Novel approaches in long term mechanical circulatory support

A thesis submitted for the degree

of Doctor of Philosophy

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Declaration of Originality

Clinical research is a collaborative exercise, and many people have been involved in the projects described in this thesis. In particular, I have worked with my supervisors and other colleagues on trial design, data collection and clinical trial management, and these contributions are acknowledged in the text. As such, all work contained herein is my own unless stated to the contrary. No part of this thesis has previously been submitted in application for a higher degree.

Copyright Declaration

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Publications and Presentations

Publications

Morley-Smith AC, Lyon AR. Challenges of Chronic Cardiac Problems in Survivors of Takotsubo Syndrome. Heart Fail Clin. 2016 Oct;12(4):551-7. Review. PubMed PMID: 27638025.

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Abstract

Long term mechanical circulatory support using left ventricular assist devices (LVADs) remains an essential tool in managing patients with advanced heart failure, and in some patients can facilitate reverse remodelling of ventricular function. However, large groups of patients remain poorly served by current technologies, and understanding of reverse remodelling, including how to assess it in vivo, remains poor.

I evaluate new approaches in long term mechanical support. Specifically, I assess: (1) the CircuLite Synergy LVAD and Sunshine Heart C-Pulse counterpulsation device as novel methods for partial LV support, considering particularly their ability to facilitate reverse remodelling and propensity to induce coagulopathy; (2) gene therapy with the AAV1.SERCA2a product as a novel adjunctive therapy, considering particularly its safety and feasibility in LVAD patients in the SERCA-LVAD trial; and (3) novel biomarkers for use in LVAD patients, with focus on strain echocardiography and circulating microRNA.

I report that (1) partial LV support is plausible as a concept and is beneficial in some patients, but there was no consistent evidence of ventricular reverse remodelling and, at the present time, high complication rates and difficulties with patient selection preclude its widespread adoption; (2) there were high rates of pump thrombosis in the Synergy LVAD, though there is no evidence of a device-specific coagulopathy to explain it; (3) gene therapy with AAV1.SERCA2a is safe and feasible in LVAD patients, though there was no evidence of significant efficacy benefit, in line with other clinical trials of this gene therapy in heart failure; (4) assessment of global circumferential strain is feasible in LVAD patients, but inconsistent image quality and effect of loading conditions limit its use; and (5) assessment of circulating microRNA profiles is feasible in LVAD patients, and preliminary data shows roles for specific miRs in patient selection and monitoring of LV function.

Contents

List of Figures

List of Tables

1: Introduction

1.1 The heart failure syndrome

1.1.1 Epidemiology and definitions

Heart failure (HF) is a broad term which implies impairment of heart muscle function and includes a wide range of aetiologies and clinical presentations. The European Society of Cardiology (ESC) HF guidelines define it as "a clinical syndrome characterised by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress"[1].

The population burden of HF in the United Kingdom (UK) and worldwide is large and increasing. The end stage of disease is associated with significant disability and extremely poor prognosis, and its personal and economic burdens are undisputed. Heart failure care in the UK consumes between 1 and 2% of the annual National Health Service (NHS) budget, equating to around £1.2 billion per annum. The personal, public health and economic costs of HF prioritise it for new approaches.

Within this diverse patient group, there is distinction between patients who have impaired function due to failure of heart muscle contraction (typically associated with dilatation of the left ventricle (LV) and impaired function in systole) and those who have failure of heart muscle relaxation (typically with normal LV size, retained radial systolic function, but increased wall thickness, and left atrial (LA) enlargement). ESC and American College of Cardiology Foundation/American Heart Association (ACCF/AHA) guidelines adopt LV ejection fraction (EF) to distinguish between these groups clinically as HF with reduced EF (HFrEF) or HF with preserved EF (HFpEF)[1,2]. The 2016 ESC guidelines require EF<40% and ≥50% for diagnosis of HFrEF and HFpEF respectively, and now recognise a crossover zone termed HF with midrange EF (HFmrEF; EF 41-49%)[1]. As well as classification by EF, there is distinction between acute and chronic HF syndromes. Chronic HF defines patients

with a long-standing abnormality of heart function, whilst acute HF includes patients presenting with de novo cardiac dysfunction and those chronic patients now presenting unwell with a decompensation of previously stable disease. The clinical approach and management priorities differ between HF settings.

1.1.2 Pathophysiology and management of HFrEF

Herein this thesis focuses exclusively on management of chronic HFrEF. The HFrEF syndrome is a progressive derangement of heart muscle function with multisystem effects. Whatever the initial insult – causes include ischaemic heart disease, valve disease, inflammatory processes, damage by cardiotoxins, inherited defects, or simply idiopathic – that loss of function in a sufficient proportion of cardiomyocytes causes an impairment of heart function which the body attempts to address with compensatory responses including upregulation of the sympathetic and renin-angiotensin systems. In the short term, these responses help to enhance heart muscle function and improve end organ perfusion, but eventually they are maladaptive, causing adverse ventricular remodelling characterised by increasing cardiac wall stress, myocardial fibrosis and progressive structural, functional and neurohormonal abnormalities. Unaddressed, the condition progresses with high morbidity burden, and ultimately death either by progressive pump failure or sudden arrhythmic death.

The bulk of evidence in contemporary HF management is focused on management of HFrEF. Therapy for HFrEF has evolved significantly over the past three decades. Drug therapy, once restricted to symptomatic relief with diuretics and nitrates, has evolved to include β-blockers[3-5], inhibitors of the renin-angiotensin-aldosterone system, including angiotensin converting enzyme (ACE) inhibitors[6,7], angiotensin receptor blockers[8] (ARBs), and mineralocorticoid receptor antagonists[9,10] (MRAs), and, more recently, the sinoatrial node modifier ivabradine[11,12] and the angiotensin-neprolysin inhibitor (ARNI) sacubitril-valsartan[13]. Device therapies have an important role in selected patients, especially intracardiac defibrillators[14] and cardiac resynchronisation therapy[15-17]. Introduction of new systems approaches such as multi-disciplinary specialist teams[18] and cardiac rehabilitation[19] have been equally important. Each plays a key role in specific patient groups at different stages of disease, but surgical intervention with cardiac transplantation or implantation of a

ventricular assist device remains the only available option for patients who have progressed to the most advanced stages[20]. Amongst these patients, cardiac transplantation remains the best definitive therapy, commanding excellent long term symptomatic and survival outcomes and low rates of complication[21]. However, increasing worldwide scarcity of donor organs demands new strategies, and mechanical circulatory support (MCS) is becoming increasingly important.

1.1.3 Outcomes of HF in the modern era

The diagnosis of HF brings significant morbidity and mortality, reducing quality of life and life expectancy[1]. The sequential introduction over the past 20 years of new medical and device therapies has significantly improved the outlook for these patients. The CONSENSUS trial reporting in 1987 recorded 1 year mortality of 52% in the placebo group (or 26% in the enalapril group)[6]. In 2001 the first large randomised control trial (RCT) comparing MCS to medical therapy showed 1-year survival of 25% in patients receiving medical therapy[22]. In the sacubitril-valsartan trial, the 1-year all-cause mortality was nearer 10%[13]. Each generation of new treatments has yielded incremental reductions in mortality, and now the benefit required for a new drug or new mechanical assist device to show superiority to best medical therapy is much greater than for previous generations. Nonetheless, there is more to be done and in particular there are certain subgroups of patients with HF, including patients with HFpEF and HF from congenital causes, who remain poorly served.

1.2 Role of mechanical circulatory support

1.2.1 Introduction

The first ventricular assist devices (VADs) developed in the 1980s as a continuum for post-cardiotomy patients unable to wean from cardiopulmonary bypass[23,24], and evolved through the 1990s as untethered, implantable devices used to sustain life until an organ became available for cardiac transplantation. Their use in this bridge to transplantation (BTT) setting allows improvements in cardiac output and blood pressure to reconstitute patients' end-organ dysfunction sufficiently to undergo transplantation safely. Technology and clinical application have continued to evolve rapidly, reducing the high morbidity associated with

early devices and now including patients whose age and co-morbidities would previously have been contraindications to VAD therapy. The burgeoning use of VADs as an independent long term treatment (destination therapy, DT) has greatly improved therapy for patients with advanced heart failure[25]. However, it remains expensive and associated with significant morbidity, and consequently the funding for such programmes remains controversial in resource-limited health economies[26].

Candidates for MCS are considered within the severity classification produced by the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS), which effectively subdivides New York Heart Classification (NYHA) levels III-IV to assist decision making about urgency of VAD implantation[25]. These are shown in Table 1.1 and referenced throughout the thesis.

Table 1.1: Judging severity of disease and urgency of intervention using the Interagency Registry for Mechanically Assisted Circulatory Support profiles.

Level	Clinical characteristics	Urgency of intervention
1	Critical cardiogenic shock despite escalating support	Within a few hours
2	Progressive decline with inotrope dependence	Within a few days
3	Clinically stable with mild to moderate inotrope dependence	Elective implantation over the next few weeks
4	Recurrent, not refractory, advanced heart failure that can be stabilized with intervention	Elective implantation over weeks to months
5	Exertion intolerant but is comfortable at rest and able to perform activities of daily living with slight difficulty	Variable: depends on nutrition, organ function, and activity
6	Exertion limited: is able to perform mild activity, but fatigue results within a few minutes of any meaningful physical exertion	Variable: depends on nutrition, organ function, and activity
7	Advanced NYHA functional class III	At this time, mechanical circulatory support is not indicated

1.2.2 Development of left ventricular assist devices

The fundamental configuration of the LVAD circuit is arranged such that blood is drawn from the heart (usually from the left ventricle) through an inflow cannula to a pump sited

within a ‘pocket’ in the thorax and/or abdomen. This pump accelerates blood to an outflow cannula (historically via pulsatile displacement pump, now almost universally by continuous flow rotary impeller) which is grafted onto a major vessel (e.g. aorta). The pump is connected to external batteries and a control unit via a percutaneous driveline, which usually exits through the anterior abdominal wall.

First generation pumps were large, pulsatile displacement pumps such as the Thoratec HeartMate I-VE-XVE series. Perioperative mortality was around 15-20% and duration of support rarely exceeded 6 months, with 60-70% of patients surviving until device explantation[27-29]. These devices were ground-breaking in their time, but their clear haemodynamic benefits were offset by high morbidity, in particular from the extensive surgical dissection due to their hefty size, high rates of infection due to the large diameter driveline and pump pocket haematomas, and limited durability necessitating reoperation[30].

Nonetheless the expanding patient numbers with an insufficient supply of donor organs led to work to reduce device-related morbidity and develop strategies for long term device therapy. As such in 2001 the prospective, randomised REMATCH trial confirmed a potential role for LVADs as DT for end stage heart failure in patients unsuitable for transplantation, with 1 year survival 52% with the HeartMate XVE versus 25% with standard therapy (p=0.002) and 23% versus 8% at 2 years respectively (p=0.09)[22]. However, morbidity remained significant including markedly increased rates of bleeding, neurological dysfunction and sepsis in the LVAD group (Rate Ratios 9.47 (CI 2.30-38.90), 4.35 (CI 1.31-14.50) and 2.03 (CI 0.99-4.13) respectively), and a high rate of suspected LVAD malfunction (0.75 events/patient-year)[22].

Throughout the 1990s the number of patients undergoing implant as BTT were increasing and work was underway to supersede the displacement pump in favour of an axial rotary impeller design that created continuous flow of blood to the aorta. These second generation devices were led by the Jarvik 2000, MicroMed DeBakey and HeartMate II, and brought benefits of smaller size, easier implant and greater durability. After initial problems with high thrombosis rates were overcome by alterations to pump design, the HeartMate II was established as a tool for BTT by Miller and colleagues[31] in 2007. They studied the HeartMate

II in an intervention-only prospective study considering a primary outcome of cardiac transplantation, myocardial recovery or ongoing support with transplant eligibility at 6 months. 133 patients were recruited and median duration of support was 126 days (IQR 1-600 days). 75% (100) reached the primary outcome and 19% (25) died. Stroke occurred in 8% (11), but accounted for 42% of deaths, and bleeding and local infection were frequent adverse effects (2.09 and 1.13 events/patient year respectively).

Subsequently, the HeartMate II was evaluated in comparison with its predecessor the pulsatile HeartMate I device as a tool for DT. Slaughter and colleagues studied 200 patients randomised 2:1, with a primary composite endpoint of survival at 2 years free of disabling stroke or reoperation to replace the device[32]. HeartMate II was superior (46% versus 11%; $p<0.001$; Hazard Ratio 0.38; CI 0.27-0.54), though there were stubbornly high rates of disabling stroke that did not differ between the study groups and overall 44% of patients implanted with the HeartMate II still suffered death or disabling stroke within 2 years. Nonetheless these studies laid the path for its widespread take up for BTT and (where funded) for DT indications, and the abandonment of first generation technology. At the time of writing, the HeartMate II remains the most frequently implanted implantable LVAD worldwide (>14,000 separate implants), though recent concerns about an abrupt increase in the rate of device thrombosis are still to be elucidated in detail[33].

Continuous flow devices are now first choice in most situations, and a third generation of VAD has introduced the centrifugal and magnetically levitated impeller. The Heartware HVAD was revolutionary for its intrapericardial placement and its use of hydrodynamic and electromagnetic forces combined to stabilise and rotate the impeller, obviating the need for any mechanical bearings[34]. An initial evaluation reported in 2011 studied NYHA class IV transplant candidates and showed 84%/79% actual survival at 1 year/2 years with a rate of bleeding (20%) lower than previous devices, reflecting the lesser magnitude of surgery, but a similar incidence of stroke (12%)[35]. The subsequent ADVANCE trial compared 140 HVAD implants to 499 contemporaneous implants for BTT as controls[36]. The composite survival outcome was achieved in 92% of HVAD patients versus 90% of controls demonstrating noninferiority of HVAD compared to contemporary therapy ($p<0.001$; 15% noninferiority margin). The two important caveats are firstly the persistence of high rates of adverse events

such as pump thrombosis[37]; and secondly the mismatch of INTERMACS profiles between study groups. Nonetheless the HVAD is already first line therapy in many European centres, and has recently received approval for use in the United States for BTT.

The concepts of centrifugal impeller and magnetic levitation have now been adopted by Thoratec (now subsumed into St Jude Medical) for the HeartMate 3, in part as response to the unwanted focus on HeartMate II and pump thrombosis[33,38,39]. The device remains in phase II evaluation. An initial single arm study in Europe enrolled 50 patients who were all treated with the HeartMate 3 (82% in INTERMACS levels 2-3 and 54/46% BTT/DT indication) with 44 (88%) continuing LVAD support at 6 months and 2 having undergone transplantation at 50 and 132 days postimplant[40]. In this study, 6 patients (12%) suffered with stroke and 19 (38%) with significant bleeding, though there were no episodes of pump malfunction including thrombosis. Notably in addition to the 12% adjudicated as stroke (with Rankin score >3), there were another 4 patients (8%) with other neurological dysfunction including transient ischaemic attack (TIA) which are likely to be device related. Subsequently, the MOMENTUM-3 study randomised 294 patients 1:1 to HeartMate II or HeartMate 3 in 69 centres in the USA[41]. The composite primary outcome measured at 6 months comprised survival free of disabling stroke, or survival free of reoperation to replace or remove the device. This was met by 86% of HeartMate 3 recipients and 77% of HeartMate II recipients, meeting the thresholds for non-inferiority (absolute difference 9.4%, 95% CI -2.1) and superiority (HR 0.55 (0.32-0.95), p=0.04). However, the primary endpoint success was powered by a large difference in need for reoperation, mainly due to suspected or confirmed pump thrombosis (1 patient for HeartMate 3 vs. 11 patients for HeartMate II (HR 0.08 (0.01-0.60), p=0.002), and actually more disabling strokes occurred in the HeartMate 3 group, though this was statistically comparable (6 patients vs. 4 patients, HR 1.31 (0.37-4.64), p=0.59). Furthermore, there was again separation in analysis of stroke from TIAs, so the rate of cerebrovascular complication may be even higher. Nonetheless the device shows promise, though given the ongoing challenges with HeartMate II around pump thrombosis, and the device's role in powering the outcome of this St Jude-funded study, perhaps the most useful trial would be a comparison of the HeartMate 3 with its direct centrifugal competitor, the HVAD.

1.2.3 Other implantable support systems

There are other modalities of support available aside from standard left ventricular support. Right sided VADs (RVADs) can be used in concert with an LVAD, or alone, in the latter case normally confined to short term use only. This is due to various factors: in post-cardiotomy RV failure, function will usually recover over days to weeks, making implantation of a long term device unnecessary; and there is difficulty balancing between high RVAD flows that can lead to overload of native LV function and low RVAD flows that increase risk of VAD thrombosis. Existing devices are being studied for potential isolated RV use, and these might have a role in post-cardiotomy and adult congenital heart disease populations. The total artificial heart (TAH) is indicated in severe biventricular failure. The failing native heart is explanted, with the TAH implanted in its place. The SynCardia TAH is the only device in clinical use, and comprises two pulsatile pneumatic pumps and four valves. Patients require anticoagulation. More than 1000 patients have been implanted worldwide, with 3.75 years as maximal duration of support and 70-80% surviving to transplantation[42]. Patients in this study were mostly (94%) INTERMACS profile 1, but excluded those with severe pulmonary hypertension. The advantages of TAH are relinquishing the difficulties of BiVAD support (such as matching pump flows), removing risk of arrhythmia or problems with worsening ventricular function, and reducing the occurrence of stroke (reported <2% during device support). Limitations to its dissemination are high cost and poor availability of portable drivers, although the new freedom driver has improved this. Furthermore, in the event of pump failure there will be complete circulatory arrest, with only immediate resolution likely to sustain life.

1.2.4 Reverse remodelling and cardiac recovery

In some patients treated with MCS, heart function improves by reverse remodelling, and if there is significant and sustained reverse remodelling then this can be termed cardiac recovery. Similar phenomena are observed in other situations, whether it be spontaneous (e.g. recovery of myocardial function after viral myocarditis or cytotoxic chemotherapy) or with specific intervention (e.g. implantation of CRT). There has been considerable interest in the possibility of harnessing MCS for use as a tool for ‘bridge to recovery’.

The mechanisms underlying recovery are complex and variably understood. The key therapeutic tool is the optimised cardiac haemodynamics and systemic circulation. Improved blood flow interrupts the processes driving the HF syndrome. There is ventricular pressure and volume unloading, with reduction in chamber size[43] and subsequent improvement in left atrial and pulmonary pressures[44]. The reduced wall stress improves subendocardial perfusion and optimises cardiomyocyte energetics. There are beneficial changes in the systemic milieu which directly follow the improved peripheral organ perfusion (e.g. reduced sympathetic activation, reduced levels of circulating renin and aldosterone[45]) and reduce the burden of adverse homeostasis.

Together, these systemic changes allow beneficial changes in cardiomyocytes and the extra-cellular matrix (ECM). Cellular changes include regression of left ventricular myocyte hypertrophy[46], upregulated expression of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA-2a) calcium pump[47] and remodelling of the t-tubule system to improve efficiency of calcium-induced calcium release[48], alterations in gene expression to a profile similar to that seen in foetal hearts. The degree of hypertrophy regression may increase with duration of LVAD support[49]. The effect of LVAD support on cellular apoptosis is less clear, with some data suggesting increased cell survival with reduced apoptosis[50], but others showing increases in mitochondrial stimulant for cell death[51]. There is variable data available on change within the ECM with LVAD support[52,53].

In concert with HF medical and device therapy these changes can drive improvements in overall myocardial function. There is a spectrum of response, with some patients showing continued decline, some patients some functional improvement. The distinct entity of myocardial recovery represents an end-point of this process not always attained, where cessation of mechanical support and return to NYHA class I symptoms is possible.

The incidence of cardiac recovery is disputed. Amongst the first trials of LVAD therapy for myocardial recovery was the demonstration of clear and sustained improvement in the first 15 patients to complete the Harefield protocol, which allied LVAD haemodynamic support with staged uptitration of heart failure drugs followed by initiation of the β_2 adrenergic

receptor agonist clenbuterol[54]. Of these 15 patients with non-ischaemic cardiomyopathy receiving pulsatile support from the HeartMate I, 11 (73%) showed sustained improvement in parameters obtained during pump 'turn-down' studies and had their device explanted. From the 11, 1 died early postoperatively, and in the remaining 10 all but 1 showed sustained improvement throughout 4 years of follow-up. The HARPS-US study was planned to use the Harefield protocol in centres in the USA to reproduce these findings, but was abandoned when the HeartMate II superseded the pulsatile technology. A follow-up study from Harefield using continuous flow HeartMate II recruited 20 patients, of whom 12 met criteria for recovery and 10 survived free of HF recurrence at 430.7±337.1 days (range 56 to 1112 days)[55]. A criticism of both studies is inclusion of several young patients with recent HF symptom onset, on the basis that these patients might have recovered anyway. Indeed, other studies have not reliably reproduced these outcomes, finding recovery in around 5-10% of patients[56], though this may be higher with an a priori recovery intent and higher in patients on HF drugs[57]. Outside clinical trials the rate is lower again (1-2% in INTERMACS Registry data, though this includes many centres who do not systematically look for it[25]). It seems likely that spontaneous recovery rates are low, but with a combination of optimised LVAD unloading with optimised medical therapy, alongside an active programme of monitoring for recovering function, higher rates of recovery are achievable[58].

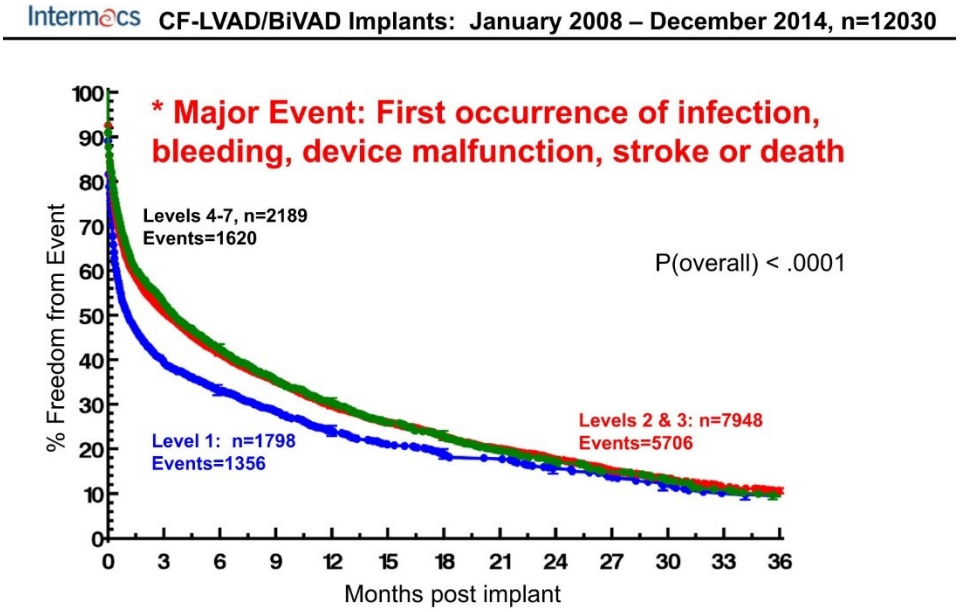
1.2.5 Current clinical use and unmet needs

The most recent INTERMACS report of new MCS implants in the USA provides a present context. In 2012-2014, 80% of new implants were for patients in INTERMACS levels 1-3 and >90% of new MCS implants were continuous flow LVADs. Of these, around two-thirds were HeartMate II and around one-third were HVAD[25], both devices providing sufficient additional blood flow to supplement cardiac output by 3-4 litres per minute and completely supplant the work of the failing LV. The adverse event rate overall for 2012-2014 was reduced from the previous period (2008-2011, all continuous flow implants), with reductions particularly in rates of infection and bleeding, but notably an increase in stroke rates, and with total burden of adverse events still 29 events per 100 patient years. At 1 year after implantation less than 40% of patients remain free of infection, bleeding, device malfunction, stroke or death (see Figure 1.1). Despite the dramatic increases in new implant numbers, evidently the use of MCS is still restricted to the sickest patients. This data likely to be

comparable in most respects to the UK, except in respect of DT. In 2014, 46% of new implants in the USA were for an intended DT indication, and in practice in the UK MCS is still restricted to BTT, or in some cases bridge to decision, indications.

Figure 1.1: Freedom from infection, bleeding, device malfunction, stroke or death in the Seventh INTERMACS Report, stratified by INTERMACS level at implantation.

At 1 year after implantation, less than 40% of patients remain free of infection, bleeding, device malfunction, stroke or death. Reproduced with permission[25]. (CF-LVAD, continuous flow left ventricular assist device; BiVAD, biventricular assist device.)



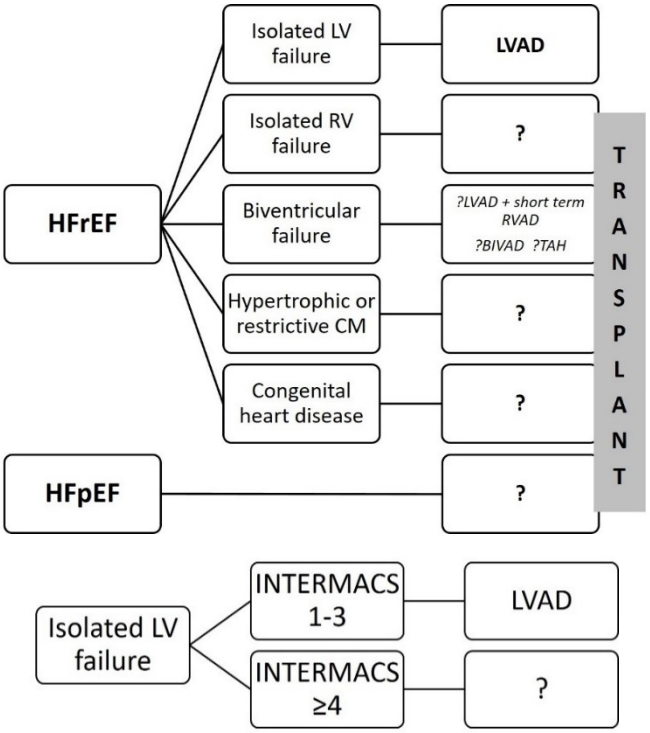
This leaves the sickest HF patients with isolated LV failure increasingly well served by current technology. However, large groups of HF patients who are on maximal tolerated medical and device therapy there are not suitable for LVAD treatment (see Figure 1.2). These groups or factors include:

1. INTERMACS levels 4-7 with LV failure. These are ambulant patients, on maximal therapy, but persistently symptomatic and declining. At present the burden of morbidity and mortality from LVAD implantation is too great to justify implantation of full support LVAD, despite burden of symptoms and quality of life impairment. The REVIVE-IT study intended to evaluate the HeartMate II in this group of patients, but was abandoned in development after the recent problems with thrombosis in that device[59].

2. Biventricular and isolated right heart failure. Increasing cardiac output with an LVAD requires the RV to show a matched increase in output due to increased venous return, and pre-existing RV failure is associated with high mortality after LVAD implantation. If present, the options are to plan for concurrent LVAD and RVAD implantation (either as a long term BiVAD system, or implantable LVAD with a short-term RVAD), total artificial heart, or cardiac transplantation. In borderline cases, pulmonary vasodilators (e.g. inhaled nitric oxide) combined with inotropes may augment native RV function sufficiently for the perioperative phase.
3. Disease aetiology. Patients with restrictive or hypertrophic cardiomyopathy can be challenging, as the disease typically involve both ventricles, and thick ventricular walls leave a small LV cavity and make LVAD implantation challenging. These patients have higher rates of RV failure post-operatively. There is little experience of MCS in patients with HFpEF, and congenital heart disease is difficult to treat with MCS.
4. Cardiac valve disease, in particular aortic valve. LVAD therapy creates a large pressure gradient from aorta to LV, transmitted across the aortic valve. This can lead to aortic regurgitation (AR), which decreases the efficacy of support and longevity of the device. All LVAD patients are at risk of acquired AR (and up to 50% will have moderate to severe AI at 18 months[60]), but implantation with existing AR is self-defeating. In these cases, the valve should be treated at the time of LVAD implantation (e.g. using central aortic valve closure[61] or a biological aortic prosthesis). Tricuspid valve incompetence may warrant concomitant repair. Mitral regurgitation is often functional and improves with LV volume unloading. Valve prostheses in the mitral position carry a theoretical risk of thromboembolism and, for biologic prostheses, deterioration of the leaflets. There is little data available, but an existing mitral valve prosthesis is usually considered a relative contraindication to LVAD therapy.
5. Other factors, including anaesthetic (e.g. lung/renal co-morbidities), surgical factors (e.g. previous sternotomy) and psychosocial (e.g. unlikely to adhere with VAD management).

Figure 1.2: Options in mechanical circulatory support for chronic heart failure.

(HFrEF/HFpEF, heart failure with reduced/preserved ejection fraction; LVAD/RVAD/BiVAD, left/right/bi ventricular assist device; CM, cardiomyopathy; INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support.)



1.3 General Aims of the Thesis

With this background, the general aims of this thesis are to address a trio of novel approaches in long term MCS for advanced HF. Firstly, I consider partial LV support as a novel approach in MCS, and consider its potential to facilitate reverse remodelling and limitations of acquired coagulopathy. Secondly, I consider cardiac gene therapy as a novel adjunctive therapy which, when combined with MCS, could facilitate reverse remodelling. Thirdly, I consider two novel approaches to the assessment of reverse remodelling.

1.4 Novel devices: the concept of partial LV support

The LVADs discussed so far are termed ‘full support’ devices, capable of completely replacing the work of the failing heart. Two novel devices have been developed with a goal to provide ‘partial’ LV support, truly ‘assisting’ the LV rather than supplanting its function, and reducing the complexity and complication of implant to justify their use in the less critical

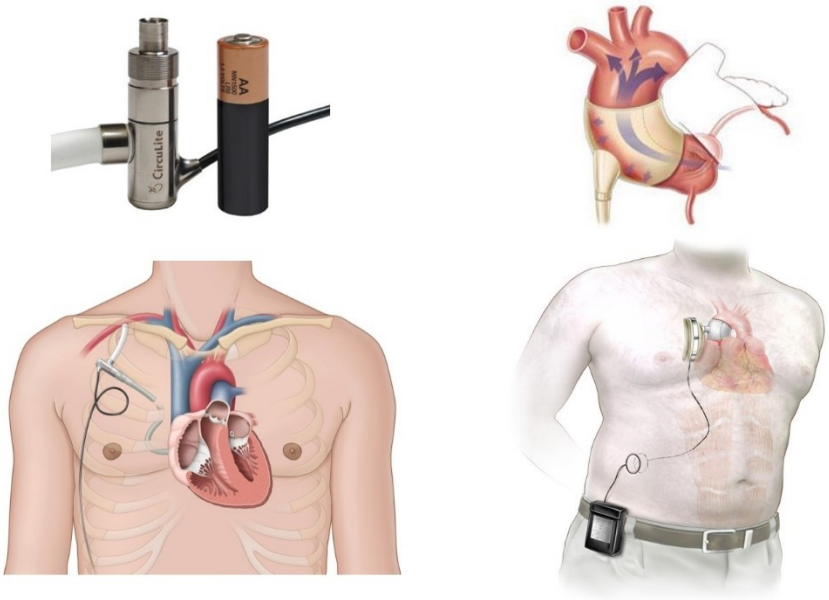
population. This approach might improve the patient's functional status, allow higher tolerance of disease-modifying HF drugs, and potentially facilitate improvement in cardiac function.

The first of these is the CircuLite Synergy LVAD, which was the first to adopt the partial support concept, drawing blood from the left atrium (LA) to a small, AA-battery sized pump unit placed in a pacemaker-like pocket under the right clavicle and discharging to the right subclavian artery[62] (Figure 1.3). It required neither sternotomy nor cardiopulmonary bypass for insertion, and was intended for exactly these ambulatory patients whose symptoms, function and quality of life are declining despite best practice medical therapy, with its low perioperative morbidity intended to adjust the balance of risk versus benefit in favour of early intervention. The actual implant was done under general anaesthesia via a right thoracotomy through which the pericardium was accessed and the pump inflow cannula passed into the LA between the upper and lower right pulmonary veins using a modified-Seldinger technique. Thereafter the inflow cannula was tunnelled through the second intercostal space to a subclavicular pocket housing the pump, and the outflow cannula anastomosed to the right subclavian artery. The device received MHRA approval in January 2012 and CE Mark in September 2012, and was adopted by several centres including Harefield Hospital.

Early results were promising, with significant improvement in cardiac index (2.0±0.4 L/min/m^2 up to 2.8±0.6 L/min/m^2; $p<0.001$) and pulmonary capillary wedge pressure (28±6 mmHg down to 18±7 mmHg; $p=0.002$) in the first 27 patients reported, 93% of whom were in INTERMACS level 4[63,64]. Although adverse events were comparable and possibly less than standard LVAD implantation, there were early problems with high rates of pump thrombosis in early iterations of the device. These were partially resolved with adjustments to the pump and its washing algorithm, but ultimately contributed to the Synergy implants being suspended in 2014.

Figure 1.3: Synergy and C-Pulse partial support devices.

Left: the Synergy device is the size of an AA battery. The inflow cannula inserted to the left atrium via right minithoracotomy and the outflow graft anastomosed to the right subclavian artery. There is no requirement for sternotomy or cardiopulmonary bypass. Right: Sunshine Heart C-Pulse, with extra-aortic cuff. The device is placed via sternotomy. Permission requested.



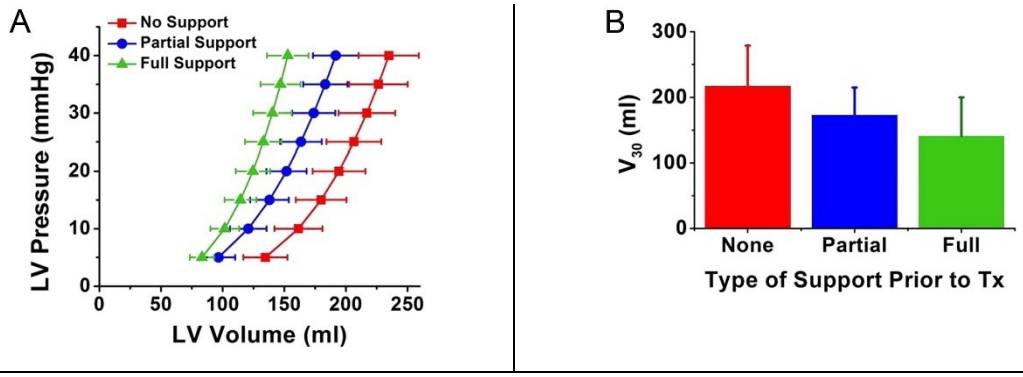
The second device was the Sunshine Heart C-Pulse, also providing partial LV assistance but using an extravascular cuff for aortic counterpulsation (Figure 1.3). Cuff inflation in early diastole increases aortic pressure and improves coronary perfusion, while cuff deflation in early systole causes relative reduction in afterload helping to reduce end diastolic pressure, wall stretch and ventricular workload[65,66]. The device is placed at sternotomy where an inflatable cuff approximately 10cm in length is wrapped around the ascending aorta, with an LV sense lead implanted to the LV epicardium. The LV sense lead is passed to the abdominal wall, alongside a large gas lead required for pneumatic cuff inflation. The procedure is short, patients are extubated immediately afterwards, and recovery is rapid. The cuff timings can be adjusted to individualise treatment, and the device can be switched off if needed. Histological studies suggested no significant injury to the aorta during support[67]. Initial studies showed moderate improvements in NYHA class and symptom scoring with small increases in 6 minute walk distance, though reduction in peak oxygen consumption and important concerns regarding mediastinal and device infection[68,69]. Like the Synergy, these problems with infection were addressed, but ultimately caused suspension of new implants and interruption of its evaluation in an international study.

1.4.1 Partial support to facilitate reverse remodelling

Partial support allied with best medical and device therapy, and potentially other adjuncts such as cardiac gene therapy, could be a new paradigm for cardiac recovery programmes. Potential advantages over full support LVADs are (1) less invasive implant and consequently an ability to promote recovery in patients earlier in their disease, when their disease may be more readily reversed; (2) easier to promote continued LV work with persistence of AV opening, to prevent myocardial atrophy; and (3) easier explant procedure in the setting of subsequent recovery[70]. This theoretical story has been supported by a study in sheep[71] and by an ex vivo study in human patients[72]. The latter studied structural remodelling in humans receiving either full support from the HeartMate II (n=15), partial support from the Synergy (n=5) or no LVAD (n=7). Hearts were examined ex vivo at the time of transplant with incremental saline infusions into an intraventricular balloon to determine the passive pressure-volume relation, indexed with V30 defined as the volume at which the intraventricular pressure was 30mmHg. The authors demonstrated mechanical support induced structural reverse remodelling as indexed by a leftward shift of the ventricular passive pressure volume relation, with the degree of remodelling related to the degree of support provided by the device (no support V30=217.5±61.7ml; partial support V30=173.1±42.7ml; full support V30=141.6±59.0ml; p=0.01 for full versus no support; see Figure 1.4). This was paralleled by B-type natriuretic peptide (BNP) measurements taken at the time of transplant (no support 3875±3248 ng/L, partial support 2201±1470ng/L, full support 1425±1030ng/L; p<0.05 for full versus no support). This pilot data instructs a key hypothesis, that partial LV support can facilitate reverse remodelling of the LV in vivo.

