁺/Cl⁻ dependent transport family (Wang Y et al., 1999). The large second extracellular loop has three sites for N-linked glycosylation which have shown to be important for synthesis, physical maturation, surface targeting and trafficking, protein stability, transport activity, and cell surface expression of NET (Stober G et al., 1996; Blakely RD et al., 1994; Eisenhofer G, 2001; Kanner BI, 1994; Melikian H et al., 1994; Melikian H et al., 1996; Meyer J et al., 1998; Nguyen T & Amara S, 1996; Zahinser NR & Doolen S, 2001; Nelson N, 1994; Hahn MK et al., 2005).

Several intracellular signalling cascades regulate NET’s activity such as PKC, cAMP/PKA, cGMP, and dephosphorylation (figure 5.2) (Mandela P & Ordway GA, 2006; Torres GE et al., 2003; Apparsundaram S et al., 1998a; Apparsundaram S et al., 1998b).
Figure 5.2: Trafficking mechanisms associated with NET

![Diagram of trafficking mechanisms associated with NET](image)

**Figure 5.2**: Schematic representation of the trafficking mechanisms associated with NET. After being synthesised, NET is delivered to the cell surface. Activation of PKC induces the phosphorylation and internalisation of NET. Adapted from (Torres GE et al., 2003).

### 5.1.3 Assessment of NET activity

Cardiac NET activity can be assessed non-invasively using either single photon emission tomography (SPECT) or positron emitting tomography (PET). It is well recognised that PET imaging provides better spatial resolution and has superior quantification accuracy as compared to SPET imaging (Langer O & Halldin C, 2002). SPECT, however, is widely available, cheaper, and has been extensively used over the years as a diagnostic and research tool (Mariani G et al., 2008; Flotats A & Carrio I, 2004).

First described in 1980 (Wieland DM et al., 1980) metaiodobenzylguanidine (MIBG), a SPECT tracer, is an analogue of the false neurotransmitter guanethidine with a molecular structure similar to NE (figure 5.3). MIBG does not undergo intracellular metabolism (Sisson JC et al., 1987) and when tagged with radiolabelled 123-Iodine, MIBG can be used to image NET in the heart with conventional planar techniques (Flotats A & Carrio I, 2004).
Cardiac $^{123}$I-MIBG uptake assessment is performed on two stages: at 15 minutes (early) and at 4 hours (delayed) after injecting $^{123}$I-MIBG. A region of interest (ROI) is drawn on the heart and the upper mediastinum and the heart-to-mediastinum (H/M) ratio, the ratio of the count density in the LV (specific activity) to that in the upper mediastinum (non-specific activity), is calculated. The H/M ratio represent a quantitative index of $^{123}$I-MIBG cardiac uptake and, consequently, cardiac sympathetic function (Patel A & Iskandarian A, 2002). The early H/M ratio (derived from 15 minutes image) reflects the integrity of presynaptic nerve terminals and NET function. The delayed H/M ratio (derived from 4 hours image) combines information on neuronal function from uptake to release through the storage vesicles at the nerve terminals. The washout rate (W/O) is an index of the degree of sympathetic drives and is calculated as the difference in H/M ratio between the early and delayed H/M ratios with respect to the early H/M ratio. An increased sympathetic activity is associated with high myocardial $^{123}$I-MIBG W/O and low myocardial $^{123}$I-MIBG delayed uptake (Agostini D et al., 2009a). Normal values for both H/M ratio and W/O rate are $2.0 \pm 0.3$ and more and $20 \pm 10\%$ and less, respectively (Patel A & Iskandarian A, 2002).

In DCM patients, $^{123}$I-MIBG uptake is reduced in proportion to the degree of HF irrespective of its aetiology (Allman KC & Lahiri A, 2002;Carrio I, 2001;Fujimoto S et al., 2004;Imamura Y et al., 1995;Merlet P et al.,
Previous studies have correlated MIBG uptake with NYHA functional class (Glowniak JV et al., 1989;Imamura Y et al., 1995;Schofer J et al., 1988), LV EF (Lotze U et al., 1999;Merlet P et al., 1999a;Merlet P et al., 1999b), and maximal oxygen consumption and maximal exercise duration (de Milliano PA et al., 2001). Others have shown that an abnormal H/M ratio is an independent predictor of death in DCM patients and is more accurate than other predictors such as LV size, functional class, and NE content (Merlet P et al., 1992;Merlet P et al., 1999a).

\[ ^{123}\text{I-MIBG} \]

imaging has been shown to provide in-vivo information regarding the potential and actual benefits of different therapies (Nakata T et al., 2005) and hence can be a very useful imaging tool such that \[ ^{123}\text{I-MIBG} \] imaging:

1) Predicted which patients with HF were likely to show the most improvement in LV function and exercise capacity following β-blocker treatment (Choi JY et al., 2001;Fukuoka S et al., 1997;Gerson MC et al., 2002;Lotze U et al., 2001;Suwa M et al., 1997;Toyama T et al., 1999;Yamazaki J et al., 2001;Agostini D et al., 2000;Cohen-Solal A et al., 2005;de Milliano PAR et al., 2002;Fujimoto S et al., 2005;Kakuchi H et al., 1999;Toyama T et al., 2003).

2) Assessed the degree of improvement of the cardiac sympathetic neuronal uptake function following ACE-I (Somsen GA et al., 1996c;Soeki T et al., 1998;Kasama S et al., 2005b;Nakata T et al., 2005;Takeishi Y et al., 1997).

3) Illustrated the benefits of AngII receptor antagonist use in DCM patients (Kasama S et al., 2005a;Kasama S et al., 2003a;Kasama S et al., 2006;Shinohara H et al., 2002).

4) Demonstrated that DCM patients treated with spironolactone had evidence of LV reverse remodelling (Kasama S et al., 2003b;Kasama S et al., 2002;Kasama S et al., 2007b).

5) Revealed that patients who respond to cardiac resynchronisation therapy (CRT) had better uptake and significantly enhanced sympathetic response (Burri H et al., 2008;Cha YM et al., 2008;Erol-Yilmaz A et al., 2005;Nishioka SA et al., 2007).
The use of $^{123}$I-MIBG to determine the response of the heart’s sympathetic innervation after LVAD implantation, however, had not yet been effectively studied to-date.

### 5.1.4 Hypothesis and aims of $^{123}$I-MIBG Scintigraphic Study

#### Hypothesis

The use of LVAD and drug combination would lead to improved sympathetic function as assessed by cardiac $^{123}$I-MIBG imaging and that this improvement would correlate with CR as evidenced by echocardiography.

#### Aims

The main aims of the present study were therefore to investigate the effects of LVAD and drug combination therapy on $^{123}$I-MIBG uptake and to correlate $^{123}$I-MIBG uptake to CR.

#### Objectives

1) Validate MIBG imaging in our hands in patients with LVAD after using a modified ROI over the heart that accounts for the inflow cannula positioned in the LV apex.
2) Determine the intra- and inter-observer reliability.
3) Assess early and delayed H/M ratios and W/O rate serially in patients:
   a. Immediately post LVAD implantation,
   b. Immediately pre-LVAD explanation for myocardial recovery or prior to listing for transplantation,
   c. Post LVAD explantation.
4) Correlate $^{123}$I-MIBG uptake parameters to CR in LVAD patients.
5) Investigate whether the changes seen in serial MIBG uptake in LVAD patients is secondary to a reduction in the LV size or solely due to alteration in the uptake mechanism itself.
5.2 Methods

5.2.1 Study Population

The study was approved by the ethics committee at the Royal Brompton and Harefield NHS Foundation Trust (Protocol Number 06/Q0404/20). All patients provided written informed consent.

$^{123}$I-MIBG scintigraphy was studied serially in 14 out of the 23 patients. Here are the studied patients: 4, 7, 8, 10, 11, 12, 14, 16, 17, 18, 19, 21, 22, 23. Reasons for excluding 9 patients are included in table 5.1.

Table 5.1: Patients excluded from the MIBG imaging study (n=10)

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Reason for exclusion</th>
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<tbody>
<tr>
<td>1</td>
<td>Explanted prior to commencing MIBG imaging study</td>
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<tr>
<td>2</td>
<td>Had already started phase II pharmacological therapy (clenbuterol) when MIBG imaging was commenced on LVAD patients ‡</td>
</tr>
<tr>
<td>3, 5, 6, 13, 15</td>
<td>Refused to take part in the study</td>
</tr>
<tr>
<td>9, 20</td>
<td>Diabetes Mellitus – studies have shown that diabetic patients have evidence of significant reduction in MIBG uptake due to autonomic dysfunction (Langer A et al., 1995; Uehara A et al., 1999; Kiyono Y et al., 2005) and sympathetic dysinnervation (Stevens MJ et al., 1998) despite normal contractile reserve (Scognamiglio R et al., 1998)</td>
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Table 5.1: Reasons for excluding 9 patients from MIBG imaging study.

‡Although, MIBG study was approved by the RBH Ethics Committee in September 2006, it took nine months to validate the technique on HF and explant patients, the use of collimators and ensure its safety. The first MIBG scan to be performed on an LVAD patient was in June 2007. Patient 2 had already started the phase II pharmacological therapy, including clenbuterol, and therefore it was felt he should be excluded from the study.
5.2.2 Patient preparation

$^{123}\text{I}$-MIBG imaging was performed serially in each of the 14 patient at two time points:

i) early post- device implantation i.e. once patients were stabilised, their inotropic support been discontinued for at least 5 days, and their mobility has been restored (Scan 1); and

ii) immediately prior to either device explantation for myocardial recovery or transplant listing for failure to recover (Scan 2).

In 4 recovered patients who had their HM II LVAD explanted for recovery a repeat scan after at least 12 months following device explantation was performed.

All patients were asked to fast and refrain from drinking caffeine products for at least 24 hours before the scan.

5.2.3 Imaging Protocol

An 18F sized cannula was inserted in the anti-cubital fossa at least 75 minutes before imaging. The cannula was used to collect blood for NE, epinephrine, and dopamine levels after at least 45 minutes of supine rest in a quiet room. See chapter 7 for liquid chromatography mass spectrometry analysis technique for these catecholamine levels.

One hour after thyroid blockade with 400mg of potassium perchlorate, $425.92 \pm 18.80$ Mega-Becquerel (MBq, range 379 - 469 MBq) of $^{123}\text{I}$-MIBG was intravenously injected through the pre-sited cannula.

Imaging was performed with a Philips Forte dual-headed gamma camera and processed using a Philips Pegasys workstation. Planar anterior images were acquired at 15 minutes and at 4 hours post $^{123}\text{I}$-MIBG injection using the following parameters: 256 x 256 x 16 matrix and 900 seconds acquisition time. All images were stored in a departmental computer.

**Use of medium energy collimator:** $^{123}$I emits 159 keV photopeak photons as well as multiple low-abundance, high-energy 529 keV photons. The latter can
penetrate the collimator septa and cause scatter detected in the 159-keV energy window. Septal penetration affects estimation of the H/M ratio if low-energy collimator was used; hence, we have used medium energy collimators to provide better quantitative accuracy (Inoue Y et al., 2003; Verberne HJ et al., 2005; Verberne HJ et al., 2009b).

**Use of dual energy windows:** Compton window subtraction is a widely used method of compensation for scatter (Jaszczak RJ et al., 1984; Chen J et al., 2006b; Chen J et al., 2006a) and is based on rectangular approximation (Matsuo S et al., 2009). In this study $^{123}$I dual energy windows were used to correct for scatter. The first window was 20% wide and was centred over the 159-keV window which represents the lower energy window whereas the second window was 19% wide and was centred over the 194-keV photopeak to represent the scatter energy window (Chen J et al., 2006b; Inoue Y et al., 2003; Kobayashi H et al., 2003; Nakajima K et al., 2007). The principle of scatter correction is shown in figure 5.4 and an example of the effects of scatter correction is depicted in figure 5.5.

**Figure 5.4: The use $^{123}$I-dual energy windows to correct for scatter**

![Figure 5.4](image)

**Fig 5.4:** The principle of the $^{123}$I dual-window method. $E_{159}$, Energy window for 159-keV imaging; $W_{159}$, width of $E_{159}$; $C_{159}$, total counts within $E_{159}$; $E_{up}$, upper energy window to estimate the 529-keV downscatter into $E_{159}$; $W_{up}$, width of $E_{up}$; $C_{up}$, total counts within $E_{up}$; $C_{scat529}$, estimated counts of the 529-keV downscatter into $E_{159}$, which is calculated as $k \times W_{159}/W_{up} \times C_{up}$. $k$ is the estimated weighting value. Finally, corrected data are obtained by subtracting $C_{scat529}$ from $C_{159}$. From (Yamashina S & Yamazaki J, 2004).
Figure 5.5: Effects of dual energy window scatter correction

Fig 5.5: Effect of improvement of imaging by the dual energy window method in our hands. MIBG planar scan of the same patient. Conventional method (left) and after scatter correction method (right).

5.2.3.1 Heart / Mediastinum ratio calculations

$^{123}$I- MIBG uptake is calculated as the ratio between the total number of counts per pixel of the ROI drawn over the heart and the total number of counts per pixel of a ROI drawn in the mediastinum. Definition of the heart ROI is operator dependent and previous studies have illustrated the importance of rigorous and uniform quantification to minimise inter- and intra-individual variations (Agostini D et al., 2009b).

In DCM patients the ROI is drawn over the entire myocardium as illustrated in figure 5.6. The presence of the inflow cannula in the LV, however, poses a major challenge on how to define the heart’s ROI and its accuracy.
Figure 5.6: An example of $^{123}$I-MIBG imaging in DCM patient

**Fig 5.6:** A) A typical MIBG imaging with a region of interest drawn over the heart in a DCM patient. B) A chest X-ray of a 17-years old male with a HM II LVAD in-situ. The presence of the inflow cannula inside the LV creates a major challenge on how to define the heart contour to manually draw the ROI.

In the LVAD patients, we have used a modified region of interest over the heart (ROI$_{h}$) which excludes the inflow cannula of the device. A 1 cm$^2$ rectangular ROI was drawn over the mediastinum (ROI$_{m}$). Both regions of interest were drawn in both lower energy and scattered windows. To determine the exact position of the inflow cannula within the myocardium, a 64-slice reconstructive CT scan or a chest X-ray was performed within 2-3 days of the nuclear scan and the position of the inflow cannula was superimposed over the MIBG planar images (figure 5.7).
Figure 5.7: Identifying the Heart ROI in LVAD patient

Fig 5.7: A) A 64-Slice reconstructed CT-scan illustrating the position of the inflow cannula within the LV apex. B) A schematic diagram showing the ROI on the anterior planar image; the ROI drawn over the heart (H) is adjusted to exclude the LVAD which is placed apically. M represents the ROI in the upper mediastinum.

After drawing both ROI\textsubscript{h} and ROI\textsubscript{m}, scatter correction was performed to correct for the high-energy photopeaks (as discussed earlier). The corrected scatter counts for both ROIs were determined using the following equation:

\[
\text{Corrected Counts}_{\text{scatter}} = \text{Counts}_{\text{scatter}} \times k \quad \text{(equation 5.1)}
\]

Where, Counts\textsubscript{scatter} were obtained from the scatter-energy window, and \( k \) is a coefficient which is equivalent to \( \frac{31.8}{36.9} \) and represents the difference in the width of the two energy windows used.
The final corrected counts for both heart and mediastinum were calculated as the counts being obtained from the lower energy window (159 keV) minus the calculated scattered counts (equation 5.2 and 5.3, respectively).

**Corrected Counts for Heart:**

\[
\text{Corrected counts}_{\text{Heart}} = \text{Counts}_{159\text{KeV}} - \text{Corrected Counts}_{\text{scatter}}
\]  
(equation 5.2)

**Corrected Counts for Mediastinum:**

\[
\text{Corrected counts}_{\text{Med}} = \text{Counts}_{159\text{KeV}} - \text{Corrected Counts}_{\text{scatter}}
\]  
(equation 5.3)

The final corrected counts were divided by the number of pixels represented within each ROI to provide the counts per pixel for both heart and mediastinum (equations 5.4 and 5.6).

**Counts per pixel for Heart:**

\[
H = (\text{Corrected Counts}_{\text{Heart}}) / \text{Number of Pixels}_{\text{ROI}_h}
\]  
(equation 5.4)

**Counts per pixel for mediastinum:**

\[
M = (\text{Corrected Counts}_{\text{Mediastinum}}) / \text{Number of Pixels}_{\text{ROI}_m}
\]  
(equation 5.5)

Early H/M (15 minutes) and delayed H/M (4 hours) activity ratios were then calculated. A high H/M ratio indicates predominant localisation of \(^{123}\text{I}\)-MIBG in the myocardium (Patel A & Iskandarian A, 2002). In normal hearts a ratio of 2.0 ± 0.3 and above is considered to be normal (Camacho V & Carrio I, 2007; Patel A & Iskandarian A, 2002).
5.2.3.2 Washout Rate calculations

W/O rate was calculated after taking iodine decay into consideration using the following equation (Ogita H et al., 2001):

\[
\text{Washout Rate (W/O)} = \frac{(H_1 - M_1)/(1/2)^{t_1} - (H_2 - M_2)/(1/2)^{t_2}}{(H_1 - M_1)/(1/2)^{t_1}} \times 100
\]

(equation 5.6)

Where, H and M are the mean counts per pixel in the heart and mediastinal ROI acquired from equations 5.4 and 5.5, respectively. \(H_1\) and \(M_1\) refer to the counts per pixel at 15 minutes (i.e. early images), whereas \(H_2\) and \(M_2\) refer to the counts per pixel at 4 hours (i.e. delayed images). \(t_1\) and \(t_2\) are the times from tracer injection to early and delayed acquisition such that \(t_1\) refers to 15 minutes and \(t_2\) refers to 4 hours, and T is the half life of 123-Iodine (13.3 hours).

W/O rate determines the rate of decrease in myocardial MIBG counts over time such that a low W/O rate reflects reduced adrenergic activity and tone (Gao DW et al., 2001). A W/O rate of less than 20 ± 10 % and less is considered to be normal (Camacho V & Carrio I, 2007; Patel A & Iskandarian A, 2002).

All images were analysed by two observers for inter-observer reproducibility. Each observer repeated the analysis at least 6 weeks from the initial analysis to determine the intra-observer reproducibility.
5.2.4 Validation of the modified region of interest

To validate the modified ROI, MIBG imaging was performed in 13 HF patients without the LVAD. The conventional ROI was drawn over the entire myocardium and the H/M ratios and W/O rate were calculated as described above. The modified ROI was then drawn on each scan missing off the apex and the H/M ratios and W/O rate were recalculated (figure 5.8). A comparison between the MIBG measured parameters derived from using the conventional and the modified ROI was performed.

Figure 5.8: Validation of the modified ROI

![Figure 5.8](image)

Figure 5.8: A) Conventional ROI drawn over the myocardium; B) modified ROI drawn over the heart missing off the apex to account for the LVAD inflow cannula in a heart failure without an LVAD.

5.2.5 Correlation with LV function and CR

To correlate MIBG uptake measurements to CR, all 14 had an echocardiographic assessment within 1 week of MIBG imaging. Echocardiographic assessment protocol was discussed in details in chapter 4. In summary, assessments were performed by one senior echocardiographer and all measurements were acquired in M-Mode. From the parasternal long axis of the LV at the level of chordal-mitral valve junction, both LVEDD and LVESD were obtained at the onset of the QRS complex and at either the peak of posterior wall motion or the nadir of septal motion, respectively. FS was calculated as the percentage change in the LV cavity dimensions with systole and EF was calculated using the cubed function formula.
of the ventricular dimensions. Correlation between echocardiographic parameters and MIBG uptake parameters acquired at both scan 1 and scan 2 was performed.

CR was assessed following the 6MW exercise test whilst the speed of the HM II LVAD was running at 6000 rpm (i.e. low speed). CR was defined as the percentage difference between EF obtained after the 6MW and after reducing the speed of the HM II LVAD to 6000 rpm for 15 minutes (see chapter 4 for more details). Correlation between CR and MIBG uptake parameters derived from scan 2 was performed.

5.2.6 The effects of LV size on MIBG uptake

To investigate whether the serial changes in MIBG uptake in LVAD patients is secondary to reduction in LV size or solely due to alteration in the uptake mechanism itself the derived measurements at scan 2 were normalised against the size of the ROI. Such that the total counts from the ROI in scan 2 were divided by the number of pixels in the ROI in the first scan. The counts per pixel were recalculated. Comparison between the H/M ratios derived from this approach and the H/M ratio from scan 1 was made.
5.2.7 Data Collection and Statistical Analysis

All data are presented as mean ± stdev and were collected prospectively. Data analysis was done using SPSS, version 16.0 for Windows, created by Lead Technologies. To validate the use of the modified ROI, $t$-test statistical tool and Bland-Altman plots were used to assess the extent of agreement between the conventional ROI, drawn over the entire heart and the modified ROI, in the 13 HF patients (Bland JM & Altman DG, 1986; Bland JM & Altman DG, 1995). Intra-observer and inter-observer reproducibility of data measurement were also assessed using Bland-Altman plots.