Figure 1.4: Passive pressure-volume relation with differing degrees of LV support.

See text for details. Reproduced with permission[72].



1.4.2 Acquired coagulopathy with assist devices

Amongst the most devastating complications of LVAD therapy are stroke, pump thrombosis and major bleeding, and overcoming these will be a key to opening partial support to wider patient groups. These challenges are common to all extracorporeal circuits and manifest as a dichotomous combination of bleeding and thrombosis, with the propensity of nonbiological surfaces to initiate platelet activation and coagulation mandating systemic anticoagulation in all patients. There are three key clinical consequences. The first and most common is non-surgical bleeding, often early post-implant but remaining a persistent risk and exacerbated by the necessity for anticoagulation. In the Slaughter trial, 81% of the HeartMate II patients required red cell transfusion for bleeding[32]. The second is pump thrombosis, which can precipitate pump stoppage. The third is stroke, either ischaemic from thromboembolism or haemorrhagic due to intracranial haemorrhage. In the HVAD trial 6 patients (12%) suffered ischaemic or haemorrhagic stroke follow-up, of whom 3 patients (50%) died[35]. Alongside infection, these three represent the most feared complications of LVAD therapy.

1.4.2.1 Bleeding and acquired von Willebrand factor deficiency

Non-surgical bleeding with extracorporeal circuits is multifactorial with underling heart failure, liver congestion, poor bone marrow perfusion and consumptive coagulopathy all involved, but particularly important is the syndrome of acquired von Willebrand factor (vWF) deficiency. vWF is a glycoprotein secreted by vascular endothelial cells and megakaryocytes with essential roles in initiating and controlling coagulation and platelet activation, adhesion and aggregation in response to damage to the vessel wall. During biosynthesis, its ≈250kDa subunits are assembled to form large molecules ranging in size from 500kDa to 20,000kDa. These large multimers are broken down in the circulation by proteases including ADAMTS13 and thrombospondin-1 into smaller multimers of differing sizes which have differing effects on propensity for coagulation, with larger multimers having the more potent effect.

Because of its large molecular size, vWF is susceptible to shear stress, which can disrupt the molecule's three-dimensional conformational structure thereby rendering binding sites for proteolysis more accessible and increasing the number of smaller vWF multimers.

Specifically, unfolding of A2 domains leads to greater ADAMTS13 activity and a loss of large vWF multimers[73]. In addition, the shear stress triggers vWF to autoassociate with platelets[74]. Zieger and colleagues compared LVAD patients with matched patients undergoing cardiac transplant and found that 100% the LVAD group (n=10) had loss of large vWF multimers compared to 17% in the transplant group (n=6)[75]. The same group went on to demonstrate that the vWF deficiency occurs early after LVAD implant, with all patients in the cohort studied affected by day 3; and, furthermore, that it is quickly reversible on explant[76,77]. Similar degrees of vWF multimer loss were seen in HeartMate II and HVAD patients, and pump speed does not seem to affect degree of vWF deficiency[78]. A small pilot study comparing acquired vWF deficiency in HeartMate II, Synergy and cardiac transplant patients confirmed the loss of large vWF multimers in Synergy patients but showed no difference from the HeartMate II cohort, except in parameters that could reflect post-operative stress[79].

Other mechanisms may contribute to the bleeding tendency, potentially related to the change from pulsatile to continuous flow and the formation of arteriovenous malformations (AVMs) in the gastrointestinal (GI) tract and cerebral vessels. AVMs may be triggered directly by thrombin generation and upregulation of angiopoietin-2 causing neoangiogenesis and resulting in intracerebral and GI bleeding[80].

1.4.2.2 Propensity to thrombosis

A multitude of factors determine the propensity to thrombus formation in an LVAD patient, but ultimately it depends on Virchow's triad of hypercoagulability, blood stasis and endothelial injury. Advanced HF patients are already hypercoagulable[81], and many will have atherosclerotic disease and be more prone to endothelial injury or have co-morbidities that increase blood stasis such as atrial fibrillation. Particular factors related to the device are (1) the interaction of the LVAD with blood components, as well as creating a bleeding tendency, causes platelet activation and impairment of fibrinolysis that induces additional hypercoagulability; (2) any blood stasis, or areas of slow flow, within the prosthetic blood path will become foci for thrombosis; and (3) the thrombogenic pump materials act to activate coagulation cascades in the same way as endothelial injury, and furthermore the continuous flow devices and lack of pulsatility may induce endothelial injury de novo.

The pump and patient work in continuum and it's difficult to differentiate patient- from pump-related factors. Blood flow characteristics are important and depend on patient-specific parameters such as degree of residual LV function, systemic blood pressure, and how the consequent delta pressure across the pump affects flow, as well as engineering within the pump. A patient with a recovering ventricle and high LV pressures, good systemic blood pressure, and hence little flow across the pump is high risk for thrombosis to due reductions in pump flow. Specific mechanical factors include impeller rotation speed (inadequate speed can mean low flow and blood stasis), heat generation within the pump[82], the degree of shear stress caused by the pump, thrombogenicity of pump materials[83] (overcome, for example, by heparin coating), and exposure time to pump components (again, closely related to pump flow quality and quantity).

There is evidence that the LVAD acts on platelets and fibrinolytic systems to induce a systemic hypercoagulable state. Platelet function in the context of HF and LVAD support is complex. Platelet aggregation is thought to be exaggerated in HF without LVAD, but other studies show impaired platelet function in HF[84] and functional platelet aggregation to arachidonic acid (ASPItest) on the MultiPlate analyser was reduced in HF patients versus controls in one recent study[85]. With LVAD support, shear stress causes platelet activation via a vWF-dependent process[74] and the number of circulating activated platelets is increased as assessed by flow-cytometric markers[86]. A mock circulation study evaluated platelet activation at different mechanical shear conditions and found that increasing the mechanical stress caused greater platelet activation (as indicated by beta-thromboglobulin and platelet factor-4 [PF4] release)[87]. However, another in vivo MultiPlate aggregometry study found impaired platelet function, and the authors suggested this might reflect chronic activation, platelet dysfunction related to vWF deficiency, and effects of antiplatelet drugs[88]. While activated platelets initiate thrombus formation, fibrin deposition is a key secondary step and is under normal conditions tightly regulated by a balance of pro- and anticoagulant processes. These are deranged during LVAD support, with enhanced thrombin generation[89] and coagulation systems most exaggerated during the first few months of therapy and then returning to baseline levels[90,91].

Pump thrombosis was a key determinant in the demise of the Synergy LVAD in its current design, and this is explored further in Chapter 3. The question arises whether the unique design of the Synergy in some way enhances this systemic hypercoagulability, and whether the extravascular position of the C-Pulse mitigates this, and this is the focus of Chapter 4.

1.5 Novel therapeutics: cardiac gene therapy

1.5.1 Introduction

The HF syndrome is a progressive dysregulation of cardiovascular function, usually initiated by a specific insult, facilitated by an individual's inherited susceptibilities, and perpetuated by maladaptive neurohormonal responses to the failing homeostasis particularly by the sympathetic nervous system and renin-angiotensin system. This dysregulation occurs at all levels from systems physiology, organ and tissue maladaptive remodelling, cellular dysfunction and ultimately dysregulation of cardiac gene expression. Extensive cell biology studies over the last thirty years have identified several proteins which appear central. In the search for new HF therapeutics to combat the growing HF epidemic[92], gene therapy could target these alterations to gene expression and return the proteome to its healthy composition, helping to regain a normal physiological milieu. But the challenge is which gene expression to select and how to deliver it to the diseased human heart in a manner which increases the levels of functional target protein to modify the underlying disease effectively.

1.5.2 Calcium as a therapeutic target

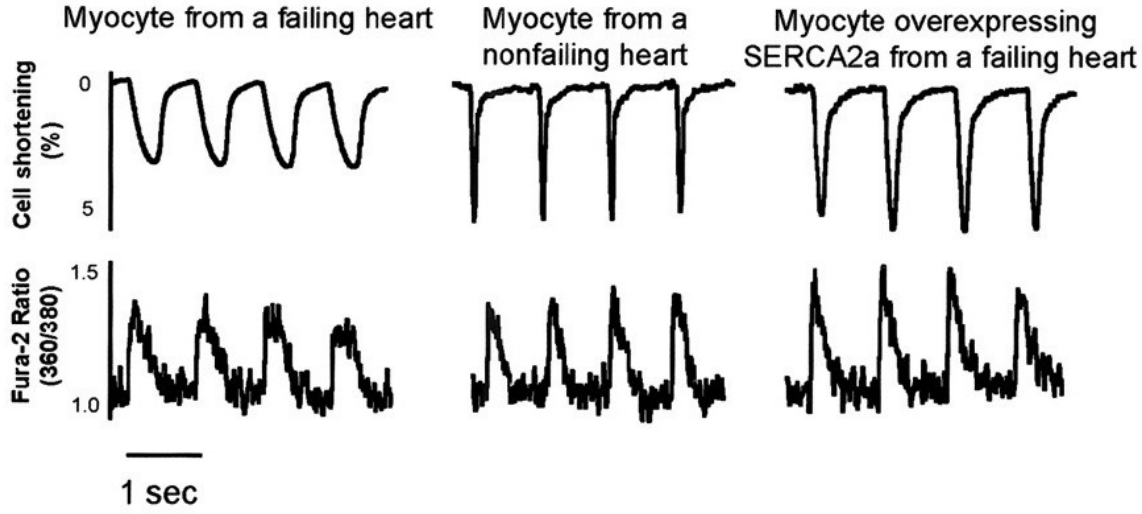
Calcium is central in excitation-contraction coupling, cellular electrophysiology, signalling and even gene expression itself. At the cellular level, the final common pathway of all HF syndromes involves malfunction of calcium handling and in particular reduced expression and activity of cardiac sarco-(endo)-plasmic reticulum calcium ATPase 2a transporter protein (SERCA; the SERCA2a isoform is predominant in cardiomyocyte SR)[93]. In healthy cardiomyocytes, depolarisation of the cardiomyocyte triggers cardiac systole by opening of voltage-gated (L-type) calcium channels and calcium influx to the cytosol. This in turn triggers opening of ryanodine receptors (RyR) in the sarcoplasmic reticulum (SR), and a much larger calcium flux from the SR into the cytosol which initiates cardiomyocyte contraction[94]. The speed and magnitude of the calcium flux determines the rate and force of

contraction. Restoration of cytosolic calcium to baseline levels occurs in cardiac diastole through a combination of active transport from cytosol back to the SR and into the extracellular space, both against Ca^{2+} concentration gradients. The former occurs by action of SERCA in the SR membrane, and the latter occurs via the Na^{+}/Ca^{2+} exchanger in the cell membrane (NCX).

Calcium reuptake to the SR is under tight physiological control to facilitate adjustments to inotropy, chronotropy and relaxation kinetics, in particular via changes to the phosphorylation status of the protein phospholamban (PLB) which associates with SERCA2a and modulates its function[95]. SR Ca^{2+} reuptake is also regulated by post-transcriptional regulation of component gene expression (e.g. by non-coding RNAs such as microRNA-25[96]), by post-translational modification to SERCA2a (e.g. by the small ubiquitin-like modifier (SUMO) proteins[97]), by protein-protein interaction (e.g. with S100A1 which regulates activity of SERCA2a and RyR[98]) and redox regulation. However, in the failing cardiomyocyte a combination of factors reduce SERCA2a activity and impairment of cytosolic calcium clearance. This leads to: (a) increased end-diastolic cytosolic calcium, causing impaired cardiomyocyte relaxation with slower rates of relaxation and stiffness; (b) depletion of SR calcium stores, causing reduced systolic RyR calcium flux and systolic impairment; and (c) compensatory mechanisms including RyR leak and upregulation of NCX that cause electrical instability and greater propensity to ventricular arrhythmia. This combination is evident in isolated cardiomyocytes as contraction of lesser amplitude and slower onset[99] (Figure 1.5). Restoring activity of SERCA2a might be one strategy to augment function in the failing cardiomyocyte. This could be achieved by (a) directly increasing expression of SERCA2a, or (b) by enhancing SERCA2a activity for example by increasing expression of factors that enhance its activity (e.g. the SUMO proteins), or by removing factors that inhibit its function (e.g. unphosphorylated state PLN). Translational gene therapy programmes in HF to date have focussed on approaches to directly increase SERCA2a levels.

Figure 1.5: Abnormal contraction profiles and calcium transients are corrected after SERCA2a gene transfer.

Human cardiomyocytes from failing (left, right) and non-failing (centre) hearts are obtained and studied in isolation. Failing cardiomyocytes show reduced velocity and amplitude of cell shortening compared to nonfailing cardiomyocytes, associated with a prolonged calcium transient. Isolated cells were treated with an experimental adenovirus containing the SERCA2a transgene, and twenty four hours after infection showed normalisation of contractile properties and calcium transient duration. Fura-2 ratio is a measure of intracellular calcium. Reproduced with permission[99].



1.5.3 Clinical trials with AAV1.SERCA2a

The laboratory data supporting strategies to directly increase SERCA2a expression have been extensively reviewed elsewhere[94,100]. In brief, studies in isolated human cardiomyocytes and animal models using viral vector transduction with the SERCA2a transgene show normalisation of calcium fluxes, correction of cell contractile properties and improvements to overall cardiac function with reduction in left ventricular (LV) volumes[99,101,102] (Figure 1.5). Furthermore, in contrast to other positive inotropes which are typically proarrhythmic, preclinical studies from our lab and others demonstrate that SERCA2a gene therapy shows improved myocyte energetics and reduction in propensity to ventricular arrhythmia[103]. Multiple mechanisms may explain the antiarrhythmic effect observed[94].

1.5.3.1 Vector choice

To achieve target protein (e.g. SERCA2a) expression at levels to influence myocardial biology and organ function, the transgene must reach the myocardium, bind to and enter individual cardiomyocytes, be trafficked to the cell nucleus, and be recognised by the host transcriptional complexes. Viruses are a natural example of these biological steps being achieved in an efficient manner, and viral vectors remain the most commonly used vector above non-viral alternatives such as microparticles (which have to be engineered to enter cells and cooperate with translation) or use of naked plasmid DNA, which, although non-pathogenic and mildly immunogenic, is largely destroyed by lytic enzymes before reaching the cell nucleus leading to low levels of efficiency[104]. Several viruses carry pre-evolved predilection to specific cell types, which can facilitate tissue targeting despite a straightforward systemic administration. Adeno-associated viruses (AAVs), adenovirus, lentiviruses and retroviruses have all been studied as candidate vectors.

Disadvantages of viruses are prominently related to their immunogenicity. Recognition of viral antigens potentially stimulates both antibody and cell mediated responses, which clinically presents in a wide spectrum from asymptomatic seroconversion to a severe systemic inflammatory response. In an early dose-escalation study for ornithine transcarbamylase (OTC) deficiency which used an adenovirus vector, one subject receiving the highest dose developed a severe inflammatory response which led to multi-organ failure and death[105]. This reflects the immunogenic effects of adenovirus as a common pathogen. Moreover, while viruses can have tropism for specific organs or cells and can be physically delivered to specific body compartments, off-target transfection of other tissues is common and has the potential to provoke inadvertent, off-target side effects. Aside from the deleterious systemic effects of an acute immune response, the genesis of anti-capsid antibodies and specific memory T cells may prevent the therapeutic virus transducing the target cell, thus limiting its effects both in subjects who have previously received the recombinant virus, and in subjects who have previously encountered a wild type virus displaying similar capsid antigens[106]. This has important implications for retreatment if required in individuals, and for potential benefit in populations with high rates of existing immunity. In general, adenoviruses have shown greater immunogenicity than other candidate vectors, in particular compared with the AAV which is non-pathogenic.

Another concern with viral vectors is potential for insertional mutagenesis resulting from recombination of the transgene with the host genome, and the risk of initiating cancer[107]. Again, clinical trials yield direct experience. Trials of a retroviral gene transfer for severe combined immunodeficiency (SCID-X1) showed initial promise but were halted by emergence of clonal T proliferation in two patients at 3 years after transfection, occurring due to transgene integration near an oncogenic promoter region[108]. This is a major concern for integrating vectors including retroviruses and some lentiviruses[104].

Despite these specific limitations, viruses are currently the best vector for administration of gene therapy. Historically the adenovirus has been favoured and is still efficient as a laboratory tool, but more recently the adeno-associated virus has (AAV) has come to the fore. AAV is a small (25nm, 4.7kb), non-enveloped virus from the Parvovirus family[104]. Twelve human serotypes have been identified (AAV 1-12). The specific characteristics which make AAV preferable in cardiac gene therapy are (1) low pathogenicity: AAV does not cause recognised disease in humans, and requires co-infection with an adenovirus or herpesvirus to replicate; (2) AAV elicits only a mild immune response which seems to be predominantly antibody-mediated; (3) the predilection of AAV serotypes 1, 6, 8 and 9 to transduce cardiac and skeletal muscle, thus targeting the transduction to the tissue of interest; (4) wild type AAV (wtAAV) can integrate into the host genome, but the recombinant AAV (rAAV) persists in the nucleus as a stable episomal concatemer[109], with very low rates of integration greatly reducing the risk of insertional mutagenesis[110]. No rAAV-induced tumours have been identified in human studies to date[111], although a recent paper described circumstances under which integration of AAV vectors in mice could result in hepatocarcinomas[112]; (5) AAV's persistence within cardiomyocytes. Once present as an episome, the transgene remains present for the lifetime of that cell, provided they do not divide or become deactivated by cell cycle machinery. This durability has been seen in animal models and in clinical trials using an AAV vector in haemophilia patients, and is likely to be related to the low level of cardiomyocyte turnover and low levels of cell-mediated immunity to AAV infection[113]; and (6) rAAV vectors have already shown a reassuring safety profile in existing clinical trials for cystic fibrosis[114] and haemophilia-B[115].

For these reasons, the AAV was chosen for the SERCA2a gene therapy clinical trials, and for the forthcoming inhibitor-1c and S100A1 gene therapy trials. The rAAV designed for

SERCA2a gene delivery in clinical studies combines the wild type capsid sequence from AAV-1 with the inverted terminal repeat sequences from AAV-2. The human SERCA2a gene (as copy DNA) with cytomegalovirus promoter are substituted in place of the wtAAV's two open reading frame elements (the Rep and Cap genes). The lack of the wtAAV's Rep and Cap genes yields the recombinant virus replication deficient, and the AAV capsid proteins are the only potentially immunogenic antigens expressed. The AAV1.SERCA2a product was manufactured by Celladon as Mydicar™.

The initial phase 1/2a studies in humans were undertaken with industry leadership from Celladon Corporation in the two stages of the CUPID-1 trial[111,116-118]. This was followed by an industry phase 2b study (the CUPID-2 trial) studying efficacy in a larger cohort[119], with two further studies, both academic led (SERCA-LVAD and AGENT-HF) running alongside. The mode of delivery for all these studies is a single intracoronary infusion procedure for delivery of the AAV1.SERCA2a product, with concurrent glyceryltrinitrate infusion aiming to prevent coronary vasospasm and achieve coronary vasodilation[120], and planned according to individual coronary anatomy to achieve maximal delivery to the LV myocardium.

1.5.3.2 The CUPID-1 Trial (Clinicaltrials.gov identifier: NCT00454818)

This study tested the first application of gene therapy as a HF therapeutic, with a primary aim to test safety as a first in man study. It was a two-stage trial in multiple centres in the United States, covering the phase 1 and 2a stages of development. All patients in CUPID-1 had advanced HF with persistent NYHA class III symptoms despite optimised medical therapy, with LVEF <30% and maximal oxygen consumption <16 ml/kg/min. A pre-existing implantable cardioverter defibrillator (ICD) was mandated for protection against the possibility of induced arrhythmia and for monitoring arrhythmia burden. Initially patients could enrol with absent (titre <1:2) or low (titre 1:2) levels of neutralising anti-AAV antibody (NAb).

Stage 1 was an open-label, single arm dose escalation study in 4 cohorts (very low-, low-, mid- and high-dose; each n=3), and the primary aim was establishing safety. The different dosing regimens correspond to the number of rAAV viral particles infused, with very low-,

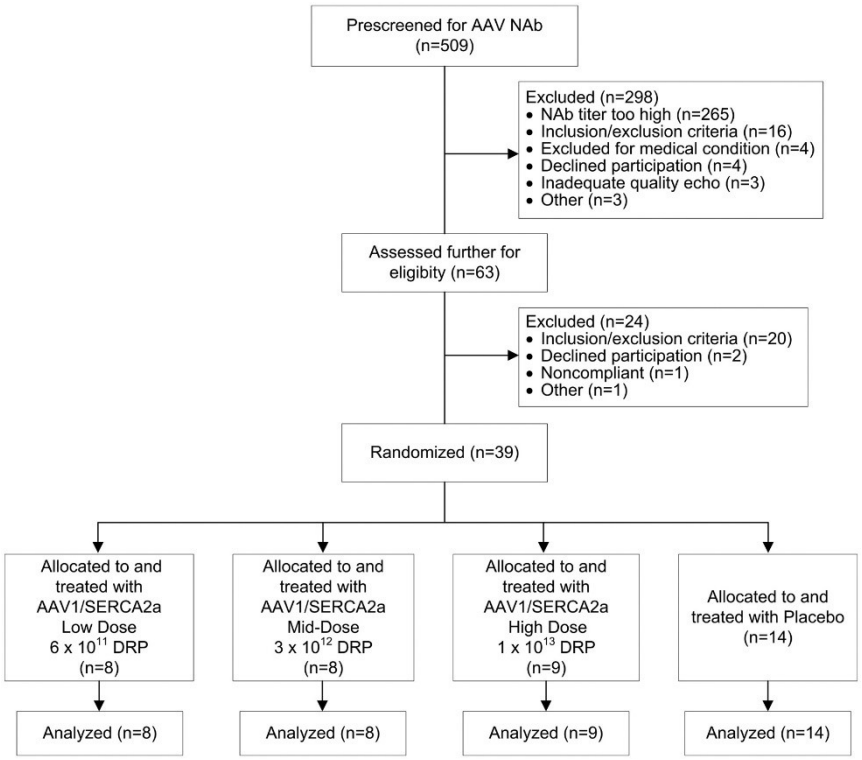
low-, mid- and high-dose regimes infusing $1.4x10^{11}$, $6x10^{11}$, $3x10^{12}$ or $1x10^{13}$ DNase-resistant particles respectively. Allocation to the high-dose group did not occur until the Data Monitoring Committee reviewed safety data from the very low-, low- and mid-dose cohorts, and this data is presented in the first report from Jaski et al[118]. This interim analysis was reassuring (there were a variety of expected adverse events (AEs) including one death adjudicated as expected and not related to the study drug) and following review by the Data Monitoring Committee authorisation was given to progress to the high-dose cohort of stage 1 and commence recruitment to stage 2[118].

This second stage was a randomised, controlled double blind study, evaluating the low-, mid- and high-dose dose cohorts and comparing to a blinded placebo control group receiving sham infusion. The LVEF criterion was relaxed to <35% (from <30%), and the maximal oxygen consumption to <20ml/kg/min (from <16%ml/kg/min). Crucially, the investigators adjusted the inclusion criteria regarding NAb status, such that henceforth only patients with absent NAb (titre <1:2) could be enrolled. This was on the basis that in stage 1, two patients had shown quickly deteriorating clinical status requiring LV assist device (LVAD) implantation and cardiac transplantation respectively, and subsequent examination of excised myocardial tissue with quantitative polymerase chain reaction (qPCR) assays had shown absence of SERCA2a transgene, suggesting neutralisation of the rAAV vector by pre-existing NAb prior to successful transduction[118]. The key result of this was a dramatic screening dropout caused by presence of NAbs. Of 509 patients screened, 265 patients (52%) were excluded due to NAbs. After other exclusions, 39 patients were enrolled in stage 2 (Figure 1.6).

The key finding was confirming safety in these small patient numbers, in that patients in all four dosing groups of the study suffered expected adverse events related to HF, without complications attributable to the gene therapy product or its administration. The occurrence of HF adverse events was lower with increasing dose of study drug, suggesting a possible dose-related efficacy. While this study was relatively small and the primary endpoint pre-specified in the methods paper was incidence and severity of adverse events[117], potential efficacy was further evaluated in the report of data from stage 2 at 12 months follow-up[116].

Figure 1.6: Screening and enrolment in the CUPID-1 trial.

For Stage 2 of this trial, the accepted titre for neutralising anti-AAV antibody was modified such that any patients with titre ≥1:2 were excluded. 52% of the 509 patients screened were ineligible on this criterion. (AAV: adeno-associated virus; NAb, neutralising anti-AAV antibodies; SERCA2a, sarcoplasmic reticulum calcium ATPase; and DRP, DNase-resistant particles.) Reproduced with permission[116].



For the efficacy analysis, the investigators studied multiple endpoints. Firstly, they studied the change at 6 months of seven markers of HF clinical efficacy by individual and by group, and a clinical outcome analysis assessed using the Kaplan-Meier approach. The seven efficacy markers are grouped into four domains of efficacy: symptomatic (NYHA class and HF questionnaire), functional (6 minute walk distance and peak oxygen consumption (peak VO_2) on cardiopulmonary exercise testing), biomarkers (natriuretic peptides) and structural (echo markers of remodelling). For the group analyses comparison of mean data was used, while a scoring system based on clinically meaningful changes was used for the individual analysis, highlighting concordant or discordant changes of the multiple variables. These parameters formed the primary efficacy endpoint success criteria, which required any of (a) improvement in at least 2 of 4 efficacy domains in the group analysis with $p<0.2$, with at least numeric superiority of active versus placebo in all domains; (b) improved score in the individual efficacy analysis with $p<0.2$; or (c) improved time to event in the Kaplan-Meier analysis. Secondly, they analysed the occurrence of recurrent clinical events as a marker of

clinical outcome, using a Joint Frailty Model (JFM) to account for factors often confounding standard outcome tests. The JFM allows the analysis to account for the number of clinical events (e.g. number of HF hospitalisations rather than time to first hospitalisation), varying severity of clinical events (e.g. cardiovascular death versus HF hospitalisation) and the effect of terminal events on risk of further clinical events (i.e. early death prevents further hospitalisations).

Using these criteria, the primary efficacy outcome was met. Patients receiving high-dose therapy showed stabilisation of their HF, compared to patients receiving placebo who showed progressive deterioration. In the individual level analysis, there was significant improvement in the individual efficacy score in high-dose versus placebo groups (which was sufficient to meet the primary outcome success in itself). In the group level analysis, there was improvement in active versus placebo groups in 3 out of 4 domains (though not exclusively in the high-dose group). The outcome endpoint was met with a significant reduction in duration of cardiovascular hospitalisations in placebo versus high-dose groups.

CUPID-1 confirmed the safety of AAV1.SERCA2a delivery in patients with advanced HF at 12 months, and this has been reiterated at 36 months in this small initial cohort[111]. This report also provided further suggestion of a persistent efficacy benefit in high-dose versus placebo groups[111], with the JFM method showing an 82% reduction in recurrent cardiovascular events adjusted for individual frailties (i.e. susceptibility to recurrent events) and correlated terminal events (HR 0.18, p=0.048). 13 of the 39 patients had died after 3 years of follow-up, none related to the gene product or its administration, and there was numerically (but not statistically) greater chance of survival in high-dose versus placebo patients[111].

There are several limitations to any efficacy conclusions from CUPID-1. Firstly, patients in the placebo group appear to have higher N-terminal pro-hormone of brain natriuretic peptide (NT-proBNP), lower LVEF, lower peak VO_2, and greater incidence of pre-existing additional cardiovascular conditions in comparison to the AAV1.SERCA2a groups (Table 1 in the main body of the Jessup et al report, and Table 10 in its Supplementary Data[116]; no statistical comparison included). This imbalance is particularly apparent in the high-dose group.

Secondly, recruitment to the high-dose group only commenced after 15 patients had already been recruited to placebo, low- and mid-dose groups. This was pre-specified safety delay due to the dose-escalation in stage 1, but high-dose patients will have been treated by operators more experienced with the procedure. Together these factors imply that any apparent clinical benefit and dose relation (i.e. added benefit of high-dose versus low- or mid-dose) may also be partly explained by baseline imbalances, procedural experience and study progression, rather than a treatment effect.

The methodology has come under some scrutiny. Proponents of the stated primary efficacy outcome would argue that its three strands (group, individual, clinical outcome) and its emphasis on concordance of efficacy outcomes provide greater specificity and allow the statistical testing to accept the less rigorous $p<0.2$ level used as the test for significance, and would argue that the JFM carries important advantages over Kaplan-Meier outcome analyses as outlined above. However detractors might comment that (1) in the symptom and functional criteria, it is the less robust measure that provides the significant result (for symptoms the subjective assessment of NYHA class is significant, but objective HF questionnaire shows no difference; in the functional testing, the submaximal 6 minute walk test shows significant change but maximal oxygen uptake as the gold standard measure shows no difference); (2) in the group level analysis, the domain success criteria generalise to active versus placebo and actually rely on the low-dose group as much as the high-dose group to meet the primary endpoint, where the authors' conclusions emphasise dose-relationship and improved efficacy in the high-dose group; (3) there is justification for using $p<0.2$ for the individual efficacy scores where there are multiple sorting criteria and statistical controls, but this is less clear for the group level analysis, where the statistical power is weaker simply due to the small number of patients.

In summary, CUPID-1 certainly provided evidence for feasibility and safety of using AAV1.SERCA2a in patients with advanced HF, and justified progression to larger clinical studies to examine the product's clinical efficacy in more detail.

1.5.3.3 The CUPID-2 (ClinicalTrials.gov identifier: NCT01643330)

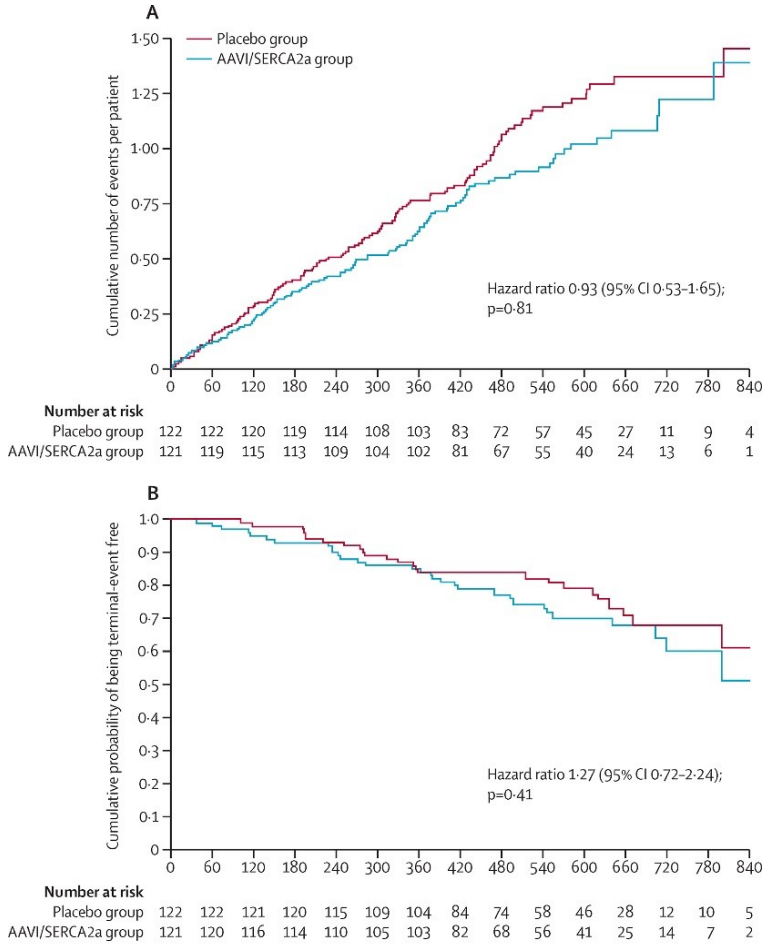
Drawing on experience in CUPID-1, the investigators set out to demonstrate clinical efficacy of AAV1.SERVCA2a in patients with advanced HF[119]. The CUPID-2 trial was a phase 2b double-blind randomised placebo-controlled trial, allocating patients 1:1 to receive placebo or high-dose AAV1.SERCA2a ($1x10^{13}$ DNase-resistant particles). All patients with NAb titres ≥1:2 were ineligible (which, similar to CUPID-1, excluded 59% (921/1558) patients initially screened). The trial used a JFM for the primary outcome to properly account for the burden of recurrent HF events, similar to CUPID-1, and there were strict HF severity criteria (LVEF<35%, hospitalisation for HF within previous 6 months, high BNP within the previous 30 days) to ensure a high rate of clinical events. The BNP criterion was added as a protocol amendment with a goal to increase the severity of HF in participants, as during the early stages of the trial there appeared to be a low rate of clinical events. Recruitment concluded in June 2014 after enrolling 250 patients in 54 centres. Altogether, 123 received AAV1.SERCA2a and 127 received placebo. Groups were well matched and baseline participants reflected a severe HF group (majority in NYHA class III, median EF 24±6.3%/23±6.5% placebo/active) who were well treated with guideline therapy. Notably more than four fifths of participants were white males, around half were ischaemic patients, and the majority were recruited in the USA.

The results from the CUPID-2 trial do not support the hypothesis that the AAV1.SERCA2a at the current dose is efficacious in patients with advanced HF on optimised medical and device therapy[121-123]. In the final modified intention to treat population, 121 patients received treatment with AAV1.SERCA2a and 122 patients received placebo (total n=243). For the primary outcome after a minimum of 12 months' follow-up there were 232 recurrent events recorded, 128 in the placebo group and 104 in the treatment group, equating to a non-significant 7% risk reduction for recurrent HF events in the presence of HF terminal events in patients treated with AAV1.SERCA2a versus placebo (HR 0.93, 95% CI 0.53, 1.65; p=0.81; Figure 1.7). Most of the recurrent events were hospital admissions (121/128 and 96/104 respectively). There was no treatment effect evident for the powered secondary endpoints ('terminal events' of all-cause death, need for a mechanical circulatory support device or heart transplant), with 27% increased risk of terminal events in the AAV1.SERCA2a group which was not significant (HR 1.27, 95% CI 0.72, 2.24), p=0.41). The exploratory efficacy

endpoints (improvement in NHYA functional class, 6 minute walk distance and quality of life by questionnaire) and pre-specified sub-group analyses all showed no significant effects. In particular, there was no sustained benefit seen in the non-ischaemic HF subgroup, patients who might have been more suitable for effective gene therapy. Importantly there were no safety concerns reported.

Figure 1.7: Primary endpoint in the CUPID-2 trial.

Kaplan-Meier curves for AAV1.SERCA2a versus placebo showing (Top) cumulative number of recurrent events per patient, and (Bottom) probability of remaining free of terminal events. Terminal events were defined as all-cause death, or need for a mechanical circulatory support device or heart transplant. Reproduced with permission[123].



1.5.3.4 Other clinical trials using AAV1.SERCA2a

Three further studies were in progress in April 2015 at the time when the initial findings from CUPID-2 were announced. However, owing to the neutral outcome, all these were closed prematurely.

Firstly, the AGENT-HF trial (Clinicaltrials.gov identifier: NCT01966887) was a single centre trial studying the reverse ventricular remodelling effects of AAV1.SERCA2a at the dose of $1x10^{13}$ DNase-resistant particles in ambulatory patients with HF[124]. The study planned to recruit 40 advanced HF patients without NAbs in a double-blind, randomised, placebo-controlled design, using the same product and method of administration as previous studies. The primary outcome was LV end-systolic volume assessed by computed tomography, and secondary outcomes included various biomarker, structural and functional tests. Before the trial was closed they had recruited 9 patients, of whom 5 were allocated to receive AAV1.SERCA2a. In this small group, the trial was negative for its primary endpoint[124] (relative change in LVESV in placebo patients -1.6% (-9.1, 11.7) versus 4.4% (3.2, 6.2) in patients receiving AAV1.SERCA2a; p=0.74). The limited recruitment led to poor matching, with important differences between study groups making findings difficult to interpret in any case (AAV1.SERCA2a patients were more likely to be ischaemic aetiology (60 vs. 25%) and more likely to be NYHA class III (100 vs. 50%) and had smaller ventricles at enrolment (LVESV 284±102ml vs. 345±131ml; no p-values for statistical difference are presented). Perhaps the most useful finding was that one patient in the study underwent cardiac transplantation at 18 months after treatment with AAV1.SERCA2a and no viral SERCA2a DNA was detected, contributing to the debate about why the CUPID-2 trial was negative (see Chapter 6 for further discussion).

Secondly, a further phase 1/phase 2 study had been initiated by Celladon Corporation in January 2015 to assess a higher dose administration of AAV1.SERCA2a (Clinicaltrials.gov identifier: NCT02346422). The study was planned in two phases similar to CUPID-1, with an initial open-label phase followed by a phase of randomised, double blind allocation. The "high dose" in the CUPID-1 and -2 studies was $1x10^{13}$ DNase-resistant particles, and dosing here was planned to be 2.5-fold greater. The rationale was based on the observed dose-response in CUPID-1, such that treatment effect could be multiplied by increasing the dose.