As the derived H/M ratios and W/O rate in scans 1 and 2 were normally distributed, paired sample Student’s $t$-test was used to determine the differences in the $^{123}$I-MIBG measured parameters (continuous variables) between the first and the second scan. Unpaired $t$-test was used to compare MIBG parameters between the recovered and the non-recovered subgroups.

To correlate the degree of change between $^{123}$I-MIBG scintigraphic findings and CR linear regression analysis was performed using the percentage change in EF following the 6MW exercise test as a dependent parameter and the H/M ratios and the W/O rate as independent parameters. Linear regression analysis was also used to correlate between the changes in the echocardiographic parameters and the changes in the $^{123}$I-MIBG scintigraphic findings with the echocardiographic changes as the dependent factors. $p < 0.05$ was considered statistically significant.
5.3 **Results**

Demographics and pre-implantation data of the 14 studied LVAD patients are presented in table 5.2. Out of these, 10 had recovered and were explanted. The remaining four patients were transplant listed.

**Table 5.2: Demographics of the studied patients (n=14)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at HM II implantation (years)</td>
<td>33.72 ± 14.58</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.51 ± 3.24</td>
</tr>
<tr>
<td>Duration of Heart Failure (months)</td>
<td>19.3 ± 24.59</td>
</tr>
<tr>
<td>Average number of pre-implant inotropes per patient</td>
<td>1.93 ± 0.27</td>
</tr>
</tbody>
</table>

**Pre-implantation Echo**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD (mm)</td>
<td>73.21 ± 9.78</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>67.29 ± 8.96</td>
</tr>
<tr>
<td>FS (%)</td>
<td>7.99 ± 4.51</td>
</tr>
<tr>
<td>EF (%)</td>
<td>21.59 ± 10.89</td>
</tr>
</tbody>
</table>

**Pre-implantation Haemodynamics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Pulmonary Artery Pressure (mmHg)</td>
<td>35.8 ± 4.94</td>
</tr>
<tr>
<td>Mean Pulmonary Capillary Wedge Pressure (mmHg)</td>
<td>28.2 ± 3.85</td>
</tr>
<tr>
<td>Cardiac Output (l/min)</td>
<td>2.75 ± 0.73</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m²)</td>
<td>1.46 ± 0.46</td>
</tr>
</tbody>
</table>

**Table 5.2:** Pre-implantation demographics of the 14 studied patients.
A list of medication taken at scan 1 and scan 2 are included in table 5.3. At the time of the first $^{123}$I-MIBG imaging all patients had already commenced on small doses of anti-failure medication. Carvedilol was administered in all 14 patients. Lisinopril was taken by 12 patients. Those who did not tolerate lisinopril were started on losartan instead. At the time of the second scan, anti-failure medication was maximal and the 10 recovered patients were on the β2-agonist, clenbuterol, after switching carvedilol to bisoprolol. The remaining 4 were considered non-recovered but eligible for transplantation and none were started on clenbuterol.

**Table 5.3: Medication list**

<table>
<thead>
<tr>
<th>MIBG</th>
<th>Drug</th>
<th>Number of Patients</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carvedilol (mg)</td>
<td>14</td>
<td>12.1 ± 3.8</td>
</tr>
<tr>
<td>Scan 1</td>
<td>Lisinopril (mg)</td>
<td>12</td>
<td>11.9 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td>2</td>
<td>87.5 ± 17.7</td>
</tr>
<tr>
<td></td>
<td>Spironolactone (mg)</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Digoxin (mcg)</td>
<td>14</td>
<td>125</td>
</tr>
<tr>
<td>Scan 2</td>
<td>Carvedilol (mg)</td>
<td>4</td>
<td>28.1 ± 6.25</td>
</tr>
<tr>
<td></td>
<td>Bisoprolol (mg)</td>
<td>10</td>
<td>9.75 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Lisinopril (mg)</td>
<td>13</td>
<td>31.35 ± 13.8</td>
</tr>
<tr>
<td></td>
<td>Losartan (mg)</td>
<td>7</td>
<td>75 ± 35</td>
</tr>
<tr>
<td></td>
<td>Clenbuterol (mcg)</td>
<td>10</td>
<td>1362 ± 807</td>
</tr>
<tr>
<td></td>
<td>Spironolactone (mg)</td>
<td>14</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Digoxin (mcg)</td>
<td>14</td>
<td>25</td>
</tr>
</tbody>
</table>

**Table 5.3**: Medications taken at the two MIBG imaging scan time points
5.3.1 Validation of the modified region of interest

As described in the methods section, the use of the modified ROI was validated in 13 heart failure patients without LVAD. The derived H/M ratios and W/O rate were calculated twice using a conventional ROI and the modified ROI (figure 5.8).

\( t \)-test analysis revealed no significant differences in the derived parameters between the different ROIs (figure 5.9). Early H/M ratio 2.49 ± 0.47 versus 2.58 ± 0.50, \( p=0.638 \); Delayed H/M ratio 2.43 ± 0.75 versus 2.52 ± 0.76, \( p=0.777 \); W/O rate 32.00 ± 22.35% versus 31.80 ± 21.69%, \( p=0.981 \).

Figure 5.10 represents Bland-Altman plots of H/M ratios and W/O derived from the conventional and the modified ROIs. There was an excellent limit of agreement between the two different regions.

From both \( t \)-test analysis and the Bland-Altman plots it can be concluded that missing off the apex makes no difference to the overall H/M ratios and the W/O rate.
Figure 5.9: Validation of the modified ROI using t-test analysis

There was no significant difference in the H/M ratios and W/O rate between the conventional and the modified ROI. Early H/M ratio 2.49 ± 0.47 versus 2.58 ± 0.50, p=0.638; Delayed H/M ratio 2.43 ± 0.75 versus 2.52 ± 0.76, p=0.777; W/O rate 32.00 ± 22.35% versus 31.80 ± 21.69%, p=0.981.
Figure 5.10: Validation of the modified ROI using Bland-Altman analysis

Fig 5.10: Bland-Altman plots determining the validity of the modified region of interest. For early H/M ratio (open rhombus), delayed H/M ratio (open triangles), and washout rate (open circles), the limits of agreement were 0.23, 0.22, and 3.38, respectively, suggesting excellent agreement between the two different techniques and hence missing off the apex does not affect the validity of using the modified ROI in the LVAD patients.
5.3.2 Intra-observer reproducibility

Figures 5.11 and 5.12 represent Bland-Altman plots for all MIBG parameters for observer 1 and observer 2, respectively.

For observer 1, the limits of agreement for early H/M ratio, delayed H/M ratio and W/O rate were 0.28, 0.29 and 18.19, respectively. 26 out of the 28 (92.86%) early and delayed H/M ratio measurements fell within the early and delayed H/M ratio limits of agreement, respectively. With regards of the W/O rate reproducibility, 27 out of the 28 of the measurements (96.43%) fell within the limits of agreement.

Similarly for observer 2, the limits of agreement for early H/M ratio, delayed H/M ratio and W/O rate were 0.33, 0.29 and 19.70, respectively. 26 out of the 28 (92.86%) early and delayed H/M ratio measurements fell within the early and delayed H/M ratio limits of agreement, respectively. 27 out of the 28 W/O rate measurements (96.43%) fell within the limits of agreement for the W/O rate.

In summary there was a strong intra-observer reproducibility of measurements for each of the observers 1 and 2.
Figure 5.11: Bland-Altman plots of MIBG parameters for observer 1. There was excellent level of agreement between the first and the second measurements for observer 1 with 92.86% of early and delayed H/M ratio measurements and 96.43% of W/O rate measurements falling within the limits of agreement.
Figure 5.12: Bland-Altman plots determining the intra-observer reproducibility of measurements for observer 2. Similar to observer 1, there was excellent level of agreement between the first and the second measurements for observer 2 with 92.86% of early and delayed H/M ratio measurements and 96.43% of W/O rate measurements falling within the limits of agreement.
5.3.3 Inter-observer reproducibility

Figure 5.13 represents a Bland-Altman plot for all MIBG measured parameters between observer 1 and observer 2.

Inter-observer analysis revealed that the limits of agreement for early H/M ratio, delayed H/M ratio and W/O rate were 0.35, 0.32 and 16.47, respectively. 26 out of the 28 (92.86%) early H/M ratio measurements and 27 out of the 28 (96.43%) delayed H/M ratio measurements fell within the early and delayed H/M ratio limits of agreement, respectively. For the W/O rate 26 out of the 28 of the measurements (92.86%) fell within the limits of agreement.
Figure 5.13: Bland-Altman plots determining the inter-observer reproducibility of measurements between observers 1 and 2. There was excellent level of agreement between the first and the second observer with 93% of early H/M ratio, 96% of delayed H/M ratio, and 93% of W/O rate measurements falling within the limits of agreement.
5.3.4 Effects of LVAD and drug combination therapy on MIBG uptake

The first MIBG imaging scan was performed early post device implantation at 35.21 ± 20.37 days. The second scan was performed at 242.57 ± 82.25 days post implantation resulting in time difference between the two scans of 208.43 ± 85.50 days.

Figures 5.14, 5.15 and 5.16 represent the effects of LVAD and drug combination therapy on each of both early and delayed H/M MIBG activity and the washout rate. There was a significant increase in the mean early H/M and the mean delayed H/M ratios by 0.65 ± 0.29 (1.56 ± 0.29 versus 2.21 ± 0.30, a rise by 42.1%, p<0.001) and by 0.80 ± 0.31 (1.46 ± 0.33 versus 2.25 ± 0.43, a rise by 54.7%, p<0.001), respectively. The mean washout rate decreased significantly from 46.96 ± 22.92% to 27.04 ± 16.71% (a reduction by 42.4% reduction, p=0.003).
Figure 5.14: The effect of LVAD and drug combination therapy on early H/M ratio

Fig 5.14: LVAD and drug combination therapy resulted in a significant increase in the early H/M ratio in all 14 patients. The mean early H/M ratio increased from 1.56 ± 0.29 to 2.21 ± 0.30, p<0.001. Red lines represent the changes in recovered patients (n=10) and the black line represent the changes in non-recovered patients (n=4).
Figure 5.15: The effect of LVAD and drug combination therapy on delayed H/M ratio

**Fig 5.15:** LVAD and drug combination therapy resulted in a significant increase in the delayed H/M ratio in all 14 patients. The mean delayed H/M ratio increased from 1.46 ± 0.33 to 2.25 ± 0.43, p<0.001. Red lines represent the changes in recovered patients (n=10) and the black line represent the changes in non-recovered patients (n=4).
Figure 5.16: The effect of LVAD and drug combination therapy on W/O rate

**Fig 5.16:** LVAD and drug combination therapy resulted in a significant decrease in the mean washout rate from 46.96 ± 22.92% to 27.04 ± 16.71%, *p*=0.003. Red lines represent the changes in recovered patients (n=10) and the black line represent the changes in non-recovered patients (n=4).
5.3.4.1 Comparison of MIBG uptake between recovered and non-recovered patients

As stated above, 10 of the 14 studied patients had recovered and were explanted. There were no significant differences in MIBG uptake parameters between the two sub-groups at either the first or the second scan. The effects of LVAD and drug combination therapy, however, were different in each subgroup. Table 5.4 represents the differences in the effects of HMII and drug combination therapy on the recovered and the non-recovered patients.

Table 5.4: Effects of LVAD and drug combination therapy between recovered and non-recovered sub-groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MIBG Parameter</th>
<th>Scan 1</th>
<th>Scan 2</th>
<th>% change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovered Group</td>
<td>Early H/M ratio</td>
<td>1.64 ± 0.29</td>
<td>2.31 ± 0.22</td>
<td>+40.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Delayed H/M ratio</td>
<td>1.53 ± 0.32</td>
<td>2.39 ± 0.22</td>
<td>+56.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Washout rate (%)</td>
<td>43.97 ± 15.96</td>
<td>23.57 ± 11.11</td>
<td>-46.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Non-Recovered Group</td>
<td>Early H/M ratio</td>
<td>1.35 ± 0.17</td>
<td>1.97 ± 0.38</td>
<td>+45.8</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Delayed H/M ratio</td>
<td>1.27 ± 0.30</td>
<td>1.90 ± 0.64</td>
<td>+50.0</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>Washout rate (%)</td>
<td>54.41 ± 37.53</td>
<td>35.71 ± 26.45</td>
<td>-34.4</td>
<td>0.352</td>
</tr>
</tbody>
</table>

Table 5.4: Both groups had a significant improvement in the H/M ratios, however, the recovered group had a more pronounced improvement also the mean ratios have normalised above the cut-off point of 2.0. The washout rate reduction was significantly better in the recovered group although the mean remained above the normal cut-off point of 20%.
5.3.4.2 Correlation between 123I-MIBG scintigraphic findings and contractile reserve

As represented in chapter 4, the HM II LVAD and drug combination therapy had resulted in a significant change in CR such that a 6MW exercise test had resulted in an absolute increase in the EF by 5.25 ± 5.30% in all 23 studied patients. In the present group (i.e. the 14 patients who had MIBG imaging) there was an absolute increase in the EF following the 6MW exercise test by 5.89 ± 6.07% (11 patients (10 recovered and 1 non-recovered) had an absolute increase and 3 non-recovered patients had an absolute reduction).

Linear regression analysis revealed a moderate but significant correlation between CR and early H/M ratio ($r=0.612$, $p=0.02$) and between CR and the delayed H/M ratio ($r=0.578$, $p=0.03$ – figure 5.17 A and B). There was no correlation between CR and the myocardial W/O rate ($r=0.414$, $p=0.142$).

Figure 5.17: Relationship between 123I-MIBG scintigraphic findings and contractile reserve

![Graph A] (Relationship between early H/M ratio and CR)

![Graph B] (Relationship between delayed H/M ratio and CR)

Fig 5.17: A) Relationship between early H/M ratio and CR. B) Relationship between delayed H/M ratio and CR.
5.3.4.3 Relationship between LV dimensions and function and $^{123}$I-MIBG scintigraphic findings

In the studied population, there was a significant reduction in LVESD ($43.36 \pm 17.81$ mm versus $47.79 \pm 17.84$ mm, $p=0.029$). The LVEDD did not change significantly throughout the study ($56.79 \pm 14.78$ mm versus $58.71 \pm 14.29$ mm, $p=0.298$), however, there was a significant increase in both FS and EF ($25.64 \pm 11.64\%$ versus $20.83 \pm 10.27\%$, $p=0.031$, and $56.07 \pm 20.17\%$ versus $48.02 \pm 20.65\%$, $p=0.024$, respectively).

There was no correlation between the changes in the LV dimensions and function, and the changes in the $^{123}$I-MIBG scintigraphic findings suggesting that the changes seen in the MIBG uptake parameters are independent of the LV dimensions and function (table 5.5).

<table>
<thead>
<tr>
<th>Δ LVEDD (mm)</th>
<th>Δ LVESD (mm)</th>
<th>Δ FS (%)</th>
<th>Δ EF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Early H/M ratio</td>
<td>Δ Delayed H/M ratio</td>
<td>Δ W/O Rate (%)</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>p-value</td>
<td>R</td>
<td>p-value</td>
</tr>
<tr>
<td>0.063</td>
<td>0.824</td>
<td>0.089</td>
<td>0.761</td>
</tr>
<tr>
<td>0.161</td>
<td>0.580</td>
<td>0.126</td>
<td>0.667</td>
</tr>
<tr>
<td>0.114</td>
<td>0.694</td>
<td>0.226</td>
<td>0.438</td>
</tr>
<tr>
<td>0.230</td>
<td>0.430</td>
<td>0.358</td>
<td>0.209</td>
</tr>
</tbody>
</table>

Table 5.5: There was no correlation between the changes in LV dimensions and function and the changes in $\Delta$I-MIBG scintigraphic parameters throughout the study. $\Delta$, delta represents the absolute change.
To further ensure that the improvements in $^{123}$I-MIBG scintigraphic findings are independent of the LV size, the measurements derived at scan 2 were normalised against the size of the ROI. The outcomes were non-significant differences between $^{123}$I-MIBG scintigraphic findings measured using the ROIs from the first scan and the findings from the second scan (early H/M ratio: 2.02 ± 0.66 versus 2.21 ± 0.30, p=0.208; delayed H/M ratio: 2.05 ± 0.79 versus 2.25 ± 0.43, p=0.236; W/O rate: 30.98 ± 21.81 versus 27.32 ± 15.80, p=0.288).
5.3.4.4 Long term explants

Out of the 10 explanted patients, 4 had a repeat scan (scan 3) 408 ± 165 days following the explant of the HM II LVAD. Following explantation there was a non-significant rise in both early H/M and delayed H/M ratios as compared to the MIBG uptake assessed just prior to device explantation (early H/M ratio: 2.59 ± 0.13 versus 2.42 ± 0.21, p=0.11; delayed H/M ratio 2.71 ± 0.36 versus 2.37 ± 0.12, p=0.090) and a non-significant reduction in washout rate (17.14 ± 9.65% versus 28.86 ± 5.23%, p=0.078) (figure 5.18 A-C).

Figure 5.18: MIBG uptake in 4 recovered LVAD patients
Fig 5.18: MIBG uptake in 4 recovered LVAD patients. There were no significant differences in MIBG uptake parameters between the second and the third scan.
5.4 Discussion

The main findings of the present study include the following:

- The use of a modified region of interest is a valid approach to assess $^{123}$I-MIBG uptake in LVAD patients;
- MIBG imaging analysis is highly reproducible with strong intra-observer and inter-observer reproducibility;
- LVAD and drug combination therapy result in a significant improvement in H/M MIBG activity;
- There were moderate correlations between MIBG uptake and CR in LVAD patients confirming that CR is linked to sympathetic function activity;
- The changes seen in MIBG uptake are irrespective of the LV size and they represent an absolute improvement in sympathetic innervation;
- There is sustained improvement in $^{123}$I-MIBG uptake following device explantation.

The involvement of cardiac SNS in HF

The role of the sympathetic nervous system in the development and progression of HF has become increasingly clear over the past decade (Schrier RW & Abraham WT, 1999). Decreased cardiac output related to LV dysfunction results in a baroreceptor-mediated increase in sympathetic tone leading to excessive exposure of the myocardium to NE (Davis D et al., 1988;Esler M et al., 1997;Kaye D & Esler M, 2005;Münch G et al., 1996) which is the trigger for the activation of the neurohormonal pathway and myocardial remodeling. In chapter 1 the effects of NE on $\alpha$- and $\beta$- ARs, RAAS, Ca$^{2+}$ handling, natriuretic peptides and endothelin have been discussed in detail.

The chronic consequences of increased sympathetic tone consist of increased LV afterload, worsening LV function, and progression of congestive HF. Atsumi et al have elegantly correlated exercise intolerance in DCM patients with enhanced cardiac
sympathetic nervous activity (Atsumi H et al., 1998). Other investigations have shown that there are elevated plasma NE levels (Cohen JT et al., 1984; Eisenhofer G et al., 1996; James KB et al., 1995), reduced NE concentration in cardiac tissue (Eisenhofer G et al., 1996), increased spillover of NE into plasma (Eisenhofer G et al., 1996; Hasking G et al., 1986; Rundqvist B et al., 1997) and increased muscle sympathetic nerve firing in patients with CHF (Narula J & Kunal S, 2003; Patel A & Iskandarian A, 2002). All these factors have been related to poor prognosis and have been reported to improve when patients had either recovered from symptoms of HF (James KB et al., 1995) or had heart transplant (Bengel FM & Schwaiger M, 2004; Hunt S, 2001; Lovric SS et al., 2004; Murphy DA et al., 2000).

Nuclear imaging and cardiac SNS

The evaluation of the SNS of the heart has been limited to either post-partum examination or invasive procedures to determine the arteriovenous difference of plasma catecholamines (Schwaiger M et al., 1990). The development of several successful sympathetic neuronal imaging agents such as the positron emitting compounds $^{11}\text{C}$-metahydroxyphedrine, $^{11}\text{C}$-epinephrine, $^{11}\text{C}$-phenylephrine, and $^{18}\text{F}$-fluorometaraminol have provided advanced information onto the role of the SNS in HF. Cardiac sympathetic innervation can also be assessed non-invasively using MIBG, a single photon emission compound with a molecular structure similar to NE. In vivo MIBG imaging is a sensitive and a specific non-invasive technique for assessing NET function and evaluation of adrenergic nerve integrity and function (Merlet P et al., 1995; Merlet P et al., 1996; Merlet P et al., 1999b; Sisson JC et al., 1987; Agostini D et al., 2008).

It is well recognised that PET imaging provides better spatial resolution and has superior quantification accuracy as compared to SPET imaging (Langer O & Halldin C, 2002).
In this study, however, MIBG SPECT analysis was used for the following reasons:

i) MIBG has the highest rate of neuronal uptake, the least leakage across neuronal membrane after NE, and does not undergo intracellular metabolism making it metabolically very stable (Raffel DM & Wieland DM, 2001)

ii) MIBG is widely used such that most studies (>400 studies) had utilised it as a sensitive tool to determine the effects of different therapeutic modalities in HF. In this study we used the outcome of those studies as a reference for the benefits of using LVAD and drug combination therapy on the SNS in LVAD patients (see text below and table 5.8 for further details),

iii) MIBG can be obtained using a “shake and break” kit and hence its production is much cheaper. PET tracers, however, are more expensive to produce and are preferred to be produced in-house where a cyclotron is available as they have a shorter half-life (Knuuti J & Sipola P, 2005),

iv) Due to the lack of PET scanner availability at Harefield, it was thought that performing PET imaging serially on LVAD patients would be very costly, time consuming, and would demand extensive labour work from VAD specialists (every VAD patient has to be accompanied by a VAD specialist during transport and at the imaging sites).