This would have been important both if CUPID-2 had shown equivocal efficacy or clear efficacy. However, owing to the neutral outcome from CUPID-2 this study was closed without results to report.

Thirdly, the SERCA-LVAD trial (Clinicaltrials.gov identifier: NCT00534703) aimed to test safety and efficacy in patients with long term LVADs for chronic HF. Recruitment commenced in June 2014, and the first patient received the investigational medicinal product at Harefield Hospital on 23 July 2014. This was the first LVAD patient in the world to receive gene therapy for HF. The design was a two-centre, double-blind randomised controlled trial enrolling 24 stable patients with chronic HF treated with an LVAD, and allocating these patients 2:1 to receive AAV1.SERCA2a or placebo. The goals of the study were fourfold. Firstly, the primary outcome was assessment of safety and feasibility in LVAD patients. Secondly, the magnitude of SERCA2a gene expression could be systematically assessed in transfected patients using tissue from elective percutaneous LV biopsy taken at 6 months. Thirdly, the trial directly assessed the hypothesis that the presence of circulating NAbs blocks viral transduction. All NAb positive patients were excluded from the CUPID-1 and CUPID-2 studies; here randomisation was stratified to include both NAb positive and NAb negative patients in equal proportions in the active and placebo arms. Finally, an exploratory hypothesis was that the effects of SERCA2a gene therapy might be magnified in the presence of optimal LV loading conditions facilitated by the LVAD. This study is discussed in Chapters 5 and 6.

1.5.3.5 Explanations for the failure to achieve efficacy

The results from these early clinical trials using AAV1.SERCA2a in HF undoubtedly represent a significant setback for the field, but reflect just the first chapter of a long story in developing gene therapy for HF. The key going forward is to understand why the CUPID-2 trial has yielded neutral results and how this understanding can be applied to future studies. This is discussed in detail in Chapter 6.

1.6 Novel biomarkers for reverse remodelling

1.6.1 Monitoring underlying heart function

One of the challenges of clinical intervention and cardiac recovery studies in LVAD patients is the lack of effective tools for monitoring heart function. Assessments are focussed on structural (imaging studies), functional (e.g. cardiopulmonary exercise testing (CPEX) or 6 minute walk test), and molecular/neurohormonal domains (e.g. natriuretic peptides), and are complemented by testing of haemodynamics at cardiac catheterisation. However, in their basic forms none of these investigations can separate the differing contributions of the heart-LVAD continuum, a physiological relation that is constantly changing but depends principally on preload, afterload and pump speed, and the assessment of LV function in a patient supported with a rotary LVAD is challenging.

Imaging options in LVAD patients are limited. Cardiac CT is limited by scatter and artefact and cardiac MRI is contraindicated due to the pump. Echocardiography is the modality most frequently in use, but images are difficult to obtain and interpret due to obstruction of standard windows and often only parasternal images of the LV can be reliably acquired. Low speed echocardiography is an established tool for heart function assessment independent of the LVAD[125]. Speed adjustments are importantly clinically to optimise pump function. If speed is too high, there is risk of LV suction events, septal bowing into the LV, and RV compromise; if speed is too low, the HF is inadequately treated. So-called 'ramp' studies are used to optimise pump speed and unloading for an individual patient, often using echo and haemodynamic parameters[126]. In contrast by lowering the rpm sequentially to the lowest safe speed in a 'turn down' study, it is possible to safely wean the heart off haemodynamic support in order to assess LV dimensions and functional parameters when the heart is working under full haemodynamic load[125,127], more reflective of physiology in the absence of the LVAD. Similar turn down can be performed for CPEX and haemodynamic assessments[128]. However, this is time consuming and carries risk of pump thrombosis. Furthermore, even if pump speed is minimised, the LV remains substantially decompressed, and if it is stopped entirely there is retrograde blood flow through the device in LV diastole which can complicate the assessment of LV function[125,129]. Development of new techniques to assess heart function independent of the LVAD and without invasive testing could be greatly beneficial clinically.

1.6.2 Speckle tracking echocardiography

Deformation imaging for estimation of multi-planar myocardial strain may facilitate native LV function assessment without necessity for turn-down. This technique exploits the three perpendicular axes of motion in the left ventricle to generate assessment of multi-dimensional myocardial mechanics including rotation, longitudinal and circumferential motion, mechanics which in HF are deranged[130]. Speckle tracking uses acoustic markers from scattering of the ultrasound beam in the myocardium to track the movement of individual points frame by frame, and in so doing provide angle-independent estimations of point position and cardiac muscle movement. Strain is the term used to quantify these deformations. Despite limitations, including insensitivity to movement out of the plane of view, deformation techniques have found various clinical roles, especially in accurate assessment of regional LV function. Within the LVAD population, strain is developing a role in risk stratification before implantation, specifically in identifying patients at risk of RV failure and requirement for RVAD placement[131], though it suffers from data heterogeneity between studies that makes it difficult to interpret.

Strain imaging is not a new technique, but to date has not been systematically evaluated as a tool for LV function assessment in existing LVAD patients. There is suggestion that it may prove to be a useful adjunct in patient selection, judging response to treatment and informing decisions about device explant, particularly for devices such as the Synergy which avoid obstruction of the apical window.

1.6.3 Circulating microRNA biomarkers

Another approach is the assessment of circulating microRNA (miR) as biomarkers in LVAD patients. miRs are small, single-stranded pieces of non-coding RNA, typically 22 nucleotides in length, that originate from non-protein coding regions of the genome. In parallel with other noncoding RNAs they are only recently identified, and their role in countless physiological and pathological processes is becoming apparent[132]. Their primary biological function is post-transcriptional regulation of gene expression by sequence-specific binding with messenger RNAs (mRNAs) causing translational repression or mRNA degradation, and they might have further roles in cell-cell interactions controlling systemic alterations to the proteome. miRs

can be isolated from circulating blood, where they remain extremely stable due to their transport in association with microvesicles[133], exosomes, or in tight association with RNA-binding proteins that protect them from ribonuclease degradation[134]. Their function outside the cell is a matter of debate, but they have potential roles as biomarkers for diagnosis, prognostication or predicting response to treatment[135].

Several studies have reported plasticity of miRs within the ventricular myocardium in response to unloading by LVAD therapy. Matkovich and colleagues[136] studied LV samples from independent groups of non-failing hearts, HF hearts and LVAD-supported hearts, and identified 28 miRs that were >50% upregulated in HF (n=17) vs. non-failing hearts (n=11; $p<0.001$), of which 20 miRs showed significant decrease or normalisation after LVAD support lasting 1.7±1.0 months (n=10; $p<0.001$). These were non-paired samples relating to a short duration of LVAD support. Another study found that greater expression of miR-23a and miR-195 in the ventricular myocardium at the time of LVAD implantation associated with subsequent LVAD dependency, while comparators with lower levels went on to reverse remodelling and LVAD explantation[137]. The levels of miR-23a and miR-195 did not change during the course of LVAD support and were similar to levels in non-failing hearts, so these may be markers of severity, rather than recovery per se[138].

For practical use, these changes would have to identifiable as changes in circulating miR. There are multiple exploratory studies which have sought to identify diagnostic and prognostic biomarkers for use in HF, with an ambition to identify a blood test biomarker with greater specificity than the natriuretic peptides. Around 30 different miRs have been proposed as candidate biomarkers for HF[139-146]. MiR-423-5p was the first candidate biomarker identified[139]. The initial microarray identified 16 candidate miRs, amongst which miR-423-5p performed best on further validation, distinguishing HF from healthy controls in a multivariate model with AUC 0.91 (CI 0.84-0.98), and correlating with NT-proBNP ($r_s=0.43$, $p=0.002$) and LVEF ($r_s=-0.34$, $p=0.002$). However, another group found no differential expression in miR-423-5p in HF vs. controls on microarray, but empirically studying miR-126 demonstrated variable expression related to levels of BNP and NYHA class, proposing this as a marker for HF severity[147]. Goren and colleagues studied 30 patients with stable chronic HF and 30 controls, and identified 10 miRs with >1.2 fold increased

expression in HF[141]. Of these they found 4 miRs (including miR-423-5p) that had significant difference between the experimental groups, and used a composite 'miR signature' score to improve differentiation. However, other data have not supported miR-423-5p as a biomarker[148], or have proposed different miR biomarkers[143]. Other miRs may distinguish HF vs. non-HF in studies of dyspnoeic patients[139,142], or distinguish HFrEF from HFpEF[144], or predict CRT responders vs. non-responders[149]. At present, there is insufficient coherence for reliable clinical biomarkers, requiring further investigation in larger cohorts and prospective studies. What is certain is that the stability and detection of circulating miRs makes their use as biomarkers a feasible enterprise.

The field of circulating miR is in an exploratory and candidate identification stage, and will need to focus in due course on large scale validation to identify whether there is a clinical role for the various miRs in play. In the realm of MCS, there is miRome plasticity within the ventricular myocardium in response to LVAD support, and circulating miRs may assist clinical management of VAD patients by providing a direct measure of native LV function.

1.7 Hypotheses

1.7.1 Chapters 3-4: Novel devices

1. We hypothesised that partial LV support with the CircuLite Synergy LVAD and Sunshine Heart C-Pulse chronic extra-aortic counterpulsation device (1) was safe and feasible in advanced HF; and (2) could facilitate functional, structural and molecular reverse remodelling of the LV.

2. We hypothesised that patients implanted with the Synergy LVAD would show (1) evidence of increased platelet activation and greater impairment of fibrinolytic pathways compared with patients implanted with the HeartMate II, and that this might help identify patients at risk of pump thrombosis; and (2) greater loss of vWF activity, with associated higher rates of bleeding.

1.7.2 Chapters 5-6: Novel therapeutics

3. In the SERCA-LVAD trial:
    a. With regard to feasibility and safety, we hypothesised that (1) AAV1.SERCA2a gene therapy was safe and feasible in patients with advanced HF and LVAD; (2) intracoronary delivery of the AAV1.SERCA2a gene product would yield detectable viral transgene in myocardial tissue from treated subjects; and (3) pre-existing NAb would not prevent successful transfection in treated subjects.
    b. With regard to clinical efficacy, we hypothesised that a single intracoronary infusion of the AAV1.SERCA2a gene product at the dose of 1x 10^{13} DNase resistant particles in patients with advanced HF with long term MCS from an LVAD would yield evidence of beneficial reverse remodelling as evidenced by functional, structural and neurohormonal measures.
    c. Further, we hypothesised that echocardiographic strain would (1) provide a novel, load-independent, low risk method to determine and monitor LV function during ongoing LVAD support; and (2) form a tool to predict clinical response to gene therapy.

1.7.3 Chapter 7: Novel biomarkers

4. We hypothesised that (1) the circulating miRome shows plasticity in patients with LVADs, and this plasticity can be quantified as a novel biomarker; (2) individual miRs or patterns within the miRome can act as novel biomarkers that to monitor native LV function in patients with LVADs, and predict which patients respond to LVAD therapy; (3) plasticity in the circulating miRome reflects plasticity of expression within the ventricular myocardium.

2: General Methods

2.1 Collection, processing and storage of blood samples

2.1.1 Blood collection

Blood samples were collected from study participants for analysis in the clinical laboratory, and for analysis in study-specific protocols. Blood was collected in Vacutainer system bottles using sterile technique and by trained staff. Prior to venepuncture the patient's verbal consent was obtained, and study consent affirmed. Clinical samples were sent to the hospital clinical laboratories by normal routes; research-specific samples were processed my me in the research lab.

2.1.2 Blood collected for clinical laboratory analysis

Blood for analysis in the clinical laboratory was sent there directly in the Vacutainer bottles. Sample processing and analysis was undertaken according to clinical laboratory protocols.

2.1.3 Processing serum and plasma specimens

The following protocol was used for all research-specific serum and plasma samples collected and analysed during this research, at the Harefield and Leuven study sites. Plasma samples were collected into 3ml EDTA bottles, and serum samples into 5ml SST bottles. Serum and plasma samples were processed identically. I performed all sample processing at the Harefield site. Processing was done within 2 hours for all specimens. Sterile technique was used to eliminate contamination as far as possible. Collection tubes were stood vertically for minimum 10 minutes and maximum 2 hours at room temperature, before centrifugation for 10 minutes at 1900g at 4ºC. Thereafter the serum/plasma was carefully extracted using sterile pipette, aliquoted into 1ml cryovials, labelled according to study format, and placed in -80ºC freezer for long term storage.

2.1.4 Processing for peripheral blood mononuclear cell specimens

Peripheral blood mononuclear cells (PBMC) were collected in patients enrolled in the SERCA-LVAD trial (Chapters 5-6) for the adeno-associated virus-1 ELISPOT test. PBMC isolation was done using reagents and methods from Cellular Technologies Limited (Shaker Heights, USA). 15ml whole blood was collected in 5 x 3ml heparinised Vacutainer system bottles using sterile technique. Samples were analysed as soon as possible, and if short term (<2 hour) storage was necessary this was done on a benchtop rocker at 20-37ºC. All processing at Harefield was done by me or a research nurse.

Isolation was performed under a sterile tissue culture hood which was sterilised with alcohol spray before each use. Heparinised whole blood was transferred to a 50ml conical tube and diluted 1:1 with sterile, room temperature Phosphate Buffer Solution, and mixed gently. The diluted whole blood solution was pipetted slowly to overlap 15ml 'Lymphoprep' Ficoll solution (Axis-Shield PoC AS, Rodeløkka, Norway) in a new 50ml conical tube. Density gradient centrifugation was undertaken at 800g at 21ºC for 30 minutes, with the centrifuge set to slowest possible acceleration and deceleration. The PBMC layer was drawn up slowly using a 1ml pipette, collected in a new conical tube, and thereafter suspended in 45ml CTL Wash Medium pre-diluted as per manufacturer instructions. After centrifugation (10 minutes at 330g/21ºC) the PBMC pellet was resuspended in 5ml CTL Wash Medium and cell counting was performed using Tryphan blue staining on a standard haemocytometer slide, to calculate the number of PBMC extracted. After counting, the cell solution was diluted with a further 45ml CTL Wash Medium (making total volume 50ml) and centrifuged (10 minutes at 330g/21ºC). The PBMC pellet was then suspended in a volume of Cryo-C solution calculated to yield 10million PBMC per 0.5ml Cryo-C. Then 0.5ml of this cell solution was then instilled slowly with 0.5ml Cryo-AB solution, yielding a final 1ml Cryo-ABC containing 10million PBMC. This was transferred to a Mr Frosty slow freeze device and placed in the -80ºC freezer for 24 hours, before long term storage in liquid nitrogen.

Analysis using ELISPOT testing was done at laboratories at Cellular Technology Limited (Shaker Heights, USA) using an IFN-γ-detecting ELISPOT assay. Specimen transfer was done after Material Transfer Agreement between Imperial College London (the SERCA-

LVAD study sponsor) and CTL, using liquid nitrogen for preservation, and in accordance with UK/EU and USA customs regulations. Results were reported to the study sponsor as 'positive' or 'negative' at 3 and 6 months, relative to baseline.

2.1.5 Specimen storage

Specimens were stored in designated facilities managed by Royal Brompton & Harefield NHS Foundation Trust. Specifically, serum and plasma specimens collected during SERCA-LVAD were stored in a dedicated -80C freezer in the Heart Science Centre, Harefield Hospital; serum and plasma specimens collected during the PAVE-UP trial (Chapter 3), and those transferred in by Material Transfer Agreement from University Hospitals Leuven, were stored in a dedicated -80C freezer within the NIHR Cardiovascular Biomedical Research Unit, Royal Brompton Hospital; and PBMC specimens were stored in liquid nitrogen storage facility in the Heart Science Centre, Harefield Hospital. All storage facilities were serviced and maintained as per manufacturer instructions. All specimens were stored in accordance with guidelines from the Human Tissue Authority, and in accordance with protocols at Royal Brompton & Harefield NHS Foundation Trust and Imperial College London.

2.2 Collection, processing and storage of myocardial tissue

2.2.1 Collection and processing

Myocardial tissue was obtained during percutaneous endomyocardial biopsies undertaken as part of the SERCA-LVAD trial (Chapters 5-6). The biopsy procedure itself is outlined in Chapter 5. Tissue specimens were extracted from the bioptome catheter and placed in a pre-labelled cryovial approved for use in liquid nitrogen. These cryovials were flash frozen in liquid nitrogen, and transferred for storage.

Myocardial tissue obtained at cardiac transplantation was processed by researchers on the tissue collection rota at Harefield Hospital. Research consent was confirmed before any tissue was retrieved. The whole heart was retrieved from the operating field immediately after explantation, and quickly processed. Specific samples for the SERCA-LVAD study were taken from the anterior wall, anteroseptum, posteroseptum and posterior wall. These were

placed in a pre-labelled cryovial approved for use in liquid nitrogen. These cryovials were flash frozen in liquid nitrogen, and transferred for storage.

Analysis for presence of viral SERCA2a DNA in myocardial tissue was done at the MPI Research Inc. (Mattawan, USA). In brief, this was studied using quantitative polymerase chain reaction experiment to identify a nucleotide sequence unique to AAV1.SERCA2a. Details of the probes are reported in Zsebo et al[111]. Data was reported as number of copies of AAV1/SERCA2a detected in ≤1 microgram of genomic DNA extracted from each specimen, based on a standard dilution curve with detection limits 20-200 single-stranded copies of AAV1/SERCA2a per μg DNA. Specimen transfer was done after Material Transfer Agreement, using liquid nitrogen for preservation, and in accordance with UK/EU and USA customs regulations.

2.2.2 Storage

Cryovials containing deep frozen myocardial tissue were stored in a liquid nitrogen facility in the Heart Science Centre, Harefield Hospital, maintained by Royal Brompton & Harefield NHS Foundation Trust. This facility is subject to regular maintenance and audit, continuous temperature monitoring and weekly resupply of liquid nitrogen. All specimens were stored in accordance with guidelines from the Human Tissue Authority, and in accordance with protocols at Royal Brompton & Harefield NHS Foundation Trust and Imperial College London.

2.3 Clinical testing and procedures

2.3.1 Sources of data

Within this thesis, I report transthoracic echocardiography (TTE), 6 minute walk testing (6MWT) and cardiopulmonary exercise (CPEX) testing data from a mixture of sources. TTE, 6MWT and CPEX studies done during the SERCA-LVAD trial (Chapters 5-6) were done according to set protocol as outlined here. Other data were collected opportunistically, and these data were recorded from standard clinical operating protocols within the institution

where the patient's care was based (either Harefield Hospital, or University Hospitals Leuven). This data is included in Chapters 3-4 and 7.

2.3.2 Echocardiography protocol including low speed assessment

This TTE protocol was conceived for use in the PAVE-UP study (Chapter 3). On this study's cessation, it was adjusted for the C-Pulse substudy (Chapter 3) and the SERCA-LVAD trial (Chapters 5-6), intending for it to be used across the different aspects of my PhD research. In fact, the only resultant trial data reported here is from the SERCA-LVAD trial. To maximise the sensitivity and specificity of the test, the TTE was performed with the LVAD at normal clinical operating speed, and with the LVAD speed turned down to the minimal safe and/or tolerated level, as detailed in Chapter 1 and discussed in Chapters 5-6.

All prospective studies undertaken as part of the SERCA-LVAD trial were performed using the Phillips IE33 (Philips Electronics, Eindhoven, The Netherlands) at Harefield Hospital. Other studies were performed using this and General Electric Vivid7 machines (GE Healthcare, Bedford, UK). All machines were serviced and maintained to clinical standard according to institutional protocols. For all the low speed assessments, there were three researchers present. The TTEs were performed by a consultant cardiologist. I organised the studies and was present to assist with the image acquisition, recording data, and managing the turn down with a VAD specialist nurse.

On arrival, the patient's study consent was checked and new verbal consent was obtained, reiterating the small risk of thromboembolism and pump thrombosis. Blood pressure and LVAD speed/power were recorded at baseline. Continuous ECG monitoring was used during the study. A core dataset of parameters was obtained with the LVAD running at normal clinical operating speed (Table 2.1).

Table 2.1: Protocol for echocardiography.

Acronyms are defined throughout the table.

1) Parasternal long axis view (PLAX):
   a) M-mode of left ventricular cavity at level of mitral valve (MV) tips
      i) LV end diastolic dimension (LVEDD)
      ii) LV end systolic dimension (LVESD)
   b) Two-dimensional (2D) assessment
      i) Aortic root dimension
      ii) LV outflow tract (LVOT) diameter
      iii) Colour flow looking for aortic regurgitation (AR)
      iv) AR jet as % LVOT diameter
      v) Vena contracta of AR
      vi) M mode aortic valve (adjust sweep speed so 10 beats are seen, and calculate % opening time)
2) Parasternal short axis view (PSAX):
   a) For strain estimation, record 3 2D loops for speckle with highest frame rate technically possible at:
      i) LV at level of mitral valve tips
      ii) LV at level of papillary muscle insertion
      iii) LV apex
3) PLAX or PSAX:
   a) Visual 2D colour flow estimation of tricuspid regurgitation (TR) severity
   b) Continuous wave Doppler estimation of TR maximal velocity (TR Vmax)
4) Apical 4 chamber view (A4C):
   a) Pulse wave Doppler trace of mitral inflow for E wave velocity, A wave velocity and E wave deceleration time
   b) Pulse wave on tissue Doppler imaging (TDI) of mitral annulus for lateral E' wave velocity
   c) Assess for mitral regurgitation (MR) on 2D colour flow. If there is visually more than mild MR, record the following parameters:
      i) Continuous wave Doppler across mitral valve, for estimation of pressure half time and MR volume time integral
      ii) Proximal isovelocity surface area (PISA) radius. Record aliasing velocity.
   d) Pulse wave Doppler trace of VAD inflow cannula and pulmonary veins
   e) Tricuspid annular plane systolic excursion (TAPSE) using M-mode at lateral aspect of tricuspid annular plane
   f) Systolic TDI of lateral tricuspid annulus.
5) Apical 5 chamber view (A5C):
   a) 2D colour flow to assess for aortic regurgitation
   b) LVOT pulse wave Doppler for LVOT velocity time integral (VTI)
   c) Aortic valve continuous wave Doppler for maximal aortic valve velocity (AV Vmax) and assessment for AR by pressure half time method
6) A4C and apical 2 chamber view (A2C):
   a) Biplane LV end diastolic volume (LVEDV), LV end systolic volume (LVESV) and ejection fraction (LVEF) by Simpsons biplane method if possible – if not, then estimate ejection fraction visually
   b) Left atrial volume by Simpsons method, measured at ventricular end-systole
   c) For strain estimation, record 3 loops of 2D speckle with highest frame rate technically possible in A4C and A2C
   d) Subcostal view: M-mode dimension of inferior vena cava in end expiration and end inspiration
   e) Suprasternal view: Continuous wave Doppler for AR assessment – measure VTI of flow reversal
7) High parasternal: Right and left high parasternal recordings (with Doppler where possible) to visualise VAD outflow

2.3.2.1 Low speed study

The low speed element of the study was completed immediately following the initial scan. To minimise risk of pump thrombosis this was done as quickly as possible, with a maximum time limit of 45 minutes. This allows 15 minutes for ramp down, 10 minutes' wait time, 5 minutes acquiring data and 15 minutes for ramp up to normal speed. Prior to any speed adjustment the anticoagulation regime was reviewed and action taken as shown in Table 2.2. If the INR was outside the patient's target range, the patient's warfarin dosing was reviewed post-procedure.

Table 2.2: Low speed echocardiogram.

(a) Management of anticoagulation. (b) Protocol for turn down. See text for details.

(a)

INR on day of procedure	***Anticoagulation management***
<1.5	Postpone procedure until INR>2
1.5-1.9	Give 10,000 unit heparin bolus before starting
2.0-2.9	Give 5,000 unit heparin bolus before starting
3.0-4.0	Proceed without heparin bolus
> 4	Postpone procedure until INR<4

(b)

(rpm = revolutions per minute)	***Thoratec HeartMate II***	***Heartware HVAD***
Typical clinical operating speeds	8,000-9,000rpm	2,500-3,200rpm
Reduce in increments of	200rpm	100rpm
To minimum speed	6,000rpm	1,800rpm

For the turn down, the LVAD speed was reduced in increments every 1 minute with BP and echo assessment at each stage. The aim was to reduce the speed to the minimum safe speed or as far as tolerated by the individual patient. The typical clinical operating speeds, size of incremental reduction and minimum safe speed vary between devices and are shown in Table 2.2b. The speed was never reduced below the minimum speed specified, and the total time below the patient's clinical operating speed never exceeded 45 minutes.

Once at minimum speed or lowest speed tolerated, there was a 10 minute equilibration period, after which BP was recorded and LV studies were repeated. The following parameters were obtained at low LVAD speed: PLAX M-mode dimensions; biplane LVEDV, LVESV and LVEF by Simpson's method; and three loops of 2D speckle with highest frame rate technically possible in PSAX, A4C and A2C, for post hoc strain assessment.

As soon as the echo studies were complete, the LVAD was returned to baseline speed, using incremental steps at 1 minute intervals using echo and BP monitoring, and by the same rpm increments used for speed reduction. At the end of the study, the pump power consumption was recorded. Thereafter the patient was instructed to check the pump power every 15 minutes for 2 hours post procedure, as a sustained increase could suggest pump thrombosis. If patient or researcher were concerned, then the patient returned the following day for blood examination for haemolysis markers.

2.3.2.2 Calculation of myocardial strain

I performed all myocardial strain analysis. Images were analysed off line using Phillips QLAB software with Automated Cardiac Motion Quantification package (Philips Electronics, Eindhoven, The Netherlands). Analysis was done during the blinded phase of the SERCA-LVAD trial so I was blinded to study grouping. Images were analysed from basal, midcavity and apical PSAX cuts through the LV. For each set of images, the region of interest (ROI) was placed over the LV in short axis and peak systolic strain identified. The QLAB package calculates circumferential strain as the change in circumference of each of region as compared to the relaxed circumference of that region.

2.3.3 Six minute walk testing

The research protocol for 6MWT adopted the same protocol used for clinical testing at Harefield Hospital, and as such underpins all data collected on that site. Two members of staff performed the 6WMT (myself plus one other). A standard corridor was used with marks delineating set distance, and cones marked this distance for the exercising patient. A chair was always available if needed by the patient, study stopwatch was used for time keeping, and a card was used for demonstrating the Borg scale. Standard statements and responses

were used asking the patient to walk as far as possible in 6 minutes, rest if needed, and not to hesitate at the turn around the cones. The Borg scale was quantified before and after the test, and the reason for stopping was documented.

2.3.4 Cardiopulmonary exercise testing

This CPEX protocol was conceived for use in the PAVE-UP study (Chapter 3). On this study's cessation, it was adjusted for the C-Pulse substudy (Chapter 3) and the SERCA-LVAD trial (Chapters 5-6), intending for it to be used across the different aspects of my PhD research. In fact, the only resultant trial data reported here is from the SERCA-LVAD trial. All the CPEX testing was done with the LVAD running at full clinical speed.

All CPEX testing was done with myself, the exercise physiologist and VAD nurse present. In practice, all testing was done at Harefield Hospital. The gas exchange analyser was an Ultima CardiO2 (MGC Diagnostics, St Paul, USA). This and the treadmill were serviced and maintained to clinical standard according to institutional protocols, including calibrated for flow and gas analysis done daily as per manufacturer instructions. Other required equipment was a Doppler probe for BP recordings, and card for demonstrating the Borg scale. If the patient was fitted with an intracardiac defibrillator there was a pacing physiologist in attendance.

On arrival, the patient's study consent was checked and new verbal consent was obtained. Blood pressure (using Doppler probe if necessary) and LVAD speed/power were recorded at baseline. Continuous ECG monitoring was used during the study. A minimum 5 minutes resting gas exchange data was obtained prior to starting exercise. Ideally the respiratory exchange ratio (RER) should be <0.7 before starting exercise, though in this patient group this is usually not achieved, and in these cases suboptimal RER would have to be accepted. If the patient had an ICD, then for patient safety, the VT therapies were turned off, and VF detection threshold to >200bpm, to avoid inappropriate therapies during the test that can precipitate collapse and falling off the treadmill. Exercise was commenced as per the modified Bruce protocol. The Borg dyspnoea scale was obtained every minute during the test. BP measurements were not attempted during the test as clinical experience states these

are unreliable during exercise. Exercise was discontinued immediately if adverse events occurred. BP measurement was obtained using the Doppler probe immediately after exercise, and the patient was monitored for 5 minutes after completion. In patients with an ICD, therapies were reprogrammed to pre-test settings before leaving the exercise room.

The following data parameters were recorded for each CPEX test: duration of exercise (minutes); final Borg score before cessation of test; stage of exercise protocol reached; peak oxygen consumption (VO_2; ml/kg/min); VE/VCO_2 slope (average of whole test); respiratory exchange ratio; reason for stopping.

2.3.5 Right heart catheterisation

Right heart catheterisation (RHC) was integrated into the PAVE-UP study and C-Pulse substudy (Chapter 3). This research protocol adopted the same protocol used for clinical testing at Harefield Hospital, and as such underpins all data collected on that site.

The procedures took place in the Catheter Laboratory at Harefield Hospital, performed by a Consultant Cardiologist. For prospective RHC studies in the research study (and some others), I was present for assistance with study protocol recording data. Patients were admitted overnight and preprocedure had INR checked. The preferred venous access route was via the right internal jugular vein. After instilling local anaesthetic and Seldinger placement of a venous sheath under ultrasound guidance, a transducer catheter was passed sequentially under fluoroscopy guidance to the right atrium, right ventricle and pulmonary artery with pressure recordings in each location. Pulmonary capillary wedge pressure was recorded by balloon inflation in a branch pulmonary artery. Cardiac output determination was done using the Fick method using assumed oxygen consumption (based on age, gender, and body surface area), main pulmonary artery saturations and peripheral pulse oximetry. Manual pressure was deployed as the sheath was removed.

2.3.6 Intra-aortic balloon pump

Implantation of an intra-aortic balloon pump (IABP) was planned as part of the C-Pulse substudy in Chapter 3. The intended protocol for implantation is included here.

The procedure was planned to take place in the Catheter Laboratory at Harefield Hospital, performed by a Consultant Cardiologist. The procedure to implant the IABP would be integrated with RHC and TTE, all occurring together in the catheter laboratory. The intention was to admit patients overnight for the assessment. Patients were admitted overnight and preprocedure had INR checked. After RHC, including cardiac output determination, the IABP would be implanted to the descending aorta via the femoral artery. Local anaesthetic would be administered prior to venous and arterial puncture. After this was complete, an intra-aortic balloon would be introduced using a percutaneous technique via the femoral artery. A 5000 IU heparin bolus would be administered via the intra-aortic balloon catheter lumen. The baseline arterial pressures (systolic/diastolic and mean) would be recorded via the catheter lumen. The balloon pump would be activated and inflation and deflation timing would be optimised. After 15 minutes of 1:1 support, the RHC/cardiac output/arterial pressure measurements would be repeated. At the end of the procedure the intra-aortic balloon and intravascular catheters would be removed and replaced with an angiography sheath which will be left in situ until such time that the APTT <70 sec. Subsequently, the sheath would be removed with manual compression of the puncture site for at least 5 minutes until haemostasis is secured (with or without a haemostatic device).

3: Partial support devices as a novel approach in advanced heart failure

3.1 Introduction

3.1.1 Background and rationale

The evolution and clinical role for partial LV support are described in detail in Chapter 1. Identifying an unmet need for patients with this subgroup of patients advanced HF, we set out to establish whether partial LV support techniques, less invasive than implantation of a standard full support LVAD, could contribute to reverse remodelling of LV function that could be beneficial with less morbidity.

The preliminary evaluation of two partial support devices is described in this Chapter, namely the CircuLite Synergy and the Sunshine Heart C-Pulse device. Their design and operation is discussed in Chapter 1. The methods and results described reflect significant evolution of the planned clinical trials throughout the research period. A prospective clinical trial studying the CircuLite Synergy device was the original concept for my PhD, and the stated goal of the British Heart Foundation Clinical Research Training Fellowship which I was awarded. This trial evolved from its original design to meet various challenges encountered early in the trial design phase, particularly the challenge of recruitment to which we responded by expanding to include collaboration with University Hospitals Leuven (UHL). When this study was closed early, focus moved to the Sunshine Heart C-Pulse system and a site-specific substudy addressing hypotheses overlapping with the Synergy trial. However, this, too, was closed early, in fact before recruitment had begun.

These events provided significant challenges. The Methods presented are the final prospective study designs, and then the designs for the retrospective work that supplanted them. The complications with the clinical studies are detailed in the Results section. In the end the research presented in this Chapter is based largely on retrospective data collected

from patients treated with the respective devices outside of the intended clinical trials. There are clear limitations, but some informative conclusions can be drawn.

3.1.2 Hypothesis

We hypothesised that partial LV support with the CircuLite Synergy LVAD and Sunshine Heart C-Pulse chronic extra-aortic counterpulsation device (1) was safe and feasible in advanced HF; and (2) could facilitate functional, structural and molecular reverse remodelling of the LV.

3.2 Methods

3.2.1 The PAVE-UP Trial

3.2.1.1 Background and Aims

This was an academic-led research study, formulated as the backbone of my PhD research, that evolved from the successful use of the CircuLite Synergy device within the clinical bridge to cardiac transplantation (BTT) programme at Harefield Hospital. The full title of the study was the 'Partial Ventricular Support in Advanced Heart Failure' trial (PAVE-UP).

The primary aims of the study were (1) to further evaluate safety of the CircuLite Synergy in the advanced HF population, (2) to quantify in vivo the degree of reverse remodelling that occurs with partial LV support from the CircuLite Synergy device using functional, structural and molecular assessments, and (3) to study novel methods of assessing reverse remodelling including strain echocardiography and circulating microRNA profiles. We planned to make functional assessments including CPEX, 6MWT and the Minnesota Living with Heart Failure questionnaire (QOL); structural assessments including echocardiography and assessment of haemodynamics at RHC; and molecular assessments (circulating miR profiles and BNP).

The secondary aim was to evaluate the acquired haematological disorders associated with use of the Synergy pump, in order to improve our clinical management of these patients. This is detailed in Chapter 4.

3.2.1.2 Study design

PAVE-UP was a prospective, multi-centre, observational study comparing patients implanted with the Synergy device with heart failure controls, enrolling patients with HF categorised by the INTERMACS at level 4-6 and who are on optimal medical and device therapy (control group) alongside patients in the same category planned to receive a Synergy pump (intervention group). The study design is summarised in the flowchart (Figure 3.1 page 70). The two study centres were Harefield Hospital and University Hospitals Leuven, Belgium (UHL).

Patients were allocated to the control or intervention groups by the clinical transplant multidisciplinary team, based on clinical criteria and independent of the research team. My role in this process was screening patients and identifying them for discussion. In addition to clinical suitability for the device, all participants were required to meet all study inclusion and no exclusion criteria (see Table 3.1, page 68).

It should be emphasised that the preferred design was a prospective, randomised study, but this was not feasible due to funding constraints. The randomised design would allow the most rigorous comparison of Synergy versus medical therapy, but required external funding for the clinical care costs, most significantly the cost of the LVAD itself. In the observational design, these costs were part of normal clinical care, funded within the transplant programme, which facilitated assessment of the study hypotheses, albeit in a study design that would ultimately require large cohort, prospective-randomised validation.

Table 3.1: Inclusion and exclusion criteria for the PAVE-UP trial.

(INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; Peak VO_2, peak oxygen consumption; RER, respiratory exchange ratio)

Inclusion criteria

1. Patients with ischaemic or dilated cardiomyopathy aged 18 to 80 years.
2. Symptoms categorised by INTERMACS at level 3-6 despite optimal tolerated medical therapy. Practically this included patients in hospital or managing at home with substantial dependence on hospital care.
3. Peak VO_2 <15ml/kg/min with RER>1 on cardiopulmonary exercise testing *or* a 6-minute walking distance <300m *or* inability to perform an exercise test due to the severity of heart failure.
4. Informed consent obtained prior to entering the study.

Exclusion criteria

1. Cause of heart failure due to or associated with uncorrected thyroid disease, obstructive cardiomyopathy, pericardial disease, amyloidosis, or active myocarditis.
2. Body surface area <1.2M^2 or >2.3M^2, or body mass index >32 kg/M^2.
3. Severe chronic obstructive pulmonary disease or severe intrinsic hepatic disease.
4. Pregnancy.
5. Occurrence of stroke within 90 days before enrolment.
6. Recent history of psychiatric disease (including chronic cognitive impairment, and severe drug or alcohol abuse) that is likely to impair compliance with the study protocol.
7. Platelet count <50 x$10^3$$mm^3$ within 24 hours before enrolment; Creatinine clearance < 30ml/min.
8. High probability of non-compliance.
9. The patient is deemed unsuitable by the clinical team for other reasons.

3.2.1.3 Study endpoints

The primary outcome was change in peak VO_2 at 6 months. Secondary outcomes were change in ventilatory equivalent for carbon dioxide (VE/VCO2) slope, change in 6-minute walk distance, change in NYHA functional class, change in BNP, change in LVEDV, change in circulating miRNA expression profile, change in haemodynamic parameters at RHC, change in QOL, number of unplanned hospital admissions for cardiovascular reasons and the incidence of significant haematological derangements. Safety outcomes were major bleeding, thromboembolism, stroke, infection, device-related morbidity and device failure including perioperative complications, right ventricular failure.

3.2.1.4 Clinical testing

Patients were scheduled to undergo assessment at baseline and during follow up as shown in Table 3.2.

Table 3.2: Proposed scheme of investigations for the PAVE-UP study.

(CPEX, cardiopulmonary exercise testing; 6WMT, 6 minute walk test; QOL, quality of life questionnaire, BNP, B type natriuretic peptide; ECHO, echocardiography; miRNA, microRNA profiling (see Chapter 7); HAEM, haematological analysis (see Chapter 4); RHC, right heart catheterisation).

TEST \ DAY	0	1	7	30	90	180
CPEX	●				●	●
6MWT	●				●	●
QOL	●				●	●
BNP	●				●	●
ECHO	●	♦	♦	♦	●	●
miRNA	●				●	●
HAEM	●	♦	♦	♦	●	●
RHC	●					●

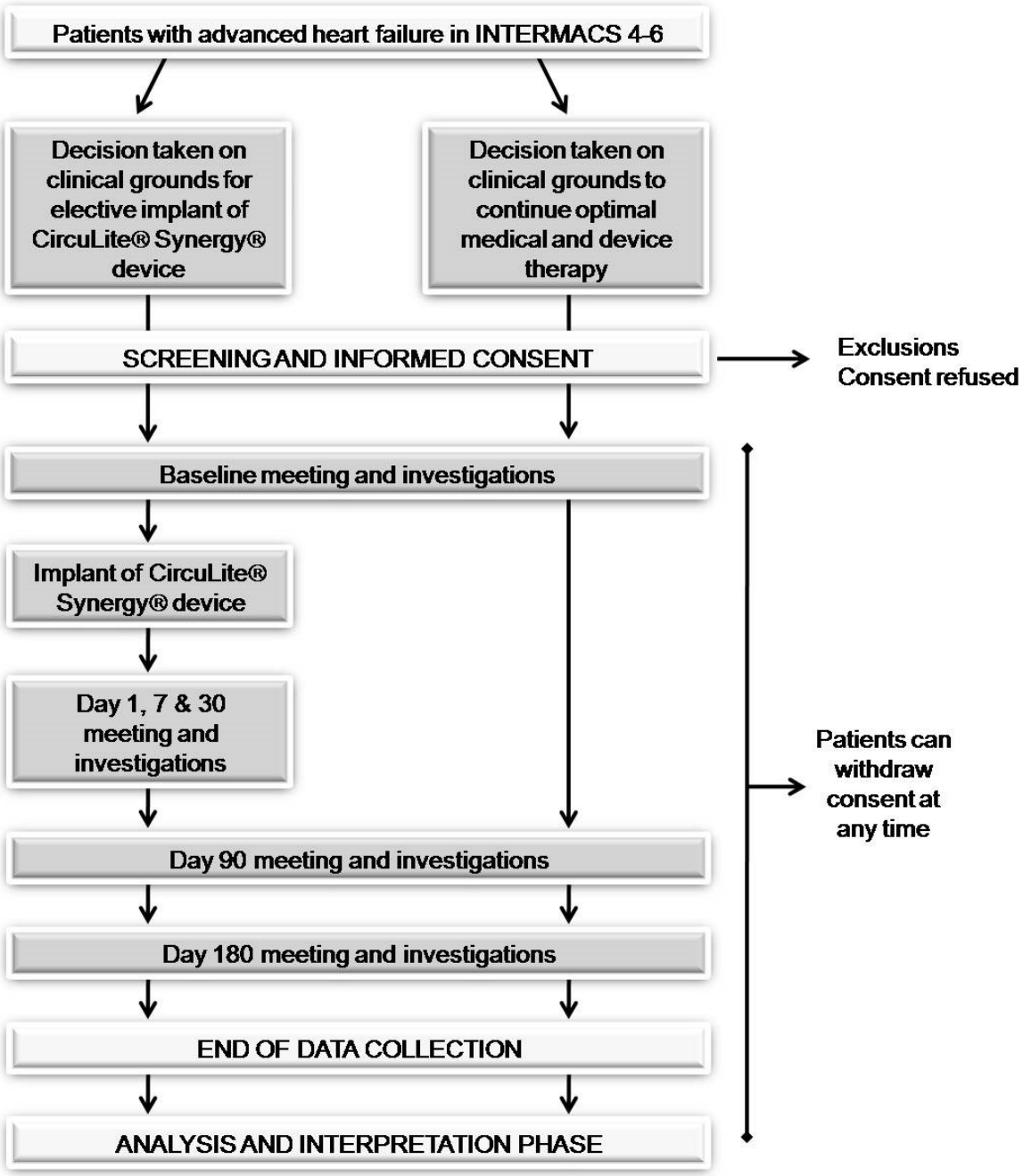
● = both groups

♦ = intervention group only

Protocols for blood sample collection, cardiopulmonary exercise testing, 6 minute walk test, echocardiography, and right heart catheterisation are described in Chapter 2. The Minnesota Living with Heart Failure questionnaire was used for quality of life assessment. Details of the intended haematology and microRNA analyses are set out in Chapters 4 and 7 respectively.

Figure 3.1: PAVE-UP study flowchart.

For details of investigations to be performed at each visit, please see Table 3.2.



3.2.1.5 Adverse events

This was an observational study, and while significant AEs or adverse events occur frequently in this patient population, all were to be recorded and analysed to assess for device-related AEs.

3.2.1.6 Sample size and statistical analysis

The proposed method was systematic enrolment of all eligible patients until recruitment targets were met. Based on internal audit, each study centre expected to be able to recruit 10-12 patients to the intervention group over 2 years. To accommodate this, we allocated patients to intervention and control at a ratio of 1:2. I made power calculations using G*Power v3.1 software[150] to detect a difference in peak VO_2 of at least 2.5ml/kg/min using paired t-tests at α=0.1 and with 80% power. We chose 2.5ml/kg/min as a minimum clinically significant difference after its validation in the RIME trial of surgical therapy for ischaemic mitral regurgitation[151]. The α=0.1 level of statistical significance was selected as this study was studying safety and efficacy, and would identify signals of efficacy to justify a larger study. This would require 19 intervention and 39 control patients to demonstrate the clinical effect. To allow for dropouts, I determined the sample size as 21 intervention and 43 controls, with a total of 64 patients to be recruited.

Statistical analysis was planned to compare baseline and 6 month data using paired *t*-tests for continuous and normally distributed data or an alternative (e.g. the Mann-Whitney test) for non-normally distributed data, and use χ^2-test for categorical variables.

3.2.1.7 Ethics and governance

The study sponsor was Royal Brompton & Harefield NHS Foundation Trust (RBHT). Permission was sought from the Research Ethics Committee and favourable opinion was granted on 3 April 2013 (13/LO/0298). RBHT governance approval was granted on 13 June 2013. Funding was through a British Heart Foundation Clinical Research Training Fellowship (FS/13/34/30173) with support from the National Institute for Health Research Cardiovascular Biomedical Research Unit at RBHT.

3.2.2 C-Pulse substudy

3.2.2.1 Background and Aims

The idea for this study developed after the premature closure of the PAVE-UP trial. The transplant and VAD team at Harefield Hospital was participating in an international study

examining efficacy of the C-Pulse device (the OPTIONS trial). The current proposal was an academic-led site-specific substudy aiming to pursue my original hypothesis regarding reverse remodelling with partial LV support, and further understand the effects of counterpulsation in chronic HF. The design was intended to overlap significantly with the PAVE-UP trial, to allow comparison of the devices.

The primary aim of the study was to quantify in vivo the degree of reverse remodelling that occurs with partial LV support from the Sunshine Heart C-Pulse device using functional, structural and molecular assessments, in line with the assessments set out above for the PAVE-UP study. The secondary aims were (1) to compare haemodynamic effects of short term support with an intra-aortic balloon pump (IABP) to long term support with the C-Pulse system to determine whether response to IABP can used to predict which patients will benefit from C-Pulse therapy, and (2) measure haematological parameters to examine the effects of C-Pulse therapy on platelets and coagulation.

3.2.2.2 Study design

The substudy was a single arm, single-centre prospective observational study aiming to recruit 6 patients. The study was based at Harefield Hospital. Detailed clinical assessments were planned at baseline, 3 months and 6 months after implantation. Patients who consented to the OPTIONS study and were planned for C-Pulse implantation were approached to participate in the additional substudy. The full OPTIONS protocols are described elsewhere in the literature[152] and at www.clinicaltrials.gov (trial identifier: NCT01872949). The OPTIONS study aimed to recruit 50 patients across all centres. Inclusion and exclusion criteria were identical between the main OPTIONS study and our substudy.

3.2.2.3 Clinical testing

Serial testing protocols are shown in Table 3.3 (page 73).

Table 3.3: Proposed scheme of investigations for the C-Pulse substudy.

(CPEX, cardiopulmonary exercise testing; 6WMT, 6 minute walk test; QOL, quality of life questionnaire, BNP, B type natriuretic peptide; ECHO, echocardiography; miRNA, microRNA profiling (see Chapter 7); HAEM, haematological analysis (see Chapter 4); RHC, right heart catheterisation; IABP, intra-aortic balloon pump).

TEST \ DAY	0	90	180
CPEX	●	●	●
6MWT	●	●	●
QOL	●	●	●
BNP	●	●	●
miRNA	●	●	●
HAEM	●	●	●
RHC+IABP+ECHO	●		
RHC+ECHO		●	●

3.2.2.3.1 Right heart catheterisation with echocardiography

We sought to collect detailed haemodynamic and echocardiographic data serially throughout the trial. Protocols for the IABP implantation, RHC and echo procedures are detailed in Chapter 2.

- Pre-implantation. We planned to perform right heart catheterisation including cardiac output calculation alongside on table echocardiography, followed by percutaneous placement of an IABP and reassessment of RHC and echo data after 15 minutes of 1:1 support.
- 3 and 6 months. We planned to perform the same procedure, minus the IABP implantation. Subsequently, we planned to record RHC and echo parameters at varying levels of C-Pulse support (e.g. 1:2, 1:3). If necessary, the inflation/deflation timings could be optimised during the study.

3.2.2.3.2 Other protocols

Protocols for blood sample collection, cardiopulmonary exercise testing and 6 minute walk test are described in Chapter 2. The Minnesota Living with Heart Failure questionnaire was used as a quality of life assessment. Details of the intended haematology and microRNA analyses are set out in Chapters 4 and 7 respectively.

3.2.2.4 Adverse events

This was an observational study, and while significant risks were identified these were related to the clinical care of the patients, rather than to the study protocol per se. Nonetheless these were to be recorded and analysed. All adverse events were also reported as per OPTIONS study requirements.

3.2.2.5 Sample size

The intended sample size of 6 patients was a pragmatic estimate based on likely recruitment over 1 year within Harefield Hospital.

3.2.2.6 Ethics and governance

The study sponsor was RBHT. Permission for the substudy was sought from the Research Ethics Committee, and favourable opinion was granted on 15 July 2014 (14/LO/1093). RBHT governance approval was granted on 10 October 2014. Principal funding was from Sunshine Heart and RBHT, with support from the National Institute for Health Research Cardiovascular Biomedical Research Unit at RBHT.

3.2.3 Retrospective data collection

After cessation of both clinical trials, I collected existing clinical data from patients in Harefield Hospital and University Hospitals Leuven. These data were collected at point of care by clinical colleagues in both hospitals. I collected the data from existing clinical records, and performed all analyses. I visited Leuven to collect data on 27 May 2014, and for consistency this date was used as cut-off for Harefield data collection as well.

Statistical analyses were done using IBM SPSS Statistics Version 23 and GraphPad Prism version 5. All data were treated as non-parametric. Comparisons of paired data used Wilcoxon signed-ranked tests, and comparisons of independent samples used Mann-Whitney U tests. In the case of multiple comparisons these were adjusted using the Bonferroni correction. Survival analysis was done using the Kaplan-Meier method.

3.3 Results

3.3.1 The PAVE-UP Trial: progress and shutdown

3.3.1.1 Screening and recruitment

The trial was open for recruitment at Harefield Hospital between 13 June 2013 and 27 July 2013. I screened 104 patients, of whom 4 gave written consent to participate in the trial. Of these, the transplant MDT elected to proceed to Synergy implantation as BTT in 1 patient (intervention group), and elected to place the remaining 3 on the routine waiting list for cardiac transplantation (medical group). Data from the Synergy patient are included in the cohort described later. The medical therapy patients did not undergo baseline testing prior to trial termination.

3.3.1.2 Decision to terminate recruitment and close the trial

Progress with the trial was halted on 27 July 2013 when CircuLite issued an Urgent Safety Notice[153] via BfARM, the German devices regulator, the UK's MHRA and institutional review boards. The issue was two episodes of inflow cannula fracture occurring in a single centre in Germany[154]. The patients involved had been admitted as emergency cases and required emergency surgery. There was no consequent mortality, and no patient at Harefield Hospital suffered the same complication. It became quickly apparent that this would be a lengthy period of withdrawal, and so recruitment to the PAVE-UP trial, and all investigations additional to normal care, were halted. The trial was terminated completely in May 2014. Owing to early cessation of recruitment, UHL was never activated as a recruitment centre.

3.3.1.3 Retrospective analysis and collaboration

Owing to early study closure and minimal recruitment there was no meaningful prospective data collection during the PAVE-UP trial. In order to provide some insight into safety and feasibility of the device, and insight that might address the core hypothesis, data were collected for retrospective analysis. Collaboration was sought with colleagues at UHL, with whom we had already shared pilot data. I was given access to clinical data that had been collected by the Leuven team in their cohort of Synergy patients (see section 3.2.3, page 74).

3.3.2 Partial support with the Synergy device

3.3.2.1 Study population and baseline characteristics

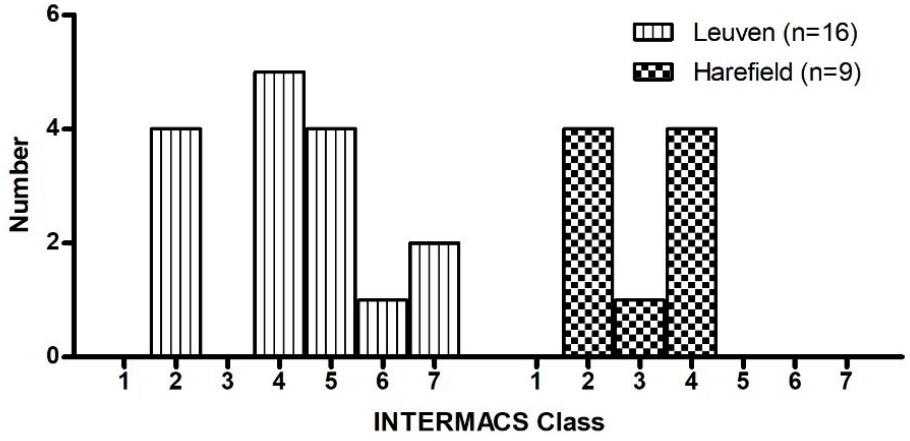
Retrospective data were available for 27 patients undergoing Synergy device implantation at Harefield Hospital (n=10) and University Hospitals Leuven (n=17) between January 2008 and July 2013. Two patients were excluded from analysis, one due to diagnosis of congenital heart disease patient with systemic RV and one as the device was compassionate use in an atypical scenario. In both patients, their unique circumstances preclude cohort comparison.

Twenty-five patients were included in the final analysis, of whom 88% (n=22) were male and 48% (n=12) had HF of ischaemic aetiology. Modal INTERMACS score was 4 (range 2-7); 64% of patients had INTERMACS score ≥4. Patients recruited at Harefield were younger (median age 26 years less than Leuven; 38 years (28, 57) vs. 64 years (48, 70), p=0.08), fell exclusively into INTERMACS class 4 or below, and were more likely current emergency inpatients needing urgent treatment (56% vs. 19%, p=0.07). Recruitment in Leuven included 7 patients in less severe HF classes (see Figure 3.2, page 77).

There were no significant differences in objective markers of HF severity between the two centres, except patients at Harefield had significantly larger LVEDD (77mm (68-80) vs. 65mm (59-67); p<0.01). In general patients were receiving good guideline based medical therapy, though fewer patients in Leuven were on angiotensin converting enzyme inhibitor or angiotensin receptor blocker (44% vs. 89%, p=0.03). Notably there was poor take up of implantable cardioverter defibrillators across the cohort (total 10/25 patients, 40%) which is unexpected as this population are at high risk of arrhythmia and sudden cardiac death. Full baseline characteristics are shown in Table 3.4 (page 78).

Figure 3.2: Numbers recruited in each study centre shown by INTERMACS class.

(INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support)



3.3.2.2 Duration of support and problems with pump thrombosis

Total duration of support was defined as time from device implantation until clinical endpoints of permanent device explant, upgrade to full support LVAD, heart transplantation or death. A key factor limiting event-free survival with ongoing support was a high rate of pump thrombosis, and to reflect this the primary uninterrupted duration of support was examined separately, defined as time from device implantation until first requirement for pump exchange due to pump thrombosis.

Table 3.4: Baseline Characteristics in the Synergy retrospective cohort.

Continuous variables are all non-parametric and presented as median (25^{th}, 75^{th} centile). Categorical variables are presented as n (%). P-values are for Mann-Whitney test for continuous variables, and Chi-squared for categorical variables. Missing data excluded by variable to maximise data use. (INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BB, beta blocker; MRA, mineralocorticoid receptor antagonist; ICD, implantable cardioverter defibrillator; CRT, cardiac resynchronisation therapy; peak VO_2, peak oxygen consumption; VE/VCO_2, minute ventilation/carbon dioxide production; LVEDD, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LA, left atrium; BNP, B-type natriuretic peptide; NT-proBNP, N-terminal pro-hormone of brain natriuretic peptide; RAP, right atrial pressure; RVP, right ventricular pressure; s, systolic; d, diastolic; PASP, pulmonary artery systolic pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output.)

	Harefield (n=9)	**Leuven (n=16)**	**Whole cohort (n=25)**	**p**
Age at implantation	38 (28, 57)	64 (48, 70)	56 (34, 67)	0.08
Male	7 (78)	15 (94)	22 (88)	0.24
Ischaemic aetiology	5 (56)	7 (44)	12 (48)	0.57
INTERMACS≥4	4 (44)	12 (75)	16 (64)	0.13
Unscheduled hospital admission at time of implantation	5 (56)	3 (19)	8 (32)	0.07
Therapy at baseline				
ACEi/ARB	8 (89)	7 (44)	15 (60)	**0.03**
BB	8 (89)	12 (75)	20 (80)	0.40
MRA	6 (67)	12 (75)	18 (72)	0.67
Diuretic	8 (89)	11 (69)	19 (76)	0.26
ICD	5 (56)	5 (31)	10 (40)	0.29
CRT	3 (33)	4 (25)	7 (28)	0.73
Previous LVAD	0 (0)	0 (0)	0 (0)	-
Baseline HF severity parameters				
Peak VO2 (ml/kg/min)	15.8 (11.1, 17.8)	11.8 (9.7, 13.6)	12.7 (9.8, 15.8)	0.12
VE/VECO2 slope	35 (31, 44)	44 (37, 49)	40 (35, 47)	0.11
6 minute walk (m)	220	392 (261, 489)	382 (220, 482)	-
LVEDD (mm)	77 (68, 80)	65 (59, 67)	67 (60, 76)	**<0.01**
LVEF (%)	19 (14, 29)	18 (15, 24)	19 (15, 24)	0.76
LA diameter (mm)	53 (48, 56)	51 (43, 55)	51 (47, 55)	0.68
BNP (ng/L)	1360 (1055, 2023)	-	-	-
NT-proBNP	-	4122 (2259, 9038)	-	-
RAP (mmHg)	15 (11, 23)	15 (11, 17)	15 (11, 19)	0.55
RVP s/d (mmHg)	69 (46, 76) / 13 (5, 20)	65 (40, 73) / 15 (12, 18)	65 (41, 75) / 15 (12, 19)	0.71 / 0.56
PASP (mmHg)	59 (40, 75)	65 (39, 73)	60 (40, 75)	0.80
PCWP (mmHg)	30 (24, 32)	32 (22, 35)	31 (24, 33)	0.74
CO (L/min)	3.98 (3.14, 4.35)	3.21 (2.39, 3.86)	3.21 (2.40, 4.18)	0.33

Within this cohort of 25 patients, the median total duration of LVAD support was 192 days (76, 640 days), and the duration of uninterrupted LVAD support was 149 days (49, 337). Pump thrombosis episodes and other key adverse events are listed in Table 3.5 (page 80). Pump exchange for thrombosis was required on 19 occasions in 11 patients (44%), of whom 8 patients suffered multiple episodes of pump thrombosis. This is an event rate of 0.82 thrombosis episodes per patient-year of Synergy support. Median time to pump exchange was 114 days (12, 282 days). Of note, two patients required pump exchange for thrombosis within the first week. Major bleeding complications are detailed in Table 4.7 (page 126). The event rate for major bleeding was 1.6 events per patient-year. Six patients suffered haemothorax immediately postoperatively due to bleeding from the insertion point of the inflow cannula to the LA. Kaplan-Meier plots for survival without meeting clinical endpoint and survival without pump thrombosis are shown in Figure 3.3 (page 81).

High severity INTERMACS profile, ongoing emergency hospital admission at time of implantation and presence of severe MR were hypothesised as factors that might impair good function of the Synergy LVAD and this adversely influence total duration of LVAD support. Patients implanted as emergencies had significantly shortened total support duration (median 392 days vs. 76 days, HR 2.22 (0.77-6.46), p=0.01). Lower INTERMACS profile (i.e. worse clinical status) was associated with shorter support duration, though this not achieve statistical significance (median 135 vs. 241 days, HR 1.53 (0.62-3.72), p=0.17), and presence of severe MR had no effect (median 162 vs. 211 days, HR 1.22 (0.53-2.83), p=0.83; Figure 3.4, page 82).

Table 3.5: Summary of pump thrombosis and clinical endpoints for individual patients.

*Pump thrombosis episodes defined as clinical suspicion plus requirement for exchange, explant or upgrade. WHF = worsening heart failure; rec, recovery; pall, palliative care. *Ongoing support at time of data collection 27/5/2014. #Explant was due to change of clinical strategy. Not suitable for full support device and Synergy was attempt to BTT, unsuccessful, explanted to short term LVAD.*

Patient ID	Number of pump thrombosis episodes	Days to first pump thrombosis	Other significant morbidity	Clinical endpoint	Total duration of support (days)
22	2	5		Transplant	50
23	2	168	Pocket haematoma	HeartMate II	483
24	0		Haemothorax	Transplant	44
25	2	114	Epistaxis	Explant/rec	192
26	1	54	Rectal bleeding, epistaxis, pocket haematoma	Death	650
27	0			Transplant	64
29	0			Transplant	174
30	2	282	Epistaxis, rectal bleeding	Death	648
31	1	767	Epistaxis	Ongoing	946 *
32	0		GI bleeding	Ongoing	648 *
33	0		Epistaxis	Explant/rec	140
34	0		Epistaxis	Transplant	392
35	0		Haemothorax, PR bleeding, epistaxis	Death	141
36	1	252	Epistaxis, subdural haemorrhage	Death	909
37	0		Haemothorax, epistaxis	Transplant	211
38	0		Haemothorax, lower gastrointestinal bleeding	Transplant	270
40	0		Haemothorax	Transplant	428
41	2	772	Phrenic nerve palsy	HVAD	995
42	0			HVAD	135
43	0		Intracranial haemorrhage	Death	92
44	2	1	Haemothorax, pocket haematoma, pocket infection	Explant/pall	75
45	0			Transplant	631
46	2	12		Explant/#	12
47	0			HVAD	5
49	2	13		HVAD	76

Figure 3.3: Kaplan-Meier plots for event free survival with the Synergy LVAD.

(a) Primary event free survival until first device complication (endpoints as listed); (b) Total event free survival (endpoints as listed); (c) Survival without requirement for pump exchange.

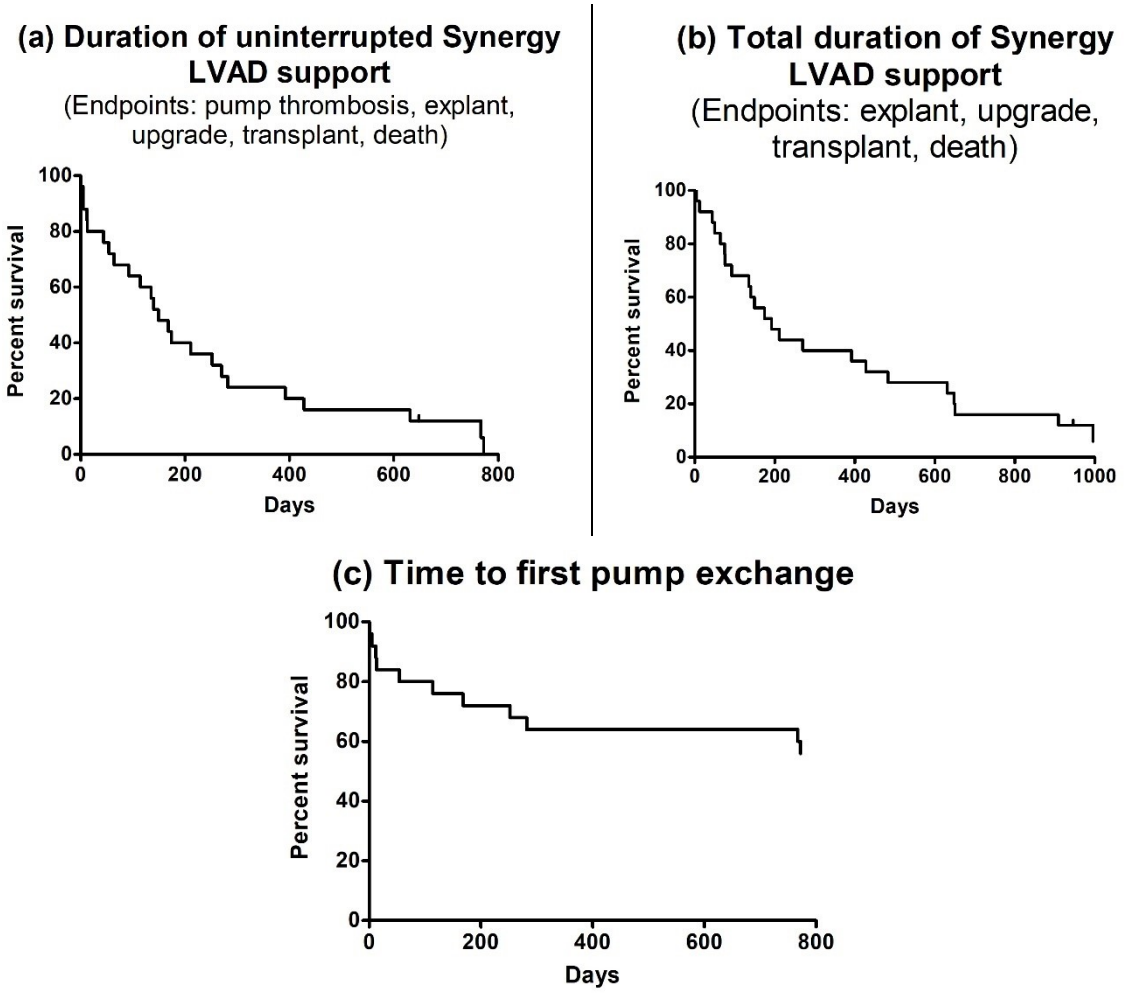
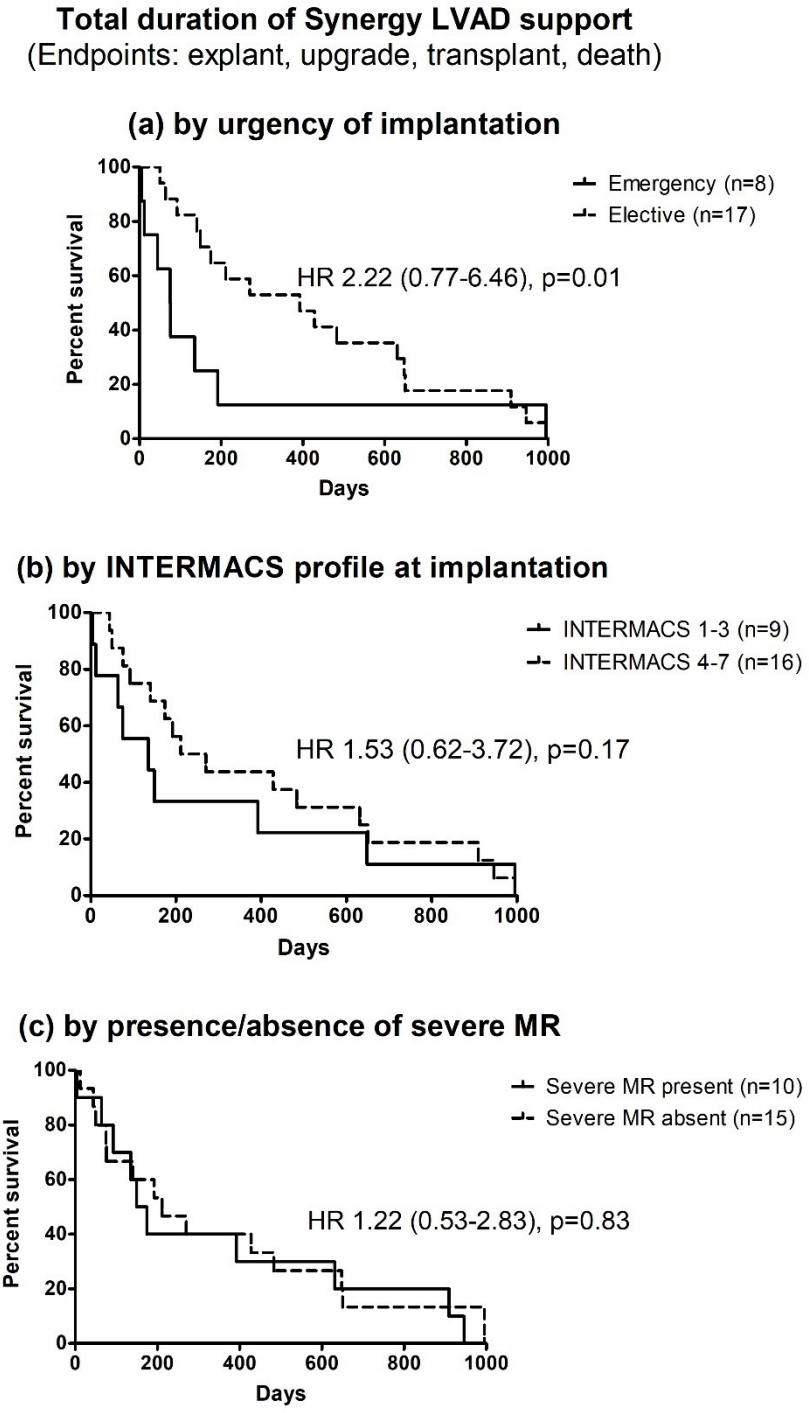


Figure 3.4: Kaplan-Meier plots for stratified event free survival with the Synergy LVAD.

Endpoints are device explant, need for upgrade to full support LVAD, cardiac transplantation or death. Event free survival stratified by (a) urgency of implantation, (b) INTERMACS profile at implantation and (c) presence of severe mitral regurgitation (MR) on preoperative echocardiogram. See text for details. (INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; HR, hazard ratio.)



3.3.2.3 Inconsistent evidence regarding ventricular performance

Functional, structural and neurohormonal markers were studied to assess the clinical efficacy of the Synergy LVAD and evidence of enhanced ventricular performance and reverse remodelling in the study population. The full data are set out in Table 3.6 (page 84) and illustrated in Figure 3.5 (page 85).

There was some evidence of improved haemodynamics in Synergy-supported patients. There was a sustained increase in calculated cardiac output (at 6 months increased by around 1.7L or 26% (6, 59%); $p<0.01$), with reduction in PCWP and PASP occurring early and sustained throughout follow-up, though without statistical significance. This was mirrored by the reduction in LA dimension (51mm (47, 55mm) to 44mm (39, 50mm); -9% (-24, -4); non-significant $p=0.01$), though by 6 months and thereafter LA size was comparable to baseline.

The specific hypothesis here is that the Synergy yields beneficial reverse remodelling in the ventricle. There was some evidence of functional improvement with significant increase in six minute walk distance at 3 months (increased by 30m or 38% (16, 102%), $p<0.01$) which was sustained to 12 months but lost statistical significance. However, there was no significant change in peak VO_2, VE/VCO_2 slope (though this trended in a beneficial trajectory), or LV end diastolic dimension, and LVEF actually worsened (at 3 months, relative reduction of 29% (13, 33%); $p=0.02$).

Legend for Table 3.6 on page 84: Clinical efficacy markers.

Serial measurements and absolute values shown in the left-hand columns. Delta changes relative to baseline shown in right-hand columns. Due to multiple comparisons, Bonferroni $\alpha=0.0125$. (Peak VO_2, peak oxygen consumption; VE/VCO_2, minute ventilation/carbon dioxide production; 6MWD, 6 minute walk distance; LVEDD, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LA dia, left atrial diameter; RAP, right atrial pressure; PASP, pulmonary artery systolic pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output.)

Table 3.6: Efficacy markers in the Synergy retrospective cohort. Complete figure legend on preceding page.

		Serial measurements					Relative change from baseline							
		Preimp.	1 month	3 months	6 months	12 months	1 month		3 months		6 months		12 months	
							Δ %	p	Δ %	p	Δ %	p	Δ %	p
Peak VO_2 (ml/kg/min)	n	19	12	16	10	8	11		13		8		6	
		12.7 (9.8, 15.8)	12.0 (10.6, 12.7)	12.2 (11.5, 13.7)	11.7 (10.5, 16.5)	12.5 (10.7-16.5)	+11 (-15, +22)	0.37	+10 (-7, +24)	0.23	-4 (-18, +31)	0.94	+5 (-15, +26)	0.56
VE/VCO_2	n	16	12	16	10	8	11		11		7		4	
		40 (35, 47)	41 (35, 48)	36 (32, 50)	36 (32, 47)	33 (27, 38)	-11 (-15, +10)	0.56	-2 (-23, +14)	0.41	+4 (-24, +32)	0.94	-23 (-29, -16)	0.10
6MWD (m)	n	15	14	9	8	4	13		9		8		4	
		382 (220, 482)	372 (319, 494)	412 (383, 552)	425 (306, 526)	414 (327, 506)	+6 (-2, +38)	0.10	+38 (+16, +102)	**<0.01**	+7 (+1, +28)	0.05	+26 (+3, +127)	0.13
LVEDD (mm)	n	23	21	18	12	8	20		17		11		7	
		67 (60, 76)	65 (61, 75)	66 (60, 71)	67 (61, 78)	74 (65, 80)	0 (-4, +9)	0.43	+1 (-8, +4)	0.60	+2 (-1, +9)	0.15	-2 (-2, +13)	0.80
LVEF (%)	n	22	18	17	12	8	16		15		10		7	
		19 (15, 24)	15 (10, 19)	13 (10, 19)	15 (10, 17)	10 (10, 14)	-21 (-35, 0)	0.03	-29 (-33, -13)	0.02	-27 (-40, 0)	0.02	-33, (-50, 0)	0.14
LA dia. (mm)	n	19	16	16	12	7	14		14		9		6	
		51 (47, 55)	44 (39, 50)	50 (45, 53)	49 (43, 53)	48 (46, 55)	-9 (-24, -4)	0.01	-4 (-12, +6)	0.25	+2 (-17, +7)	0.59	+3 (-16, +29)	0.67
RAP (mmHg)	n	22	9	14	8	6	8		12		8		6	
		15 (11, 19)	9 (8, 12)	10 (7, 11)	10 (8, 14)	11 (5, 14)	-33 (-53, +20)	0.23	-41 (-52, +3)	0.07	-43 (-52, +26)	0.06	-32 (-50, -14)	0.09
PASP (mmHg)	n	25	9	14	8	6	9		14		8		6	
		60 (40, 75)	42 (34, 53)	40 (34, 48)	46 (33, 57)	41 (32, 56)	-26 (-47, -11)	0.02	-35 (-52, -20)	0.02	-46 (-51, -9)	0.04	-30 (-53, -17)	0.03
PCWP (mmHg)	n	23	9	14	8	6	9		13		8		6	
		31 (24, 33)	19 (15, 23)	18 (9, 24)	22 (11, 27)	21 (15, 29)	-35 (-50, -19)	0.02	-45 (-66, -19)	0.05	-40 (-66, -9)	0.06	-25 (-48, -15)	0.03
CO (L/min)	n	23	9	14	8	6	9		13		8		6	
		3.21 (2.40-4.18)	4.25 (3.73-5.09)	4.95 (3.52-5.62)	4.07 (3.58-4.39)	4.15 (3.43-4.46)	+11 (+7, +51)	**<0.01**	+22 (+17, +71)	**<0.01**	+26 (+6, +59)	**<0.01**	+27 (+21, +37)	0.03

Figure 3.5: Summary of clinical efficacy markers in the Synergy retrospective cohort.