**MIBG and cardiac SNS in HF**

HF animal model studies revealed that impairment of NET binding sites is neither mediated by a decreased NET gene expression nor by a loss of noradrenergic nerve terminals, however, it is due to posttranscriptional downregulation of NET per neuron (Backs J et al., 2001). NET-deficient mice demonstrated a significant rise in resting mean arterial blood pressure and heart rate as compares to controls (Keller NR et al., 2004).

Decreased $^{123}$I-MIBG uptake has been shown to be associated with cardiac and sudden death in ischaemic and non-ischaemic DCM patients (Imamura Y et al.,
2001; Kasama S et al., 2008; Matsui T et al., 2002; Merlet P et al., 1999a; Nakata T et al., 1998; Ogita H et al., 2001; Tamaki S et al., 2009; Verberne HJ et al., 2008a; Wakabayashi T et al., 2005; Merlet P et al., 1994) and other events such as hospitalisation and arrhythmia (Fujimoto S et al., 2004; Fujimoto S et al., 2005; Paul M et al., 2006).

Furthermore, MIBG scintigraphy has shown to be a more reliable predictor of overall mortality than heart rate variability measurements (Anastasiou-Nana M et al., 2005; Yamada T et al., 2003; Somsen G et al., 1997), a more reliable tool in determining the progression of HF (Arimoto T et al., 2005; Matsuo S et al., 2002; Agostini D et al., 2008), a strong predictor for DCM patients with increased risk for potentially fatal arrhythmias (Arora R et al., 2003; Jacobson AF et al., 2009), and an accurate and an independent predictor of cardiac transplantation or death than other standard clinical tests such as mVO₂, radionuclide LV EF and plasma NE levels (Gerson MC et al., 2003).

**Technical issues with MIBG imaging**

The choice of collimator substantially influences estimation of H/M ratios in cardiac ¹²³I-MIBG imaging. Previous studies have compared the use of medium energy collimators to low energy collimators and found that the former provides higher quantitative accuracy and enhances reliability in the evaluation of cardiac sympathetic nerve function (Inoue Y et al., 2003; Verberne HJ et al., 2005; Verberne HJ et al., 2009b). Scatter correction using dual energy windows has also been shown to provide extra reliability and accuracy of data such that it standardises the H/M ratios (Fletcher AA et al., 2007; Kobayashi H et al., 2003; Yang YW et al., 2007; Matsuo S et al., 2009; Nakajima K et al., 2007; Imamura Y et al., 2001). In our study placing a scatter window at 194 keV has enhanced the quality of the images (figure 5.5).

The first part of this study was to decide on how to draw the heart ROI. Naruse et al have recommended drawing the ROI over the entire myocardium (Naruse H et al., 2000). Others have, selectively, drawn the ROI by excluding the blood pool
(Anastasiou-Nana M et al., 2005). In the latter approach, Anastasiou-Nana et al have excluded the presynaptic neurones allocated in the anterior and the posterior walls of the myocardium and therefore the calculated H/M ratios do not reflect the whole presynaptic neurones concentrated in the heart.

We have opted to use a ROI over the entire myocardium despite the challenge presented by the presence of the inflow cannula. Therefore, the ROI had to be modified to account for the inflow cannula. Hence, a horse-shoe shaped ROI was used (George R et al., 2009). To validate the use of the modified ROI, the conventional ROI drawn over the entire myocardium was compared to the modified ROI in 13 HF patients. Both early and delayed H/M ratios and W/O rate were derived from each ROI. Bland-Altman analysis revealed excellent limits of agreements between the conventional ROI and the modified ROI suggesting that missing off the apex will not compromise the results. Hence, the use of the modified ROI throughout the study was considered to be valid.

The most common ROI used as a reference to calculate the ratio is the mediastinum (Verberne HJ et al., 2008b). Other investigators have used different reference organs other than the mediastinum, such as the liver, the lungs, or the left ventricular cavity (Carrio I, 2001; Somsen GA et al., 1995; Somsen GA et al., 1996a; Verberne HJ et al., 2009a; Somsen GA et al., 1996b). They have all shown that MIBG uptake is reduced while the washout is increased in patients with HF in proportional to their degree of HF irrespective of the aetiology.

Similar to previous studies (Kasama S et al., 2003b; Ogita H et al., 2001; Yamada T et al., 2003) there were strong intra- and inter-observer reproducibility of all MIBG measured parameters (George R et al., 2009).

**The effects of LVAD on MIBG uptake**

LVAD unloading has shown to have a therapeutic effect in patients with end-stage HF. Unloading has a significant impact at the cellular and molecular levels in both humans and experimental (Bruckner BA et al., 2001; Bruggink AH et al., 2006; Dipla
K et al., 1998b; Khan N et al., 2003b; Xydas S et al., 2006; Zafeiridis A et al., 1998b) and this becomes far more significant if combines with specific drug combination therapy to promote a sustainable reverse remodeling followed by physiologic hypertrophy (Birks EJ et al., 2001; Birks EJ et al., 2005; Birks EJ et al., 2006; Latif N et al., 2007; Terracciano CM et al., 2007; Terracciano CMN et al., 2003; Terracciano CMN et al., 2004).

The main findings of the present study are the positive effects of the HM II LVAD and drug combination therapy on $^{123}$I-MIBG uptake. We have shown that LVAD support alongside uptitration of the anti-failure medication has resulted in a significant increase in both early and delayed H/M ratios by 42.1% and 54.7%, respectively, and a significant reduction in the W/O rate by 42.4%. These significant improvements were much higher than those demonstrated by other groups. Table 5.6 summarises the changes in H/M MIBG activity following various therapeutic interventions that targeted either one or a combination of the following: β-receptors, angiotensin converting enzymes, aldosterone, and angiotensin II receptor. Although a meta-analysis was not performed the percentage increase in both early and delayed H/M ratios ranged from 4% to 10.5% and from 2.4% to 23.1%, respectively. The percentage reduction in the W/O rate ranged from 2% to 27.5%.

These findings suggest a significant improvement in sympathetic innervation following LVAD and drug combination therapy and the possibility for NET to be used as direct target for pharmacologic treatment of HF in the future (George RS et al., 2009).
<table>
<thead>
<tr>
<th>Author / Source</th>
<th>Therapeutic option</th>
<th>Number of patients</th>
<th>Study duration (months)</th>
<th>Percentage Change in MIBG parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Toyama T et al., 2003)</td>
<td>Carvedilol</td>
<td>15</td>
<td>12</td>
<td>NR</td>
</tr>
<tr>
<td>(Toyama T et al., 2003)</td>
<td>Metoprolol</td>
<td>15</td>
<td>12</td>
<td>NR</td>
</tr>
<tr>
<td>(Kasama S et al., 2007a)</td>
<td>Carvedilol</td>
<td>30</td>
<td>12 ± 1</td>
<td>NR</td>
</tr>
<tr>
<td>(de Milliano PAR et al., 2002)</td>
<td>Metoprolol</td>
<td>59</td>
<td>6</td>
<td>NR</td>
</tr>
<tr>
<td>(Lotze U et al., 2001)</td>
<td>β-blockers *</td>
<td>11</td>
<td>12</td>
<td>NR</td>
</tr>
<tr>
<td>(Gerson MC et al., 2002)</td>
<td>Carvedilol</td>
<td>10</td>
<td>7.2 ± 2.7</td>
<td>↑ 4</td>
</tr>
<tr>
<td>(Toyama T et al., 1999)</td>
<td>Metoprolol</td>
<td>12</td>
<td>12</td>
<td>NR</td>
</tr>
<tr>
<td>(Toyama T et al., 1999)</td>
<td>Enalapril</td>
<td>12</td>
<td>12</td>
<td>NR</td>
</tr>
<tr>
<td>(Kasama S et al., 2006)</td>
<td>Valsartan ‡</td>
<td>25</td>
<td>6</td>
<td>NR</td>
</tr>
<tr>
<td>(Kasama S et al., 2006)</td>
<td>Enalapril ‡</td>
<td>25</td>
<td>6</td>
<td>NR</td>
</tr>
<tr>
<td>(Kasama S et al., 2003a)</td>
<td>Valsartan</td>
<td>16</td>
<td>6</td>
<td>NR</td>
</tr>
<tr>
<td>(Shinohara H et al., 2002)</td>
<td>ARB **</td>
<td>34</td>
<td>6</td>
<td>↓ 0.9</td>
</tr>
<tr>
<td>(Kasama S et al., 2005a)</td>
<td>Candesartan</td>
<td>25</td>
<td>6</td>
<td>NR</td>
</tr>
<tr>
<td>(Soeki T et al., 1998)</td>
<td>Enalapril</td>
<td>10</td>
<td>3 – 15</td>
<td>↑ 10.5</td>
</tr>
</tbody>
</table>
Table 5.8: (continue)

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<tr>
<th>Author / Source</th>
<th>Therapeutic option</th>
<th>Number of patients</th>
<th>Study duration (months)</th>
<th>Percentage Change in MIBG parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Early H/M ratio</td>
</tr>
<tr>
<td>(Takeishi Y et al., 1997)</td>
<td>Enalapril</td>
<td>19</td>
<td>9.1 ± 3.0</td>
<td>†↑ 8.1</td>
</tr>
<tr>
<td>(Kasama S et al., 2005b)</td>
<td>Perindopril †</td>
<td>20</td>
<td>6</td>
<td>NR</td>
</tr>
<tr>
<td>(Kasama S et al., 2005b)</td>
<td>Enalapril †</td>
<td>20</td>
<td>6</td>
<td>NR</td>
</tr>
<tr>
<td>(Kasama S et al., 2002)</td>
<td>Spironolactone</td>
<td>15</td>
<td>6</td>
<td>NR</td>
</tr>
</tbody>
</table>

| PRESENT STUDY                 | LVAD & DRUG COMBINATION THERAPY | 14 | 242.57 ± 82.25 days | †↑ 42.1 | †↑ 54.7 | †↓ 42.4 |

Table 5.6: Percentage change in MIBG measured parameters following various therapeutic interventions. All percentage changes highlighted in bold represent a significant change as compared to the MIBG baseline measurements taken at baseline prior to commencing any specific treatment. Percentage changes highlighted in red represent a non-significant change compared to the MIBG baseline measurements taken at baseline prior to commencing any specific treatment.

**NR**, not reported.

* refers to a cocktail of different β-blockers used in the studied population (metoprolol, n=5; bisoprolol n=1; carvedilol, n=4)

‡ A comparison between enalapril and valsartan with valsartan having a better effect on MIBG uptake as compared to enalapril

** Patients received either losartan or candesartan

† Comparison between perindopril and enalapril. Perindopril had better effects on MIBG uptake as compared to enalapril.
MIBG, LVAD and contractile reserve

The improvements in MIBG uptake following LVAD and drug combination therapy correlated moderately but significantly (r=0.612, p=0.02) with CR.\(^8\) Previously Ohshima et al have shown that abnormal myocardial \(^{123}\)I-MIBG accumulation in DCM patients is related to an impaired myocardial CR (Ohshima S et al., 2005). Three years later the same group have used dobutamine stress testing \(^***\) to correlate MIBG uptake with CR (Kobayashi M et al., 2008). They concluded that patients who had greatly reduced \(^{123}\)I-MIBG uptake had a reduced adrenergic myocardial CR. As described in chapter 4, CR has improved significantly in the LVAD recovered patients implying improvement in the cardiac SNS function. Coupled with the \(^{123}\)I-MIBG scintigraphic findings, it becomes the first time that MIBG uptake and CR to be positively correlated in LVAD patients suggesting that LVAD unloading combined with pharmacological therapy aiming to reverse remodelling and induce physiological hypertrophy results in a significant improvement in sympathetic innervation. Improved MIBG uptake throughout the LVAD support period reflects an improvement in NET functional property which itself is defected in HF (Liang C, 2007).

Dividing the studied population into two subgroups, recovered (n=10) and non-recovered (n=4), revealed that the uptake has improved in both groups but the percentage changes in the delayed H/M ratio and the W/O rate were more significant in the recovered patients. Also the mean values for both early and delayed H/M ratios were above the cut-off point in the recovered patients and not so for the non-recovered group (table 5.4).

Although never highlighted in previous studies, reduction in the LV size following therapeutic intervention may result in concentrating the number of NET uptake sites in a smaller area / volume and hence the counts per pixel measured by drawing the ROI will be high. Therefore the improvements seen in \(^{123}\)I-MIBG

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\(^8\) Contractile reserve was determined as the percentage change in the EF following the 6MW exercise test whilst the HM II LVAD running at 6000 rpm.

\(^***\) Reasons for not using dobutamine stress echocardiography to determine CR were described in details in chapter 4.
uptake could have been a consequence of reduction in the LV size rather than a true improvement in the uptake mechanism itself.

Previously, Kassama et al have identified, in most of their studies, a correlation between changes in the end-diastolic volume and $^{123}$I-MIBG scintigraphic findings after the treatment period with spironolactone (Kasama S et al., 2003b; Kasama S et al., 2007b), angiotensin II antagonists (Kasama S et al., 2003a; Kasama S et al., 2005a; Kasama S et al., 2006), and ACE inhibitors (Kasama S et al., 2005b) such that the significant increase seen in delayed H/M ratio and the significant decrease seen in the W/O rate were significantly correlated to significant reductions in the end-diastolic volume and significant increase in the LV EF (see table 5.6 for the percentage rises in delayed H/M ratio and percentage reduction in the washout rate for each of these studies). Other groups have also identified a correlation between changes in LV EF and improvements in MIBG uptake following the treatment with β-blockers (Choi JY et al., 2001; Fukuoka S et al., 1997; Yamazaki J et al., 2001). In two of their studied, Toyama et al have found an association but not a correlation between changes in echocardiographic measurements and $^{123}$I-MIBG scintigraphic findings (Toyama T et al., 1999; Toyama T et al., 2003). None of those groups, however, had commented on the possibility that a reduction in LV size may result in increased concentration of the NET uptake sites.

In the present study similar to others (Agostini D et al., 2000; de Milliano PAR et al., 2002; Shinohara H et al., 2002; Soeki T et al., 1998) we did not find a correlation between the changes in the echocardiographic parameters throughout the study duration and the changes in $^{123}$I-MIBG scintigraphic findings (table 5.5). Furthermore, to ensure that the changes seen in the LV size do not exhibit a significant effect on $^{123}$I-MIBG uptake, we normalised the LV size by superimposing the ROI drawn in the first scan over the second scan without changing the mediastinal ROI. Theoretically this entails that a bigger myocardial ROI area will have a lesser number of counts per pixels and hence early and delayed H/M ratios would decrease. The analysis, however, revealed a non-significant change in the ratios. A possible explanation is that improvements seen in $^{123}$I-MIBG scintigraphic findings are genuine secondary to improvements in the
uptake mechanism itself irrespective and independent of the size of the LV following LVAD and drug combination therapy. It is worth noting that it was not possible to accurately measure the end-diastolic volume due to the presence of the LV cannula at the LV apex.

Interestingly, the improvements seen in the H/M MIBG activity were sustainable after a mean of 408 ± 165 days after device explantation. The four studied patients were maintained on anti-failure medication. We have previously reported that recovery after the LVAD and drug combination therapy protocol is sustainable with excellent quality of life (George RS et al., 2008b; Birks EJ et al., 2006; George RS et al., 2008a).

The pronounced improvement in the H/M MIBG activity whilst on LVAD support and the sustained normalisation following LVAD explanation raise the possibility for NET as a therapeutic target in the treatment of HF. Although, no human studies have directly targeted NET in the treatment of HF as yet, some animal studies have shown that NET might represent a novel therapeutic principle. In 2005, Münch et al have shown that local overexpression of the uptake-1 NET using recombinant adenoviruses in rabbits with pacing induce HF has resulted in increased NE uptake, reversal of SERCA and β-adrenergic receptors downregulation, improvement in LV dimensions and function, and enhanced CR (Münch G et al., 2005). Others have targeted NET indirectly by injecting nerve growth factor reduced in HF secondary to overexpression to NE (Kaye DM et al., 2000b; Qin F et al., 2002) in the left stellate ganglia of rats with HF produced by transverse aortic constriction, resulting in enhanced uptake of NE, repleted cardiac NE stores, and increased FS without affecting the number of cardiac sympathetic nerves (Kreußer MM et al., 2006).
Clinical implications of MIBG imaging in LVAD patients

Determining myocardial recovery in LVAD patients depends on rigorous and continuous assessment of the native LV function in the absence of LVAD support. As described in Chapter 2, the Harefield Bridge-to-Recovery Protocol utilises the following parameters as clinical guides for explanation (table 5.7).

Table 5.7: Explantation Criteria

<table>
<thead>
<tr>
<th>Parameter with the speed of HM II reduced to 6000 rpm for 15 minutes</th>
<th>Parameter value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end-diastolic diameter (mm)</td>
<td>&lt; 60</td>
</tr>
<tr>
<td>LV end-systolic diameter (mm)</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>&gt; 45</td>
</tr>
<tr>
<td>Maximal VO₂ (mls/kg/min)</td>
<td>&gt; 16</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mmHg)</td>
<td>&lt; 12</td>
</tr>
<tr>
<td>Cardiac Index (l/min/m²)</td>
<td>&gt; 2.8</td>
</tr>
</tbody>
</table>

Table 5.7: The explantation criteria as been previously described (Birks EJ et al., 2006).

Despite the above 6 criteria, the decision to explant remains a serious issue and the need for more explantation guidelines to assist clinicians is mandatory. The main purpose of MIBG imaging was to attempt to utilise it as an additional explantation tool. Unfortunately, LVAD and drug combination therapy has resulted in a significant increase in MIBG uptake in both recovered and non-recovered patients, although the percentage increase in the recovered patients was higher. The results, however, need to be interpreted cautiously due to the small number of the non-recovered patients. It can also be argued that MIBG imaging is not a useful tool to guide explantation and that this study turned out to be an observational study which demonstrated the direct positive effects of unloading and combination therapy on MIBG uptake and the indirect improvements of NET and the SNS.
Limitations

A major limitation of this study is the small number of included patients which means that concrete conclusions regarding the potential benefit of $^{123}$I-MIBG imaging in LVAD patients should be drawn cautiously. Another limitation was the unavailability of pre-implant MIBG scan such that all patients were on multiple inotropic supports which interfere with the uptake mechanism (Appendix A for the pre-implant medication and Appendix E for the drugs that interact with MIBG uptake).

However, our results are encouraging to suggest the need for a trial with a large number of patients to provide the opportunity to assess MIBG uptake at different set time points from the time of implantation such as on quarterly basis and prior to commencing clenbuterol. This would also allow the correlation of the changes seen in MIBG uptake with the uptitration of the medication in the LVAD patients. A fourth limitation is the lack of standardisation of imaging procedure such that the setting of the ROI is a major factor affecting MIBG H/M ratios. A final limitation was the inability to study regional MIBG washout rate as suggested by Yamazaki et al (Yamazaki J et al., 1997) due to the presence of the LVAD inflow cannula which exerted immense attenuation on polar images.

Conclusion

In summary, this study has shown that LVAD and drug combination therapy has directly improved H/M MIBG uptake in patients with end-stage HF. The degree of improvement was independent of the changes in the LV dimensions. Moreover, the improvement in MIBG uptake was correlated to the change in CR and hence improvements in CR are related to improvement in cardiac sympathetic nerves activity. Interestingly, the enhanced $^{123}$I-MIBG uptake was noted in both recovered and non-recovered subgroups but more significant in the former group. Furthermore, explanted patients had sustained normalisation of MIBG uptake. The use of a modified ROI to account for the inflow cannula is a validated technique with strong intra- and inter-observer reliability. Finally, these results suggest that NET could act as a potential therapeutic target for the treatment of patients with end-stage HF.
CHAPTER 6 –
Immunohistochemistry Analysis of Various Nerve Markers
6.1 Background

Sympathetic fibres leave the spinal cord at segments T1 to L2-3. Preganglionic sympathetic fibres consist of small myelinated fibres that come off the spinal roots as white rami communicantes and synapse in the paravertebral ganglia. Adrenergic fibres that innervate the heart originate in the left and right stellate ganglia. The left stellate innervates the right ventricle, whereas the right stellate innervates the anterior and lateral portions of the heart. Parasympathetic innervation originates from the medulla and follows through the right and left vagus nerves which then divide into superior and inferior cardiac nerves. Animal studies have previously confirmed heterogenicity in myocardial nerve distribution.

Activation of the SNS results in cardiac adrenergic stimulation with changes in the contractile and electrophysiologic status of the heart. In the heart, parasympathetic innervation controls the heart rate. In HF, both systems tend to fail with the SNS being over-activated (Esler M et al., 1997) and the parasympathetic nervous system being under-activated (Olshansky B et al., 2008).

By means of specific antigen/antibody reactions immunohistochemistry (IHC) combines anatomical, immunological and biochemical techniques for the identification of specific tissue components. IHC makes it possible to visualize the distribution and localization of specific cellular components within a cell or tissue.