Each point represents an individual patient. Lines represent median and interquartile range. (a) Peak VO_2, peak oxygen consumption; (b) VE/VCO_2, minute ventilation/carbon dioxide production; (c) 6MWD, 6 minute walk distance; (d) LVEDD, left ventricular end diastolic dimension; (e)LVEF, left ventricular ejection fraction; (f) LA, left atrium; Continued on following page. (g) RAP, right atrial pressure; (h) PASP, pulmonary artery systolic pressure; (j) PCWP, pulmonary capillary wedge pressure; (k) CO, cardiac output.)

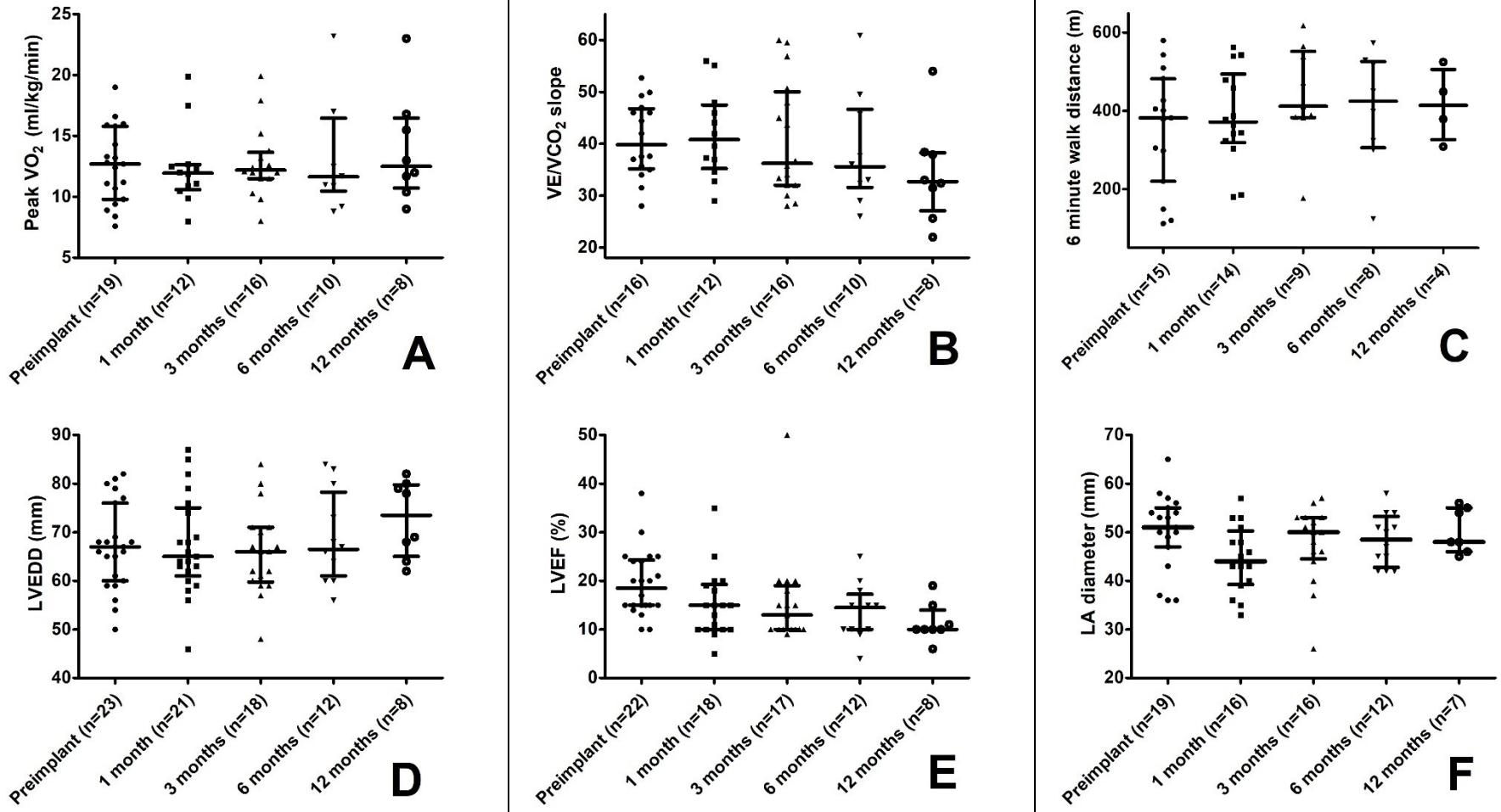
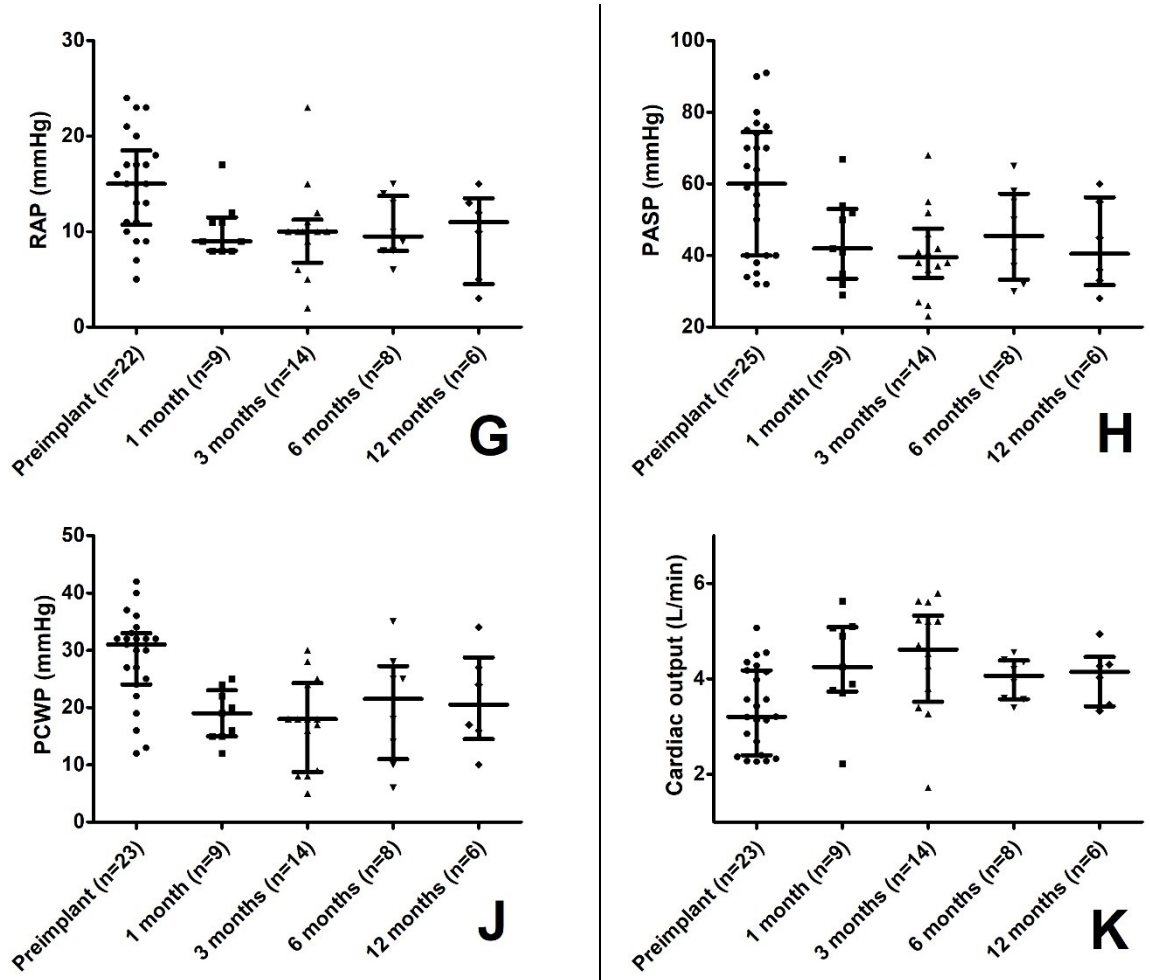


Figure 3.5: Summary of clinical efficacy markers. (continued)



There was a relative reduction in levels of natriuretic peptides across the group. The two centres used different assays for natriuretic peptides (RBHT using BNP, UHL using NT-proBNP). To facilitate cohort analysis, relative change from baseline was calculated. There was a 68% reduction (38, 74%) at 6 months) reflecting beneficial remodelling of the neurohormonal milieu (Table 3.7 and Figure 3.6).

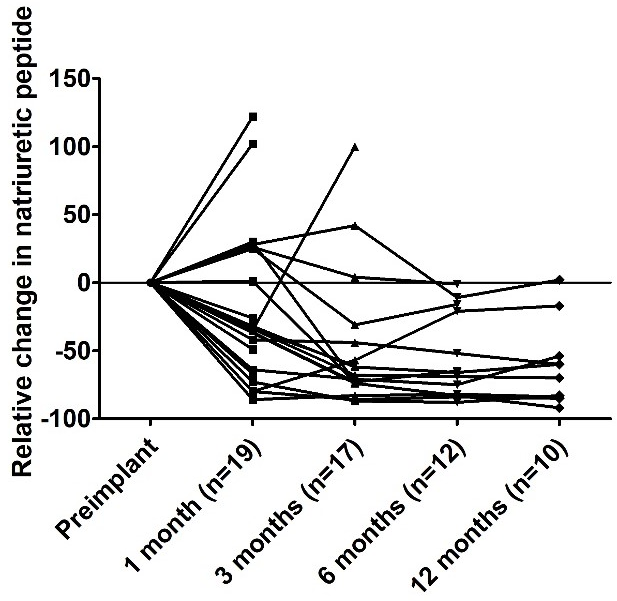
Table 3.7: Serial measurements and relative changes of natriuretic peptides.

(BNP, B-type natriuretic peptide; NT-proBNP, N-terminal pro-hormone of brain natriuretic peptide.)

		Serial measurements				
		Preimplant	**1 month**	**3 months**	**6 months**	**12 months**
BNP (ng/L)	n	8	4	4	2	3
		1359 (1055-2023)	381 (173-508)	375 (177-787)	240 (147, 332)	133 (129, 221)
NT-proBNP (ng/L)	n	16	15	13	10	7
		4122 (2259, 9038)	3424 (1709, 6198)	3098 (1288, 3809)	2872 (1820, 4980)	3380 (1490, 7203)
		Relative change from baseline				
Change in natriuretic peptide	n	-	19	17	12	10
	%	-	-34 (-67, +26)	-68 (-74, -38)	-66 (-80, -17)	-65 (-84, -45)

Figure 3.6: Relative change in natriuretic peptides with Synergy LVAD support.

Change relative to baseline, shown as zero. See Table 3.7.



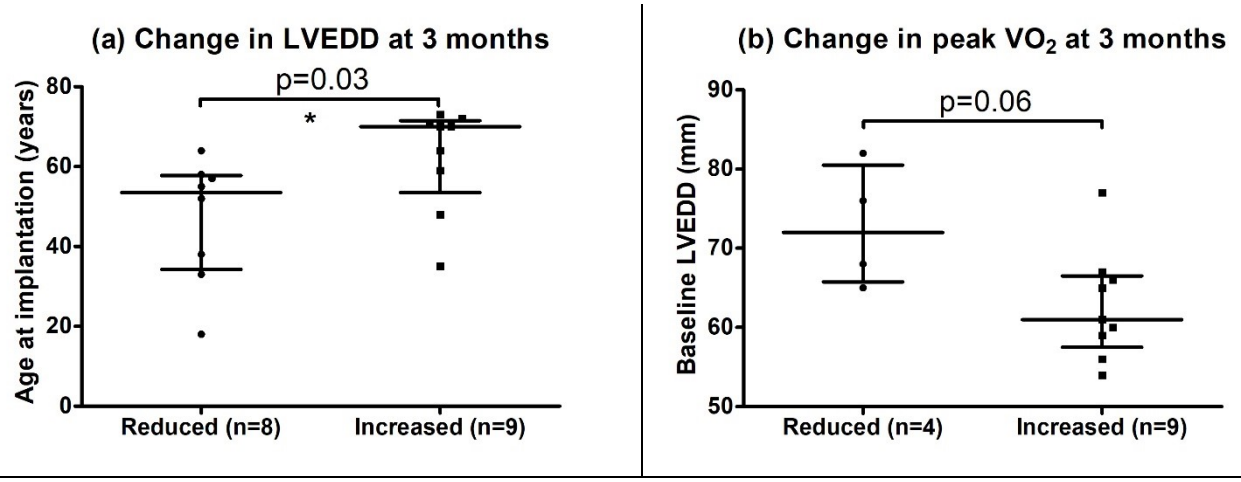
In summary, there was some evidence of enhanced ventricular performance (68% reduction in natriuretic peptides, 38% increase in 6 minute walk distance) but other evidence to suggest no change (peak VO_2, VE/VCO_2 slope, LV dimensions), and in fact some suggestion of adverse remodelling (29% relative reduction in LVEF at 3 months).

3.3.2.4 Reverse remodelling in a subgroup of patients

To understand what factors might determine positive response the cohort was stratified by various baseline characteristics. There are paired preimplant versus 3 month LVEDD measurements for 17 patients, of whom 8 showed reduction in LVEDD (median -8% (-15, -3%), p=0.01) and 9 showed increase in LVEDD (median +3%, (+2, +8), p<0.01). Patients in the former subgroup were significantly younger at time of device implantation (54 years (34, 58 years) vs. 70 years (54, 72 years), p=0.03; Figure 3.7). There was no demonstrable effect of aetiology, gender, medical therapy, baseline haemodynamics, or baseline functional parameters. Similarly, there are paired preimplant versus 3 month peak VO_2 measurements for 13 patients, of whom 3 patients experienced reduction (median -13%, (-40, -3%), p=0.11) and 9 showed increase (+20%, (10, 31%), p<0.01; Figure 3.7). Patients who showed improved peak oxygen consumption tended to have smaller LV dimensions at the time of implantation (72mm (66, 81mm vs. 61mm (58, 67mm)) though this did not reach statistical significance (p=0.06). Again, there was no demonstrable effect of aetiology, gender, medical therapy, baseline haemodynamics, or baseline functional parameters.

Figure 3.7: Younger age and smaller LV size associate with clinical benefit at 3 months.

The cohort was stratified by change in LVEDD and peak VO2 at 3 months (see text for details). Younger age at implantation (a; p=0.03) and smaller LVEDD (b; p=0.06) associated with beneficial response.



Two patients showed evidence of significant reverse remodelling sufficient to justify explantation of the device. Both patients were initially in INTERMACS class 4 and yielded significant haemodynamic improvements from baseline (e.g. PCWP decreased in both patients to <10mmHg). Subject 33 showed functional improvement (at 3 months peak VO_2 increased from 7.6 to 12.4ml/kg/min, 6 minute walk distance increased 112 to 384m), structural remodelling (LVEDD decreased from 60 to 48mm and LVEF increased from 20 to 50%). This female patient was 33 years old with non-ischaemic dilated cardiomyopathy and notably had a recent diagnosis of HF, initially presenting 6 weeks prior to device implantation. As such it is unclear how much of this reverse remodelling was due to the device and how much might have been attributable to the natural history of her disease. Subject 25 also showed convincing functional improvement (at 3 months peak VO_2 increased from 12.4 to 17.0 ml/kg/min, 6 minute walk distance 482 to 574m), though structural remodelling was less evident (LVEDD marginally increased in size from 54 to 56mm, and LVEF mildly worse at 15%). This second patient was also a younger female (48 years old) though ischaemic and unknown preceding symptom duration.

In summary, there is some evidence of structural and functional reverse remodelling, including ventricular recovery, in a subgroup of patients. Younger age and lesser degrees of LV dilatation may be factors favouring successful reverse remodelling. In contrast, in other patients there was progression of the HF syndrome, despite Synergy support.

3.3.2.5 Left atrial pressure as a potential determinant of pump thrombosis

Contemporaneous clinical observation suggested that at least one episode of pump thrombosis was related to LA collapse and low pump flow in the context of a hypovolaemic patient in the immediate post-operative phase. We hypothesised that patients with high LA pressures (as inferred by high PCWP or greater LA diameter) preimplantation may be relatively protected from pump thrombosis and may be better candidates for Synergy support. Furthermore, if low PCWP is an important precipitant of pump thrombosis then good response to therapy with progressive lowering of PCWP and PASP could increase risk of thrombosis with duration of successful treatment, and presence of significant MR may protect against pump thrombosis.

Among these 25 patients, there was no difference in preimplantation PCWP or LA diameter between patients who required pump exchange and those who did not (preimplantation: PCWP 32mmHg (24, 33mmHg) vs. 31mmHg (21, 34mmHg), p=0.78; LA diameter 51mm (47, 54mm) vs. 52mm (40, 57mm), p=0.83). Furthermore, there was no correlation between days to pump exchange or total duration of support and baseline PCWP (R=0.28, p=0.40; R=0.24, p=0.25) or LA diameter (R=-0.14, p=0.76; R=0.08, p=0.25). Patients with severe MR appeared to be relatively protected from pump thrombosis. Two-way contingency showed that absence of severe MR gave a non-significant but increased relative risk of pump thrombosis of 3.0 (95% CI 0.8-11.1, p=0.10). Segregation of patients who required pump exchange into those who required exchange early (<60 days) or late (>60 days) suggested there were lower preimplantation PCWP values in those patients suffering pump thrombosis early (within 60 days) with a trend towards significance (PCWP 24mmHg (17, 32mmHg) vs. 33mm (29, 38mm), p=0.08). However, this could be confounded by progressive lowering of PCWP with therapy with duration of treatment. Thus, the lowest PCWP and LA observation were identified from each patient's dataset, to identify the hypothesised highest risk of thrombosis. There was no difference in the lowest PCWP or LA diameter between patients requiring pump exchange and those not, or between patients requiring early vs. late pump exchange. See Table 3.8.

Table 3.8: No influence of PCWP or LA diameter on pattern of pump thrombosis.

Showing the pre-implant measurement or the lowest measurement recorded during follow-up. See text for details. P-values refer to Mann-Whitney tests of early vs. late and yes vs. no.

		Required pump exchange			No pump exchange	p early vs. late	p yes vs. no
		Early	Late	All			
Preimplantation							
PCWP (mmHg)	n	5	6	11	12		
		24 (17, 32)	33 (29, 38)	32 (24, 33)	31 (21, 34)	0.08	0.78
LA diameter (mm)	n	3	4	7	12		
		53 (47, 54)	51 (45, 55)	51 (47, 54)	52 (40, 57)	0.86	0.83
Lowest observed							
PCWP (mmHg)	n	5	6	11	13		
		22 (14, 28)	17 (7, 20)	18 (10, 24)	18 (10, 26)	0.23	0.98
LA diameter (mm)	n	4	6	10	14		
		43 (38, 50)	42 (42, 48)	42 (40, 48)	46 (36, 50)	0.76	0.86

3.3.3 The C-Pulse substudy: progress and shutdown

3.3.3.1 Recruitment and decision to close the trial

The international C-Pulse study, upon which this substudy was dependent, was paused for recruitment after regulatory bodies withdrew approval for new device implants. The reason for device withdrawal was device infection complications occurring in patients in other centres internationally. We were unable to recruit any patients to the C-Pulse substudy before recruitment was halted.

3.3.3.2 Retrospective analysis

One patient underwent C-Pulse implantation during the period before we had ethical and governance approval for the substudy, but before the international study was closed. Data from this patient were analysed retrospectively.

3.3.4 Single patient experience with Sunshine Heart C-Pulse

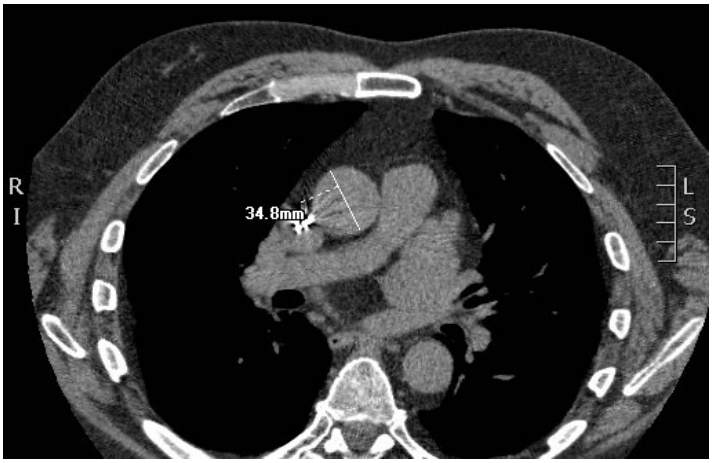
There were data available from one patient who underwent C-Pulse implantation at Harefield Hospital. The data are presented qualitatively, highlighting specific aspects of the case. Dr Chris Bowles was closely involved in management of this patient, and he and I acquired much of the data presented here in collaboration.

The patient was male and 54 years old on day of implantation. He had ischaemic HF, having presented initially in 2002 with acute myocardial infarction and cardiac arrest due to ventricular fibrillation, with successful resuscitation and primary angioplasty to the right coronary artery and left anterior descending (LAD), but complicated by LAD dissection and total occlusion. His most recent angiogram showed patent RCA stents, unobstructed circumflex vessel, with chronic total occlusion of LAD distal to the first diagonal. He was in permanent atrial fibrillation (AF) with previous AV node ablation and >99% biventricular pacing from a CRT-D device. Other medical history included type 2 diabetes, and previous thyrotoxicosis after amiodarone (now euthyroid on carbimazole and thyroxine replacement). Baseline characteristics are shown in Table 3.10. He was referred to Harefield Hospital for cardiac transplant assessment. He was in NYHA class III. His peak VO_2 was 12.8ml/kg/min

with 6 minute walk distance 205m, and he had no absolute contraindications to transplantation. He was deemed suitable for transplantation, and enrolled into the international C-Pulse study using the device as BTT. Specific preoperative workup included CT aorta to exclude aortic disease (Figure 3.8). During the transplant assessment period, he received an appropriate ICD shock for ventricular tachycardia.

Figure 3.8: Preoperative work-up before C-Pulse implantation.

Investigation includes computed tomography imaging of the aorta to exclude aortic pathology.



Implantation was off-pump and without complication, and the patient was discharged from intensive care on day 1 post operatively. He was treated with empiric antibiotics for a spike in inflammatory markers without clear source. He was discharged from hospital on day 18 post operatively.

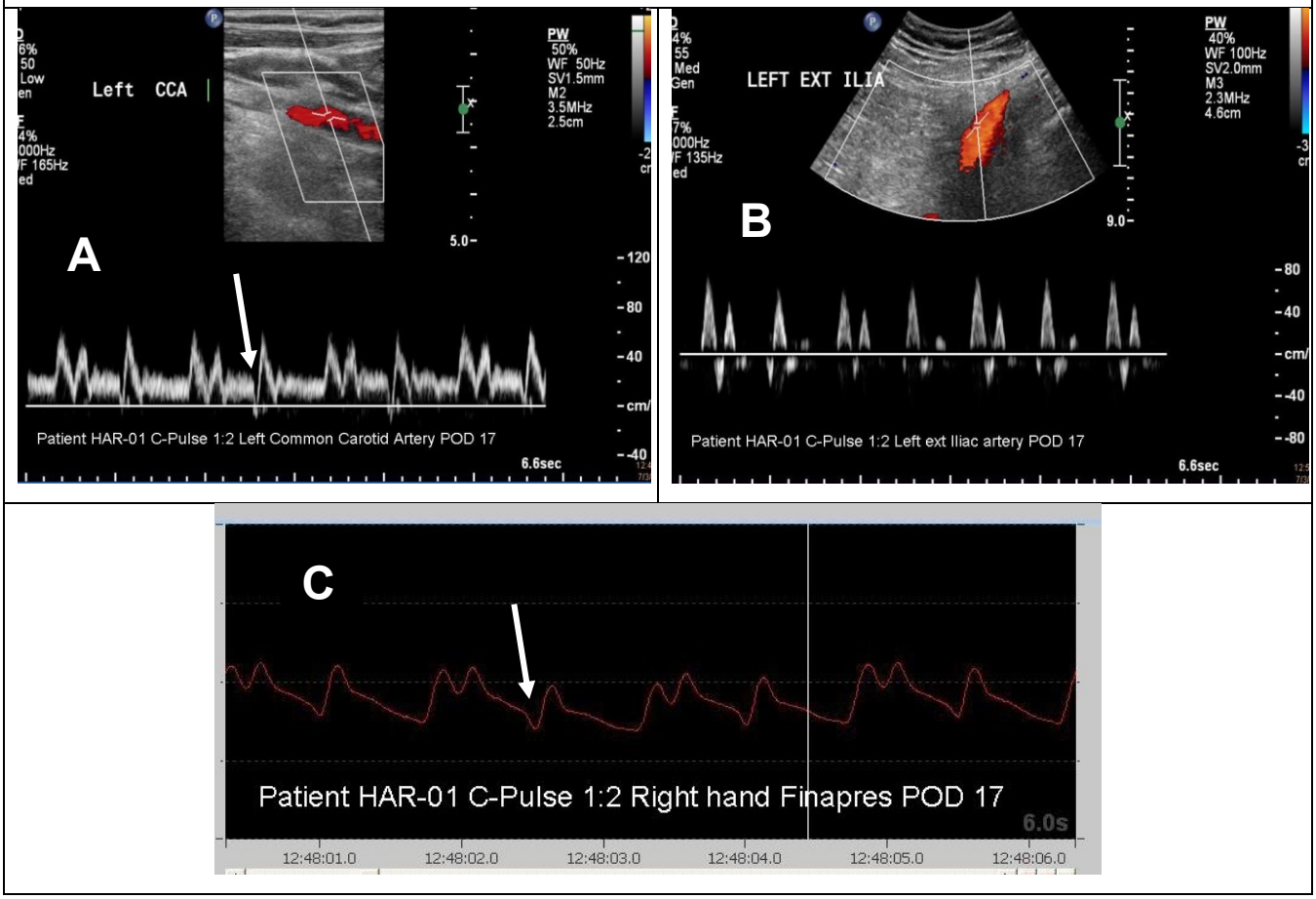
3.3.4.1 Optimisation of device function

We invested significant effort to ensure the C-Pulse device was optimised to best possible augmentation of cardiac dynamics. The principle variable is control of sleeve inflation and deflation, which is triggered by the R wave and set to standard parameters after implantation. This patient was initially >99% biventricular paced, and another important variable was paced heart rate. This was complicated by increasing ventricular ectopy burden, control of which provided further device optimisation.

Firstly, optimisation of device timing was performed with the aim of maximising the degree of pre-systolic augmentation provided by the device. In the perioperative phase, the requirement was verified by examination of the radial arterial blood velocity profile. After cessation of invasive monitoring, this was also attempted with echocardiography of LVOT and ascending aorta, but poor quality images precluded accurate assessment. Ultrasound Doppler of carotid, iliac and femoral vessels provided interpretable data (Figure 3.9). Setting the device to 1:2 allowed comparison of assisted vs. non-assisted beats. This technique required a radiologist and was time consuming. An alternative was use of the Finometer PRO non-invasive blood pressure monitoring system (Finapres Medical Systems, Enschede, The Netherlands) which provides similarly high resolution data but with greater ease.

Figure 3.9: Optimisation of the C-Pulse.

Techniques used were ultrasound Doppler of (a) left common carotid artery and (b) left external iliac artery; and (c) continuous blood pressure assessment using the Finometer PRO. Each of these images was acquired with the device running 1:2 (i.e. cuff inflation every other beat). Note the presystolic dip on assisted beats (white arrows).



Secondly, echocardiography with measurement of LVOT VTI was used for optimisation of pacer rate. Heart rate had been empirically adjusted postoperatively to 90 beats per minute. By sequential assessment the heart rate delivering best cardiac index in this patient was found to be 80 beats per minute (see Table 3.9 and Figure 3.10).

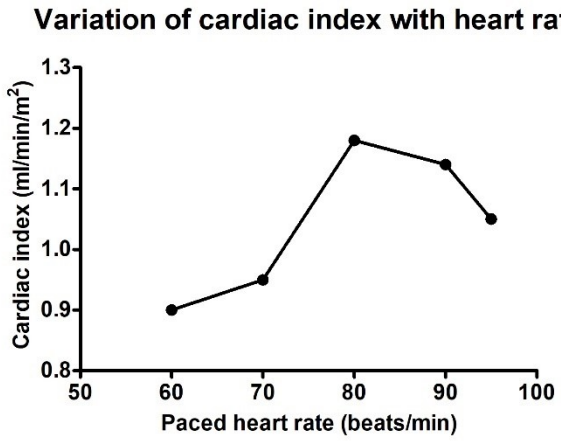
Table 3.9: Heart rate modulation for C-Pulse optimisation.

The velocity-time integral of flow in the left ventricular outflow tract (LVOT VTI) was calculated using echocardiography and pulse-wave Doppler. Stroke volume = VTI x LVOT area, calculated from measured LVOT diameter; Cardiac output = Stroke Volume x Heart Rate. Cardiac index normalises for body surface area. See Figure 3.10.

Heart rate (/min)	LVOT VTI (cm)	Stroke volume (ml)	Cardiac output (L/min)	Cardiac index (L/min/m^2)
60	11.7	33	2.0	0.90
70	10.7	30	2.1	0.95
80	11.7	33	2.6	1.18
90	9.8	28	2.5	1.14
95	8.6	24	2.3	1.05

Figure 3.10: Cardiac index variation with heart rate during C-Pulse support.

See Table 3.9. Calculated cardiac index varied with heart rate and was optimised at 80 beats per minute. Units of cardiac index are L/min/m^2.



Finally, optimisation of cardiac rhythm was needed. After around 2 months of device support a high ventricular ectopy burden (12%) and short runs of non-sustained VT were

desynchronising the device and impairing augmentation. Increased dose of beta-blocker controlled the arrhythmic burden and facilitated improved cardiac augmentation.

3.3.4.2 Device efficacy

The device was not successful in this patient in achieving BTT. On POD 112 the patient underwent upgrade to HVAD after four readmissions with worsening heart failure, rising BNP and persistently low cardiac output. Nonetheless, there was some evidence of clinical benefit (Table 3.10). Six minute walk distance increased by 128% (205 to 467m), though this was diminished during the period of frequent ectopy around the 2 month follow-up period. There was a rise in cardiac index (1.49 to 1.69L/m^2/min; Table 3.11 and Figure 3.11, page 96), though notably to a lesser extent than with milrinone with or without the counterpulsation. Mitigating against any significant improvement to cardiac function was a persistent rise in BNP throughout period, increasing from 155 at baseline to 412ng/L by 3 months. We did not observe any symptoms, signs or investigation results that would support a diagnosis of mediastinal infection, as was seen in some previous C-Pulse patients.

Table 3.10: Serial efficacy parameters in the C-Pulse.

Single patient data shown from preimplantation and serial timepoints thereafter. (Peak VO_2, peak oxygen consumption; VE/VCO_2, minute ventilation/carbon dioxide production; LVEDD, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; ALP, alkaline phosphatase; ALT, alanine transaminase; INR, international normalised ratio.)

Parameter	Preimplant	Weeks after implantation			
		2	4	8	12
Peak VO_2 (ml/kg/min)	12.8	-	11.9	-	-
$VE/VECO_2$ slope	60	-	43	-	-
6 minute walk (m)	205	316	403	330	467
LVEDD (mm)	68	61	60	59	63
LVEF (%)	29	24	11	25	16
LVOT VTI (cm)	7	12	9	9	10
BNP (ng/L)	155	346	217	434	412
eGFR (ml/min)	73	78	85	65	73
Bilirubin (µmol/L)	13	10	9	17	15
ALP (U/L)	122	346	217	169	188
ALT (U/L)	29	39	21	14	20
INR	1.1	1.2	1.2	1.5	1.3

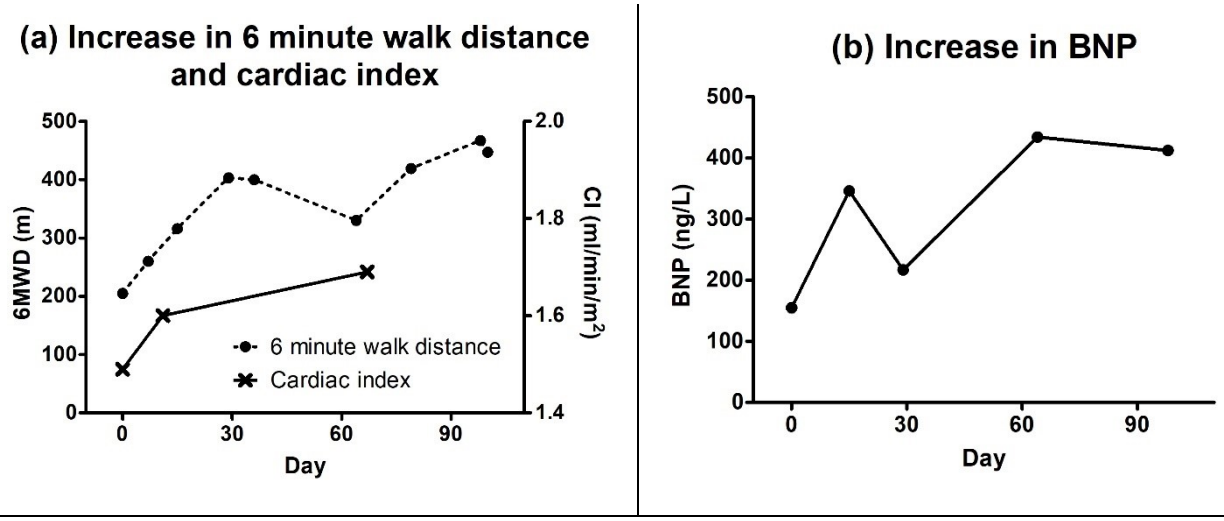
Table 3.11: Serial haemodynamic parameters in C-Pulse.

Single patient data shown from preimplantation and serial timepoints thereafter, with addition of milrinone (Mil) or device activation as indicated. (BP, blood pressure; s, systolic; d, diastolic; m, mean; HR, heart rate; RAP, right atrial pressure; RVP, right ventricular pressure; PASP, pulmonary artery systolic pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; W, Wood units; CI, cardiac index.)

	Preimplantation		**Post-operative day 11**			**2 months**			
	Baseline	**+Mil**	**Device on 1:1**	**Device off**	**Device on 1:1 +Mil**	**Device on 1:1**	**Device off**	**Device on 1:1 +Mil**	**Device off +Mil**
BP s/d/m (mmHg)	99/81/88	100/77/84	117/87	98/84	100/81	102/72	95/74	104/66	87/63
HR (/min)	60	60	90	90	90	80	80	80	80
RAP (mmHg)	23	-	14	-	11	11	-	-	-
RVP s/d (mmHg)	32/14	-	40/6	-	33/2	33/3	-	-	-
PASP s/d/m (mmHg)	34/17/23	28/8/13	39/21/29	39/21/29	33/22/23	34/19/25	36/21/27	37/20/26	33/18/23
PCWP (mmHg)	16	6	21	21	15	10	22	18	16
PVR (W)	2.08	1.85	2.55	2.85	2.02	1.68	1.40	1.42	1.20
CI ($L/min/m^2$)	1.49	2.23	1.60	1.48	2.02	1.69	1.69	2.00	2.75

Figure 3.11: Serial changes in walk distance and natriuretic peptides.

After 3 months' support with the C-Pulse there are (a) beneficial increases in 6 minute walk distance (6MWD) and cardiac index (CI), but also (b) increase in B-type natriuretic peptide (BNP).



3.4 Discussion

This chapter presents novel data pertaining to two devices designed for partial LV support in advanced HF. The devices are contrasting, one being a rotary blood pump and the other an entirely extravascular aortic sleeve for chronic counterpulsation. For both devices, we had planned prospective studies to evaluate their potential for reverse remodelling the ventricle under optimised haemodynamic conditions, but for reasons beyond our control both these studies were abandoned. The data presented are retrospective data collected from the clinical programmes at Harefield Hospital and University Hospitals Leuven.

3.4.1 The unmet need

Current MCS strategies serve well those patients with isolated LV failure who are likely to deteriorate inexorably without LVAD implantation, typically patients in INTERMACS classes 1-3. In this group the incumbent risks of LVAD implantation – as discussed in Chapter 1, but particularly perioperative death, stroke, bleeding, device thrombosis, infection – are sufficiently justified by the high risk of morbidity and mortality without treatment. We know that patients in INTERMACS 1-3 inevitably develop multi-organ dysfunction that compounds these risks, and device implantation earlier in the disease process improves outcomes. The trajectory of HF can be interrupted by medical and device therapy, and the low usage of renin-angiotensin axis blockers (in the Leuven cohort) and of ICD (at both centres) is interesting to note. This reflects the important role of the HF cardiologist, working alongside surgical colleagues, to ensure the correct early strategies have been exhausted prior to surgical therapies being considered.

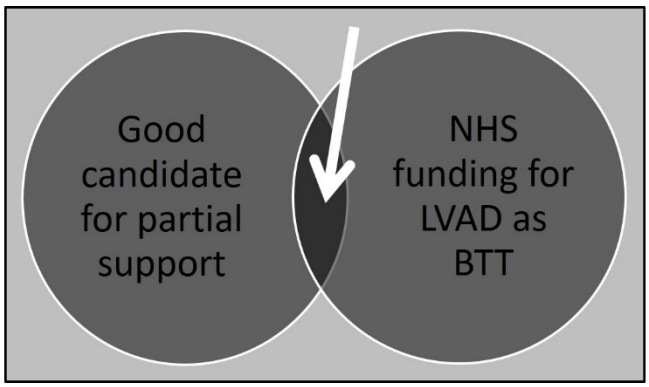
The concept of partial LV support, provided by smaller devices implanted off-pump by minimally invasive surgery, was proposed to address this challenge and justify MCS device implantation in these less gravely ill patients by markedly reducing the risks. The Synergy partial support LVAD and the C-Pulse chronic counterpulsation device were two devices introduced to meet this objective.

3.4.2 Synergy: progress, problems and promise

At the time of initiating the PAVE-UP trial, the Synergy device had been awarded its CE mark and was in use within the clinical MCS programme at Harefield. Initial single arm studies had established the device's safety and feasibility, and we sought specifically to study its propensity to induce beneficial reverse remodelling in an observational, controlled trial comparing patients implanted with the Synergy device to patients continuing standard medical therapy. One of the key challenges we anticipated was achieving sufficient recruitment into the intervention group, given the logistical challenge of meeting Synergy candidacy with indications and funding for BTT within the UK healthcare system (Figure 3.12). To overcome this, we collaborated with colleagues in Leuven, instrumental in developing the Synergy device and the centre with the largest clinical experience, to recruit at their centre.

Figure 3.12: Finding the ideal Synergy candidate.

Small patient group meeting criteria for BTT funding and suitability for partial support.



3.4.2.1 Device withdrawal

Regulatory suspension of new device implants cut this trial short. The specific problem that caused device withdrawal was episodes of inflow cannula fracture, described in a case report from Schmack and colleagues[154] in Heidelberg. Their patient presented 55 days post-implantation with a pump alarm, and was found to have pump stoppage due to thrombosis affecting the impeller and extending into the inflow cannula, and complete fracture of the inflow cannula around 4cm from the pump. Bleeding was prevented by the thrombus, which may have been lifesaving. The material fatigue may have been related to mechanical stress from contact with ribs as the cannula passed the intercostal space, and it's possible that a

specific aspect of the implant procedure was being done differently in some centres (e.g. use of second vs. third intercostal space changing stress pattern). The safety notice banned further implants and required screening of all current patients[153]. Reportedly some patients were found to have defective circuits, though this data has not been formally reported. No patients at Harefield were affected.

In the background were ongoing concerns about high rates of pump thrombosis. The data from this cohort of 25 patients shows survival free of pump thrombosis around 72% at 6 months and 64% at 1 year. This compares to 95% and 93% respectively in 9808 patients receiving HeartMate II from April 2008 to June 2014 from INTERMACS registry data[38]. Like thrombosis in the HeartMate II and HVAD, Synergy thrombosis is preceded by blood markers of haemolysis such as raised LDH and plasma haemoglobin. We did not have any instances where conservative treatment (e.g. intravenous heparin) resolved the issue without the need for pump exchange.

The first iterations of the device were prone to very early pump thrombosis, with 8 of the first 12 patients implanted requiring pump exchange[63], and in this cohort the rate of pump thrombosis was 0.82 events per patient-year. Revision of a washing channel in the pump, increasing the recommended target INR from 2.0-3.0 to 2.5-3.5, and, later, introduction of alternating speed algorithms to improve washout, were all strategies to tackle this, and were successful to some extent. The relative ease of pump exchange (access through a prepectoral incision akin to a pacemaker box change, compared to major cardiac surgery to exchange a standard LVAD) reduced the morbidity, but, as with pacemakers, pocket infection can be devastasting[155] and there remains the risk of sudden haemodynamic compromise from device stoppage. Furthermore, the requirement for dual antiplatelet therapy and warfarin with INR 2.5-3.5 led to bleeding complications in follow-up including epistaxis and pocket haematomas (see section 4.3.6, page 124).

The aetiology of the high rates of pump thrombosis is likely to be multifactorial. Firstly, the long and narrow calibre inflow cannula undoubtedly increase propensity to thrombosis due to flow characteristics in the narrower tube, perhaps exacerbated by episodes of extrinsic

compression or kinks with manipulation of the right arm. The inflow cannula length on the HVAD is a few centimetres, and on the HeartMate II around 10cm, while on the Synergy system typically around 30cm. Similarly, the diameter of the Synergy inflow is approximately 8mm compared to approximately 20mm for HVAD and HeartMate II. Secondly, it might be that the LA to arterial concept itself predisposes to thrombosis. In standard LV to aorta LVADs suction episodes and LV collapse are relatively rare to the stiffness of the LV. However, the LA walls are more compliant, and as the pump draws blood from the LA and the intrachamber pressure can quickly drop with a propensity to chamber collapse and suction episodes, where flow through the pump drops to zero creating conditions for thrombus formation. This may be propagated by early reduction in LA size with treatment (Figure 3.5f). The data here show no systematic relation between low PCWP and occurrence of pump thrombosis, but observational experience suggests than in the hypovolaemic patient postoperatively this can be important. Thirdly, the Synergy uses a high impeller rotation speed, potentially creating procoagulant effects from blood trauma and platelet activation. The Synergy was designed as a micropump and consequently the pump and in turn the impeller were significantly smaller than other LVADs. To achieve the necessary flow, this small impeller rotated at around 23,000-25,000rpm (compared to e.g. 2500-3500 for the HVAD). Mechanical disruption of blood is known to induce coagulopathy and platelet activation, and it may be that the Synergy was more prone to these abnormalities due to the high rotation speed. This is examined in Chapter 4. Conversely, anecdotal experience suggests that achieving impeller speeds ≥25,000rpm actually reduces propensity to thrombosis, by increasing flow. Finally, there is thrombogenicity of the materials and efficiency of blood washout in the pump. All artificial surfaces potentiate thrombosis, and engineers redesigning the pump will be exploring how materials properties, in-pump flow characteristics and speed algorithms can be optimised further. Other LVADs have been hindered by high thrombosis rates in early stages of clinical use, and it's possible that these setbacks could be overcome.

3.4.2.2 Effects on ventricular performance and reverse remodelling

These difficulties aside, our hypothesis was that partial LV support with novel circulatory support devices could facilitate functional, structural and molecular reverse remodelling of the LV. It is well understood that MCS can induce ventricular recovery in some patients[54,55],

and previous reports have shown that hearts explanted from patients supported with the Synergy device show improved pressure-volume relation[72]. Partial support devices could be a recovery strategy that improves ventricular loading conditions and allows greater uptitration of disease modifying therapy, but requiring less invasive implant procedure than current devices.

In this group of 25 patients receiving partial LV support with a Synergy LVAD, there is evidence of haemodynamic benefit with optimisation of ventricular loading conditions, with increased cardiac output, reduction in PCWP and reduction in PASP. Furthermore, there is neurohormonal remodelling, with significant and sustained reductions in natriuretic peptide levels. Together this pressure/volume unloading and improved neurohormonal milieu creates good conditions for LV reverse remodelling, and within this group there is some suggestion of improved functional capacity as measured with the 6 minute walk test. However, across the group as a whole, there is no improvement in peak oxygen consumption, and no suggestion of structural reverse remodelling seen on echocardiography. Indeed, LVEF seems to deteriorate during support with the Synergy pump. A subgroup of patients did show evidence of functional and structural reverse remodelling. Indeed, two patients showed signs of ventricular recovery which facilitated device explant with no requirement for other LVAD support or cardiac transplantation. Younger patients showed greater propensity to beneficial structural remodelling, while in contrast patients with larger ventricles tended to show decreases in ventricular performance measured by peak VO_2. This probably reflects the progression of adverse remodelling including myocardial fibrosis at the time of implantation., in line withexisting evidence that younger patients with shorter duration of illness have greater propensity to recovery, though how much is due to the LVAD and how much the natural course of disease is difficult to discern.

Overall, the data here show the primary haemodynamic benefit is direct preload reduction, resulting, as seen in this cohort, in sustained reductions in PCWP and consequently PASP. The consequent optimised cardiac dynamics, alongside cardiac output increased by between 1.5-2.0L per minute, can improve exercise capacity. The degree of systemic blood pressure elevation is less than seen with full support devices, helping to avoid undesirable increases in afterload and helping to perpetuate continued aortic valve opening. Previous experience has

suggested that LV recovery requires the combination of significant LV offloading to promote structural reverse remodelling on the one hand, yet some training of the LV to prevent disuse atropy[54,55]. It may be that the Synergy inherently meets the latter but not the former objective.

3.4.3 Unclear role for chronic counterpulsation

The C-Pulse device was another mode of partial support examined here. Intra-aortic balloon counterpulsation has long been used in the BTT setting and acute cardiogenic shock after myocardial infarction. The results from the SHOCK-2 trial have challenged standard practice in the latter situation, such that extracorporeal membrane oxygenation and percutaneous temporary circulatory support devices have largely superseded it in this setting[156,157]. Ambulatory IABP is challenging due to vascular access, and the C-Pulse sleeve was conceived as an implantable device to deliver the benefits of counterpulsation in the outpatient setting. However, unlike the Synergy device the C-Pulse does require sternotomy for implantation, though avoiding cardiopulmonary bypass, and as such is still major surgery to justify to a patient in INTERMACS≥4. At Harefield, we were recruiting patients for the international study funded and sponsored by the manufacturer and intended to gain CE mark classification for the device. Our substudy intended to provide a more detailed assessment of ventricular function, focussing on evidence of reverse remodelling and as such paralleling some aspects of the PAVE-UP study. Like the Synergy, the device was withdrawn and complete assessment of the hypothesis was not possible.

Infection was a major problem during the initial C-Pulse study with 3 of 5 patients suffering systemic sepsis due to mediastinitis or other device-related infection[68]. The patient with mediastinitis in the initial study had a fall with pneumatic driveline fracture, and subsequently infective pneumomediastinum. After modifications just 1 of 20 patients in the first safety study suffered major infection (though 40% had some driveline site infection)[69], but nonetheless it was infection that again drew the CE mark study to its halt, though this is not reported in a preliminary data paper from the study[152]. The driveline is a nidus for infection on any implantable MCS device, but particularly for the C-Pulse due to its large size, required to accommodate pneumatic control over the aortic sleeve. The sleeve's interaction with the aorta is another limitation. Reports to date suggest there is inflammatory response

causing some adventitial injury, and certainly in our experience at Harefield there were dense adhesions between cuff and aorta that had developed after 120 days of support[158].

There were some signs of clinical benefit in our patient, with increased walk distance and cardiac index, though there were contradictory changes in BNP (large increase) and LVEF (worsened), and four HF hospitalisations in the 3 months of device support. It's unclear whether, on balance, this patient derived significant benefit from the device. Published data have suggested the C-Pulse can yield clinical benefit, though again in small numbers and in a single-arm design open to bias[68,152]. We found that onset of ventricular ectopy significantly impaired device function, and rhythm stability remains another key factor in patient selection. Optimising the inflation and deflation timings was a challenge, and in our experience the manufacturer's protocol did not sufficiently address this. Any device design revisions would need to develop a more coherent approach.

Key advantages of the C-Pulse which remain relevant were firstly its extravascular operation, thereby limiting blood trauma and thereby limiting coagulopathy and platelet activation, and secondly the ability to disconnect or switch off the device for long periods without danger (e.g. for showering, or for clinical assessment of underlying heart function). In a future study with the C-Pulse, the clinical benefit could be evaluated by a crossover design, where patients are implanted and observed in settings of both active counterpulsation and switched off. Despite this, at present it's not clear that the benefits derived from the C-Pulse are sufficient to justify the risks incurred in its implantation.

3.4.4 Suitability of partial support as bridge to transplantation

The results from these devices have been mixed so far, and there is much refinement to come before they will be widely adopted for ambulant HF patients who are symptomatic but remain stable. While the Synergy per se was fundamentally flawed, it's legacy is demonstration of proof of principle of the LA to right subclavian artery partial support concept.

The key issue is identifying the appropriate substrate for partial support. Undoubtedly one problem studying these devices and integrating them into MCS programmes within the UK is their limitations in severely unwell patients in INTERMACS classes 1-2. At present, NHS funding for long term MCS is restricted to the BTT indication, and, while there is some flexibility and a move to bridge to candidacy (e.g. treating reversible pulmonary hypertension), ultimately most patients considered for long term MCS are strictly too advanced for partial support (Figure 3.12). Previously reported cohorts suggest the Synergy can work in critically ill patients[159], and this is supported in our cohort showing no significant difference in support duration between INTERMACS classes 1-3 vs. 4-7. The C-Pulse is similarly limited in a BTT capacity by its limited augmentation of haemodynamics and cardiac function.

Aside from younger age, less degree of LV dilatation, and elective implantation it's unclear from this small cohort which factors are key determinants of long term success for Synergy implants, both for duration of successful support and reverse remodelling. Specific reasons for using Synergy rather than a standard full support device in more advanced patients within our cohort included previous LVAD making redo apical device a challenging and high risk surgery, and requirement for support in a young patient and potential to avoiding sternotomy with Synergy. Both patients eventually required alternative approaches. The key should be to prioritise the likely support requirements above other considerations.

Candidates for partial support will be ambulant HF patients, and a priority should be establishing best medical therapy prior to consideration for LVAD implantation. Across our cohort there was lower than expected usage of ICD and CRT, and in the Leuven patients there was low use of core drug therapy such as ACE inhibitors. Any benefit from partial support will be incremental, on top of the significant benefit delivered by current guideline medical and device therapies. This is important prior to implant, and as well during LVAD support, for example by systematically uptitrating drug doses.

To assist patient selection and optimal management within our institution in advance of the PAVE-UP trial, I and others proposed selection criteria and a perioperative management

algorithm which was agreed by the programme leads (see Table 3.12, page 107 and Figure 3.13, page 108). The goals were to ensure patient selection was appropriate and carried full multidisciplinary team support, and ensure perioperative team including anaesthetic and colleagues on the intensive care unit understood the peculiarities of the device. The criterion stating that severe MR is an absolute contraindication followed an experience in one patient in the initial Harefield cohort who had very poor outcome, with increasing reliance on the LVAD, cessation of aortic valve opening, and quickly deteriorating HF, supposedly related to the MR allowing reflux from the LV to LA and severely impaired native LV function. The data from this cohort suggest severe MR is not an important factor determining duration of successful support, so this criterion could be revised.

3.4.5 Limitations

The absence of prospective, controlled datasets and reliance on a small group of heterogeneous and retrospective data significantly limit the validity of conclusions that can be drawn. Nonetheless, the inclusion of data from multiple centres helps to mitigate these deficiencies. Practically, retrospective data collection yields incomplete datasets, and in addition to missing parameters (identified throughout by consistent quotation of n numbers), there are several missing parameters that could have been included. These include (1) systematic recording of adverse events, (2) data on mean arterial blood pressure and pump impeller speed serially throughout the period, and (3) detailed analysis of acquired aortic regurgitation in the cohort. Furthermore, recruitment of a control group would strengthen the interpretation.

3.5 Conclusions

Partial support is a new concept in long term MCS strategies and there is good rationale for its inclusion in advanced HF programmes for patients with symptomatic severe HF who do not yet meet criteria for transplantation. Two devices with differing mechanisms of action have been studied in this Chapter, both of which were hindered by significant drawbacks that limit their widespread adoption, and both of which were associated with limited evidence of benefit in some patients. The Synergy LVAD delivers incremental haemodynamic benefits with some evidence of improved functional status, and in a subgroup there evidence of

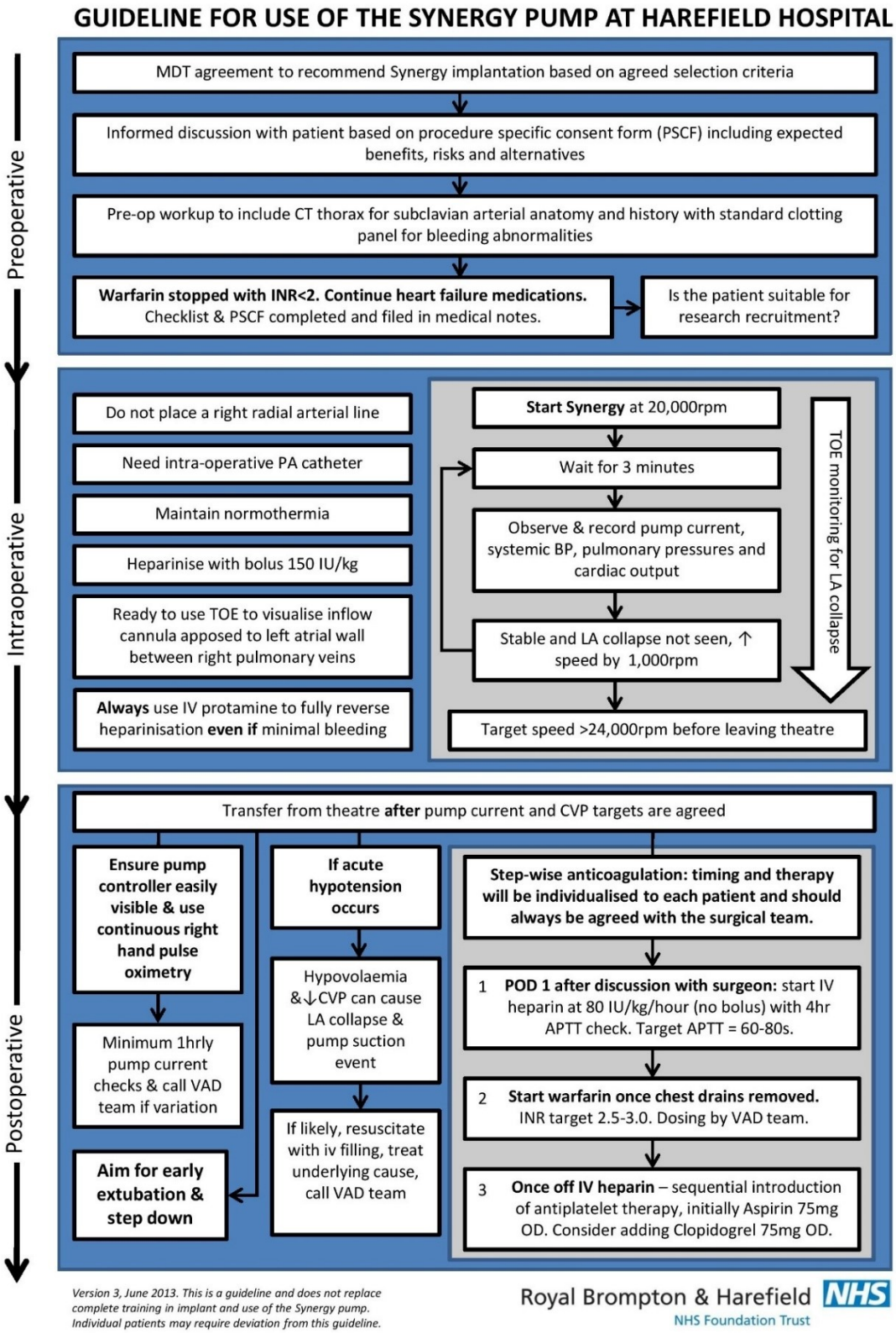
reverse remodelling, but progress has been halted while the device is re-engineered taking account of problems with pump thrombosis and inflow cannula mechanical failure. The concept of LA to arterial conduit shows promise, particularly in patients with high PCWP and pulmonary hypertension, and should be explored further in controlled trials. Benefit from the C-Pulse chronic counterpulsation device is less clear, and the device was associated with high burden of infection which ultimately precipitated its withdrawal. It is less clear whether chronic counterpulsation is a tool which will be beneficial in the HF clinic in the future.

Clinical research in advanced HF and surgical therapies is important but challenging, and an absence of large, controlled studies can force clinicians to rely on anecdote over evidence. My experiences in this section of my PhD underline the significant challenges of clinical research in this field.

Table 3.12: Agreed clinical criteria for suitability for Synergy implantation at Harefield Hospital as agreed in June 2013.

1. Advanced heart failure and eligibility to receive left ventricular assist device as bridge to cardiac transplantation (or alternative funding source agreed).
2. Persistently poor functional status and/or quality of life and/or end organ function despite maximal tolerated medical therapy and cardiac resynchronisation therapy where indicated. This could be manifested by:
    a. Frequent hospital admissions due to heart failure;
    b. Requirement to reduce doses of HF medications due to intolerance;
    c. Symptomatic status persistently less than INTERMACS 6;
    d. Deteriorating renal function expected to improve with partial support device (i.e. eGFR 20-50mls/min).
3. True cardiovascular inotrope dependency is an absolute contraindication. However, exceptions include the use of milrinone (or any other agent) for the purposes of reducing pulmonary vascular resistance/PA pressure, enhancing renal function or cardiac recompensation.
4. Patients with resting heart rate >100bpm in the absence of positively chronotropic drugs are unsuitable.
5. Absence of severe functional mitral regurgitation and a 6 minute walk distance of >175m are mandatory.
6. Cardiac index < 2L/min/m^2 is a relative contraindication.
7. Absence of surgical contraindications to Synergy implantation (e.g. subclavian artery stenosis).
8. Elevated pulmonary artery and pulmonary capillary wedge pressures are not contraindications to implantation.

Figure 3.13: Clinical protocol for perioperative management of patients undergoing Synergy LVAD implantation at Harefield Hospital as agreed in June 2013.



4: Comparative coagulopathy in implantable assist devices

4.1 Introduction

4.1.1 Background and rationale

Bleeding and thrombotic complications continue to provide a high burden of morbidity in LVAD patients, frequently manifesting as stroke, gastrointestinal bleeding or pump thrombosis[25]. Bleeding is at least in part related to an acquired von Willebrand factor (vWF) deficiency syndrome, as discussed in Chapter 1. Pump thrombosis is multifactorial, related to a multitude of factors reflecting the interaction between pump and patient and particularly affected by particulate ingestion, thrombogenic materials, prothrombotic aspects of pump mechanics such as flow stasis or component heating, and activation of platelets and coagulation cascades by shear stress on blood constituents. There has been recent focus on pump thrombosis after an increase in incidence in patients with HeartMate II devices[33,38], and the CircuLite Synergy pump, discussed in Chapter 3, was significantly affected by occurrence of pump thrombosis.

In the case of the Synergy, multiple factors are likely to be involved (see section 3.4.2.1, page 98), but one hypothesis is that the high rotation speed of the Synergy impeller coupled with the narrow cannula and pump cavity dimensions causes higher degrees of shear stress to blood components than would be expected in other devices. In turn this might cause greater degrees of platelet activation and greater impairment of fibrinolytic pathways, and greater breakdown of large vWF multimers compared with other LVADs. Markers of prothrombotic tendency could be used to identify patients at risk of pump thrombosis for early instigation of targeted therapy such as enhanced anticoagulation regimes. This Chapter sets out to test this hypothesis by studying specific markers of these processes.

4.1.2 Hypotheses

We hypothesised that patients implanted with the Synergy LVAD would show (1) evidence of increased platelet activation and greater impairment of fibrinolytic pathways compared with patients implanted with the HeartMate II, and that this might help identify patients at risk of pump thrombosis; and (2) greater loss of vWF activity, with associated higher rates of bleeding.

4.2 Methods

4.2.1 Aims

The aims of the study were to quantify markers of platelet activation, fibrinolysis and vWF activity during the first 6 months after LVAD implantation (1) in the advanced HF condition vs. published control data, (2) in the Synergy LVAD vs. the HeartMate II LVAD, and (3) in those Synergy implants going on to suffer pump thrombosis vs. those remaining free of pump thrombosis. Biomarkers were selected and analysed as shown in Table 4.1.

Table 4.1: Haematological assays.
(vWF, von Willebrand factor.)

Markers of platelet activation	Platelet factor-4 (PF4), platelet microparticles (MP)
Markers of impaired fibrinolysis	Plasminogen activator inhibitor-1 (PAI-1)
Markers of vWF function	vWF collagen binding activity (vWF:CBA)

4.2.2 Study design

Human plasma samples were obtained from patients who underwent implantation of a Thoratec HeartMate II or CircuLite Synergy LVADs at University Hospital Leuven, Belgium (UHL) between November 2009 and September 2011. All samples were collected with informed consent and ethical approval as part of a previous research project (Study number S-52-659; Ethics approval number ML-6832). Plasma samples were collected prior to LVAD implantation, at 14 days after implantation in some patients, at 3 months and at 6 months.

From the larger cohort of samples available patients were prioritised who had baseline and 6 month samples available. Samples were obtained and processed as set out in Chapter 2.

Clinical data were obtained contemporaneously with sample collection. Assays of NT-proBNP and estimation of LVEF were performed respectively by the clinical laboratories and core echocardiography laboratory at UHL. Transfer and use of clinical specimens and confidential clinical data from UHL was done under a Material Transfer Agreement agreed by UHL and RBHT dated January 2014.

4.2.3 Laboratory work

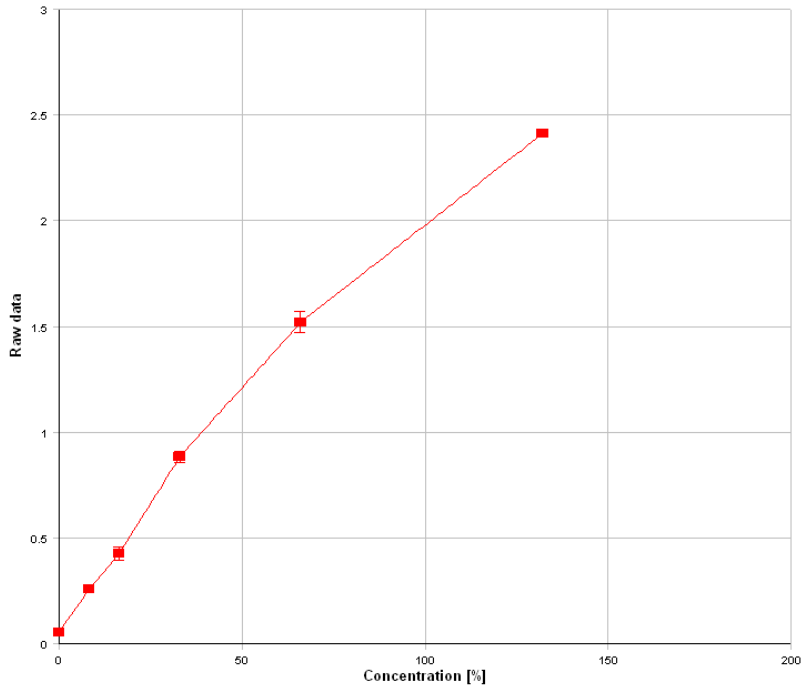
I performed all bench work in the Haematology Laboratory at Royal Brompton Hospital under the supervision of Mr. Simon Davidson, Consultant Clinical Scientist. All the molecular quantification was done using enzyme-linked immunosorbent assay (ELISA) as per manufacturers' instructions. The kits used were Zymutest PF4 (RK006A), Zymutest vWF:CBA (RK038A), Zymutest MP-Activity (521096) and Zymutest PAI-Antigen (RK012A) (all Hyphen Biomed, Neuville sur Oise, France).

The amount of colour developed in the ELISA reaction is directly proportional to the concentration of the assay target in question, and light absorbance is measured at wavelength 450nm. Quantification for each assay was done using a calibration curve based on a serial dilution of a supplied control sample of defined concentration (see Table 4.2 and Figure 4.1, page 112). Manufacturer recommended dilutions varied by assay. Plate reading and data quantification was done using Magellan ELISA analysis software (Tecan, Männedorf, Switzerland). Calibration curve and control specimens were run in duplicate. Each clinical sample was analysed once. There was a negative control included on each reaction plate. Where an experimental result was above or below the limits of the calibration curve it was not possible to calculate an accurate concentration/amount for that sample. In these cases, the result was substituted with the highest (or lowest) value from the dataset on that assay.

Table 4.2: Illustrative dilutions for the calibration curve.						
Desired concentration	C	C/2	C/4	C/10	C/20	0
Volume of calibrator control (ml)	1	0.5	0.25	0.1	0.05	0
Volume of diluent (ml)	0	0.5	0.75	0.9	0.95	1

Figure 4.1: Example calibration curve.

The raw light absorbance data is calbrated to known concentrations of assay target based on the serial dilution, and then extrapolated to form a standard curve. Each calibration concentration is analysed in duplicate, and points represent mean of the two data outputs (error bars are standard error). This was the calibration curve for von Willebrand factor collagen binding activity.



4.2.4 Prospective study

The original intention was that this hypothesis would be integrated into the PAVE-UP study and C-Pulse substudy as set out in Chapter 3. Analyses planned in addition to those set out here were (1) systematic platelet aggregometry testing using the MultiPlate analyser, for functional testing of platelet activation and function; (2) comprehensive assessment of vWF biology including electrophoresis for WF multimers, in collaboration with Professor Barbara Zieger in Freiberg, Germany. However, with the early cessation of these trials this was not possible.

4.2.5 Statistical analysis

Statistical analyses were done using IBM SPSS Statistics Version 23 and GraphPad Prism version 5. All data were treated as non-parametric. Comparisons of paired data used Wilcoxon signed-ranked tests, and comparisons of independent samples used Mann-Whitney U tests. In the case of multiple comparisons these were adjusted using the Bonferroni correction.

4.3 Results

4.3.1 Study population and baseline characteristics

There were 79 serial plasma specimens from 25 patients with severe advanced heart failure who underwent LVAD implantation, of whom 17 received a CircuLite Synergy partial support LVAD and 8 received a Thoratec HeartMate II LVAD. Patients' baseline characteristics are summarised in Table 4.3 (page 114). The cohort was predominantly male (88%) with evidence of severe HF (44% in INTERMACS profiles 1-3, NT-proBNP concentration 2735 (1492, 9038) ng/l and LV end diastolic dimension 63mm (56, 67mm). As would be expected, patients selected for full LVAD support with HeartMate II were in more severe INTERMACS class than patients selected for partial support with the Synergy (88% vs. 24% respectively in INTERMACS classes 1-3, $p<0.01$). There was no difference in platelet count or INR (International Normalised Ratio) between study groups.

4.3.2 Antiplatelet and anticoagulant therapy

Patients' preimplantation medication records were obtained as far as possible, though complete records were available for 9 patients of whom 8 were recipients of the HeartMate II. Amongst these 9 patients, 5 were on Aspirin and 3 on Clopidogrel at the time of implantation. Median INR was 1.3 (1.2, 1.4) before device implantation, though details of heparinisation were not available. After device implant, patients receiving the Synergy LVAD were universally treated with Aspirin, Clopidogrel and Warfarin (target INR 2.5-3.5). Patients receiving HeartMate II were treated with Aspirin and Warfarin (target INR 2.0-3.0).

Table 4.3: Baseline characteristics in the haematology analysis.

Data shown for the whole cohort, and split by type of LVAD. Continuous variables are all non-parametric and presented as median (25^{th}, 75^{th} centile). Categorical variables are presented as n (%). P-values are for Mann-Whitney test for continuous variables, and Chi-squared for categorical variables. (INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; NT-proBNP, N-terminal pro-hormone of brain natriuretic peptide; IQR, interquartile range; LV, left ventricular.)

	Whole cohort (n=25)	**Synergy (n=17)**	**HeartMate II (n=8)**	**p-value**
Gender, n (%)				
Female	3 (12)	1 (6)	2 (25)	0.23
Male	22 (88)	16 (94)	6 (75)	
Age in years, median (IQR)	58 (49, 70)	64 (49, 71)	57 (50, 64)	0.56
Aetiology, n (%)				
Ischaemic	14 (56)	8 (47)	6 (75)	0.23
Non-ischaemic	11 (44)	9 (53)	2 (25)	
Medications, n (%)				
Aspirin	5 (20)	1 (6)	4 (50)	
Clopidogrel	3 (12)	1 (6)	2 (25)	-
Other platelet inhibitor	0 (0)	0 (0)	0 (0)	
Incomplete drug history	16 (64)	16 (94)	0 (0)	
Platelet count	225 (170, 268)	225 (179, 253)	221 (136, 327)	0.85
International normalised ratio	1.3 (1.2, 1.4)	1.3 (1.1, 1.4)	1.3 (1.2, 1.4)	0.64
Pump thrombosis	8 (32)	8 (48)	0 (0)	0.03
Heart Failure severity markers				
INTERMACS profiles 1-3, n (%)	11 (44)	4 (24)	7 (88)	<0.01
NT-proBNP (ng/l), median (IQR)	2735 (1492, 9038)	2744 (2241, 8935)	1582 (1405, 6900)	0.4
LV end diastolic dimension (mm)	63 (56, 67)	63 (57, 67)	61 (51, 69)	0.77

4.3.3 Parameters in advanced heart failure before LVAD implant

Four parameters were studied in the advanced HF cohort from before LVAD implantation. Parameters are shown in Table 4.4 (page 120).

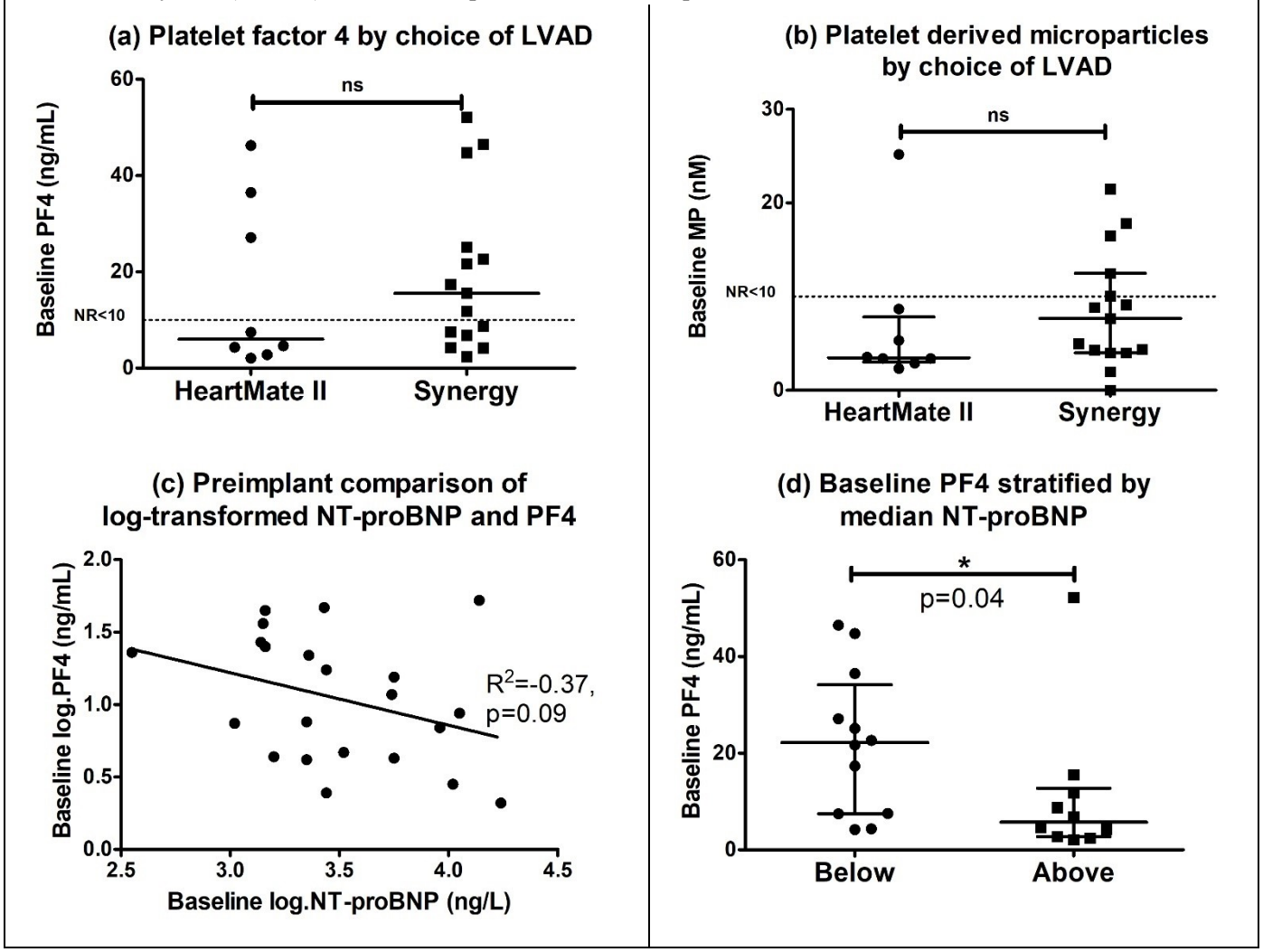
4.3.3.1 Platelet activation

There was general elevation of PF4 across the cohort, with 52% (41 of 79) individual results above the quoted normal range (>10ng/mL). There is suggestion that baseline PF4 varied with severity of HF, but in the contrary direction to what might be expected. Plotted linearly, NT-proBNP and baseline PF4 appear to show an inverse exponential relation, with PF4

decreasing as HF severity increases. After log transformation, there is a weak bivariate correlation with R=-0.37, p=0.09), though dividing the cohort at the median value for NT-proBNP (2735ng/L) does show that patients with NT-proBNP above the median (i.e. with greater severity of HF) had significantly lower PF4 levels than those below the median (5.74ng/mL (2.80-11.82) vs. 22.19ng/mL (7.50-34.14), p=0.04; see Figure 4.2c-d). There was no significant variation in PF4 between patients implanted with HMII vs. Synergy (6.05ng/mL (3.19-34.1) vs. 15.54ng/mL (6.85-25.12), p=0.44; see Figure 4.2a) nor variation with INTERMACS class (classes 1-3, 13.18ng/mL (3.86, 38.93) vs. classes 4-7, 11.82ng/mL (7.16-23.89), p=0.85).