The principle of IHC has existed since the 1930s, but it was not until 1942 that the first IHC study was reported. Methods utilizing enzyme conjugated antibodies were developed in the 1960’s (Nakane PK & Pierce GB Jr, 1966). After the antigen-antibody reaction, the enzyme label is reacted with a substrate to yield an intensely coloured product that can be analyzed with an ordinary light microscope.

The aims of the present study were firstly to examine the localisation and distribution of specific nerve markers in LV cores from patients with end-stage HF prior to device implantation. Secondly, to quantify these nerve markers and attempt to correlate the findings with myocardial recovery.
6.2 Materials

6.2.1 Patient population

Out of the 23 studied patients, LV core tissues were available from 16 patients only. Core tissue from patients 1, 2, 3, 9, 18, 21, and 22 were not collected at implantation. Out of the 16 patients, 11 had recovered (Rec group) and 5 did not recover (NRec group). A tissue from the posterior wall of a normal heart was used as control.

6.2.2 Tissue collection in LVAD patients

During implantation a circular coring knife was used to remove the apex as been previously described (Slater JP et al., 1999). Core tissue was cut longitudinally into eight sections each measuring approximately 0.25 x 0.25 x 1.0 cm, wrapped into foil, stored in eppendorfs, snap frozen, and stored at – 80°C until sectioning.

6.2.3 Sectioning, fixation and immunostaining

Frozen tissues were supported in optimum cutting tissue (OCT) medium (R A Lamb Ltd, Eastbourne, UK) to allow optimum orientation. Using cryostat at -20°C, 15µm thickness of frozen sections were collected onto poly-L-lysine coated glass slides (VWR Ltd., Lutterworth, Leics., UK) and allowed to dry for 30 minutes. Sections were then fixed in freshly prepared, 4% w/v paraformaldehyde in PBS (0.1M phosphate; 0.9% w/v saline; pH 7.3) for a further 30 minutes. For immunoperoxidase staining endogenous peroxidase was blocked by incubation in 0.3% w/v hydrogen peroxide in methanol. After rehydration with PBS, sections were incubated overnight at 4°C with primary antibodies (Table 6.1) diluted in PBS containing bovine serum albumin (BSA 0.05% w/v), 3% w/v normal serum (from donor of second antibodies) and sodium azide (0.1% w/v) preservative. On the following day sections were rinsed in PBS and biotinylated secondary antibodies to either rabbit or mouse IgG applied for 60 min at ambient temperature. After a further rinse in PBS, sections were incubated for 90 min with avidin-biotin peroxidase complex (ABC; Elite ABC Vector Labs, Peterborough, UK) prepared in advance according to manufacturer’s instructions.
Sites of primary antibody attachment were revealed using nickel-enhanced, diaminobenzidine (DAB) and nascent hydrogen peroxide (Shu et al. 1989).††† Positive immunoreactivity was revealed as a blue/black product at primary antibody attachment sites. Nuclei were counterstained with 0.1% w/v aqueous neutral red and sections allowed to dry before mounting with glass coverslips using a xylene based mountant (DPX; R A Lamb Ltd, Eastbourne, UK). Controls included omission of primary antibodies, or their replacement with pre-immune serum.

Table 6.1: Primary antibodies

<table>
<thead>
<tr>
<th>Antibodies to</th>
<th>Host</th>
<th>Source (Ref#)</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerve marker - Neurofilaments cocktail</td>
<td>Mouse</td>
<td>Dakocytomation (M0762)</td>
<td>1:500/10000</td>
</tr>
<tr>
<td>Sodium Channel (Na,1.7)</td>
<td>Rabbit</td>
<td>GlaxoSmithKline (K241)</td>
<td>1:200 - 300</td>
</tr>
<tr>
<td>Sodium channel (Na,1.8)</td>
<td>Rabbit</td>
<td>GlaxoSmithKline (K107)</td>
<td>1:200 - 400</td>
</tr>
<tr>
<td>Sodium channel (Na,1.9)</td>
<td>Rabbit</td>
<td>GlaxoSmithKline (K186)</td>
<td>1:200 - 300</td>
</tr>
<tr>
<td>Norepinephrine Transporter (NET)</td>
<td>Rabbit</td>
<td>Atlas Antibodies (HPA004057)</td>
<td>1:50 - 100</td>
</tr>
<tr>
<td>Substance P</td>
<td>Rabbit</td>
<td>Millipore (AB1566)</td>
<td>1:4000 - 8000</td>
</tr>
<tr>
<td>VIP</td>
<td>Rabbit</td>
<td>Millipore (AB1581)</td>
<td>1:5000 – 10,000</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>Mouse</td>
<td>Novocastra (Clone HR2)</td>
<td>1:750</td>
</tr>
<tr>
<td>Choline Acetyltransferase</td>
<td>Mouse</td>
<td>Novocastra (Clone 38B12)</td>
<td>1:250</td>
</tr>
</tbody>
</table>

Table 6.1: Primary antibodies for different nerve markers used for immunostaining each sample. All antibodies were used at two different concentrations except for neurofilaments, acetylcholinesterase, and choline acetyltransferase.

††† ABC complex was made by diluting 5 μl of Avidin and 5 μl of Biotinilated peroxidase in 1 ml of PBS-A and then left to set for at least 30 minutes.
6.2.4 Analysis

For image analysis, images were captured using an Olympus DP70 camera mounted to an Olympus BX50 microscope and analysed using analySIS (Olympus version 5.0) software. Positive immunostaining in images was measured by setting grey-level detection limits to highlight positive nerve fibre immunoreactivity. Five different fields per section were randomly chosen and scanned at the same magnification (x20 objective). Analysis was performed by two independent observers and immunoreactivity for nerve fibres was quantified by either the percentage area or the direct counting techniques:

**Percentage area technique.** This technique was used to calculate the amount of norepinephrine transporter in each section. After adjusting the threshold ‡‡‡, the red colour was separated to obtain black and white images. The amount of black immunostaining is calculated as a percentage of the total area. Figures 6.1 and 6.2 are examples of images pre- and post-red colour separation, respectively.

**Counting technique.** This technique was used to count the neurofilaments in each section.

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‡‡‡ Threshold adjustment ensures minimal background interference
Figure 6.1: A captured image pre-colour separation

Fig 6.1: Nerve fibres in a control sample prior to separation of the red colour.

Figure 6.2: A captured image post-colour separation

Fig 6.2: Nerve fibres after separation of the red colour.
6.2.5 Statistical Analysis

All data are presented as mean ± stdev. Observer two was blinded to the recovery status of the patients. Intra-observer and inter-observer reliability was assessed using intraclass correlation coefficient (ICC) analysis. Non-parametric Mann-Whitney U test was used for inter-group (recovered versus non-recovered) analysis. Linear regression analysis was performed to determine predictors of myocardial recovery where recovered patients were used as a dependent parameter and percentage abundance of norepinephrine transporter immunoreactive nerve fibers and counts of neurofilaments immunoreactive nerve fibers as independent parameters. A p-value < 0.05 was considered to be statistically significant.
6.3 Results

Immunoreactivity was not detected for substance P, vasoactive intestinal peptide, acetylcholinesterase, and choline acetyltransferase. Antibodies to norepinephrine transporter (NET) nerve fibers revealed abundance of NET-immunoreactive nerve fibers in the control (n=1) and the LV core samples (figure 6.3). Mild innervation in single fibres and fascicles was also detected throughout the LV core samples using the nerve marker neurofilaments (NF; figure 6.4). Immunoreactivity was much less abundant for sodium channels (Na\textsubscript{v}1.7, Na\textsubscript{v}1.8, and Na\textsubscript{v}1.9). Therefore, analyses focused on NET \textsuperscript{***} and neurofilaments.

Figure 6.3: NET immunostaining

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\textsuperscript{***} Image analysis for NET-immunoreactivity was performed on section immunostained with 1:100 NET antibody since the higher concentration (1:50) demonstrated excessive background immunostaining.
Figure 6.3 (cont.): NET immunostaining

**Fig 6.3:** NET immunostaining in control and LV core samples. Magnification x20 objective.
Figure 6.4: An example of neurofilament immunostaining

Fig 6.4: An example of neurofilament immunostaining in an LV core sample. Magnification x10 objective.
6.3.1 Inter-observer reliability

Figure 6.5 represents a scatter plot of the percentage of NET-immunoreactive nerve fibers per total area between observer 1 and observer 2. ICC between the two observers revealed a moderate but significant reliability of 45.8% with correlation of 0.464 (p=0.028).

Figure 6.5: Scatter plot for NET between observer 1 and observer 2

**Fig 6.5:** Scatter plot for NET percentage in total area (ICC between both observers was 45.8%).
Similarly, inter-observer reliability for the mean counts of neurofilament immunoreactive nerve fibers was moderate but significant; ICC was 42.7% and intraclass correlation was 0.424 (p=0.039; figure 6.6 for the scatter plot).

Figure 6.6: Scatter plot for neurofilament between observer 1 and observer 2

Fig 6.6: Scatter plot for neurofilament average counts (ICC between both observers was 42.7%).
6.3.2 Recovered versus Non-Recovered

Of the 16 studied patients who had their LV core studied, 11 had recovered (patients 4, 7, 8, 10, 11, 12, 13, 15, 19, 20, and 23) and 6 did not recover (patients 5, 6, 14, 16, and 17).

The percentage of NET-immunoreactive nerve fibers per area in the control sample was 1.91%.

A comparison between the recovered and the non-recovered subgroups revealed that the percentage of NET-immunoreactive nerve fibers per area was significantly higher in the 11 recovered patients in the LV core at the time of implantation (1.04 ± 0.30% versus 0.65 ± 0.43%, \( p=0.048 \), figure 6.7).

**Figure 6.7: NET concentration – Recovered versus Non-recovered**

![Graph showing NET concentration comparison](image)

Fig 6.7: NET concentration was significantly higher in the LV core samples of the recovered subgroup as compared to the non-recovered subgroup (\( p=0.048 \)).
There was no difference in the neurofilament immunoreactive counts in the LV cores between the two sub-groups (6.95 ± 3.02 counts versus 7.83 ± 2.75 counts, p=0.591; figure 6.8).

**Figure 6.8: Counts of Neurofilaments – Recovered versus Non-Recovered**

![Bar chart showing counts of neurofilaments for recovered (Rec) and non-recovered (NRec) groups.](image)

**Fig 6.8:** There was no significant difference in the neurofilament reactive counts in the LV core samples between the recovered and the non-recovered subgroups (p=0.591).
6.3.3 Correlation between LV core immunostaining and myocardial recovery

Figure 6.9A represents a moderate correlation between the percentage of NET-immunoreactive nerve fibers per total area in the pre-implanted LV core and myocardial recovery ($r=0.513$, $p=0.035$) suggesting that the abundance of NET prior to device implantation could be related to myocardial recovery. No correlation was identified between neurofilament immunoreactive counts and the recovered patients ($r=0.152$, $p=0.560$)

**Figure 6.9: Correlation between nerve markers and myocardial recovery**

![Graph A: Correlation between NET immunoreactive fibers and myocardial recovery](image1)

![Graph B: Correlation between neurofilament counts and myocardial recovery](image2)

**Fig 6.9:** There was a moderately significant correlation between NET-immunoreactive nerve fibers and myocardial recovery (A) and no correlation between neurofilament immunoreactive counts and myocardial recovery (B).
6.4 Discussion

The main findings of the present study include:

- NET-immunoreactive nerve fibers and non-specific neurofilament immunoreactive nerve fibers were abundant in the LV apex (core tissue) as compared to other nerve markers;
- Patients who had recovered on LVAD and drug combination therapy had significantly higher percentage of NET-immunoreactive nerve fibers per total area in the LV apical core excised at implantation as compared to non-recovered patients;
- The percentage of NET-immunoreactive nerve fibers correlated significantly with myocardial recovery.

IHC technique and nerve markers

In this study ABC immunoperoxidase method was important in IHC. Staining intensity is a function of the enzyme activity and improved sensitivity can be achieved by increasing the number of enzyme molecules bound at the immunoreactive sites. Hsu et al, has previously demonstrated that the multiple binding sites between the tetravalent avidin and biotinylated antibodies (bound to the antigen) are ideal for achieving this amplification (Hsu SM et al., 1981a;Hsu SM et al., 1981b). As illustrated in figure 6.1 the nerve fibres immunostained black and were clear to identify under light microscope.

Na\textsubscript{v}1.7, Na\textsubscript{v}1.8, and Na\textsubscript{v}1.9 are voltage-gated sodium channels that play critical role in the generation and conduction of action potentials in sensory fibers. To our knowledge, this is the first report attempting to identify voltage-gated sodium channels in the LV apex. However, immunoreactive fibres for these channels were too few in the LV apex of DCM patients precluding image analysis.

Immunostaining for neuropeptides such as Substance P and VIP is not optimal in post fixed tissues which might explain their absence in the studied samples. Hence, for the future, tissue would need to be immersion fixed once collected to
improve their preservation. In the present study, this was not practical at implantation time.

AChE and ChAT were not very abundant and very difficult to analyse. Animal studies have confirmed regional variation in the distribution of AChE and ChAT in the heart (Brown OM et al., 1985; H.Criss Hartzell, 1980; Slaviková J & Tušek S, 1982; Vlk J et al., 1961). For example atrial areas receive richer parasympathetic innervation than ventricular areas, with the right portions receiving more than the left. The nodal areas were the most abundantly innervated regions examined. In our studied samples none were detected in the LV apex in parallel with the animal studies.

**NET-immunoreactive nerve fibers**

The heart is densely innervated with sympathetic nerves. Previous studies using non-invasive approaches such as $^{123}$I-MIBG have identified spatial variation in NET-immunoreactive nerve fibers distribution in the myocardium (Somsen GA et al., 2004; Verberne HJ et al., 2008a). Others have identified heterogenic distribution on regional basis in the LV wall thickness (Dae MW et al., 1997). However, no group has commented on the presence of sympathetic nerve fibers in the LV apex. The present study is the first to report that NET is localised in the LV apex using IHC techniques.

Interestingly, NET-immunoreactive nerve fibers were quite abundant in the LV apex in all patients. Recovered patients, however, demonstrated a significantly greater percentage per area of fibers as compared to the non-recovered subgroup. Furthermore, there was a moderate but significant correlation between NET-immunoreactive nerve fiber concentration in the LV apex and myocardial recovery. Unfortunately, the biopsies taken during right heart catheterisation prior to explant were too small to detect NET-immunoreactive nerve markers. Further, biopsies taken from the LVAD insertion site (i.e. around cannula) were scarred as been previously described (Moon JCC et al., 2003).

In chapter 5, MIBG uptake at the apex could not be investigated as the LV cannula had replaced the core. In the present study a correlation between NET, as assessed using IHC techniques, and the first MIBG scans performed (early post
device implantation) was not attempted. This is due to the fact that there was an average of 35.21 ± 20.37 days difference between the removal of the LV core at implantation and the first MIBG scan to be performed.

With these observations it has to be cautiously concluded that NET concentration in the LV apex may act as an early predictor of myocardial recovery.

**Limitations**

The number of LV cores is very small to conclude whether the presence of NET in LV apex at implantation is a strong predictor of myocardial recovery or not and therefore the results should be interpreted with caution. Another limitation is the availability of only one control sample which precluded direct comparison between HF cores and control cores.

Also the lack of a paired transmural sample from the LV at the time of either explantation or transplantation has acted as a limiting factor to determine the direct effects of the LVAD and drug combination therapy on NET-immunoreactive fibers.

**Conclusions**

This study has demonstrated that NET-immunoreactive nerve fibers and non-specific neurofilament immunoreactive nerve fibers were abundant in the LV apex (core tissue) as compared to other nerve markers. At pre-implantation patients who had recovered on LVAD and drug combination therapy had higher percentage of NET-immunoreactive nerve fibers per total area in the LV apex as compared to non-recovered patients. This was significantly correlated to myocardial recovery and hence assessment of NET-immunoreactive nerve fibres percentage per area of samples from the LV apex acquired at implantation could act as predictor of recovery.
CHAPTER 7 – Catecholamine Levels
7.1 Background

HF patients have increased sympathetic efferent neuronal activity which initially supports but ultimately harms the failing heart (Davis D et al., 1988; Eisenhofer G et al., 1996; Esler M et al., 1997). Several investigations have shown that there are elevated plasma NE levels (Cohen JN et al., 1984; Eisenhofer G et al., 1996; James KB et al., 1995), reduced NE concentration in cardiac tissue (Eisenhofer G et al., 1996), increased spillover of NE into plasma (Eisenhofer G et al., 1996; Hasking G et al., 1986; Rundqvist B et al., 1997), and increased muscle sympathetic nerve firing in patient with HF (Narula J & Kunal S, 2003; Patel A & Iskandarian A, 2002). All these factors have been related to poor prognosis and have been reported to improve when patients have either recovered from symptoms of heart failure or had had transplant (James KB et al., 1995).

Catecholamines are synthesised from tyrosine as represented in figure 7.1.

**Figure 7.1:** The pathway of catecholamine synthesis.

![Figure 7.1](image-url)

**Fig 7.1:** Synthesis of dopamine, NE and epinephrine from tyrosine. Two major breakdown by-products, normetanephrine and metanephrine, reflect catecholamine turnover and could potentially be used as markers of catecholamine turnover in HF.
Determination of catecholamines, however, is challenging due to the low circulatory concentration. The best approach is to use mass spectrometry (MS) to analyse the circulatory amount. To achieve this liquid chromatography (LC) is used to generate the charged ions needed for detection by MS. LC transforms samples injected into sampling orifice into a beam of fine aerosol droplets. The droplets are charged using a fine capillary which holds 3-5 kV of electrical energy. Once the solvents evaporate from the droplets, the charges remain on the analytes for detection using MS (figure 7.2 and 7.3).

**Figure 7.2: Generation of ions from injected samples.**

![Figure 7.2](image1.png)

**Fig 7.2:** Samples are injected into sample orifice and guided using two separate quadropoles towards the MS detector.

**Figure 7.3: The charging process of the analytes**

![Figure 7.3](image2.png)

**Fig 7.3:** Once samples are charged, evaporation results with charged analytes ready for MS detection. The quadropoles shown in figure 7.2 are used to direct specific weighed charges (the chosen weight is predetermined by the analyser) towards the MS analyser.
The high speed collision cell results in breaking down the large charged analytes to smaller substrates with different molecular weights. For example, NE has a molecular weight of 170 Dalton (Da) which breaks down to two compounds of molecular weights 152 Da and 107 Da. Epinephrine (EPI) has a molecular weight of 184 Da and during ionisation, it becomes very unstable and is converted to a molecule with 166 Da, EPI 166, which gives off two by-products with molecular weights of 77 Da and 107 Da. Figure 7.4 illustrates the chromatograms of seven different catecholamines and their breakdown products in our laboratory.

Figure 7.4: The chromatogram of human catecholamines measured in serum

Fig 7.4: Both epinephrine and normetanephrine have similar molecular weights and produce by-products with the same molecular weights. Metanephrine and dopamine have molecular weights of 198 Da and 154 Da, respectively, and each produce one ionised fragment. Norepinephrine has a molecular weight of 170 Da which produces two by-products with molecular weights of 107 Da and 152 Da. 3,4-dihydroxybenzylamine is used as an internal standard.

The aims of this study is to investigate the effects of LVAD and drug combination therapy on different catecholamine levels and to attempt to correlate the changes in their level with myocardial recovery, contractile reserve, and MIBG uptake.
7.2 Methods

7.2.1 Patient population

Blood sampling was performed serially in 14 out of the 23 patients (this is the same sub-population who had had the MIBG study, discussed in chapter 5) at two time points on the same day of MBIG nuclear imaging:

iii) early post-device implantation i.e. once patients were stabilised, their inotropic support been discontinued for at least 5 days, and their mobility has been restored; and

iv) immediately prior to either device explantation for myocardial recovery or transplant listing for failure to recover.

Reasons for excluding 9 patients have been already discussed in chapter 5 (table 5.1). Involved patients were asked to fast and refrain from drinking caffeine products for at least 24 hours before blood sampling.

7.2.2 Sample collection and preparation

An 18F sized cannula was inserted in the anti-cubital fossa at least 75 minutes before sample collection. The cannula was primed with 0.8 mls of heparin (5000 units/ml). 36 mls of blood (24 mls serum and 12 mls plasma) were collected from the pre-cited cannula from each patient at each time point after at least 45 minutes of supine rest in a quiet room. The samples were immediately transferred to a fridge for storage at 4\textdegree{}C for a maximum of four hours before being centrifuged. Each sample was spun at 2000 rpm for 10 minutes after which the supernatant was removed and the cell pellet discarded. Both plasma and serum samples were then stored at –80\textdegree{}C until formal analysis could be performed as a batch process.
7.2.3 Assessment of catecholamines using liquid chromatography mass spectrometry (LCMS)

Analysis was performed using liquid chromatography mass spectrometry (described above). After thawing the frozen serum samples, 0.5 ml was spiked with 2µl of 0.1 mM 3,4-dihydroxybenzylamine as an internal standard. Each sample was then added to 10 mg of activated alumina in an Eppendorff tube for adsorption. Sample was vortexed for 5 min. Tubes were then centrifuged at 13000 rpm and supernatant discarded. Pellet was then washed twice with water and 0.02 ml of 0.5 M acetic acid was used for elution (extraction).

The eluate was then diluted three times before injecting at a flow rate of 0.2 ml/min into a 1.8 µm diameter Agilent Eclipse Plus analytical column. Through the column each sample was allowed to pass a gradient from 0% to 70%.

The total run time with re-equilibration was 12 minutes and the catecholamines quantified included: NE, EPI 166, Dopamine (DA), Normetanephrine (NorMET), and Metanephrine (MET). The peak of each chromatogram is multiplied by the molecular weight and the dilution factor to yield the final concentration in nmol/l.

7.2.4 Data Collection and Statistical Analysis

All data are presented as mean ± stdev and were collected prospectively. Data analysis was done using SPSS, version 16.0 for Windows, created by Lead Technologies. For serial analysis of catecholamine concentrations between the two time points, paired t-test and Wilcoxon Signed Ranks test were used for parametric and non-parametric measurements, respectively. Linear regression analysis was performed using the catecholamine measurements in the LVAD patients as independent parameters and myocardial recovery, CR, and MIBG uptake values as dependent parameters. p < 0.05 was considered statistically significant.
7.3 Results

7.3.1 Effects of LVAD and drug combination therapy on catecholamine concentrations

The first blood sample was taken early post device implantation at 35.21 ± 20.37 days. The second sample was performed at 242.57 ± 82.25 days post implantation resulting in time difference between the two time points of 208.43 ± 85.50 days.

Demographics and pre-implantation data for the 14 studied LVAD patients have been previously represented in table 5.2. Also a list of medication taken at each blood sampling was presented in table 5.3.

Figure 7.5 represents the effects of LVAD and drug combination therapy on serial catecholamine levels. There was a significant reduction in NE by 47.6% (3.14 ± 1.17 nmol/l versus 1.65 ± 0.46 nmol/l, p<0.001), EPI 166 by 32.0% (0.37 ± 0.15 nmol/l versus 0.25 ± 0.11 nmol/l, p=0.028), DA by 27.6% (0.27 ± 0.12 nmol/l versus 0.19 ± 0.07 nmol/l, p=0.050). The reduction in both NorMET and MET were not significant (NorMET: 0.41 ± 0.25 nmol/l versus 0.36 ± 0.16 nmol/l, p=0.517; MET: 0.99 ± 0.46 nmol/l versus 0.92 ± 0.42 nmol/l, p=0.705).
Fig 7.5: There was a significant reduction in NE, EPI 166 and DA after a mean of 208 days of LVAD support.
### 7.3.2 Catecholamines and myocardial recovery

Out of the 14 HM II LVAD studied patients, 10 had recovered and were explanted whilst four patients were transplant listed. Comparing the effects of LVAD and drug combination therapy on the trend of catecholamine levels in the studied subgroup showed that the recovered patients had a significant reduction in NE (3.46 ± 1.15 nmol/l versus 1.56 ± 0.48 nmol/l, p<0.001) and EPI 166 (0.41 ± 0.16 nmol/l versus 0.22 ± 0.11 nmol/l, p=0.007, figure 7.6) and no significant change in DA, NorMET and MET levels.

**Figure 7.6: Effects of LVAD and drug combination therapy on catecholamine levels in the recovered patients (n=10)**

![Graph showing the effects of LVAD and drug combination therapy on catecholamine levels](image)

**Fig 7.6:** Both NE and EPI 166 decreased significantly in the recovered patients. There were no significant differences in DA, NorMET, and MET levels.
The non-recovered patients showed a reduction in NE, DA, NorMET and MET and an increase in EPI 166 between the two time points. However, none of the changes reached statistical significance (figure 7.7).

**Figure 7.7: Effects of LVAD and drug combination therapy on catecholamine levels in the non-recovered patients (n=4)**

**Fig 7.7:** There was no significant difference in catecholamine levels in the non-recovered patients. These data, however, need to be cautiously interpreted due to the low sample number.
Further, the percentage reduction in NE and EPI 166 levels were significantly higher in the recovered patients as compared to the non-recovered patients. NE decreased by 51.7% in the recovered patients as compared to 14.2% in the non-recovered patients. EPI 166 decreased by 39.5% in the recovered patients as compared to a rise by 26.2% in the non-recovered patients. The percentage reduction in DA was higher in the non-recovered patients but did not reach statistical significance (figure 7.8).

**Figure 7.8: Percentage change in catecholamine levels**

![chart](image_url)

**Fig 7.8:** There was a significant reduction in NE and EPI 166 in the recovered patients as compared to the non-recovered patients.
Linear regression analysis has revealed a strong correlation between percentage changes in NE and EPI 166 and myocardial recovery such that a higher percentage decrease in either was significantly associated with recovery (figures 7.9 A-E). No correlation between myocardial recovery and changes in DA, NorMET and MET has been identified.

Figure 7.9: Correlation between percentage changes in catecholamines and myocardial recovery

A)  

B)
Fig 7.9: A-E correlation analysis between percentage change in catecholamine levels and myocardial recovery. There was a significant correlation between percentage reduction in NE and EPI and myocardial recovery.
7.3.3 Catecholamines and CR

As represented in chapter 4, the HM II and drug combination therapy had resulted in a significant change in CR such that a 6MW exercise test had induced an absolute increase in the EF by $5.25 \pm 5.30\%$ in all 23 studied patients. In the 14 sub-population the absolute increase in the EF following the 6MW exercise test was $5.89 \pm 6.07\%$ with 11 patients (10 recovered and 1 non-recovered) had had an absolute increase and 3 non-recovered patients had an absolute reduction.

Linear regression analysis revealed a significant negative correlation between CR and the percentage change in both NE and EPI 166 such that as the circulating levels of these catecholamines had decreased the CR increased. Figure 7.10 illustrates the correlation between CR and the percentage change in NE and EPI 166 levels between the two time points. There was no correlation between the other three catecholamines measured and CR.

**Figure 7.10: Relationship between percentage change in NE and CR**

![Relationship between percentage change in NE and CR](image)

Fig 7.10: A) Relationship between early percentage change in NE and CR. B) Relationship between percentage change in EPI 166 and CR.
7.3.4 Correlation between catecholamine levels and $^{123}$I-MIBG uptake parameters

There was a moderate positive correlation between NE and $^{123}$I-MIBG measured early H/M and delayed H/M ratios (see chapter 5) in the 14 studied patients (figures 7.11A and 7.11B). As the NE levels decreased, both ratios have increased (early H/M: $r=0.522$, $p=0.004$; delayed H/M: $r=0.497$, $p=0.007$). There was also a weak but a significant negative correlation between W/O rate and NE levels ($r=0.404$, $p=0.033$) (figure 7.11C).

No correlation was identified between other catecholamines and MIBG uptake parameters.

Figure 7.11: Relationship between NE concentrations and $^{123}$I-MIBG uptake parameters

![Graph showing the relationship between NE concentration and early H/M ratio]
Fig 7.11: There was a negative correlation between NE concentration and EHM and DHM ratios (figures A and B, respectively) and a positive correlation with W/O rate (figure C).
7.4 Discussion

The main findings of the present study include:

- LVAD and drug combination therapy resulted in a significant reduction in NE, EPI 166, and DA,
- The percentage change in NE and EPI 166 concentrations were more significant in the recovered group as compared to the non-recovered patients and correlated significantly with myocardial recovery,
- The percentage changes in NE and EPI 166 concentrations exhibited a significant negative correlation with contractile reserve,
- There was a significant correlation between NE concentrations and $^{123}$I-MIBG uptake measured parameters.

Role of catecholamines in HF

To overcome the deficit in cardiac output in HF the body compensates by an enhanced activation of the SNS (Rundqvist B et al., 1997). The adaptations in the neurohormonal pathway play an important role in determining the signs and symptoms of HF such that elevated levels of NE and epinephrine contribute to:

i) the maintenance of perfusion of vital organs,
ii) preservation of the systemic pressure by increasing the vascular resistance, and
iii) the restoration of cardiac output by increasing myocardial contractility of the heart and by expansion of the extracellular volume.

These compensatory mechanisms however, lead to a further deterioration in cardiac performance which includes the development of pulmonary congestion and peripheral oedema secondary to elevation in diastolic pressure, depression in cardiac function due to an increase in LV afterload which is induced by the rise in peripheral resistance, and coronary ischaemia which is induced by increased heart rate (Novo G et al., 2009). Furthermore, the rise of NE has been shown to correlate with the severity of cardiac dysfunction and inversely with survival (Cohen JN et al., 1984;Anand IS et al., 2003) and improve when patients have
either recovered from symptoms of HF using different therapeutic interventions (Anand IS et al., 2003; Liang C, 2003) or had had transplant (James KB et al., 1995).

**Determining catecholamine concentrations – technical aspects**

The effects of LV unloading on catecholamine levels in patients who have recovered using LVAD without the need of transplantation, however, have not been investigated to date.

Determination of catecholamines, however, is challenging due to the low circulatory concentration. In 1995, James et al have used radioenzyme assay technique to detect NE levels in plasma (James KB et al., 1995). Their approach and others such as ELISA have shown to be very time consuming, involve complicated multiple steps, and sometimes non-reproducible (Mishra A et al., 2009). LCMS has been previously validated as a technique with high sensitivity and specificity in determining NE levels in either plasma or serum (Mishra A et al., 2009).

Although NE was the main molecule of interest, we have also studied the effects of LVAD and drug combination therapy on four other catecholamines that are produced throughout the catecholamine synthesis pathway. To ensure that the collected blood samples were not contaminated from other endogenous sources of NE (such as from the adrenal gland), all patients were laid supine in a quiet environment for at least 45 minutes with a pre-cited cannula. Once samples were collected, they were processed rapidly to avoid biodegradation of the catecholamines.

Interestingly, epinephrine was very unstable once ionised and yielded EPI 166, a smaller molecule with a molecular weight of 166 Da. The latter was more stable for analysis. In the present study we have used the Agilent Quadropole Analyser to measure the concentrations of NE, EPI 166, DA, NorMET, and MET levels in LVAD patients and hence to determine the impact of LVAD and drug combination therapy on these catecholamine levels.
LVAD and catecholamine levels

All 14 studied patients exhibited a reduction in catecholamine levels following LVAD and drug combination therapy. Interestingly, the recovered patients exhibited the most significant reductions in NE and EPI 166 as compared to the non-recovered patients who did not show any significant change following LVAD support. Furthermore, the percentage decrease in both NE and EPI 166 was significantly higher in the recovered patients as compared to the non-recovered patients and correlated significantly to myocardial recovery.

To link the relationship between CR and catecholamine / neurohormonal pathway, significant negative correlations between NE and EPI 166 levels and CR were identified suggesting that high levels of circulating neurotransmitters NE and EPI result into poor CR and thus poor prognosis. In conjunction with the correlation between $^{123}$I-MIBG uptake and CR (illustrated in chapter 5), this correlation analysis between catecholamine levels and CR is an extra supporting evidence that CR is an important clinical marker / parameter in determining the degree of myocardial recovery.

Limitations

A major limitation of this study is the small number of included patients which means that the results, especially in the non-recovered patients, should be drawn cautiously. Another limitation was the unavailability of pre-implant catecholamine concentrations such that all patients were on multiple inotropic supports which would interfere with the analysis (Appendix A for the pre-implant medication).

The reduction in catecholamine levels using LVAD and drug combination therapy, however, is encouraging to suggest the need for a trial with a large number of patients and to obtain serial measurements at different preset time points from the time of implantation such as on biweekly basis and prior to commencing clenbuterol. This would also allow the correlation of the changes seen in catecholamine levels with the uptitration of the medication in the LVAD patients.
Conclusion

In summary, this study has shown that LVAD and drug combination therapy has directly improved NE and EPI levels in patients with end-stage HF. The percentage improvement was more significant in the recovered patients and correlated significantly with recovery, CR and MIBG uptake parameters.
CHAPTER 8 – General Discussion and Future Directions
HF is a complex syndrome that is associated with enhanced neurohormonal activation. The pathophysiology of HF is initiated by over-stimulation of the cardiac SNS. The synaptic NE abundance in HF is secondary to excessive release from the afferent neurons and failure of NET uptake mechanism at the presynaptic membrane. Activation of the inflammatory axis and different pathways of the ECM will result into the pathological and remodeling features seen in HF. Clinically cardiac sympathetic dysfunction has been correlated to impaired myocardial contractile reserve, impaired contractility, and reduced exercise tolerance.

LV unloading using LVAD has been shown to induce significant improvement in many pathophysiologic pathways involved in HF. Clinically these improvement were translated into significant advancements in haemodynamic and echocardiographic parameters, exercise tolerance, and QOL. The use of the Harefield BTR protocol in patients supported with pulsatile flow devices has resulted in an enhanced degree of myocardial recovery and made recovery more sustainable.

For the first time, this thesis reports the effects of continuous flow LVADs combined with aggressive pharmacologic regimen in the induction of myocardial recovery. This protocol has resulted in myocardial recovery in 65% of patients with end-stage non-ischaemic HF, as evidenced by significant improvements in echocardiographic, haemodynamic, and exercise tolerance parameters.

Despite the efficiency of the continuous flow device in providing equivalent degree of haemodynamic support, LV unloading, end-organ perfusion, and exercise capacity to pulsatile LVADs, it is believed that continuous monitoring of myocardial recovery, the hallmark in the recovery protocol, would be impossible since continuous flow device provide less pulsatility and generate negative pressure in the inflow cannula as a result of the suctioning effects of the rotary pump. Therefore, device cessation (normally performed with pulsatile devices to assess the native LV function) would result in severe retrograde filling of the LV masking the real function of the native ventricle. In chapter 3, time modelling analysis, bench work experiment and clinical studies have determined that speed reduction unmask the underlying LV function allowing “near” accurate
assessment of the native LV. A speed of 6000 rpm was found to be safe, tolerable by all patients with no ill-side effects, sufficient to assess the native LV function and would not result in massive retrograde filling that would hinder LV assessment.

To assess the effects of LVAD on direct LV unloading and tolerance to physiological “day-to-day activity” exercise, CR following six-minute walk was chosen as an objective quantification to assess the effects of LVAD on LV contractility. The inhibition of CR associated with exercise intolerance has been shown to represent important clinical dilemmas in the pathophysiology of HF where both are induced by excessive stimulation of cardiac SNS. In our studied population, those who had recovered had better exercise tolerance and CR as illustrated by significant improvement in their echocardiographic and haemodynamic parameters when compared to the non-recovered patients. Improvements in both CR and exercise tolerance in the recovered patients were consistent throughout the duration of the LVAD support. Interestingly, those who were supported with the continuous flow HM II LVAD had a better response to exercise than those supported with the pulsatile HM I LVAD.

In this thesis, $^{123}$I-MIBG nuclear imaging was used as a direct measure of the importance of NET in the pathophysiology of HF and recovery. For the first time, the use of $^{123}$I-MIBG imaging has been validated in LVAD patients. The presence of the inflow cannula in the LV apex posed major challenges in the technical aspect of imaging. This has led to the development of a specific region of interest that accounts for the inflow cannula without compromising the final outcome of the results. Furthermore, there was high intra- and inter-observer reproducibility in the studied population.

LVAD and drug combination therapy has resulted in a significant improvement in MIBG uptake parameters independent of the LV size suggesting that improvements in uptake measurements are merely due to positive changes in NET function rather than reduction in LV size secondary to unloading.

The main purpose of MIBG imaging, however, was to try to utilise it as an additional explantation tool. Alas, LVAD and drug combination therapy has
resulted in a significant increase in MIBG uptake in both recovered and non-recovered patients. Although, the results need to be interpreted cautiously due to the small number of the non-recovered patients, it can still be argued that MIBG imaging is not a useful tool to guide explantation and that this component of the thesis turned out to be an observational study which demonstrated the direct positive effects of unloading and combination therapy on MIBG uptake and the indirect improvements of NET and the SNS. The enhancements in MIBG uptake parameters, however, were much higher in the recovered than the non-recovered patients. In addition, MIBG nuclear imaging was a useful tool to demonstrate that LV unloading using LVAD and drug combination therapy has resulted in much higher uptake levels as compared to patients who were treated with different oral medications. In addition the improvements in MIBG uptake parameters were sustained in explanted patients.

In chapter 6 immunohistochemistry analysis was utilised as an approach to study the correlation between NET and myocardial recovery. Again this is the first time to correlate the concentration of NET-immunoreactive nerve fibers at the time of implantation to myocardial recovery suggesting that analysis of NET from the LV core could “pre-determine” which patients shall recover and will have their device explanted. Again another novel finding of this thesis was the abundance of NET in the LV apex. Based on search in the English literature, this was the first time to demonstrate that NET-immunoreactive nerve fibers could be visualised in the LV apex.

LVAD and drug combination therapy have also been shown to reduce catecholamine levels with the percentage reduction being more significant in the recovered patients. Similar to MIBG uptake, NE and epinephrine levels have correlated significantly with recovery.

To illustrate the importance of CR as an important explanation tool / guide, a correlation analysis revealed moderate correlations between MIBG uptake and CR and between the percentage change in catecholamine levels and CR suggesting an important association between CR and SNS in HF and recovery.
Future Directions

The ability to produce a rate of 65% of recovery in patients with end-stage non-ischaemic heart failure using continuous flow LVAD and drug combination therapy is very encouraging to conduct a randomised multi-centre trial. It is also encouraging to reproduce the results using third generation devices such as HeartWare. Furthermore, it is of paramount importance to attempt recovering patients with ischaemic origin once the extent of viability has been assessed.

It is of paramount importance to define more explanation tools / guidelines. One approach is to use PET imaging to determine the viability of different LV regions during LVAD support and to device a score to add to the current explantation guidelines.

Results from the MIBG study are encouraging to suggest the need for a trial with a large number of patients to provide the opportunity to assess MIBG uptake at different set time points from the time of implantation such as on quarterly basis and prior to commencing clenbuterol. This would also allow the correlation of the changes seen in MIBG uptake with the uptitration of the medication in the LVAD patients.

Although LV unloading combined with anti-failure medication was sufficient to induce sustained myocardial recovery, gene therapy may still have a major role. For example, overexpression of NET using targeted gene therapy should be looked at. This could improve SNS function and ameliorate the negative effects of dysfunctional NET. Studying the heterogeneity of NET distribution using immunohistochemistry, however, will be a preceding step to determine the target areas where NET carrying vectors could be delivered and how.
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Acknowledgments

In the first instinct, I would like to praise God for giving the strength to complete this exciting endeavour. I would like to thank our patients and their families whose courage in desperate circumstances and ongoing fortitude has been an inspiration to see and has helped me grow as a human being.

I am indebted to my principal supervisor, Professor Emma Birks (Transplant and Artificial Heart Consultant) for her unrelenting commitment, advice and guidance throughout this research and my career. Without her implacable support I would have not been able to acquire my specialist training number in cardiothoracic surgery. I am also very grateful for her helpful recommendations and suggestions on the text. It has also been an honour to work for Professor Sir Magdi Yacoub who has been an unending source of inspiration during the development of the thesis. Professor Yacoub has supervised my progress with the greatest interest and has offered valuable suggestions on numerous occasions. Without his principle concept about myocardial recovery using LVAD and drug combination therapy, this thesis would not have not been possible. I would also like to thank my third supervisor, Dr Andrew Kelion, Consultant Nuclear Cardiologist, for making his support available on number of ways. Dr. Kelion has continuously helped, challenged and motivated the MIBG study.

I am also thankful to Dr Christopher Bowles, senior VAD coordinator for his much thoughtful scientific input. Carole Webb deserves a special mention for her dedication to our patients and implacable commitment. I also appreciate the expertise, help, and goodwill of all VAD team members at Harefield throughout this project in particular Kate Absalom, Robert Dean, Rachel Hards, Michael Hedger, Mandy Hipkin, Louise Mitchinson, and Christine Saunders.

I would also like to thank all members of the Nuclear Cardiology Department at Harefield, in particular Andrew Cheetham who has conducted most of the MIBG nuclear scans and was the second observer for the inter-observer reliability study. Rommel Manlapig, Fabrice Ghiotto, Suzie Hinton-Taylor, and Gabriella Bolgona deserve my deepest gratitude for facilitating the imaging process at Harefield.
throughout the study. For financial support, I thank Thoratec Corporation for their research grant that had supported my salary and I also thank GE Healthcare for providing $^{123}$I-MIBG free of charge.

I am most grateful to Dr Nikant Sabharwal, Consultant Cardiology at Radcliffe Hospital, for measuring the forward flows across the HM II LVAD echocardiographically. Dr Derek Robinson, statistician at Sussex University, has assisted in developing the time-modelling analysis. Dr Tom Smolenski (Head of Metabolic Research) has kindly analysed catecholamine levels using the cut-edge technology the Quadropole Agilent Liquid Chromatography Mass Spectrometry. Another appreciation goes to Professor Praveen Anand, Head of Neuropathy Unit at Hammersmith Hospital, for allowing me to conduct all immunohistochemistry staining in his laboratory. A special thanks goes to Paul Facer, his senior laboratory technician, for teaching me the principles of immunostaining and his unstinting technical assistance. I am also grateful to Professor Kim Parker who has given me the opportunity to perform the low speed experiments in his Bioengineering laboratory at Imperial College. I would like to thank Mr. Niraj Hirani, a MEng graduate for assisting me throughout the low flow experiments and teaching me how to use MatLab to analyse the flow and pressure readings.