Figure 4.2: Markers of platelet activation before LVAD implantation.

*There were elevated levels of platelet factor-4 (PF4, a) and platelet-derived microparticles (MP, b) in some patients, but no significant differences by type of LVAD. Patients with higher NT-proBNP at implant tend to have lower levels of PF4 (c and d). ns indicates p>0.05, * indicates p<0.05.*

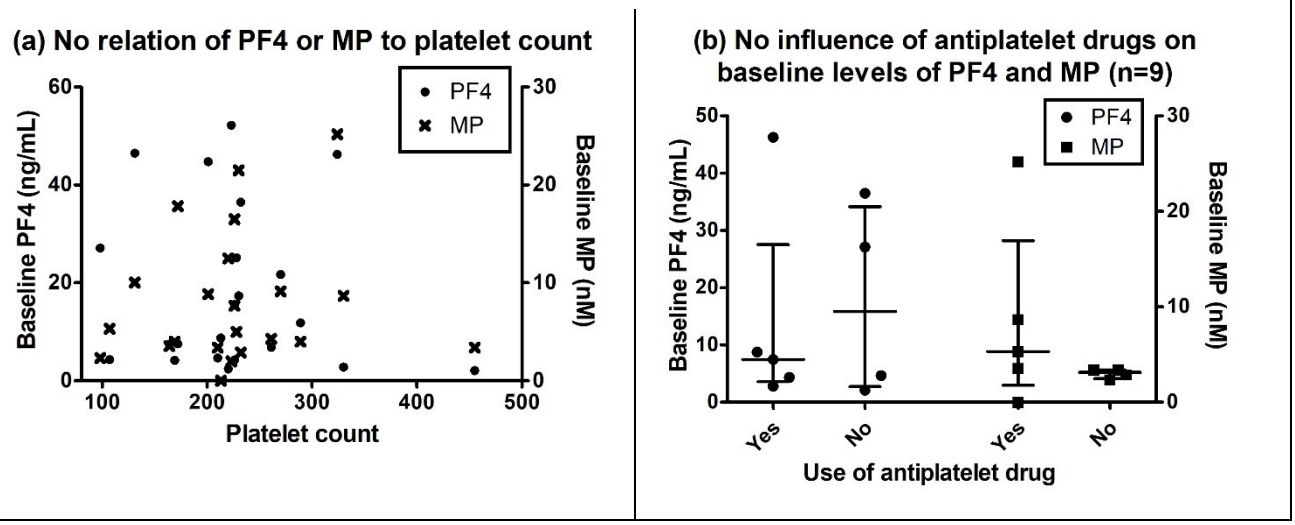


There was evidence of elevated MP in some patients implanted with Synergy vs. HeartMate II, but this was not consistent nor statistically significant (7.66nM (4.00-12.48) vs. 3.46nM (3.01-7.84), p=0.20; see Figure 4.2b). There was no similar relation with NT-proBNP (above median 4.13nM (3.03-9.63) vs. below median 5.15nM (3.65-9.80), p=0.32), and no significant correlation with PF4 (R=0.06, p=0.62). The levels of PF4 and MP showed no relation with total platelet count at implantation (respectively, R=0.14, p=0.53 and R=-0.10, p=0.67; see Figure 4.3a).

Limited information on use of antiplatelet drugs precluded detailed analysis of the effect of pharmacotherapy on levels of PF4 or MP prior to LVAD implantation. Full medication history was available for 9 patients, 8 of whom were HeartMate II recipients. Within this group, there was a suggestion that use of antiplatelet drug may increase levels of MP (5.31nM (1.78, 16.93) vs. 3.14nM (2.46, 3.39), p=0.19). There was no clear variation in PF4 between patients taking or not taking an antiplatelet drug (7.46ng/mL (3.57, 27.51) vs. 15.88ng/mL (2.71, 34.14), p=1.00; Figure 4.3b).

Figure 4.3: Possible confounders of platelet activation markers.

Analysis for confounders shows no influence of platelet count (a) or use of antiplatelet drugs (b) on levels of plate factor-4 (PF4) and platelet-derived microparticles (MP) at time of LVAD implantation.

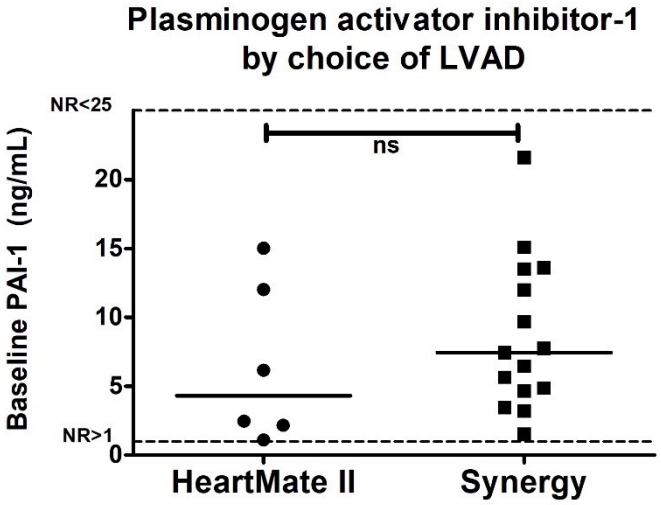


4.3.3.2 Fibrinolytic activity

PAI-1 was studied as a marker of fibrinolytic activity in the cohort. There was no significant difference in PAI-1 between the device cohorts (Synergy: 7.44ng/mL (4.65, 13.53) vs. HMII: 4.32ng/mL (1.91, 12.77); p=0.31; see Figure 4.4), nor any significant relation at the time of implantation of PAI-1 with NT-proBNP (Spearman R=0.38, p=0.10) or INTERMACS class (classes 1-3, 6.80ng/mL (2.25, 12.15) vs. classes 4-7, 6.48ng/mL (4.06, 12.78), p=0.56).

Figure 4.4: Fibrinolytic activity before LVAD implantation.

There was no significant difference in levels of plasminogen activator inhibitor-1 by subsequent choice of LVAD. ns indicates p>0.05.

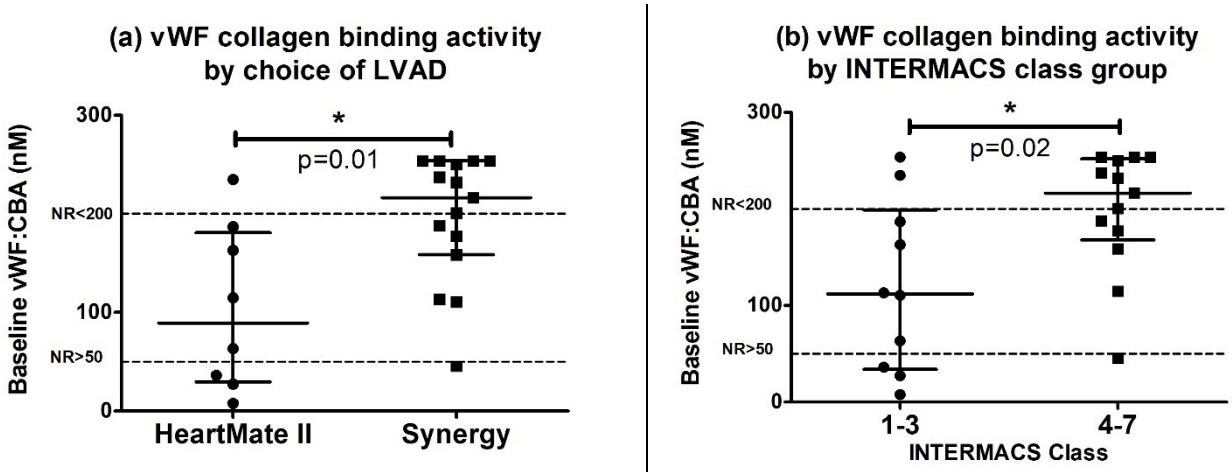


4.3.3.3 von Willebrand factor activity

vWF:CBA was higher in patients with less severe HF presentations, suggesting increasing impairment of vWF function with worsening HF syndrome, possibly related to inflammation. Preimplantation vWF:CBA was higher in patients who underwent Synergy implantation than in those receiving HeartMate II (216.42% (158.63-253.69) vs. 89.26% (29.58-180.99), p=0.01), and similarly higher in patients in higher INTERMACS classes (classes 1-3, 111.95% (33.98-198.92) vs. classes 4-7, 216.42 (167.95-251.91), p=0.03; see Figure 4.5). vWF is higher in women than men which can causing confounding of vWF:CBA. There was a heavy male predominance in the cohort, but with these small numbers no significant difference was seen (male n=20, female n=3; 187.45% (111.75-246.94) vs. 113.23% (60.54-174.02), p=0.31).

Figure 4.5: von Willebrand factor collagen binding activity before LVAD implantation.

*At baseline patients with more severe heart failre syndromes as evidenced by choice of LVAD (a) and preoperative INTERMACS profile (b) have lower vWF collagen binding activity (vWF:CBA). * indicates p<0.05. (INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support.)*



4.3.4 Variation with duration of support

The levels of the four markers were studied sequentially throughout the 6 month period after LVAD implantation, assessing for differing effects of the HeartMate II versus the Synergy LVADs. The serial measurements and delta changes are shown in Table 4.4 and Figure 4.6 (on page 120 and 121 respectively) with details of the n numbers at each timepoint[a].

4.3.4.1 Comparison of devices during the first 6 months after implantation

There was no significant variation in markers of platelet activation throughout the 6 month period. In patients with the Synergy LVAD, levels of PF4 appeared to decrease with ongoing LVAD support (at 6 months -41% (-70, +13), p=0.26) whereas in patients with the HeartMate

[a] There were variable numbers of data points available for individual patients, resulting in varying n numbers across the study timepoints. These are shown clearly in Table 4.4. Delta changes were calculated based on paired data only, and in some cases this creates discordance where the delta change does not reflect the apparent change in calculated medians for two timepoints calculated individually. For example, for PAI-1, the delta change at 3 months in Synergy patients (n=11) is +14 (-29, +124), whereas the calculated median at 3 months is less than the preimplant figure (7.22ng/mL (4.08, 16.67; n=13) from 7.44ng/mL (4.65, 13.53; n=15), suggesting a negative delta change. Here, the delta change is calculated using data from the 11 patients for whom there is paired preimplant to 3 month comparison data, discarding data for which there is no paired comparator.

II there was an increase (at 6 months +74% (-78, +386), p=0.78), though not with any statistical significance in either case. MP levels remained within reported normal limits with no significant variation between devices or with duration of LVAD support (at 6 months +47% (+7, +65; p=0.58) in HeartMate II patients and +41% (-18, +160); p=0.26) in Synergy patients).

Likewise, there were no significant changes in PAI-1 in either Synergy or HMII patients during 6 months of LVAD support. Both groups showed positive delta changes (Synergy +41% (-51, +143), p=0.48; HeartMate II +138 (-10, +236), p=0.23) though neither was statistically significant and median values remained within the reported normal limits at all time points.

Regarding vWF function, Synergy patients showed early and sustained decreases in vWF:CBA which were statistically significant (at 6 months -62% (-80, -26), p=0.02; due to Bonferroni correction, α=0.025). This was noticeably due to the significantly higher vWF:CBA in Synergy patients before LVAD implantation, as noted above. There were no significant changes in vWF:CBA in HMII patients (at 6 months -31% (-76, +144, p=0.48), nor were there differences between patients with Synergy vs. HMII at the 3 or 6 months timepoints.

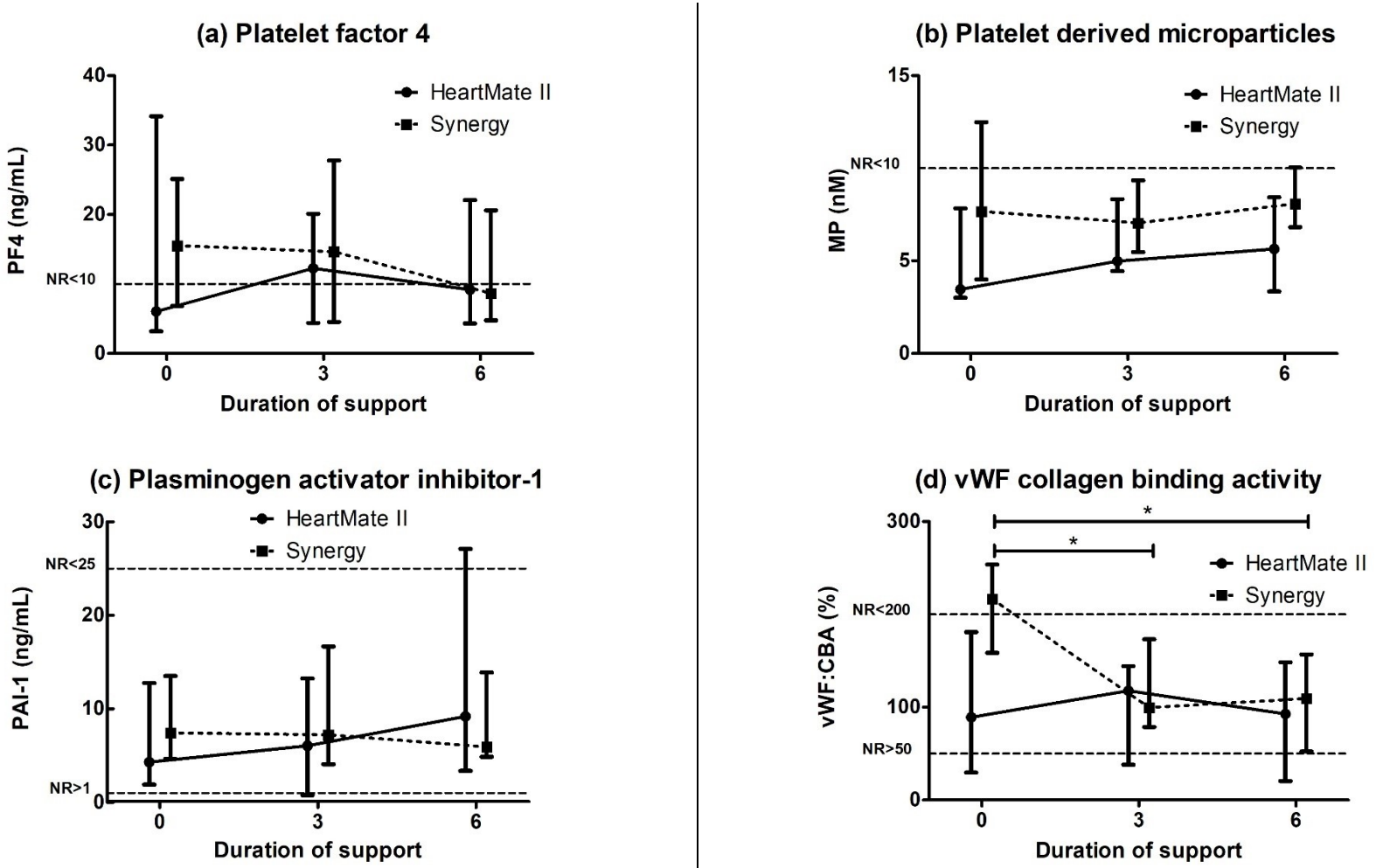
Table 4.4: Serial measurements of haematology markers with relative changes from baseline.

N numbers varied and are specified. P-values on the right, above the double line (top part of columns labelled #) refer to Mann-Whitney tests for baseline vs. the delta timepoint. Due to multiple comparisons, the Bonferroni-corrected level for statistical significance is p=0.025 (2 comparisons). P-values in the bottom section (below the double line) labelled $ refer to Kruskal-Wallis test for variation between device types for each assay at each study time point (with Bonferroni α=0.017 (3 comparisons)) or to Mann-Whitney tests comparing the delta change at 3 or 6 months (with Bonferroni α=0.025 (2 comparisons)). (PAI-1, plasminogen activator inhinitor-1; PF4, platelet factor-4; MP, platelet derived microparticles; vWF:CBA, von Willebrand factor collagen binding activity.)

Device	**Assay**		**Serial measurements**			**Relative change from baseline**			
			Preimplantation	**3 months**	**6 months**	**3 months**		**6 months**	
						Δ %	**p#**	**Δ %**	**p#**
HeartMate II	PAI-1	n	6	6	5	6		5	
			4.32 (1.91, 12.77)	6.06 (0.79, 13.25)	9.20 (3.38, 27.11)	+23 (-76, +273)	0.60	+138 (-10, +236)	0.23
	PF-4	n	8	7	8	7		8	
			6.05 (3.19, 34.1)	12.26 (4.41, 20.10)	9.18 (4.33, 22.09)	-26 (-66, +420)	0.74	+74 (-78, +386)	0.78
	MP	n	8	7	8	7		8	
			3.46 (3.01, 7.84)	4.98 (4.45, 8.33)	5.65 (3.35, 8.44)	+47 (-11, +76)	0.31	+47 (+7, +65)	0.58
	vWF:CBA	n	8	7	8	7		8	
			89.26 (29.58, 180.99)	117.79 (38.00, 144.22)	92.71 (20.46, 148.36)	+23 (37, +299)	0.31	-31 (-76, +144)	0.48
Synergy	PAI-1	n	15	13	9	11		8	
			7.44 (4.65, 13.53)	7.22 (4.08, 16.67)	5.93 (4.88, 13.90)	+14 (-29, +124)	0.48	+41 (-51, +143)	0.48
	PF-4	n	15	13	9	11		8	
			15.54 (6.85, 25.12)	14.66 (4.54, 27.79)	8.65 (4.77, 20.62)	+140 (-68, +473)	0.53	-41 (-70, +13)	0.26
	MP	n	15	13	9	11		8	
			7.66 (4.00, 12.48)	7.04 (5.48, 9.35)	8.08 (6.82, 10.04)	-20 (-29, +48)	0.37	+41 (-18, +160)	0.26
	vWF:CBA	n	15	13	9	11		8	
			216.42 (158.63, 253.69)	99.58 (78.66, 173.18)	109.39 (59.92, 119.17)	-45 (-67, +1)	**0.02**	-62 (-80, -26)	**0.02**
p-value$ HMII vs. Synergy	PAI-1		0.31	0.38	1.00	0.84		0.31	
	PF-4		0.44	0.45	0.89	0.62		0.72	
	MP		0.20	0.17	0.04	0.25		0.96	
	vWF:CBA		**0.01**	0.97	0.50	0.03		0.38	

Figure 4.6: Device-specific changes of haematology markers with duration of LVAD support.

*Device-specific changes in (a) platelet factor-4, (b) platelet derived microparticles, (c) plasminogen activator inhibitor-1 and (d) vWF collagen binding activity. * indicates p<0.05 (see Table 4.4).*



4.3.4.2 Early variation in Synergy patients

Additional data at 14 days post LVAD implant were available in patients receiving the Synergy LVAD. These were scrutinised for any early variation in the parameters tested and data are shown in Table 4.5. There was no significant change in any of the studied parameters, with a trend towards significance in reduction of vWF:CBA (-30% (-47, +17), p=0.05).

Table 4.5: Early variation in patients receiving the Synergy LVAD.

N numbers varied and are specified. P-values refer to Mann-Whitney tests for baseline vs. the delta timepoint.

		Serial measurements		**Relative change from baseline**	
		Preimplantation	**14 days**	**14 days**	
				Δ %	**p**
PAI-1	n	15	16	14	
		7.44 (4.65, 13.53)	6.16 (4.47, 8.58)	-11 (-51, +73)	0.33
PF-4	n	15	16	14	
		15.54 (6.85, 25.12)	8.53 (7.14, 18.92)	-40 (-66, +88)	0.30
MP	n	15	16	14	
		7.66 (4.00, 12.48)	5.28 (4.22, 11.80)	-4 (-54, +96)	0.59
vWF:CBA	n	15	16	14	
		216.42 (158.63, 253.69)	134.02 (121.27, 176.28)	-30 (-47, +17)	0.05

4.3.5 Factors associated with LVAD thrombosis

We evaluated if any of the parameters studied could predict which patients would later suffer LVAD thrombosis. Eight of the 25 patients (32%) suffered pump thrombosis at least once during their period of LVAD support. These 8 patients were all recipients of the Synergy LVAD (47% of the Synergy patients). There were no reported episodes of HeartMate II thrombosis. Incidence of pump thrombosis in the Synergy patients is reported in detail in section 3.3.2.2 (page 77).

Including all time points, measurements of PAI-1 and MP were significantly higher in patients who required pump exchange for thrombosis (PAI-1: 9.70ng/mL (6.12, 14.31) vs. 5.18ng/mL (2.77, 9.15), p<0.01; MP: 7.66nM (5.24, 10.39) vs. 5.34nM (4.13, 8.61), p=0.04).

Notably the levels of MP were higher in Synergy patients overall with trend towards significance, which may be a confounder (7.08nM (4.38, 10.03) vs. 4.98nM (3.39, 8.33), p=0.05). PAI-1 did not vary between device types (Synergy 6.73ng/mL (4.70, 12.32) vs. HMII 6.17ng/mL (2.32, 13.52); p=0.43). There was no significant difference in levels of PF4 (requiring 8.48ng/mL (4.77, 19.90) vs. not requiring 12.20ng/mL (4.62, 23.05); p=0.61) or vWF:CBA (requiring 134.91% (99.58, 216.42) vs. not requiring 120.23% (63.52, 177.27); p=0.19).

If device-induced changes in the measured parameters cause propensity to thrombosis, then the measurements after implantation would particularly influence its occurrence. Including only measurements from 14 days onwards, there remains a significantly higher PAI-1 (9.40ng/mL (6.21, 15.96) vs. 4.98ng/mL (3.05, 9.04), p<0.01) and MP (8.25nM (5.84, 11.56) vs. 5.42 (4.37, 7.78), p<0.01) in patients requiring pump exchange than not, now independent of device type in both cases. PF4 and vWF:CBA continued to show no significant difference (requiring vs. not requiring, respectively 7.76ng/mL (4.57, 18.16) vs. 12.20ng/mL (5.01, 20.10), p=0.36, and 109.68% (80.28, 172.22) vs. 120.23% (74.72, 146.48), p=0.99).

Receiver-Operator Characteristic (ROC) analysis determined that PAI-1 measurements during device support could be used to identify patients at risk of pump thrombosis (AUC 0.76, p<0.01). Best discrimination was obtained with a cut-off of >5.92ng/mL (sensitivity 81% (60-95), specificity 63% (44-80; see Figure 4.7, page 124). Using this cut-off, patients with PAI-1>5.92ng/mL at 14 days after LVAD implantation were on average 21 times more likely to suffer pump thrombosis (Odds Ratio 21.00 (95% CI 1.5-293.3), p=0.04). ROC analysis for MP showed this was less discriminatory (AUC 0.66, p=0.03; see Table 4.6, page 124).

Figure 4.7: Receiver-Operator Characteristics Analysis.

This analysis includes only results from after LVAD implantation, i.e. 14 days onwards. Pump thrombosis defined as need for pump exchange due to suspected thrombosis. See text for details.

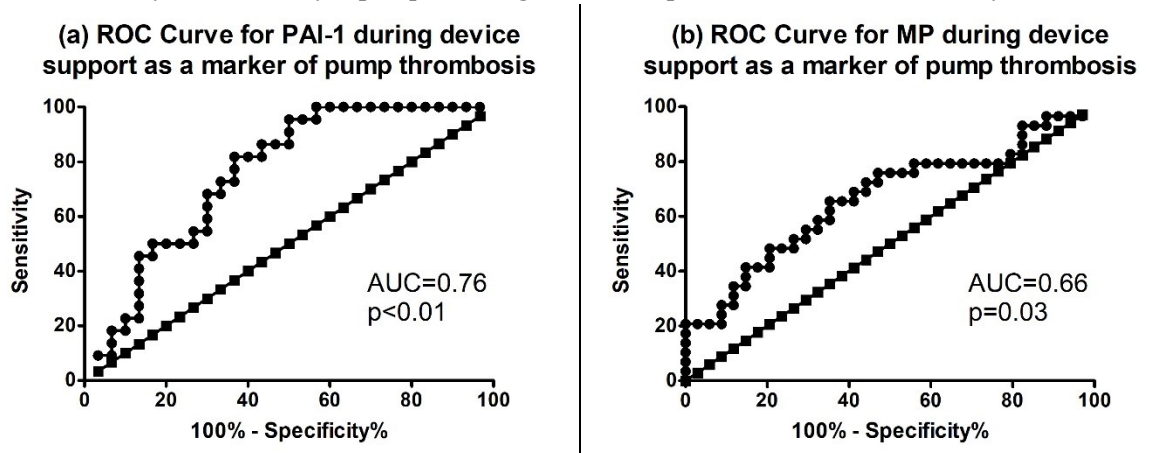


Table 4.6: Contingency table for PAI-1 and Synergy pump thrombosis.

Positive PAI-1 is defined as PAI-1>5.92ng/mL at 14 days after LVAD implantation (n=16). Pump thrombosis defined as need for pump exchange due to suspected thrombosis.

			Positive PAI-1		
			Yes	No	Totals
Pump thrombosis		Yes	6	1	7
		No	2	7	9
		Totals	8	8	16
Odds of requiring pump exchange			**3.00**	**0.14**	**Odds Ratio 21.00** (95% CI 1.5-293.3, p=0.04)

4.3.6 Relation of vWF activity to bleeding events

Clinical bleeding events are detailed in Table 4.7 (page 126), including surgical (e.g. haemothorax) and non-surgical (e.g. epistaxis) bleeding. Major non-surgical bleeding was defined as any bleeding requiring urgent clinical assessment or unplanned admission to hospital. The hypothesis is that a greater loss of vWF collagen binding activity might confer a greater risk of non-surgical bleeding during device support.

There was a significant burden of surgical and non-surgical bleeding in patients treated with both devices. A greater proportion of Synergy patients suffered with non-surgical bleeding compared with HeartMate II patients, though this was not statistically significant (71% (12/17) vs. 38% (3/8), Odds Ratio 4.00 (95% CI 0.68-23.52, p=0.19)).

In the whole cohort (HeartMate II and Synergy patients), there were 18 patients for whom we had paired baseline versus 3 month data. In these patients, those patients suffering non-surgical bleeding had greater loss of vWF:CBA at 3 months (-41% (-66, +63) vs. +17% (-30, +94), p=0.15), by when vWF:CBA was lower in bleeders vs. non-bleeders with a trend towards significance (98.50% (70.37, 131.16) vs. 142.65% (113.55, 198.89), p=0.13). Considering just the Synergy patients, as observed previously all patients showed marked reduction in vWF:CBA at 14 days. There was no difference in the delta change at 14 days between bleeders and non-bleeders. However, by 3 months, those patients suffering major non-surgical bleeding had reduced vWF:CBA activity versus those not suffering bleeding, with a trend towards statistical significance in this small group (bleeders, n=11: 97.4% (75.4, 171.3) vs. non-bleeders, n=2: 196.2% (138.7, 253.7), p=0.15). This might indicate that a subgroup of patients who have relatively preserved vWF activity are protected against bleeding complications. See Table 4.8 (page 127) and Figure 4.8 (page 128).

Table 4.7: Bleeding events in HeartMate II and Synergy LVADs.

HeartMate II (n=8): patient IDs 2-21, shaded light grey. Synergy (n=17): patient IDs 22-38, no shading. (POD, postoperative day.)

Patient ID	Bleeding event	POD	Days to first non-surgical bleed	Number of bleeding events
2	Tamponade Tamponade Haemothorax Upper GI haemorrhage	0 3 3 19	19	4
3	Epistaxis	25	25	1
16	*None*	-	-	-
17	Intracranial haemorrhage Lower GI haemorrhage	5 13	5	2
18	*None*	-	-	-
19	Tamponade	4	-	1
20	*None*	-	-	-
21	*None*	-	-	-
22	*None*	-	-	-
23	Pocket haematoma	0	-	1
24	Haemothorax Haemothorax	1 3	-	2
25	Epistaxis Epistaxis	12 37	12	2
26	Lower GI haemorrhage Epistaxis Epistaxis Lower GI haemorrhage Pocket haematoma Pocket haematoma	17 294 301 425 682 691	17	6
27	*None*	-	-	-
28	Haemothorax Haemothorax Haematuria	0 3 463	463	3
29	*None*	-	-	-
30	Epistaxis Lower GI haemorrhage	14 549	14	2
31	Epistaxis Epistaxis Epistaxis	8 96 111	8	3
32	Upper GI haemorrhage Upper GI haemorrhage	463 542	463	2
33	Epistaxis	20	20	1
34	Epistaxis Epistaxis	4 10	4	2
35	Haemothorax Lower GI haemorrhage Epistaxis Lower GI haemorrhage	1 78 78 109	78	4
36	Epistaxis Subdural haemorrhage	13 838	13	2
37	Haemothorax Epistaxis	10 17	17	2
38	Haemothorax Lower GI haemorrhage Lower GI haemorrhage Lower GI haemorrhage	0 104 171 184	104	4

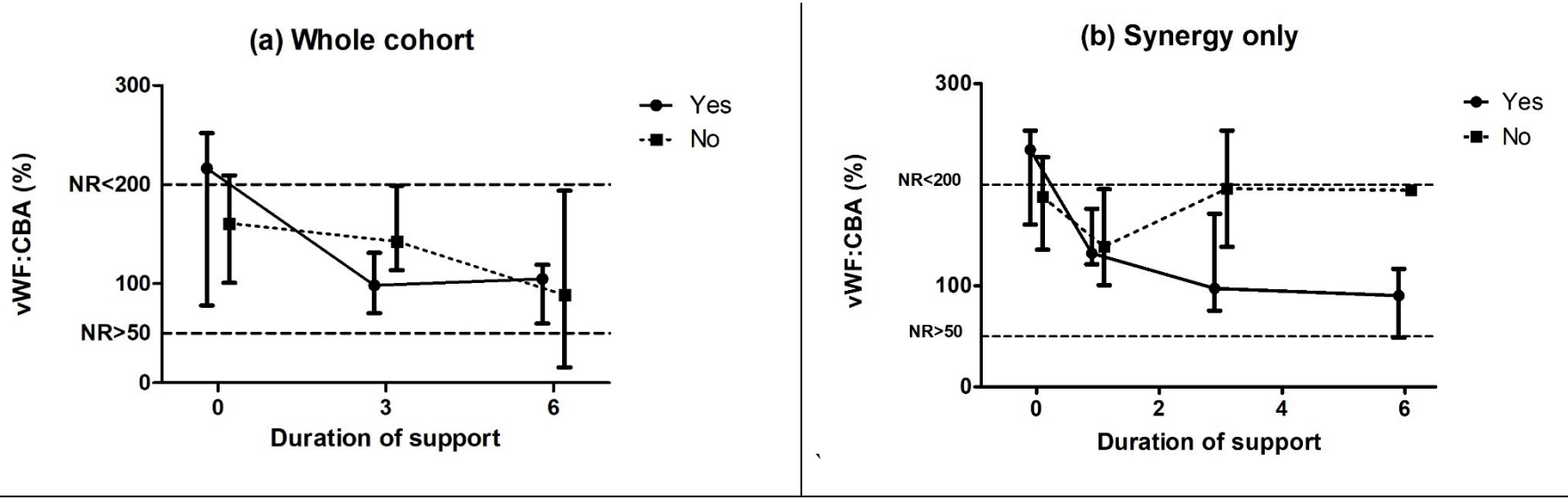
Table 4.8: vWF collagen binding activity relates to incidence of major non-surgical bleeding.

N numbers varied and are specified. 14 day results in the whole cohort are greyed out, as they were not available in HeartMate II patients. P-values on the right, in columns labelled #, refer to Mann-Whitney tests for baseline vs. the delta timepoint. Due to multiple comparisons, the Bonferroni-corrected level for statistical significance is p=0.025. P-values in the bottom line of each section (labelled $) refer to Mann-Whitney tests for variation between bleeders vs. non-bleeders at each study time point (p<0.025 for significance) or to Mann-Whitney tests comparing the delta change at 14 days, 3 or 6 months (p<0.017 for significance).

Occurrence of major non-surgical bleeding			**Serial measurements**				**Relative change from baseline**					
			Preimp.	**14 days**	**3 months**	**6 months**	**14 days**		**3 months**		**6 months**	
							Δ %	**p#**	**Δ %**	**p#**	**Δ %**	**p#**
Whole cohort (n=25)	Yes	n	13		14	11			12		10	
			216.4 (78.1, 251.9)		98.5 (70.4, 131.2)	104.9 (59.9, 119.2)			-41 (-66, +63)	0.04	-48 (-78, +49)	0.05
	No	n	10		6	6			6		6	
			160.9 (100.8, 209.4)		142.7 (113.5, 198.9)	104.9 (59.9, 119.2)			+17 (-30, +94)	0.35	-47 (-80, -15)	0.03
	p-value$		0.45$		0.13$	0.80$			0.15$		0.79$	
Synergy only (n=17)	Yes	n	10	12	11	8	10		9		7	
			234.6 (160.6, 253.7)	132.3 (121.3, 176.3)	97.4 (75.4, 171.3)	90.4 (48.5, 116.9)	-32 (-50, +8)	0.07	-61 (-72, -15)	0.02	-72 (-81, -33)	0.03
	No	n	5	4	2	1	4		2		1	
			188.0 (135.9, 227.3)	138.9 (100.6, 195.6)	196.2 (138.7, 253.7)	194.4	-10 (-41, +24)	0.47	+1 (-26, +26)	0.66	-23	-
	p-value$		0.44$	1.00$	0.15$	0.44$	0.54$		0.22$		0.50$	

Figure 4.8: Changes in vWF collagen binding activity with duration of support.

Presence ('Yes') or absence ('No') of major non-surgical bleeding in (a) the whole cohort and (b) in Synergy patients only. (vWF:CBA, von Willebrand factor collagen binding activity.)



4.4 Discussion

This Chapter focuses on acquired coagulopathy in implantable assist devices, and specifically the hypothesis that high rates of pump thrombosis in the Synergy LVAD might be accounted for by heightened activation of pro-thrombotic pathways due to increased shear on passage through the pump. We hypothesised that patients implanted with the Synergy LVAD would (1) show evidence of increased platelet activation and greater impairment of fibrinolytic pathways compared with patients implanted with the HeartMate II, and that this might help identify patients at risk of pump thrombosis; and (2) would show greater loss of vWF activity associated with higher rates of non-surgical bleeding.

This cohort of 25 patients had markers of severe HF and were treated either with the partial support Synergy LVAD (n=17) or with the full support HeartMate II (n=8). Appropriately, patients in more severe INTERMACS classes were more likely to receive the HeartMate II, though unusually these more severe patients actually had, on average, lower NT-proBNP at baseline than the Synergy patients, perhaps due to confounders such as inotrope use and intensive optimisation of cardiac haemodynamics prior to operation. Plasma was tested for levels of PAI-1, PF4 and MP, and for vWF:CBA activity, serially at preimplantation, 3 months and 6 months (and, in the Synergy patients, an additional timepoint at 14 days). These data were interrogated looking for evidence of acquired coagulopathy in the advanced HF condition prior to LVAD implant, in the Synergy LVAD compared with the HeartMate II LVAD during periods of support, and as markers of pump thrombosis in those Synergy implants going on to suffer this important complication. Furthermore, the rates of non-surgical bleeding were assessed in relation to vWF activity.

4.4.1 Platelet activation

Activation of platelets is a key step in thrombus formation. Activation occurs in response to local chemical or mechanical factors, and once activated the platelets integrate with other proteins such as vWF and fibrinogen to initiate fibrin deposition. Platelets are exposed to high shear stress in the LVAD which can trigger platelet activation, though these are damped by counterregulatory mechanisms and by other aspects of platelet dysfunction. Early studies of platelets in extracorporeal circuits suggested platelet function might be impaired by

concomitant haemolysis and ADP release[160], and functional aggregation studies have shown impaired platelet aggregation during LVAD support[161]. Red cell haemolysis may play a role in platelet activation by increasing preponderance of reactive oxygen species, with platelet activation alongside inflammatory effectors and endothelial dysfunction. Once activated, there is conformational change and release of various effector molecules including PF4 and release of MP from alpha-granules[87].

Within this cohort, the PF4 level before LVAD implantation was inversely related to NT-proBNP as a marker of HF severity and, before LVAD implantation, patients with higher NT-proBNP had significantly lower levels of PF4. MP did not show this relation to NT-proBNP. The unusual finding earlier that NT-proBNP was higher in patients in less severe INTERMACS classes could reflect confounding of NT-proBNP's relation to actual HF severity in this cohort. Indeed, previous reports have suggested increased platelet activation in HF even in the absence of LVAD support[84]. Neither parameter was confounded by platelet count. Lack of complete medication history precluded full analysis of the effect of concurrent antiplatelet therapy, but within the 9 patients there was no discernible effect. Particularly relevant information that is unavailable are details on heparin use, as heparin releases PF4 from storage in endothelial cells causing marked increase in plasma levels[162].