I am most grateful to my brother for proof reading the thesis. Most of all, I am most grateful to my wife, Sherine, and my two daughters for their endless comfort and for their support with great kindness and understanding. Finally, among the many people to whom I owe my deepest gratitude, respect and affection, my parents deserve the most special place. Their love, dedication, prayers, as well as their continuous support, both morale and financial, were a foundation for my work. I would like to dedicate this work to my family.
Personel Contribution

As the thesis involves multiple disciplinaries and departments, I would like to highlight my personel contribution:

- Involved in LVAD implantation and core tissue collection.
- Post-operative and out-patient management of LVAD patients.
- Set up the mock circuit in chapter 3 and performed all the benchwork experiments with analysis of the data points acquired and the videos captured. Wrote the computer script shown in Appendix D.
- Organised patient’s monthly follow-up and managed them during low-speed testing (chapter 4) including testing below 6000 rpm to identify optimal low-speed for native LV assessment (chapter 3 – part 3).
- Obtained ethics approval from Royal Brompton and Harefield NHS Foundation Trust and funding from GE Healthcare for the MIBG nuclear imaging on LVAD and heart failure patients. Set up the MIBG imaging protocol with full validation in Harefield. Acquired and analysed all serial images from the 14 collaborating patients. Evolved the modified ROI to account for the inflow cannula in the apex.
- Performed the immunohistochemistry experiments on the LV core tissue collected during implantation including slide analysis on the analySIS software.
- Collected and processed blood samples for liquid chromatography mass spectroscopy and analysed the results.
- Apart from the time-modelling, I have performed all the statistical analysis within this thesis.
Appendices
Publications and Presentations

Peer-Reviewed Papers and Case Studies

1) Emma J. Birks, Robert S. George, Mike Hedger, Toufan Bahrami, Christopher Bowles, Carole Webb, Mohamed Amrani, Magdi H. Yacoub, Giles Dreyfus, Asghar Khaghani
Reversal of severe heart failure using a continuous flow left ventricular assist device and pharmacologic therapy: A prospective study
Accepted by Circulation – In press

2) Robert S. George, Magdi H. Yacoub, Carole Webb, Christopher T. Bowles, Asghar Khaghani, Emma J. Birks
Continuous flow LVADs provide better response to six-minute walk exercise as compared to pulsatile flow LVADs
In publication

3) Robert S. George, Emma J. Birks, Andrew Cheetham, Rommel, Manlapig, Suzie Hinton-Taylor, Fabrice Ghiotto, Magdi H. Yacoub, Andrew Kelion
The effect of left ventricular assist device support on myocardial sympathetic activity in patients with dilated cardiomyopathy
In publication

4) Emma J. Birks and Robert S. George
Molecular changes occurring during reverse remodelling following left ventricular assist device support
Journal of Cardiovascular Translational Research 2010;3(6):635-42

5) Robert S. George, Nikant K. Sabharwal, Carole Webb, Magdi H. Yacoub, Christopher Bowles, Mike Hedger, Giles Dreyfus, Asghar Khaghani, Emma J. Birks
Echocardiographic evaluation of flow across HeartMate II axial flow LVADs at varying low speeds
Journal of Heart and Lung Transplantation 2010;29(11):1245-1252

*equal contribution
The impact of acute reduction of continuous-flow left ventricular assist device support on cardiac and exercise performance
Heart 2010;96(17):1390-1395

7) Djordje G Jakovljevic, Robert S George, Gay Donovan, David Nunan, Keiran Henderson, Robert S Bougards, Magdi H Yacoub, Emma J Birks, David A Brodie
Comparison of cardiac power output and exercise performance in patients with left ventricular assist devices, explanted (recovered) patients and those with moderate to severe heart failure
American Journal of Cardiology 2010;105(12):1780-85
8) Robert S. George, Claire Khaghami, Christopher T. Bowles, Asghar Khaghami, Emma J. Birks  
Sustained Myocardial Recovery Five Years following in-situ Disconnection of a Jarvik 2000 Device  
*Journal of Heart and Lung Transplantation* 2010;29(5):587-588

9) Leanne E. Felkin, Enrique Lara-Pezzi, Robert George, Magdi H. Yacoub, Emma J. Birks, Paul J. R. Barton  
Expression of extracellular matrix genes during myocardial recovery from heart failure following left ventricular assist Device (LVAD) Support  
*Journal of Heart and Lung Transplantation* 2009;28(2):117-122

Quality of Life Following LVAD Removal for Myocardial Recovery  
*Journal of Heart and Lung Transplantation* 2008;27(2):165-72

Haemodynamic and Echocardiographic Responses to Acute Interruption of Left Ventricular Assist Device Support: Relevance to Assessment of Myocardial Recovery.  
*Journal of Heart and Lung Transplantation* 2007;26(10):967-73

12) E. Birks, P. Tansley, J. Hardy, R. S. George, C. Bowles, M. Burke, N. Banner, A. Khaghami, M. Yacoub.  
Left Ventricular Assist Device and Drug Therapy for the Reversal of Heart Failure.  

**Book Chapter**

**Title:** Advances in Tissue Engineering  
Robert S George and Emma J Birks  
Book Chapter: Myocardial Recovery Following LVAD Support  
Editor: Julia Polak

**Abstracts**

1) **R. S. George**, M. H. Yacoub, A. Cheetham, A. Kelion, E. J. Birks  
LVAD Support Results in a Significant Improvement in the Myocardial Sympathetic Nervous System, as Assessed by $^{123}$I-Metaiodobenzylguanidine ($^{123}$I-MIBG), Independent of Ventricular Function  
*Journal of Heart and Lung Transplantation* 2010;29(2 Suppl 1): S180

2) **R. S. George**, M. H. Yacoub, H. Lister, C. Webb, A. Khaghami, E. J. Birks  
The Effects of Clenbuterol on the Left Ventricular Wall Thickness and Function in Patients Recovered on Left Ventricular Assist Device (LVAD)  
*Journal of Heart and Lung Transplantation* 2010;29(2 Suppl 1): S182
3) E. J. Birks, R. S. George, M. Hedger, A. Firozzi, T. Bahrami, M. Amrani, M. H. Yacoub, G. Dreyfus, A. Khaghani
Myocardial Recovery from Advanced Heart Failure Using the HeartMate II LVAD with Drug Combination Therapy: Results from a Prospective Study
*Journal of Heart and Lung Transplantation* 2010;29(2 Suppl 1): S66

4) E. J. Birks, R. S. George, M. Noor, T. Bahrami, M. Amrani, J. Pepper, G. Dreyfus, M. Petrou, M. H. Yacoub, A. Khaghani
Long Term Outcome of Bridge to Recovery versus Bridge to Transplantation
*Journal of Heart and Lung Transplantation* 2010;29(2 Suppl 1): S176-7

5) J. Beale, R. S. George, J. Smith, A. Khaghani, M. H. Yacoub, E. J. Birks, P. Barton
Myocardial Recovery is Associated with Low Levels of circulating MMP8 at LVAD Implantation
*Journal of Heart and Lung Transplantation* 2010;29(2 Suppl 1): S65-6

6) Robert S George, Magdi H Yacoub, Carole Webb, Christopher Bowles, Asghar Khaghani, Emma J Birks
The Extent of Myocardial Recovery as Assessed by the Response of Echocardiographic Parameters to Exercise in Patients With Heartmate I and Heartmate II Left Ventricular Assist Devices (LVADs)
*Circulation, Nov 2009; 120: S798*

7) Robert S George, Magdi H Yacoub, Carole Webb, Asghar Khaghani, Emma J Birks
Contractile Reserve Assessment in DCM Patients Supported With Continuous Flow Left Ventricular Assist Device (LVAD)
*Circulation, Nov 2009; 120: S726*

The Effects of Left Ventricular Assist Device (LVAD) Unloading on Cardiac Sympathetic Innervation
*Journal of Heart and Lung Transplantation* 2009;28(2 Supplement 1):SI29

Echocardiographic Evaluation of Flow across HeartMate II Axial Flow LVADs at Varying Low Speeds
*Journal of Heart and Lung Transplantation* 2009;28(2 Supplement 1):S244

10) E.J. Birks, R.S. George, M. Hedger, T. Bahrami, M. Amrani, M.H. Yacoub, G. Dreyfus, A. Khaghani
Myocardial Recovery from Advanced Heart Failure Using the Heartmate II LVAD Combined with Drug Therapy: Early Results from a Prospective Study
Robert S. George, Magdi H. Yacoub, Carole Webb, Christopher Bowles, Robert Dean, Mike Hedger, Giles Dreyfus, Asghar Khaghani, Emma J. Birks
Serial Assessment of Pulsatile and Non-Pulsatile Left Ventricular Assist Devices on Left Ventricular Unloading
*Circulation, Oct 2008; 118: S1016*

Sustained Normalisation of $^{123}$I-MIBG Uptake after Explantation of Left Ventricular Assist Device (LVAD)s
*Journal of Heart and Lung Transplantation 2008;27(2 Supplement 1):S94-95*

Complete discontinuation of mechanical support and assessment of inotropic reserve in patients with left ventricular assist devices
*Journal of Heart and Lung Transplantation 2007;26(2 Supplement 1):S61*

R.S. George, P. Rogers, C. Hallas, M. Petrou, N.R. Banner, G. Dreyfus, A. Khaghani, M.H. Yacoub, E.J. Birks
Quality of life two or more years following LVAD removal
*Journal of Heart and Lung Transplantation 2007;26(2 Supplement 1):S161*

Robert George, Christopher Bowles, Patrick Tansley, James Hardy, Carole Webb, Asghar Khaghani, Mgdi Yacoub, Emma Birks
Cessation of Left Ventricular Assist Device (LVAD) to study cardiac function is a safe method to monitor myocardial recovery
*Circulation, Nov 2006;114:II_661*
Presentations and Scientific Meetings

1. “Assessment of Myocardial Recovery in Patients with Left Ventricular Assist Device”
   Invited Speaker
   

2. “Higher Implant Norepinephrine Transporter Levels in the Myocardium are Associated with Myocardial Recovery during Left Ventricular Assist Device Support” – Poster
   
   Robert S. George, Paul Facer, Magdi H. Yacoub, Anand Praveen, Emma J. Birks
   Meeting: The American Heart Association, Chicago, USA (November 2010)

3. “The Effects of Clenbuterol on the Left Ventricular Wall Thickness and Function in Patients Recovered on Left Ventricular Assist Device (LVAD)” - Poster
   
   Meeting: The International Society for Heart and Lung Transplantation Paris, France (Chicago 2010)

4. “LVAD Support Results in a Significant Improvement in the Myocardial Sympathetic Nervous System, as Assessed by 123I-Metaiodobenzylguanidine (123I-MIBG), Independent of Ventricular Function” - Poster
   
   Robert S. George, M.H. Yacoub, A. Cheetham, A. Kelion, E.J. Birks
   Meeting: The International Society for Heart and Lung Transplantation Paris, France (Chicago 2010)

5. “Myocardial Recovery from Advanced Heart Failure Using the Heartmate II LVAD Combined with Drug Therapy: Results from a Prospective Study”
   
   E.J. Birks, R.S. George, M. Hedger, A. Firouzi, T. Bahrami, M. Amrani, M.H. Yacoub, G. Dreyfus, A. Khaghani
   Meeting: The International Society for Heart and Lung Transplantation Paris, France (Chicago 2010)

6. “Long Term Outcome of Bridge to Recovery Versus Bridge to Transplantation”
   
   E.J. Birks, R.S. George, M. Noor, T. Bahrami, M. Amrani, J. Pepper, G. Dreyfus, M. Petrou, M.H. Yacoub, A. Khaghani
   Meeting: The International Society for Heart and Lung Transplantation Paris, France (Chicago 2010)
7. “Myocardial Recovery Is Associated with Low Levels of Circulating MMP8 at LVAD Implantation”

J. Beale, R.S. George, J. Smith, A. Khaghani, M.H. Yacoub, E.J. Birks, P. Barton

Meeting: The International Society for Heart and Lung Transplantation Paris, France (Chicago 2010)

8. “A Safe and a Cost-Effective Approach for Managing Patients with Levitronix CentriMag® Short Term Devices” - Poster

A. Firouzi, R.S. George, K. Absalom, S.M. Panther, H. Doyle, A. Bashford, C. Bindoff, A. Khaghani, E.J. Birks

Meeting: The International Society for Heart and Lung Transplantation Paris, France (Chicago 2010)

9. “The Extent of Myocardial Recovery as Assessed by the Response of Echocardiographic Parameters to Exercise in Patients with HeartMate I and HeartMate II left ventricular assist devices (LVADs).”

Robert S. George, Magdi H. Yacoub, Carole Webb, Christopher Bowles, Asghar Khaghani, Emma J. Birks

Meeting: The American Heart Association, Orlando, USA (November 2009)

10. “Contractile reserve assessment in DCM patients supported with continuous flow Left Ventricular Assist Device (LVAD)”

Robert S. George, Magdi H. Yacoub, Carole Webb, Asghar Khaghani, Emma J. Birks

Meeting: The American Heart Association, Orlando, USA (November 2009)

11. “Clinical Aspects of Myocardial Recovery Following LVAD Treatment”

Invited Speaker

Meeting: The Mechanoreceptor and Heart Symposium. Imperial College, Harefield (October 2009)

12. “What happens to myocardial sympathetic function in heart failure patients who recover on Left Ventricular Assist Device (LVAD) therapy?”

Robert S. George, Andrew Cheetham, Aandrew Marshall, Rommel Manlapig, Suzie Hinton-Taylor, Magdi H.. Yacoub, Emma J. Birks, Andrew Kelion

Meeting: 9th Biannual International Conference in Nuclear Cardiology, Barcelona, Spain (May 2009)

13. “Reproducibility of Myocardial 123I-Metaiodobenzylguanidine (123I-MIBG) Scintigraphy in Patients with Left Ventricular Assist Device (LVAD).”

Robert S George, Nikant K Sabharwal, Carole Webb, Mike Hedger, Magdi H. Yacoub, Gilles Dreyfus, Asghar Khaghani, Emma J. Birks
Meeting: The International Society for Heart and Lung Transplantation Paris, France (April 2009)

15. “Effects of Left Ventricular Assist Device (LVAD) Unloading on Cardiac Sympathetic Innervation.”

Robert S George, Magdi H. Yacoub, Andrew Cheetham, Rommel Manlapig, Asghar Khaghani, Andrew Kelion, Emma J. Birks
Meeting: The International Society for Heart and Lung Transplantation Paris, France (April 2009)

16. “Myocardial Recovery from Advanced Heart Failure Using the Heartmate II LVAD Combined with Drug Therapy: Early Results from a Prospective Study”

Emma J. Birks, Robert S. George, Mike Hedger, Toufan Bahrami, Mohamed Amrani, Magdi H. Yacoub, Gilles Dreyfus, Asghar Khaghani
Meeting: The International Society for Heart and Lung Transplantation Paris, France (April 2009)

17. “Limited surgical approaches for LVAD explant following myocardial recovery are associated with low morbidity and improved outcome.”

Saleem Haj-Yahia, Robert S. George, Szymon Waligorski, Gilles Dreyfus, Magdi H. Yacoub, Emma J. Birks, Asghar Khaghani
Meeting: The International Society for Heart and Lung Transplantation Paris, France (April 2009)


Christopher Bowles, Mohamed Borhani, Ann van der Horst, Robert S. George, Kim H. Parker, Emma J. Birks
Meeting: The International Society for Heart and Lung Transplantation Paris, France (April 2009)
19. “What happens to myocardial sympathetic function in heart failure patients who recover on left ventricular assist device (LVAD) therapy?”
Robert S. George, Andrew Cheetham, Romel Manlapig, Fabrice Ghiotto, Suzie Hinton-Taylor, Andrew Marshall, Magdi H. Yacoub, Emma J. Birks, Andrew Kelion
Meeting: British Nuclear Cardiology Society, NHLI, London (December 2008)

20. “Serial Assessment of Pulsatile and Non-Pulsatile Left Ventricular Assist Devices on Left Ventricular Unloading.”
Robert S George, Magdi H. Yacoub, Carole Webb, Christopher Bowles, Robert Dean, Mike Hedger, Giles Dreyfus, Asghar Khaghani, and Emma J. Birks
Meeting: The American Heart Association, New Orleans, USA (November 2008)

21. “Sustained Normalisation of $^{123}$I-MIBG Uptake after Explantation of Left Ventricular Assist Devices (LVAD)”
Robert S George
Meeting: Postgraduate Student Presentation Day, NHLI, Imperial College (June 2008)

22. “Role of MIBG imaging in Heart Failure, Left Ventricular Assist Device and Recovery Patients”
Robert S. George, A Cheetham, S Hinton-Taylor, R Manlapig, F Ghiotto A Kelion, MH Yacoub, EJ Birks
Meeting: The International Society for Heart and Lung Transplantation, Boston, USA (April 2008)

23. “Normalisation of Autonomic Dysfunction in Patients following Left Ventricular Assist Device Combination Therapy.”
David Nunan, Robert S. George, Gavin R. Sandercock, Robert S. Bougard, James Hardy, Asghar Khaghani, Magdi H. Yacoub, David A. Brodie, Emma J. Birks
Meeting: The International Society for Heart and Lung Transplantation, Boston, USA (April 2008)

Invited Speaker
Meeting: 4th Lugano Cardiosurgical Postgraduate Course. The Failing Heart: a Paradigm of Multidisciplinary Approach. Lugano, Switzerland (October 2007)
25. “Investigation of the Neuronal Uptake System in Patients with Heart Failure and Patients Recovered using Left Ventricular Assist Device” - Poster

Robert S. George, Asghar Khaghani, Enrique Lara-Pezzi, Nadia Rosenthal, Andrew Kelion, Magdi H Yacoub, Emma J Birks
Meeting: Postgraduate Student Presentation Day, NHLI, Imperial College (June 2007)

26. “Complete Discontinuation of Mechanical Support and Assessment of Inotropic Reserve in Patients with Left Ventricular Assist Devices.”

Robert S. George, Giordano Tasca, Christopher Bowles, Carole Webb, Gilles Dreyfus, Aasghar Khaghani, Magdi H. Yacoub, Emma J. Birks
Meeting: The International Society for Heart and Lung Transplantation, San Francisco, USA (April 2007)

27. “Quality of Life Two or More Years Following LVAD Removal.” - Poster

Robert S. George, Paula Rogers, Claire Hallas, Mario Petrou, Nicholas Banner, Gilles Dreyfus, Asghar Khaghani, Magdi H. Yacoub, Emma J. Birks
Meeting: The International Society for Heart and Lung Transplantation, San Francisco, USA (April 2007)

28. “Patient Outcome Following LVAD Bridge to Recovery Compared to Bridge to Transplantation.”

Emma J. Birks, Robert S. George, Paula Rogers, John R. Pepper, Gilles Dreyfus, Magdi H. Yacoub, Asghar Khaghani
Meeting: The International Society for Heart and Lung Transplantation, San Francisco, USA (April 2007)

29. “Cessation of Left Ventricular Assist Device (LVAD) to study cardiac function is a safe method to monitor myocardial recovery.”

Robert S. George, Christopher Bowles, Patrick Tansley, James Hardy, Carole Webb, Asghar Khaghani, Magdi H. Yacoub, Emma J. Birks
Meeting: The American Heart Association, Chicago, USA (November 2006)
### Appendix A

<table>
<thead>
<tr>
<th>Box 1: INTERMACS level of Limitation at Time of Implant</th>
<th>INTERMACS profile description</th>
<th>Time frame for intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Profile 1: Critical cardiogenic shock</strong></td>
<td>Patients with life-threatening hypotension despite rapidly escalating inotropic support, critical organ hypoperfusion, often confirmed by worsening acidosis and/or lactate levels. “Crash and burn”</td>
<td>Definitive intervention needed within hours.</td>
</tr>
<tr>
<td><strong>Profile 2: Progressive decline</strong></td>
<td>Patient with declining function despite intravenous inotropic support. May be manifest by worsening renal function, nutritional depletion, inability to restore volume balance “Sliding on inotropes.”</td>
<td>Definitive intervention needed within few days.</td>
</tr>
<tr>
<td><strong>Profile 3: Stable but inotrope dependent</strong></td>
<td>Patient with stable blood pressure, organ function, nutrition, and symptoms on continuous intravenous inotropic support (or a temporary circulatory support device or both), but demonstrating repeated failure to wean from support “Dependent stability.”</td>
<td>Definitive intervention elective over a period of weeks to few months.</td>
</tr>
<tr>
<td><strong>Profile 4: Resting symptoms</strong></td>
<td>Patient can be stabilized close to normal volume status but experiences daily symptoms of congestion at rest. Doses of diuretics generally fluctuate at very high levels. More intensive management and surveillance strategies should be considered. Some patients may shuttle between 4 and 5.</td>
<td>Definitive intervention elective over period of weeks to few months.</td>
</tr>
<tr>
<td><strong>Profile 5: Exertion intolerant</strong></td>
<td>Comfortable at rest but unable to engage in any other activity, living predominantly within the house. May have underlying refractory elevated volume status, often with renal dysfunction.</td>
<td>Variable urgency.</td>
</tr>
<tr>
<td><strong>Profile 6: Exertion limited</strong></td>
<td>Patient without evidence of fluid overload is comfortable at rest, and with activities of daily living and minor activities outside the home but fatigues after the first few minutes of any meaningful activity. “Walking wounded.”</td>
<td>Variable urgency.</td>
</tr>
<tr>
<td><strong>Profile 7: Advanced NYHA III</strong></td>
<td>A placeholder for more precise specification in future, this level includes patients who are without current or recent episodes of unstable fluid balance, living comfortably with meaningful activity limited to mild physical exertion.</td>
<td>Transplantation or circulatory support may not currently be indicated.</td>
</tr>
</tbody>
</table>
Box 2: Definition of advanced HF (Swedberg K et al., 1990)

1. Symptoms of NYHA class III or IV;
2. Episodes of fluid retention or reduced cardiac output at rest;
3. Objective evidence of severe cardiac dysfunction, shown by at least one of the following:
   a. Low left ventricular ejection fraction (<30%),
   b. Severe abnormality of cardiac function on Doppler echocardiography with pseudonormal or restrictive mitral inflow pattern,
   c. High left ventricular filling pressure (mean pulmonary capillary wedge pressure > 16 mmHg, or mean atrial pressure > 12 mmHg by pulmonary artery catheterisation),
   d. High brain natriuretic peptide or N-terminal pre-brain natriuretic peptide plasma levels, in the absence of non-cardiac causes,
4. Severe impairment of functional capacity shown by one of the following:
   a. Inability to exercise,
   b. 6-minute walk test distance < 300 m or less in women or patients aged ≥ 75 years,
   c. Peak oxygen consumption < 12 to 14 ml/min/kg,
5. History of at least one heart failure hospitalisation in the past 6 months;
6. Presence of all previous features despite “attempts to optimise” therapy, including diuretics, inhibitors of renin-angiotensin-aldosterone system, and β-blockers, unless these are poorly tolerated or contraindicated, and cardiac resynchronisation therapy, when indicated.
Table A1 represent the pre-implantation demographics, clinical parameters and reasons for exclusion of the 13 excluded patients.

Tables A2 and A3 represent the pre-implantation demographics and clinical parameters of each individual patient. All patients were inotrope dependent, 5 patients required IABP, 2 were intubated and 2 were haemofiltered.

Study end date refers to the time point up to which patient’s echocardiographic and haemodynamic parameters are included in the thesis. For the non-recovered patient it was assigned as the date when they were transplant listed. For the recovered patients the study end date refers to the date when the device was either explanted or the decision to explant was made.

As stated in chapter 2, 15 patients recovered of whom 14 were explanted and 8 did not show signs of myocardial recovery and were either transplanted or transplant listed.
Table A1: Excluded patients (n=13)

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Diagnosis</th>
<th>Gender</th>
<th>Implant Date</th>
<th>Implant Age (yrs)</th>
<th>Support Duration (d)</th>
<th>Global EF (%)</th>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>IHD</td>
<td>M</td>
<td>20/07/2006</td>
<td>60.48</td>
<td>62</td>
<td>42</td>
<td>Ischaemic Origin</td>
</tr>
<tr>
<td>E2</td>
<td>HOCM</td>
<td>M</td>
<td>28/08/2006</td>
<td>41.07</td>
<td>Ongoing</td>
<td>12</td>
<td>HOCM</td>
</tr>
<tr>
<td>E3</td>
<td>PPM DCM</td>
<td>F</td>
<td>16/10/2006</td>
<td>35.09</td>
<td>312</td>
<td>28</td>
<td>Developed chest pain every time the speed of the HM II was reduced to assess the underlying myocardial function</td>
</tr>
<tr>
<td>E4</td>
<td>IHD</td>
<td>F</td>
<td>12/02/2007</td>
<td>45.43</td>
<td>166</td>
<td>30</td>
<td>Ischaemic Origin</td>
</tr>
<tr>
<td>E5</td>
<td>DCM</td>
<td>M</td>
<td>26/02/2007</td>
<td>57.46</td>
<td>311</td>
<td>10</td>
<td>Apical VSDs</td>
</tr>
<tr>
<td>E6</td>
<td>DCM</td>
<td>M</td>
<td>03/03/2007</td>
<td>27.51</td>
<td>23</td>
<td>10</td>
<td>Died 23 days after device implantation</td>
</tr>
<tr>
<td>E7</td>
<td>DCM</td>
<td>M</td>
<td>26/06/2007</td>
<td>47.20</td>
<td>9</td>
<td>-</td>
<td>Died 9 days after device implantation</td>
</tr>
<tr>
<td>E8</td>
<td>Becker’s MD</td>
<td>M</td>
<td>10/07/2007</td>
<td>18.67</td>
<td>674</td>
<td>-</td>
<td>Becker’s muscular dystrophy</td>
</tr>
<tr>
<td>E9</td>
<td>DCM</td>
<td>M</td>
<td>11/07/2007</td>
<td>21.87</td>
<td>Ongoing</td>
<td>24</td>
<td>Severe MR</td>
</tr>
<tr>
<td>E10</td>
<td>IHD</td>
<td>M</td>
<td>13/10/2007</td>
<td>56.04</td>
<td>276</td>
<td>25</td>
<td>Ischaemic Origin</td>
</tr>
<tr>
<td>E11</td>
<td>DCM</td>
<td>F</td>
<td>29/12/2007</td>
<td>28.85</td>
<td>246</td>
<td>15</td>
<td>Severe MR and prolonged ITU stay prevented from recovery assessment</td>
</tr>
<tr>
<td>E12</td>
<td>PPM DCM</td>
<td>F</td>
<td>30/09/2008</td>
<td>41.94</td>
<td>240</td>
<td>20</td>
<td>Poor clinic attendance and compliance with medication</td>
</tr>
<tr>
<td>E13</td>
<td>IHD</td>
<td>F</td>
<td>09/02/2009</td>
<td>32.08</td>
<td>Ongoing</td>
<td>-</td>
<td>Ischaemic Origin</td>
</tr>
</tbody>
</table>

Table A1: Basic demographics of the 13 excluded patients with reasons for exclusion.
Table A2: Pre-implantation demographics and clinical parameters of each individual patient.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Diagnosis</th>
<th>Gender</th>
<th>Disease Duration (mths)</th>
<th>Symptoms Duration (mths)</th>
<th>Implant Date</th>
<th>Implant Age (yrs)</th>
<th>Support Duration (d)</th>
<th>Outcome</th>
<th>Outcome as of 1st Oct 2009</th>
<th>Study end date</th>
<th>Study Duration (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCM</td>
<td>M</td>
<td>2</td>
<td>2</td>
<td>27/02/2006</td>
<td>16.88</td>
<td>219</td>
<td>Recovered</td>
<td>Explanted; Alive</td>
<td>04/10/2006</td>
<td>219</td>
</tr>
<tr>
<td>2</td>
<td>DCM</td>
<td>M</td>
<td>2</td>
<td>2</td>
<td>06/02/2007</td>
<td>18.89</td>
<td>204</td>
<td>Recovered</td>
<td>Explanted; Alive</td>
<td>29/08/2007</td>
<td>204</td>
</tr>
<tr>
<td>3</td>
<td>DCM</td>
<td>M</td>
<td>48</td>
<td>6</td>
<td>15/02/2007</td>
<td>41.41</td>
<td>Ongoing</td>
<td>Non-Recovered</td>
<td>Awaiting Tx</td>
<td>12/08/2008</td>
<td>544</td>
</tr>
<tr>
<td>4</td>
<td>DCM</td>
<td>F</td>
<td>4</td>
<td>4</td>
<td>11/05/2007</td>
<td>58.26</td>
<td>215</td>
<td>Recovered</td>
<td>Explanted</td>
<td>12/12/2007</td>
<td>215</td>
</tr>
<tr>
<td>5</td>
<td>DCM</td>
<td>M</td>
<td>132</td>
<td>2</td>
<td>07/06/2007</td>
<td>42.86</td>
<td>776</td>
<td>Non-Recovered</td>
<td>Tx; Dead</td>
<td>03/02/2009</td>
<td>607</td>
</tr>
<tr>
<td>6</td>
<td>DCM</td>
<td>M</td>
<td>50</td>
<td>9</td>
<td>19/06/2007</td>
<td>27.14</td>
<td>Ongoing</td>
<td>Non-Recovered</td>
<td>Awaiting Tx</td>
<td>29/05/2008</td>
<td>345</td>
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<tr>
<td>7</td>
<td>DCM</td>
<td>M</td>
<td>2</td>
<td>2</td>
<td>22/06/2007</td>
<td>29.71</td>
<td>474</td>
<td>Recovered</td>
<td>Explanted; Dead</td>
<td>08/10/2008</td>
<td>474</td>
</tr>
<tr>
<td>8</td>
<td>Familial DCM</td>
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<td>48</td>
<td>4</td>
<td>27/06/2007</td>
<td>21.04</td>
<td>260</td>
<td>Recovered</td>
<td>Explanted; Dead</td>
<td>13/03/2008</td>
<td>260</td>
</tr>
<tr>
<td>9</td>
<td>DCM</td>
<td>M</td>
<td>108</td>
<td>12</td>
<td>20/08/2007</td>
<td>55.89</td>
<td>363</td>
<td>Non-Recovered</td>
<td>Tx; Dead</td>
<td>17/08/2008</td>
<td>363</td>
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<tr>
<td>11</td>
<td>DCM</td>
<td>M</td>
<td>1.5</td>
<td>1.5</td>
<td>15/12/2007</td>
<td>35.23</td>
<td>387</td>
<td>Recovered</td>
<td>Alive</td>
<td>05/01/2009</td>
<td>387</td>
</tr>
<tr>
<td>12</td>
<td>DCM</td>
<td>M</td>
<td>1.5</td>
<td>1.5</td>
<td>20/01/2008</td>
<td>21.55</td>
<td>213</td>
<td>Recovered</td>
<td>Explanted; Alive</td>
<td>20/08/2008</td>
<td>213</td>
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</tbody>
</table>
Table A2 (cont.):

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Diagnosis</th>
<th>Gender</th>
<th>Disease Duration (mths)</th>
<th>Symptoms Duration (mths)</th>
<th>Implant Date</th>
<th>Implant Age (yrs)</th>
<th>Support Duration (d)</th>
<th>Outcome</th>
<th>Outcome as of 1st Oct 2009</th>
<th>Study end date **</th>
<th>Study Duration (d)</th>
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</thead>
<tbody>
<tr>
<td>13 §</td>
<td>DCM</td>
<td>M</td>
<td>84</td>
<td>6</td>
<td>01/03/2008</td>
<td>48.10</td>
<td>439</td>
<td>Recovered</td>
<td>Explanted; Alive</td>
<td>14/05/2009</td>
<td>439</td>
</tr>
<tr>
<td>14</td>
<td>DCM</td>
<td>M</td>
<td>12</td>
<td>12</td>
<td>01/04/2008</td>
<td>23.90</td>
<td>Ongoing</td>
<td>Non-Recovered</td>
<td>Awaiting Tx</td>
<td>14/08/2009</td>
<td>500</td>
</tr>
<tr>
<td>15</td>
<td>DCM</td>
<td>F</td>
<td>9</td>
<td>9</td>
<td>19/05/2008</td>
<td>33.92</td>
<td>317</td>
<td>Recovered</td>
<td>Explanted; Alive</td>
<td>01/04/2009</td>
<td>317</td>
</tr>
<tr>
<td>16</td>
<td>DCM</td>
<td>M</td>
<td>78</td>
<td>12</td>
<td>06/10/2008</td>
<td>51.66</td>
<td>Ongoing</td>
<td>Non-Recovered</td>
<td>Awaiting Tx</td>
<td>27/08/2009</td>
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<tr>
<td>17</td>
<td>DCM</td>
<td>M</td>
<td>30</td>
<td>1</td>
<td>23/11/2008</td>
<td>24.18</td>
<td>Ongoing</td>
<td>Non-Recovered</td>
<td>Awaiting Tx</td>
<td>15/08/2009</td>
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</tr>
<tr>
<td>18 §</td>
<td>DCM</td>
<td>M</td>
<td>1.5</td>
<td>1.5</td>
<td>27/12/2008</td>
<td>16.58</td>
<td>138</td>
<td>Recovered</td>
<td>Explanted; Alive</td>
<td>14/05/2009</td>
<td>138</td>
</tr>
<tr>
<td>19 ††</td>
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<td>M</td>
<td>5</td>
<td>5</td>
<td>02/01/2009</td>
<td>42.37</td>
<td>235</td>
<td>Recovered</td>
<td>Explanted; Alive</td>
<td>25/08/2009</td>
<td>235</td>
</tr>
<tr>
<td>20</td>
<td>DCM</td>
<td>M</td>
<td>14</td>
<td>14</td>
<td>06/01/2009</td>
<td>53.23</td>
<td>Ongoing</td>
<td>Recovered</td>
<td>Awaiting Explant</td>
<td>31/10/2009</td>
<td>298</td>
</tr>
<tr>
<td>21</td>
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<td>M</td>
<td>36</td>
<td>3</td>
<td>12/01/2009</td>
<td>58.82</td>
<td>Ongoing</td>
<td>Non-Recovered</td>
<td>Awaiting Tx</td>
<td>15/08/2009</td>
<td>215</td>
</tr>
<tr>
<td>22</td>
<td>DCM</td>
<td>M</td>
<td>1.5</td>
<td>1.5</td>
<td>07/03/2009</td>
<td>32.39</td>
<td>205</td>
<td>Recovered</td>
<td>Explanted; Alive</td>
<td>28/09/2009</td>
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</tr>
<tr>
<td>23</td>
<td>DCM</td>
<td>M</td>
<td>1.5</td>
<td>1.5</td>
<td>19/03/2009</td>
<td>16.72</td>
<td>202</td>
<td>Recovered</td>
<td>Explanted; Alive</td>
<td>01/10/2009</td>
<td>196</td>
</tr>
</tbody>
</table>

* Patient 1 and 2 were intubated prior to device implantation
† Patients 2 and 5 were haemofiltered prior to device implantation
†† Patients 1, 5, 6, 8, and 19 had IABP prior to device implantation
§ Patients 2, 6, 13, and 18 had pre-implant short term support (patients 2, 6, and 13 had BiVAD Levitornics® and patient 18 had ECMO)

** Study end date refers to the time point up to which patient’s echocardiographic and haemodynamic parameters are included in the thesis. For the non-recovered patient it was assigned as the date when they were transplant listed and for the recovered patients as the either explant date or the decision to explant
Table A3: Pre-implantation demographics and clinical parameters of each individual patient

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>BMI (kg/m²)</th>
<th>BSA (m²)</th>
<th>PMH</th>
<th>Number of Inotropes</th>
<th>Pre-Implant Haemodynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sys PA (mmHg)</td>
</tr>
<tr>
<td>1</td>
<td>20.68</td>
<td>1.83</td>
<td>Nil</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>25.62</td>
<td>2.04</td>
<td>Nil</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>23.77</td>
<td>1.96</td>
<td>ICD</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>19.01</td>
<td>1.38</td>
<td>Nil</td>
<td>2</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>22.86</td>
<td>1.84</td>
<td>Nil</td>
<td>2</td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>25.57</td>
<td>1.95</td>
<td>ICD</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>22.52</td>
<td>2.10</td>
<td>Nil</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>19.14</td>
<td>1.76</td>
<td>Nil</td>
<td>2</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>28.85</td>
<td>1.74</td>
<td>ICD, Type II DM, AF</td>
<td>1</td>
<td>72</td>
</tr>
<tr>
<td>10</td>
<td>25.94</td>
<td>1.85</td>
<td>Asthma</td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td>11</td>
<td>26.85</td>
<td>2.09</td>
<td>Multiple PE’s</td>
<td>2</td>
<td>38</td>
</tr>
<tr>
<td>12</td>
<td>21.30</td>
<td>1.73</td>
<td>Mild asthma</td>
<td>2</td>
<td>43</td>
</tr>
<tr>
<td>13</td>
<td>24.98</td>
<td>1.77</td>
<td>DVT</td>
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<tr>
<td>14</td>
<td>26.84</td>
<td>2.07</td>
<td>Nil</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>15</td>
<td>19.83</td>
<td>1.57</td>
<td>Nil</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>24.09</td>
<td>1.78</td>
<td>ICD, Hypercholesterolaemia</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>17</td>
<td>20.63</td>
<td>1.79</td>
<td>ICD</td>
<td>2</td>
<td>53</td>
</tr>
<tr>
<td>18</td>
<td>26.73</td>
<td>1.96</td>
<td>Nil</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>21.50</td>
<td>1.74</td>
<td>Nil</td>
<td>2</td>
<td>57</td>
</tr>
<tr>
<td>20</td>
<td>23.05</td>
<td>1.81</td>
<td>CRT-D, restless leg syndrome, Type II DM</td>
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<td>39</td>
</tr>
<tr>
<td>21</td>
<td>24.21</td>
<td>1.91</td>
<td>ICD, Aortic regurgitation</td>
<td>2</td>
<td>53</td>
</tr>
<tr>
<td>22</td>
<td>29.32</td>
<td>2.18</td>
<td>Mild asthma</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>21.05</td>
<td>1.74</td>
<td>Nil</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>
Table A3 (cont.):

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Pre-Implant Echocardiography</th>
<th>Pre-Implant Serum Biochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVEDD (mm)</td>
<td>LVESD (mm)</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>1</td>
<td>73</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>65</td>
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<tr>
<td>4</td>
<td>67</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
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<td>69</td>
<td>67</td>
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<td>8</td>
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<td>9</td>
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<td>72</td>
</tr>
<tr>
<td>10</td>
<td>85</td>
<td>78</td>
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<tr>
<td>11</td>
<td>63</td>
<td>61</td>
</tr>
<tr>
<td>12</td>
<td>63</td>
<td>59</td>
</tr>
<tr>
<td>13</td>
<td>74</td>
<td>67</td>
</tr>
<tr>
<td>14</td>
<td>78</td>
<td>73</td>
</tr>
<tr>
<td>15</td>
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<td>65</td>
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<td>71</td>
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<td>91</td>
<td>82</td>
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<td>54</td>
</tr>
<tr>
<td>23</td>
<td>83</td>
<td>75</td>
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</tbody>
</table>
Appendix B

Multi-Level Time Model Analysis

To ensure that the echocardiographic and haemodynamic parameters are not due to data averaging, a multi-level time model analysis was performed to determine the trends in the echocardiographic and haemodynamic parameters over a 1-year period.

The model assumes that for each patient there is a linear relationship between the response and time since device implantation. In the thesis the response has been defined as one of the following:

1) The change in absolute value in the echocardiographic measured parameters (LVEDD, LVESD, FS, and EF) at:
   a. 15 minutes of off-pump
   b. Post 6-minute walk exercise test

2) The percentage change in echocardiographic parameters from 15 minutes of off-pump testing to post 6-minute walk exercise test.

Model 1

Model 1 is a basic model which allows for a linear change in the response with time since operation. In this model recovered and non-recovered patients were considered separately.

The intercept and the slope of the linear relationship are assumed to vary randomly. In detail, if \( y_{ij} \) is the response for Patient \( j \) at Occasion \( i \), and \( t_{ij} \) is the time (years after operation) of Occasion \( i \) for Patient \( j \), then

\[
y_{ij} = \beta_{0j} + \beta_{1j} t_{ij} + e_{ij}
\]
Where:

\[ e_{ij} = \Sigma(0, \sigma_e^2), \]
\[ \beta_{0j} = \Sigma(\beta_{00}, \sigma_{u0}^2), \]
\[ \beta_{1j} = \Sigma(\beta_{01}, \sigma_{u1}^2), \]
\[ \text{cov}(\beta_{0j}, \beta_{1j}) = \sigma_{u01}. \]

So, \( \beta_0 \) is the overall mean intercept (i.e. mean response at time 0) and \( \beta_1 \) is the overall mean slope (i.e. mean increase in response per year). \( e_{ij} \) and \( \sigma_e^2 \) relate to within-patient variability, while \( \sigma_{u0}^2, \sigma_{u1}^2, \) and \( \sigma_{u01} \) relate to between-patient variability.

It is the mean increase in the measured parameters after the response has occurred per year which is the main quantity of interest. The mean slope of the relationship refers to the change in the measured parameter over a period of time such that a positive slope indicates an increase in the value and a negative slope indicates a reduction in the value.

**Model 2**

When comparing the trends of a response, for example the absolute change in EF following the 6-minute walk exercise test, between two subgroups of a certain population such as recovered versus non-recovered patients with HM II LVAD, the same equation is used:

\[ y_{ij} = \beta_{0j} + \beta_{1j}t_{ij} + e_{ij} \]

Where:

\[ e_{ij} = N(0, \sigma_e^2), \]
\[ \beta_{0j} = \beta_{00} + \beta_{01}x_j + u_{0j}, \{ x_j \text{ is the value of the “recovered” for patient } j \} \]
\[
\beta_{1j} = \beta_{10} + \beta_{11}x_j + u_{1j}, \quad \sigma_u^2
\]
\[
u_{0j} = N(0, \sigma_u^2)
\]

So, \(\beta_{00}\) is the overall mean intercept (i.e. notional mean response at time 0) for non-recovered patients and \(\beta_{01}\) is the increase in mean intercept for recovered patients (i.e. the intercept for recovered patients is \(\beta_{00} + \beta_{01}\)). \(\beta_{10}\) is the coefficient of \(t\) (i.e. the slope) for non-recovered patients, while \(\beta_{11}\) is the increase in the coefficient of \(t\) for recovered patients (i.e. the coefficient of \(t\) for recovered patients is \(\beta_{10} + \beta_{11}\)).

When comparing two sub-groups, the key quantities are therefore \(\beta_{11}\) and \(\beta_{10}\); such that if \(\beta_{11} = 0\), then the slope is the same for the two groups; if \(\beta_{01} = 0\), then the notional mean response at time 0 is the same for the two groups; if both \(\beta_{11} = 0\) and \(\beta_{01} = 0\), then the relationship between the response and time since operation is the same for the two sub-groups.

**Model 3**

Similar to model 2, this model compares two groups but two different populations such as HM I LVAD versus HM II LVAD. In this model, the coefficients are allowed to differ between the HM I and HM II patients. The group is indicated by the variable “HM2”, which takes the value 1 for HM II patients and 0 for HM I patients.

The same relationship is considered:

\[
y_{ij} = \beta_{0j} + \beta_{1j}t_{ij} + e_{ij}
\]

Where:

\[
e_{ij} = N(0, \sigma_e^2),
\]

\[
\beta_{0j} = \beta_{00} + \beta_{01}x_j + u_{0j}, \quad \{ x_j \text{ is the value of the “HM II” for patient } j \}\
\]
\[ \beta_{ij} = \beta_{i0} + \beta_{i1}x_j + u_{ij}, \]

\[ u_{ij} = N(0, \sigma_{u0}^2) \]

\[ u_{ij} = N(0, \sigma_{u1}^2) \]

So, \( \beta_{00} \) is the overall mean intercept (i.e. notional mean response at time 0) for HM I patients and \( \beta_{01} \) is the increase in mean intercept for the HM II patients (i.e. the intercept for HM II patients is \( \beta_{00} + \beta_{01} \)). \( \beta_{10} \) is the coefficient of \( t \) (i.e. the slope) for the HM I patients, while \( \beta_{11} \) is the increase in the coefficient of \( t \) for HM II patients (i.e. the coefficient of \( t \) for HM II patients is \( \beta_{10} + \beta_{11} \)).

When comparing two sub-groups from two different populations, the key quantities are therefore \( \beta_{11} \) and \( \beta_{10} \); if \( \beta_{11} = 0 \), then the slope is the same for the two groups; if \( \beta_{01} = 0 \), then the notional mean response at time 0 is the same for the two groups; if both \( \beta_{11} = 0 \) and \( \beta_{01} = 0 \), then the relationship between the response and time since operation is the same for the two sub-groups of the two populations.
Appendix C

**Viscosity calculations**

A Cannon-Fenske Routine Viscometer for transparent liquids size 50 (Poulten Selfe & Lee Ltd, United Kingdom) was used.

Steps to calculate viscosity:

1. The viscometer was cleaned using suitable solvents, and by passing clean, dry, filtered air through the instrument to remove the final traces of solvents.

2. The instrument was inverted and suction was to tube L after immersing tube N in the glycerol-water mixture. Liquid was then drawn to mark F.

3. After cleaning arm N, the instrument was turned to its normal vertical position and was placed into a holder. Since the experiments were to run at
room temperature, the viscometer was kept in the straight position at room temperature and not immersed in a temperature regulated bath.

4. The liquid sample was then allowed to flow freely down past mark F, measuring the efflux time for the meniscus to pass from mark E to mark F.

5. Steps 1-4 were repeated three times on each experimental day and at the end of the experiment to ensure no alteration had occurred in the viscosity throughout the experiment.

6. The kinematic viscosity in mm²/s(cSt) of the sample was calculated by multiplying the efflux time in seconds by the viscometer constant. Such that:

\[ \text{Viscosity} = Ct \]

Where:

C is the calibrating factor which is 0.003962 (mm²/s²). The value is based upon a value for the kinematic viscosity of distilled water at 20°C of 1.0038 mm²/s; \( t \) is the efflux time taken in seconds.
Appendix D

Computer Script for Matlab®

Below is the computer script that was run in the computer programme MatLab® R2007b to produce ventricular and aortic pressure traces and flow trace. The generated graphs were then transported as JPEG images.

% INSTRUCTIONS:

% Open File and get the file:

% 0 mmHg

% 40 mmHg

% 80 mmHg

% 120 mmHg

% 0 mmHg “off”

% subplot to plot 7 by 2: ventricular pressure

% subplot to plot more than 1 plot

% matrix used is two rows by 7 columns

% 7 different speeds from 10,000 rpm to 4,000 rpm

% Column "1" = time (sec) - 10001Hz:16000Hz (3 sec)

        subplot(2,7,1);plot(((rpm10000off(10001:16000,1)/1000)-5),

% Average trace = all trace period

% Filter = sgolay filter: 11=ventricular pressure;
% Column "2" = ventricular pressure (mmHg)
% b=blue;
    sgolayfilt((averagetrace(rpm10000off,2)),2,11,'b')
%
% X AXIS and Y AXIS
AXIS([0 3 -40 60])
    grid on
    YLABEL('Pressure(mmHg)')
    TITLE('10000 rpm')
    hold on
%
% subplot to plot 7 by 2: aortic pressure
% subplot to plot more than 1 plot
% matrix used is two rows by 7 columns
% 7 different speeds from 10,000 rpm to 4,000 rpm
% Column "1" = time (sec) - 10001Hz:16000Hz (3 sec)
    plot(((rpm10000off(10001:16000,1)/1000)-5)
% Average trace = all trace period
% Filter = sgolay filter: 351=aortic pressure;
% Column "3" = aortic pressure (mmHg)
% b=blue
% linewidth = 1.5 pts
    sgolayfilt((averagetrace(rpm10000off,3)),2,351,'r'
    ,'linewidth',1.5);
% repeat the runs for each speed at the different
ventricular pressure 40 mmHg, 80 mmHg, and 120 mmHg
subplot(2,7,2);plot(((rpm9000off(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm9000off,2)),2,11 ),'b');

AXIS([0 3 -40 60])

grid on

TITLE('9000 rpm')

hold on

plot(((rpm9000off(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm9000off,3)),2,35 1),'r','linewidth',1.5);

subplot(2,7,3);plot(((rpm8000off(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm8000off,2)),2,11 ),'b');

AXIS([0 3 -40 60])

grid on

TITLE('8000 rpm')

hold on

plot(((rpm8000off(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm8000off,3)),2,35 1),'r','linewidth',1.5);

subplot(2,7,4);plot(((rpm7000off(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm7000off,2)),2,11 ),'b');

AXIS([0 3 -40 60])

grid on

TITLE('7000 rpm')

hold on

plot(((rpm7000off(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm7000off,3)),2,35 1),'r','linewidth',1.5);
subplot(2,7,5); plot(((rpm6000off(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm6000off,2)),2,11),'b');

AXIS([0 3 -40 60])
grid on
TITLE('6000 rpm')
hold on
plot(((rpm6000off(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm6000off,3)),2,35 1),'r','linewidth',1.5);

subplot(2,7,6); plot(((rpm5000off(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm5000off,2)),2,11),'b');

AXIS([0 3 -40 60])
grid on
TITLE('5000 rpm')
hold on
plot(((rpm5000off(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm5000off,3)),2,35 1),'r','linewidth',1.5);

subplot(2,7,7); plot(((rpm4000off(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm4000off,2)),2,11),'b');

AXIS([0 3 -40 60])
grid on
TITLE('4000 rpm')
hold on
plot(((rpm4000off(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm4000off,3)),2,35 1),'r','linewidth',1.5);
% Average trace = all trace period
% Filter = sgolay filter: 351=flow;
% Column "$4" = flow (l/min)
% k=black;

subplot(2,7,8);plot(((rpm10000off(10001:16000,1)/1000)
- 5),sgolayfilt((averagetrace(rpm10000off,4)),2,351),'k');

AXIS([0 3 0 8])
grid on
YLABEL('Flow(l/min)')
XLABEL('Time(sec)')

subplot(2,7,9);plot(((rpm9000off(10001:16000,1)/1000)
- 5),sgolayfilt((averagetrace(rpm9000off,4)),2,351),'k');

AXIS([0 3 0 8])
grid on
XLABEL('Time(sec)')

subplot(2,7,10);plot(((rpm8000off(10001:16000,1)/1000)
- 5),sgolayfilt((averagetrace(rpm8000off,4)),2,351),'k');

AXIS([0 3 0 8])
grid on
XLABEL('Time(sec)')

subplot(2,7,11);plot(((rpm7000off(10001:16000,1)/1000)
- 5),sgolayfilt((averagetrace(rpm7000off,4)),2,351),'k');
% 40 mmHg

subplot(2,7,1);plot(((rpm10000off(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm10000off,4)),2,11),'b');
AXIS([0 3 -40 60])
grid on
YLABEL('Pressure (mmHg)')
TITLE('10000 rpm')
hold on
plot(((rpm1000040(10001:16000,1)/1000) -
     5),sgolayfilt((averagetrace(rpm1000040,3)),2,351),'','linewidth',1.5);
subplot(2,7,2);plot(((rpm900040(10001:16000,1)/1000) -
     5),sgolayfilt((averagetrace(rpm900040,3)),2,11),'');
AXIS([0 3 -40 60])
grid on
TITLE('9000 rpm')
hold on
plot(((rpm900040(10001:16000,1)/1000) -
     5),sgolayfilt((averagetrace(rpm900040,3)),2,351),'','linewidth',1.5);
subplot(2,7,3);plot(((rpm800040(10001:16000,1)/1000) -
     5),sgolayfilt((averagetrace(rpm800040,3)),2,11),'');
AXIS([0 3 -40 60])
grid on
TITLE('8000 rpm')
hold on
plot(((rpm800040(10001:16000,1)/1000) -
     5),sgolayfilt((averagetrace(rpm800040,3)),2,351),'','linewidth',1.5);
subplot(2,7,4); plot(((rpm700040(10001:16000,1)/1000) - 5).sgolayfilt((averagetrace(rpm700040,2)),2,11), 'b');

AXIS([0 3 -40 60])

grid on

TITLE('7000 rpm')

hold on

plot(((rpm700040(10001:16000,1)/1000) - 5).sgolayfilt((averagetrace(rpm700040,3)),2,351), 'r', 'linewidth', 1.5);

subplot(2,7,5); plot(((rpm600040(10001:16000,1)/1000) - 5).sgolayfilt((averagetrace(rpm600040,2)),2,11), 'b');

AXIS([0 3 -40 60])

grid on

TITLE('6000 rpm')

hold on

plot(((rpm600040(10001:16000,1)/1000) - 5).sgolayfilt((averagetrace(rpm600040,3)),2,351), 'r', 'linewidth', 1.5);

subplot(2,7,6); plot(((rpm500040(10001:16000,1)/1000) - 5).sgolayfilt((averagetrace(rpm500040,2)),2,11), 'b');

AXIS([0 3 -40 60])

grid on

TITLE('5000 rpm')

hold on

plot(((rpm500040(10001:16000,1)/1000) - 5).sgolayfilt((averagetrace(rpm500040,3)),2,351), 'r', 'linewidth', 1.5);
subplot(2,7,7); plot(((rpm400040(10001:16000,1)/1000)-
5), sgolayfilt((averagetrace(rpm400040,2)),2,11)
,'b');

AXIS([0 3 -40 60])
grid on
TITLE('4000 rpm')
hold on

plot(((rpm400040(10001:16000,1)/1000)-
5), sgolayfilt((averagetrace(rpm400040,3)),2,351
),'r','linewidth',1.5);

subplot(2,7,8); plot(((rpm1000040(10001:16000,1)
/1000)-
5), sgolayfilt((averagetrace(rpm1000040,4)),2,351
),'k');

AXIS([0 3 0 8])
grid on

YLABEL('Flow(l/min)')

XLABEL('Time(sec)')

subplot(2,7,9); plot(((rpm900040(10001:16000,1)/1000)-
5), sgolayfilt((averagetrace(rpm900040,4)),2,351
),'k');

AXIS([0 3 0 8])
grid on

XLABEL('Time(sec)')

subplot(2,7,10); plot(((rpm800040(10001:16000,1)/1000)-
5), sgolayfilt((averagetrace(rpm800040,4)),2,351
),'k');

AXIS([0 3 0 8])
grid on
XLABEL('Time (sec)')

subplot(2,7,11); plot(((rpm7000040(10001:16000,1)/1000) - 5), sgolayfilt(average_trace(rpm7000040,4)), [2, 351], 'k');

AXIS([0 3 0 8])
grid on
XLABEL('Time (sec)')

subplot(2,7,12); plot(((rpm6000040(10001:16000,1)/1000) - 5), sgolayfilt(average_trace(rpm6000040,4)), [2, 351], 'k');

AXIS([0 3 0 8])
grid on
XLABEL('Time (sec)')

subplot(2,7,13); plot(((rpm5000040(10001:16000,1)/1000) - 5), sgolayfilt(average_trace(rpm5000040,4)), [2, 351], 'k');

AXIS([0 3 0 8])
grid on
XLABEL('Time (sec)')

subplot(2,7,14); plot(((rpm4000040(10001:16000,1)/1000) - 5), sgolayfilt(average_trace(rpm4000040,4)), [2, 351], 'k');

AXIS([0 3 0 8])
grid on
XLABEL('Time (sec)')

figure;

80 mmHg
subplot(2,7,1); plot(((rpm1000080(10001:16000,1)/1000)-5), sgolayfilt((averagetrace(rpm1000080,2)),2,351),'b');

AXIS([0 3 -40 100])
grid on
YLABEL('Pressure(mmHg)')
TITLE('10000 rpm')
hold on
plot(((rpm1000080(10001:16000,1)/1000)-5), sgolayfilt((averagetrace(rpm1000080,3)),2,351),'r','linewidth',1.5);

subplot(2,7,2); plot(((rpm900080(10001:16000,1)/1000)-5), sgolayfilt((averagetrace(rpm900080,2)),2,351),'b');

AXIS([0 3 -40 100])
grid on
TITLE('9000 rpm')
hold on
plot(((rpm900080(10001:16000,1)/1000)-5), sgolayfilt((averagetrace(rpm900080,3)),2,351),'r','linewidth',1.5);

subplot(2,7,3); plot(((rpm800080(10001:16000,1)/1000)-5), sgolayfilt((averagetrace(rpm800080,2)),2,351),'b');

AXIS([0 3 -40 100])
grid on
TITLE('8000 rpm')
hold on
plot(((rpm800080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm800080,2)),2,351),'r','linewidth',1.5);

subplot(2,7,4);plot(((rpm700080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm700080,2)),2,351),'b');

AXIS([0 3 -40 100])
grid on
TITLE('7000 rpm')
hold on
plot(((rpm700080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm700080,2)),2,351),'r','linewidth',1.5);

subplot(2,7,5);plot(((rpm600080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm600080,2)),2,351),'b');

AXIS([0 3 -40 100])
grid on
TITLE('6000 rpm')
hold on
plot(((rpm600080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm600080,2)),2,351),'r','linewidth',1.5);

subplot(2,7,6);plot(((rpm500080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm500080,2)),2,351),'b');

AXIS([0 3 -40 100])
grid on
TITLE('5000 rpm')
hold on
plot(((rpm500080(10001:16000,1)/1000) - 5), sgolayfilt((averagetrace(rpm500080,3)),2,351), 'r', 'linewidth', 1.5);

subplot(2,7,7); plot(((rpm400080(10001:16000,1)/1000) - 5), sgolayfilt((averagetrace(rpm400080,2)),2,351), 'b');

AXIS([0 3 -40 100])
grid on
TITLE('4000 rpm')
hold on
plot(((rpm400080(10001:16000,1)/1000) - 5), sgolayfilt((averagetrace(rpm400080,3)),2,351), 'r', 'linewidth', 1.5);

subplot(2,7,8); plot(((rpm1000080(10001:16000,1)/1000) - 5), sgolayfilt((averagetrace(rpm1000080,4)),2,351), 'k');

AXIS([0 3 -5 15])
grid on
YLABEL('Flow(l/min)')
XLABEL('Time(sec)')

subplot(2,7,9); plot(((rpm900080(10001:16000,1)/1000) - 5), sgolayfilt((averagetrace(rpm900080,4)),2,351), 'k');

AXIS([0 3 -5 15])
grid on
XLABEL('Time(sec)')
subplot(2,7,10);plot(((rpm800080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm800080,4)),2,351),'k');
AXIS([0 3 5 15])
grid on
XLABEL('Time(sec)')

subplot(2,7,11);plot(((rpm700080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm700080,4)),2,351),'k');
AXIS([0 3 5 15])
grid on
XLABEL('Time(sec)')

subplot(2,7,12);plot(((rpm600080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm600080,4)),2,351),'k');
AXIS([0 3 5 15])
grid on
XLABEL('Time(sec)')

subplot(2,7,13);plot(((rpm500080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm500080,4)),2,351),'k');
AXIS([0 3 5 15])
grid on
XLABEL('Time(sec)')

subplot(2,7,14);plot(((rpm400080(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm400080,4)),2,351),'k');
AXIS([0 3 5 15])
grid on

XLABEL('Time(sec)')

% 120 mmHg

figure;

subplot(2,7,1);plot(((rpm10000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm10000120,2)),2,351),'b');

AXIS([0 3 -60 120])

grid on

YLABEL('Pressure(mmHg)')

TITLE('10000 rpm')

hold on

plot(((rpm10000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm10000120,3)),2,351),'r','linewidth',1.5);

subplot(2,7,2);plot(((rpm9000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm9000120,2)),2,351),'b');

AXIS([0 3 -60 120])

grid on

TITLE('9000 rpm')

hold on

plot(((rpm9000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm9000120,3)),2,351),'r','linewidth',1.5);

subplot(2,7,3);plot(((rpm8000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm8000120,2)),2,351),'b');
AXIS([0 3 -60 120])
grid on
TITLE('8000 rpm')
hold on
plot(((rpm8000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm8000120,3)),2,35 1),'r','linewidth',1.5);
subplot(2,7,4);plot(((rpm7000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm7000120,2)),2,35 1),'b');

AXIS([0 3 -60 120])
grid on
TITLE('7000 rpm')
hold on
plot(((rpm7000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm7000120,3)),2,35 1),'r','linewidth',1.5);
subplot(2,7,5);plot(((rpm6000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm6000120,2)),2,35 1),'b');

AXIS([0 3 -60 120])
grid on
TITLE('6000 rpm')
hold on
plot(((rpm6000120(10001:16000,1)/1000) - 5),sgolayfilt((averagetrace(rpm6000120,3)),2,35 1),'r','linewidth',1.5);
subplot(2,7,6);plot(((rpm5000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm5000120,2)),2,351),'b');

AXIS([0 3 -60 120])
grid on
TITLE('5000 rpm')
hold on
plot(((rpm5000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm5000120,3)),2,351),'r','linewidth',1.5);

subplot(2,7,7);plot(((rpm4000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm4000120,2)),2,351),'b');

AXIS([0 3 -60 120])
grid on
TITLE('4000 rpm')
hold on
plot(((rpm4000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm4000120,3)),2,351),'r','linewidth',1.5);

subplot(2,7,8);plot(((rpm10000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm10000120,4)),2,351),'k');

AXIS([0 3 -5 25])
grid on
YLABEL('Flow(l/min)')
XLABEL('Time(sec)')
subplot(2,7,9);plot(((rpm9000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm9000120,4)),2,351),'k');

AXIS([0 3 -5 25])
grid on
XLABEL('Time(sec)')

subplot(2,7,10);plot(((rpm8000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm8000120,4)),2,351),'k');

AXIS([0 3 -5 25])
grid on
XLABEL('Time(sec)')

subplot(2,7,11);plot(((rpm7000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm7000120,4)),2,351),'k');

AXIS([0 3 -5 25])
grid on
XLABEL('Time(sec)')

subplot(2,7,12);plot(((rpm6000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm6000120,4)),2,351),'k');

AXIS([0 3 -5 25])
grid on
XLABEL('Time(sec)')

subplot(2,7,13);plot(((rpm5000120(10001:16000,1)/1000)-5),sgolayfilt((averagetrace(rpm5000120,4)),2,351),'k');
AXIS([0 3 -5 25])
grid on
XLABEL('Time(sec)')

subplot(2,7,14);plot(((rpm4000120(10001:16000,1)/1000) - 
5),sgolayfilt((averagetrace(rpm4000120,4)),2,35 
1),'k');

AXIS([0 3 -5 25])
grid on
XLABEL('Time(sec)')
# Appendix E

## Medicines known to interact with MIBG uptake

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Medicine</th>
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<tbody>
<tr>
<td>Amitriptyline</td>
<td>Methylephedrine</td>
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<td>Amoxapine</td>
<td>Mianserin</td>
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<td>Benperidol</td>
<td>Nicardipine</td>
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<td>Bethanidine</td>
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