We hypothesised that higher shear stress may result in greater degrees of platelet activation to account for greater propensity to pump thrombosis in Synergy patients. However, the data refute this hypothesis in showing that after initiation of LVAD support, both PF4 and MP showed no significant changes during the 6 month follow-up period in either study group. There was a nonsignificant reduction in PF4 levels in the Synergy cohort, which could reflect the mandated dual antiplatelet therapy with Aspirin and Clopidogrel. PF4 showed no variation between patients suffering pump thrombosis and those not, and although MP did show a difference it seems that this was confounded by LVAD type as the same difference was observed in a Synergy vs. HeartMate II comparison.

4.4.2 Impaired fibrinolysis

In health, there is active balancing between procoagulant and opposing anticoagulant processes in vivo, and interruption of this balance can manifest as thrombosis. PAI-1 is one factor involved in maintaining this balance, regulating fibrinolysis by inhibition of tissue plasminogen activator. Existing studies have shown PAI-1 to be increased in HFrEF and HFpEF, causing suppression of fibrinolytic activity and a procoagulant effect[163], and have shown marked elevations in PAI-1 after LVAD implantation sustained to 45 days[91]. In this cohort, prior to LVAD implantation, we saw no increase of PAI-1 outside the published normal range, and no variation based on HF severity or choice of LVAD implant. Furthermore, there was no significant plasticity of PAI-1 with duration of support in either Synergy or HeartMate II patients, though in the latter there was a numerically large but statistically insignificant increase. In the Synergy group, there was no increase in the 14 day data to corroborate the early and transient increase seen previously[91].

Importantly, levels of PAI-1 did vary significantly between patients suffering pump thrombosis and those not. Furthermore, by ROC analysis it was possible to determine a cut-off of 5.92ng/mL which gave 81% sensitivity and 63% specificity for detecting future pump thrombosis, and patients with PAI-1>5.92ng/mL in samples from 14 days after LVAD implantation were 21 times more likely to suffer pump thrombosis than those <5.92ng/ml. Biologically, this could reflect a patient-specific response to LVAD implantation that renders a marked impairment to fibrinolysis that persists. There is suggestion from previous studies that PAI-1 levels were increased around the time of pump thrombosis, though this was in a predominantly pulsatile device cohort[91]. Notably, this analysis included all results from the whole cohort, including patients supported with HeartMate II, while the pump thrombosis episodes were exclusively in the Synergy group. This introduces the possibility of confounding, though there was no difference in PAI-1 between devices across the cohort, mitigating against this.

4.4.3 von Willebrand factor activity

Acquired vWF deficiency is an important and well described complication in LVAD recipients that accounts for some of the increased bleeding propensity in this patient group[164].

High shear stress causes conformational change in large vWF multimers and allows cleavage by ADAMTS13 and other factors with a consequent loss of the largest and most biologically active vWF multimers, and impairments to vWF-mediated procoagulant and thrombotic processes.

The hypothesis was that the Synergy device's characteristics cause greater shear stress on blood components, and this would increase the degree of acquired vWF deficiency. The most interesting finding is the differences in vWF:CBA between HeartMate II versus Synergy patients prior to LVAD implantation. Patients who go on to have Synergy support – predominantly in INTERMACS classes 4-7, with less severe HF – have better preserved vWF:CBA compared to patients with more severe HF who receive HeartMate II. This could be directly consequent to the more severe HF syndrome (which has been described previously in subsets of patients[165]), or could be confounded, for example by use of haemodialysis or extracorporeal support devices in the sickest patients prior to HeartMate II implantation. The detailed clinical information about for this cohort is not available to account for these in analysis.

From this better-preserved baseline, the Synergy recipients show an early (onset at 14 days) and sustained (out to 6 months) reduction in vWF:CBA activity. After the initial drop, there is then no significant difference between vWF:CBA levels in HeartMate II versus Synergy patients. This is consistent with previous studies which have found no major differences in vWF activity between modes of rotary support, comparing HeartMate II versus HVAD[78] or Synergy[79].

Isolated analysis of vWF:CBA is insufficient to make a complete assessment of acquired vWF deficiency, this only being possible when additionally equipped with levels of vWF antigen (to account for changes in total amount of vWF present) and multimer analysis (to identify large multimer loss and classify the subtype). This is underlined by the finding that, despite clear LVAD-induced changes in activity levels, vWF:CBA remains in the quoted normal range throughout follow-up. It is likely that the large multimer activity is more profoundly affected due to the mechanical shear, and the small multimer activity remains

preserved on this assay. The relation between vWF large multimer loss and vWF:CBA remains unquantified in this study. However, vWF:CBA does give an indication of severity. Notably, a greater loss of vWF:CBA at 6 months seems to be accompanied by higher chance of significant non-surgical bleeding, and Synergy patients avoiding significant bleeding seem to have better preserved vWF:CBA at 3 and 6 months.

4.4.4 Overall assessment of acquired coagulopathy

This work sought evidence for an acquired coagulopathy in LVAD recipients. In summary, (1) within this cohort we have not found evidence for increased platelet activation in patients receiving Synergy LVAD support versus full LVAD support, nor have we demonstrated a role for PF4 or MP as important harbingers of pump thrombosis. There is an interesting finding that PF4 levels may decrease with increasing HF severity, but there may be confounding issues in this small cohort and further studies would be required to confirm this; (2) the data do not support the hypothesis that LVAD support causes universally impaired fibrinolysis across this cohort, as measured by levels of PAI-1. However, high levels of PAI-1 may indicate higher risk of pump thrombosis, and this may reflect significant impairment in fibrinolysis in a subset of patients; (3) in this cohort the Synergy patients show a significant reduction in vWF:CBA activity from 14 days and sustained throughout follow-up, whereas HeartMate II recipients show suggestion of impaired vWF:CBA pre-implant which is sustained throughout follow-up. The evidence does not support a hypothesis that increased shear from the Synergy LVAD causes greater impairment of vWF:CBA; and (4) patients with greater loss of vWF:CBA may be at greater risk of non-surgical bleeding during LVAD support.

4.4.5 Limitations and future work

These findings should be interpreted mindful of limitations in the study methodology, and thoughtful to future work. The original intention was for these hypotheses to be integrated into the PAVE-UP study and C-Pulse substudy as set out in Chapter 3. This would have allowed prospective data and sample collection, and facilitated functional platelet aggregation testing and a more comprehensive assessment of vWF biology including assessment of large multimer loss.

However, due to early cessation of both studies, this aspect of my PhD project relied on retrospective analysis of existing plasma samples. These were obtained from colleagues in University Hospitals Leuven. The small sample size reduces the statistical power for drawing conclusions on the hypothesis. Disadvantages of retrospective studies include reliance on data collected outside a set protocol, and frequently absence of key data in analysis. This was complicated further here by the difficulty obtaining clinical information from a hospital overseas. Key parameters not included in analysis here are (1) full medication history, (2) details of preimplantation clinical status, (3) data on prothrombin time, activated partial thromboplastic time and fibrinogen for full assessment of coagulation at baseline (INR is included as a surrogate), (4) data on serial plasma haemoglobin as marker of haemolysis, (5) pump speed, (6) pump power consumption, and (7) follow-up clinical data.

The retrospective design may have affected plasma sample quality. The collection of blood may not have been done with subsequent platelet activity testing in mind, and platelet activation may have occurred at the time of venesection. Repeated freeze/thaw cycles may have destabilised some targets of the analysis. Finally, the ELISA kits recommend double-spinning the samples on acquisition, to ensure they are free of platelets before storage, and these samples were spun once as is standard for most plasma analysis.

The platelet activation studies presented here are limited by lack of antiplatelet drug history, and reliance on PF4 and MP as surrogate markers of platelet activation, rather than physiological measurements of platelet aggregation. The original prospective study design included direct physiological measurement of platelet aggregation using the MultiPlate analyser, for example in in response to collagen or arachidonic acid. Future studies could include this functional test, including tests for Aspirin and Clopidogrel hyporesponsiveness, and in addition consider other markers of platelet activation such as beta-thromboglobulin.

The absence of corroborative clinical data after the baseline assessment is important, particularly in relation to the pathophysiology of pump thrombosis. These plasma samples were from set time points, rather than associated with clinical thrombosis events, and it might be that analysis of these markers in proximity to a thrombosis episode could provide more

information about mechanism, or for consideration of diagnostic biomarkers. Furthermore, changes in serial measurements (for example the suggestion of increased PAI-1 in HeartMate II) could be due to worsening HF just as much as pump related dysfunction. The key to address this is a prospective clinical study with detailed parallel assessments of haematological and clinical status frequently throughout the follow-up period.

Finally, central to a future study would be complete assessment of acquired vWF deficiency in these cohorts, including multimer analysis, levels of vWF antigen and the vWF:CBA studied here. The data here suggest variable levels of vWF:CBA in different severity of HF, and a systematic study of vWF activity in HF would inform our understanding of surgical risk during LVAD implantation. A prospective study could examine the effect of different modes of LVAD support on the individual HF patient's bleeding diathesis, and further interventional studies might consider a role for therapeutic vWF in LVAD patients.

4.5 Conclusions

High rates of thrombosis in the Synergy LVAD were identified and discussed in Chapter 3. The data in this Chapter addresses the hypothesis that mechanics or materials specific to Synergy pump (high shear stress caused by rapid impeller rotation and narrow blood flow channels) might cause greater degrees of platelet activation and greater impairment to fibrinolysis that increases propensity to thrombosis. While accepting the markedly greater thrombosis risk compared to other contemporary LVADs, the data presented here do not support an increased systemic propensity to thrombosis, except in a subset of patients with impaired fibrinolysis. Indeed, in the Synergy patients there is some evidence of reduced platelet activation with LVAD support, perhaps reflecting benefits of partial support with preserved aortic valve opening and actually reductions in overall shear to platelets. In the absence of an acquired coagulopathy, alternative explanation for high rates of Synergy thrombosis should centre on pump mechanics and flow, discussed in detail in Chapter 3. These including pump materials and their thrombogenicity, length and blood flow in the inflow graft and heating within the Synergy pump. Understanding and navigating the balance between thrombosis and bleeding in mechanical assist devices, in both implantable and extracorporeal systems, remains a key challenge for the field going forwards.

5: The SERCA-LVAD Trial – Feasibility and Safety

5.1 Introduction

5.1.1 Background and Rationale

The scientific basis and clinical rationale for modulation of cardiomyocyte calcium cycling by AAV1.SERCA2a gene therapy was outlined in detail in Chapter 1. Twenty years of accumulated preclinical data yielded the CUPID programme, a series of related clinical trials investigating AAV1.SERCA2a in patients with heart failure. Data from the CUPID-1 trial demonstrated feasibility and safety of the gene product and clinical delivery protocol, and further carried preliminary signs of possible efficacy[111,116]. The CUPID-2 trial was a larger, randomised, double blind, placebo-controlled randomised study to evaluate the clinical efficacy of the gene product in a phase 2b trial[119]. This reported initial results in April 2015, confirming safety but neutral for clinical efficacy[123]. Three other clinical trials were running parallel with CUPID-2, as discussed in Chapter 1, one of these being the SERCA-LVAD trial, studying the higher dose gene product in patients with LVADs to establish safety and feasibility in this patient group. All three of these parallel trials were suspended for recruitment after the CUPID-2 trial results were announced, and ultimately closed without completing planned recruitment. The SERCA-LVAD trial is the focus of Chapters 5-6.

5.1.2 Study questions

Discussion of the SERCA-LVAD trial outcomes is split between two Chapters, divided according to the three study questions:

1. Is the intracoronary delivery of AAV1.SERCA2a feasible and safe in patients with stable chronic HF and an LVAD? (Chapter 5)
2. To what extent is intracoronary delivery of AAV1.SERCA2a an effective method of achieving cardiomyocyte expression of SERCA2a DNA, and what is the effect of pre-existing neutralising anti-AAV antibodies? (Chapter 5)

3. Does AAV1.SERCA2a delivered at this dose by intracoronary infusion result in clinical benefit for patients with HF? (Chapter 6)

5.1.3 Hypotheses

We hypothesised that (1) AAV1.SERCA2a gene therapy was safe and feasible in patients with advanced HF and LVAD; (2) intracoronary delivery of the AAV1.SERCA2a gene product would yield detectable viral transgene in myocardial tissue from treated subjects; and (3) pre-existing NAb would not prevent successful transfection in treated subjects.

5.2 Methods

5.2.1 Study overview

5.2.1.1 Design

The SERCA-LVAD was a randomised, double blind, placebo-controlled, multi-centre study intending to recruit 24 stable patients with an existing LVAD for advanced heart failure, and randomise them 2:1 in favour of active gene therapy. The cohort was stratified 50:50 by neutralising antibody status.

The aims were (1) to study the feasibility and safety of a single intracoronary infusion of AAV1.SERCA2a gene therapy as a treatment for advanced heart failure patients with MCS from an implantable LVAD; (2) to study the magnitude of transgene expression in the transfected heart; and (3) to understand the influence of pre-existing circulating NAbs on viral gene transfer. The primary endpoint was overall feasibility and safety of delivering the gene therapy in this patient group. Secondary endpoints were presence of transgene in myocardial tissue obtained at endomyocardial (EM) biopsy or cardiac transplantation and functional clinical outcomes including echocardiography and cardiopulmonary exercise testing. Predefined subgroup studied were planned to examine differences between NAb positive and negative patients.

5.2.1.2 Eligibility

Inclusion and exclusion criteria are listed in Table 5.1.

Table 5.1: SERCA-LVAD inclusion and exclusion criteria.

Inclusion criteria

1. Patients that have had a left ventricular assist device (LVAD) implanted for chronic heart failure, where chronic heart failure is defined as at least 6 months
2. Patients are clinically stable in the opinion of the clinical team looking after the patient
3. Written informed consent

Exclusion criteria

1. <18 or >70 years of age at the time of consent
2. Pregnancy or within 6 months of giving birth
3. Women of child-bearing potential not using an effective method of contraception
4. Men not using an effective method of contraception
5. Suspected or active viral, fungal or parasitic infection within 48 hours prior to administration of IMP, in the opinion of the investigator*.
6. Patients at a high risk of thrombosis in the opinion of the investigator
7. Patients with a previous episode of LVAD thrombosis on their current device
8. Patients with persistently raised lactate dehydrogenase (LDH >2.5 ULN)
9. Patients requiring triple anticoagulation i.e. warfarin and dual anti-platelet
10. Patients participating in another clinical trial
11. Patients unable to comply with the protocol mandated procedures for social or other reasons, in the opinion of the investigator and primary care physician

* Eligible, enrolled and randomised patients who develop an infection will have study treatment delayed until 7 or more days after the time point when infection is no longer clinically evident.

5.2.1.3 Sample size

Sample size was planned to be 24 patients. This was determined by a pragmatic estimate balancing the likely recruitment over two years at two sites versus the need for group sizes to yield meaningful comparison between the four outcome groups.

5.2.1.4 Study protocol

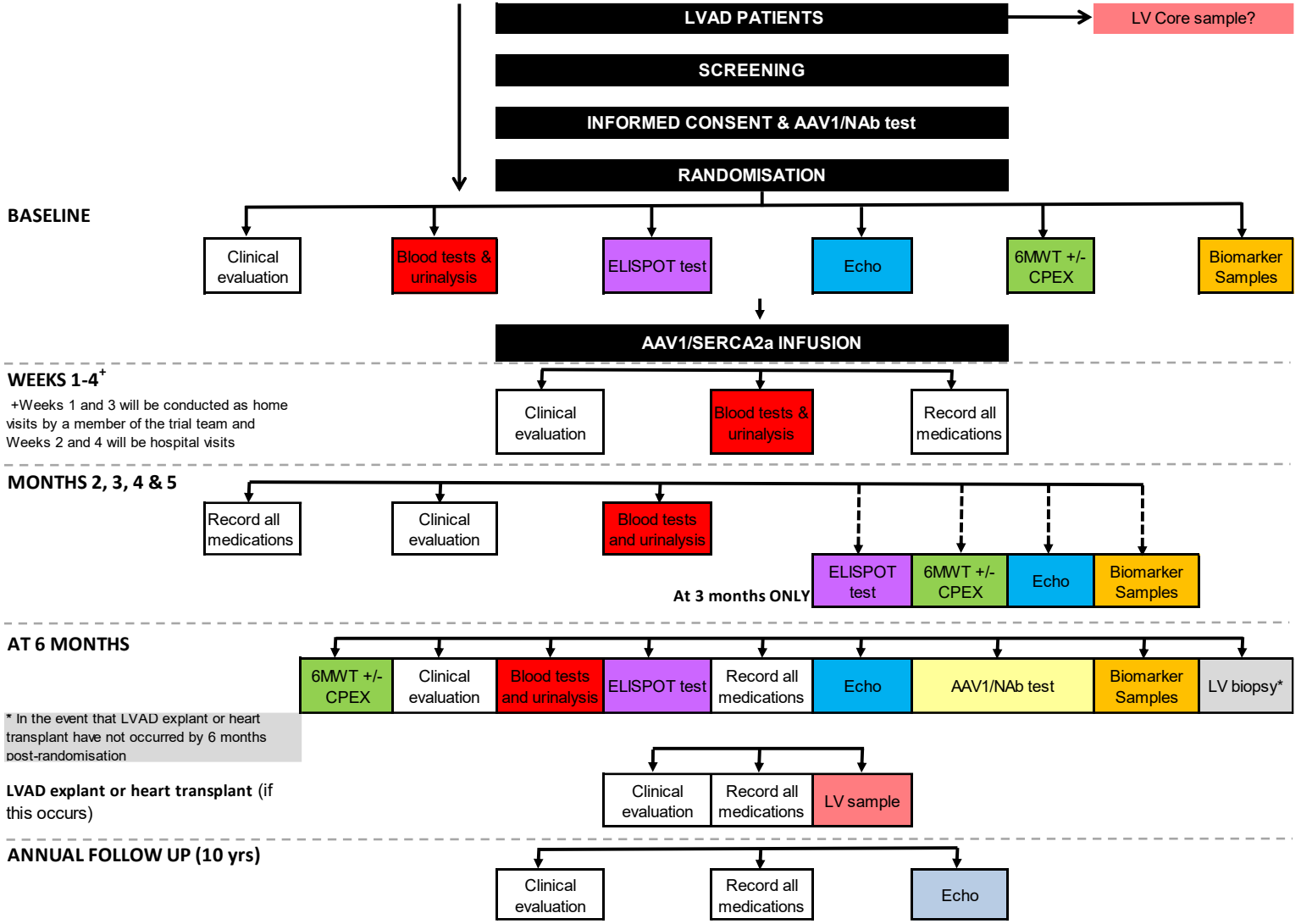
The study period was 6 months. Patients progressed through the study protocol as illustrated in Figure 5.1 (page 140), with clinical testing and procedures as per Table 5.2.

Table 5.2: SERCA-LVAD Trial – Clinical testing and procedures by study visit.

(NAb, anti-AAV1 neutralising antibody; ELISPOT, AAV1- specific enzyme-linked ImmunoSpot test; Echo, echocardiography; CPEX, cardiopulmonary exercise testing; 6MWT, six minute walk test; AE/SAE, (serious) adverse event; LV, left ventricular)

Pre-baseline	Baseline	Weeks 1-3	Months 1-2	Month 3	Months 4-5	Month 6
Clinical evaluation Medications NAb titre ELISPOT Laboratory blood tests Urinalysis Biomarker samples Echo (incl. low speed) CPEX 6MWT AE/SAE monitoring	IMP infusion AE/SAE monitoring	Clinical evaluation Medications Laboratory blood tests Urinalysis AE/SAE monitoring	Clinical evaluation Medications Laboratory blood tests Urinalysis AE/SAE monitoring	Clinical evaluation Medications ELISPOT Laboratory blood tests Urinalysis Biomarker samples Echo (incl. low speed) CPEX 6MWT AE/SAE monitoring	Clinical evaluation Medications Laboratory blood tests Urinalysis AE/SAE monitoring	Clinical evaluation Medications NAb titre ELISPOT Laboratory blood tests Urinalysis Biomarker samples Echo (incl. low speed) CPEX 6MWT LV biopsy AE/SAE monitoring

Figure 5.1: Flow chart of study investigations in the SERCA-LVAD trial. (see Table 5.2 legend for abbreviations)



Suitable patients were identified from within the LVAD programmes at participating centres. After informed consent, patients underwent baseline testing. Patients were admitted to hospital for the infusion of investigational medicinal product (IMP). Thereafter patients attended weekly for the first month, and then monthly until 6 months. Comprehensive visits were at baseline, 3 and 6 months; other visits were focussed on safety monitoring. At 6 months patients had a further hospital admission for EM biopsy.

Procedures associated with risk of LVAD thrombosis due to low speed (echocardiography) or reduction in anticoagulation (IMP infusion, EM biopsy) were spaced by at least 1 week to minimise additive risk. Serum and plasma were stored for future biomarker studies, with processing as detailed in section 2.1, page 54.

5.2.1.5 Investigational medical product

The IMP under study was the AAV1.SERCA2a gene product, manufactured and provided by Celladon Corporation as Mydicar. It was delivered to study participants in aliquots of 1x 10^{13} DNase resistant particles. The placebo aliquots had the same composition except they lacked the active ingredient.

5.2.1.6 Safety monitoring

There was systematic reporting of adverse events and adverse reactions during the study period. Adverse event (AE) was defined as any untoward medical occurrence in a patient administered an investigational medicinal product. Adverse reaction (AR) was defined as any untoward and unintended response to an investigational medicinal product related to any dose administered. Serious adverse events (SAE) or reactions (SAR) are defined as any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing inpatients hospitalisation, results in persistent or significant disability or incapacity, or causes a congenital anomaly or birth defect. Suspected unexpected serious adverse reaction (SUSAR) was defined as an AR that is classed as serious, is suspected to be caused by the investigational medicinal product and is unexpected i.e. not consistent with the information about the investigational medicinal product in the

study documentation. In this group of advanced heart failure patients there is a high rate of morbidity and mortality related to the underlying disease process, with consequently a series of expected SAEs defined a priori, including risks of death, myocardial infarction, stroke, worsening heart failure, heart transplantation, LVAD, driveline or indwelling line infection, LVAD mechanical failure, LVAD thrombus, bleeding, tamponade following LV biopsy, and others. It was estimated that participants would have a 20-30% risk of non-fatal SAE related to their underlying disease during the 12 month study follow-up period.

All AE, AR, SAE and SUSAR were reported to the study team within 24 hours, adjudicated by the CI, and if necessary unblinding and further reporting to MHRA and GTAC as per study protocols.

5.2.1.7 Study title, oversight and funding

The full title of the study was 'Investigation of the safety and feasibility of AAV1/SERCA2a gene transfer in patients with chronic heart failure and a left ventricular assist device', abbreviated here to SERCA-LVAD trial. The lead investigators were Dr Alexander Lyon (lead clinical) and Professor Sian Harding (lead scientific), Principal Investigator at Harefield Hospital was Dr Nick Banner, the sponsor was Imperial College London, and funding was from the British Heart Foundation, Celladon Corporation and NIHR Cardiovascular Biomedical Research Unit at Royal Brompton & Harefield NHS Foundation Trust. Ethical approval for the study was granted by the UK Gene Therapy Advisory Committee of the National Research Ethics Service and the trial was conducted according to the principles of the Declaration of Helsinki and the EU Clinical Trial Directive. The ClinicalTrials.gov identifier was NCT00534703.

5.2.2 Clinical procedures

5.2.2.1 Blood collection and analysis

Procedures for blood collection and analysis, including methods for isolation of peripheral blood mononuclear cells, are detailed in section 2.1 (page 54).

5.2.2.2 Preparation prior to infusion procedure and endomyocardial biopsy

Patients were admitted to the transplant ward 3 days before the procedure for optimisation of anticoagulation. On admission to hospital, patients were assessed for clinical stability and evidence of new infection. Patients reaffirmed their study consent prior to all clinical procedures. For the IMP infusion and LV EM biopsy, patients completed specific informed consent process including written consent forms for the individual procedures.

5.2.2.3 Infusion procedure

Patients were admitted to a cardiac catheterisation laboratory with institutional approval for gene therapy research. At Harefield Hospital this was catheter lab 4. The IMP infusion procedure was led by a consultant cardiologist who led a team briefing before the patient arrived. Initial haemodynamic monitoring was based on non-invasive (cuff) blood pressure measurement and derived parameters from the LVAD console (calculated flow). As soon as the arterial sheath was placed arterial blood pressure was monitored continuously. A baseline 12-lead ECG was obtained on arrival to the catheter lab.

GTN was administered to all patients during the IMP procedure, starting at 5mcg/min and increasing in 5mcg/min increments every 3-5 minutes until a criterion was met that defined maximum tolerated dose. These criteria were (i) LVAD flow dropping by >0.5 litres/min, (ii) MAP falling below 65mmHg, or (iii) MAP falling >10mmHg from baseline. Once the maximum tolerated dose was reached, this was continued until the end of the procedure and then stopped before the patient left the catheter lab.

Vascular access was obtained via the femoral artery. After local anaesthetic, a 6 French vascular sheath was placed to the right femoral artery (preferentially) or left femoral artery using ultrasound guidance. Continuous blood pressure monitoring was initiated using an arterial transducer. Pre-specified 5 French catheters approved for use with the IMP were used for coronary catheterisation (JL4 and JR5 from Cordis Infinity and Boston Scientific Expo ranges). Blood was obtained for a baseline measurement of activated clotting time (ACT). Intra-arterial unfractionated heparin was given with target ACT of 200 seconds.

Diagnostic angiography was performed to confirm coronary anatomy and disease. Based on coronary anatomy, the infusion strategy was agreed by the study team based on left-, co- or right-dominance and in accordance with the standard algorithm for administration. This is included in the CUPID-2 trial methods paper[119]. Where infusion was directed to one coronary artery (e.g. non-obstructed coronary arteries with left dominance), 50ml of IMP was infused directly via the coronary catheter at a constant rate over 10 minutes (300ml/hour). Where infusion was directed to two coronary territories (e.g. non-obstructed coronary arteries with right- or co-dominance), 35ml was infused to the left main stem over 7 minutes, and 15 ml was infused to the right coronary artery over 3 minutes (both at constant rate 300ml/hour).

The ACT was repeated to confirm >200 seconds. Catheter position was confirmed at the coronary ostium, the catheter was flushed, and the infusion was delivered as planned based on anatomy. In all cases the total volume of the infusion was 50ml and duration of the infusion was 10 minutes. Catheter position was checked intermittently during the infusion using radiographic screening.

After the infusion, the coronary catheters were removed. ACT was rechecked. The strategy for managing the arterial access site was decided on a case by case basis, guided by the location of femoral puncture and ACT at the end of the procedure. Options included deployment of a vascular closure device, early removal and pressure, or late removal and pressure. A 12-lead ECG was repeated and reviewed before leaving the catheter lab.

5.2.2.4 Endomyocardial biopsy and cardiac transplantation

In the event of cardiac transplantation, explant heart specimens were collected for analysis of transgene persistence. If this had not occurred, six months after IMP infusion trial subjects were re-admitted for EM biopsy. Preparation on arrival to the catheter laboratory was the same as for the infusion procedure. Vascular access was obtained via the femoral artery after local anaesthetic and using the micropuncture kit in all cases. The vascular sheath was placed and a guidewire passed across the aortic valve (AV) into the LV. In one patient, it was not

possible to cross the AV and the procedure was abandoned. A bioptome was introduced using the guidewire, and biopsy specimens were obtained from the LV endocardial surface. Tissue specimens were extracted and processed (details in section 2.2, page 56).

5.2.2.5 Analysis of specimens for transgene persistence and ELISPOT

Tissue samples for presence of SERCA2a DNA and peripheral blood mononuclear cells for ELISPOT testing were collected and stored, and analysed in bulk at the end of the trial (see section 2.2.1, page 56 and section 2.1.4, page 55 respectively).

5.3 Results

5.3.1 Trial course and early termination

The trial opened for recruitment at Harefield Hospital in June 2014. Recruitment was suspended by the TSC on 1 May 2015. This interruption was due to results from the CUPID-2 trial, preliminary findings from which were announced on 27 April 2015. This announcement indicated that AAV1.SERCA2a delivered no clinical benefit in the HF patients studied within CUPID-2, and in this context the risks incumbent in SERCA-LVAD became more challenging to justify. All patients already enrolled in the trial completed the primary 6 month follow-up period after receiving IMP. Patients completed all aspects of the study protocol, except for remaining EM biopsy and low speed echo procedures, which were felt to carry unjustified risk in the context. However, no further patients were enrolled, and the trial closed in July 2016 without meeting its pre-specified recruitment targets.

5.3.2 Numbers recruited

At the time of halting recruitment, 82 patients with implantable circulatory assist devices had been screened, of whom 18 had been approached with 6 consenting to participation. One patient subsequently withdrew for personal reasons before undergoing baseline assessment. Five patients underwent baseline assessment, were randomised and received the IMP.

5.3.3 Baseline characteristics

Due to small numbers of study subjects, all clinical data is displayed as patient-by-patient breakdowns for fuller exposition. Baseline characteristics are shown in Table 5.3. Median age was 36 years (range 29-69 years). Four patients were male, and one female (80% male); four patients were white ethnicity while one was Afro-Caribbean (80% white). All patients had chronic, severe heart failure requiring therapy with an implanted LVAD. Median time from LVAD implant to study consent was 48 months (range 18-153 months). One patient had a Thoratec HeartMate II while the remainder had Heartware HVAD devices. One patient had HF from pre-existing valvular disease (congenital quadricuspid aortic valve with severe stenosis), and the remainder had forms of dilated cardiomyopathy. All patients were clinically stable at the time of enrolment, and no participant had history of hypertension, dyslipidaemia, diabetes, thyroid disorder or prior thoracic radiation.

Table 5.3: SERCA-LVAD Trial – subject demographics and clinical history.

(DCM, dilated cardiomyopathy; peak VO_2, peak oxygen consumption; BNP, B-type natriuretic peptide)

	Patient number				
	002	**003**	**004**	**005**	**007**
Core details					
Age (years)	36	69	29	49	30
Gender	Male	Male	Male	Male	Female
HF aetiology	Valvular	Idiopathic DCM	Familial DCM	Idiopathic DCM	Familial DCM
Details of LVAD implantation and study consent					
Type of LVAD	Heartware HVAD	Heartware HVAD	Thoratec HeartMate II	Heartware HVAD	Heartware HVAD
Date of LVAD implant	11/07/2012	01/12/2001	06/10/2009	17/11/2010	07/08/2013
LVAD Speed (rpm)	2800	2900	9200	2900	2860
LVAD Power (W)	4.8	4.9	6.5	5.1	4.9
Date of study consent	23/06/2014	27/06/2014	04/09/2014	28/10/2014	21/01/2015
LVAD implant to study enrolment (months)	24	153	60	48	18

Table 5.3 continued

	002	003	004	005	007
Medical history					
Time since last unscheduled HF admission (months)	31	24	60	27	16
Time on current medical therapy (days)	1	19	3	27	14
Transplant waiting list	Yes	Yes	Yes	Yes	Yes
Hypertension	No	No	No	No	No
Dyslipidaemia	No	No	No	No	No
Thyroid disorder	No	No	No	No	No
Thoracic radiation	No	No	No	No	No
Smoking history	Never	Never	Never	Never	Never
Alcohol (units/week)	1	1	0	1	1
Ethnicity	White	White	White	Black	White
Height (cm)	170	185	180	173	164
Weight (kg)	62.0	79.4	87.7	102.5	63.1
Body mass index (kg/m^2)	21.5	23.2	27.1	34.3	23.5
Baseline HF severity parameters					
Peak VO_2 (ml/kg/min)	28.3	19.6	20.3	13.5	15.6
6 minute walk distance (metres)	623	627	480	563	397
BNP (ng/L)	58	314	37	155	359

5.3.4 Neutralising antibody status at enrolment, and outcome of randomisation

Presence of anti-AAV neutralising antibodies is thought to impair delivery of gene therapy. All patients were screened for anti-AAV NAb as part of baseline screening. Unlike previous clinical trials with AAV1.SERCA2a gene therapy, SERCA-LVAD intended to include all patients, regardless of anti-AAV NAb status, with the intention to stratify patients 50:50 within the placebo and active treatment groups.

At enrolment to the trial, one patient had pre-existing anti-AAV NAbs (titre >1:16), and the remaining four had undetectable titres (titre <1:2). Double blinded randomisation to placebo or AAV1.SERCA2a occurred for the five patients enrolled to the study, stratified by NAb status. One patient received placebo, and four received active treatment. The single patient with pre-existing anti-AAV NAbs received active treatment (see Table 5.4).

The intended (open circles) and actual (filled circles) trial recruitment to the different study arms is shown in Figure 5.2.

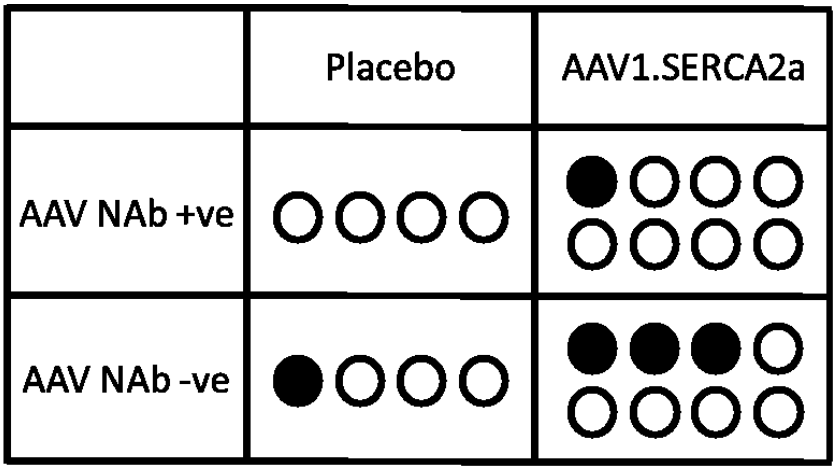
Table 5.4: Trial subject NAb status and study arm allocation.

(NAb, anti-AAV1 neutralising antibody)

	Patient number				
	002	003	004	005	007
NAb status	Negative	Positive	Negative	Negative	Negative
NAb titre	<1:2	>1:16	<1:2	<1:2	<1:2
Randomisation	AAV1.SERCA2a	AAV1.SERCA2a	AAV1.SERCA2a	Placebo	AAV1.SERCA2a

Figure 5.2: Intended (open circles) and actual (filled circles) trial recruitment.

(NAb, anti-AAV1 neutralising antibody)



5.3.5 Feasibility and safety of invasive procedures

5.3.5.1 Infusion of the investigational medicinal product

Five intracoronary infusion procedures were undertaken in five patients. Catheter lab 4 at Harefield Hospital was used for all IMP infusion procedures. Vascular access was achieved via the left (n=1) or right femoral artery (n=4). Ultrasound guidance was used in all cases. The single patient (subject 002) in whom left femoral access was used was due to old scar tissue and unusual anatomy, most likely related to previous arterial access (e.g. placement of intra-aortic balloon pump). During the trial, there was ongoing review and improvement of techniques for vascular access. The first case (subject 002) was performed with standard bore vascular access equipment; subsequent cases were done using paediatric micropuncture access kits, which facilitated greater precision with lower risk of bleeding.

All patients were treated with intravenous GTN during the infusion procedure. The dose was uptitrated based on blood pressure and calculated LVAD flow, until any criterion was met to define "peak" dose (see section 5.2.2.3, page 143). As expected, there was variability in peak GTN infusion dose reached (range 20-60 mcg/min, median 55mcg/min) and in the duration of peak GTN dose (range 14-28 minutes, median 21 minutes; see Table 5.5, page 150). There were no complications related to the use of GTN.

All patients had unobstructed coronary arteries, two with right dominant, two with co-dominant and one patient with left dominant coronary arteries. There was successful delivery of IMP in all cases, with the entire volume delivered to the LMS in the left dominant case, and distributed according to protocol between LMS and RCA in the remainder (Table 5.5, page 150). There were no serious adverse events related to the infusion procedure. Subject 002 had a small superficial haematoma at the groin puncture site after the procedure, which was managed conservatively. This was reported as an AE (see section 5.3.9.1, page 159). There were no other adverse events related to the infusion procedure.

Table 5.5: Procedure details from delivery of investigational medical product (IMP).

(LFA/RFA, left/right femoral artery; LMS, left main stem; RCA, right coronary artery; LAD, left anterior descending; LCx, left circumflex, ACT, activated clotting time; GTN, glyceryl trinitrate.)

		Patient number				
		002	003	004	005	007
Length of hospital stay (nights)		5	5	5	4	4
Vascular access		LFA	RFA	RFA	RFA	RFA
Dominance		Left	Co	Right	Right	Co
Unobstructed coronaries?		Yes	Yes	Yes	Yes	Yes
Volume of IMP delivered to… (ml)	LMS	50	35	35	35	35
	RCA	0	15	15	15	15
	LAD	0	0	0	0	0
	LCx	0	0	0	0	0
ACT pre-infusion		212	285	161	184	207
GTN rate reached (mcg/min)		60	55	20	55	40
Total time at peak GTN dose (minutes)		38	14	31	18	21

5.3.5.2 Endomyocardial LV biopsy

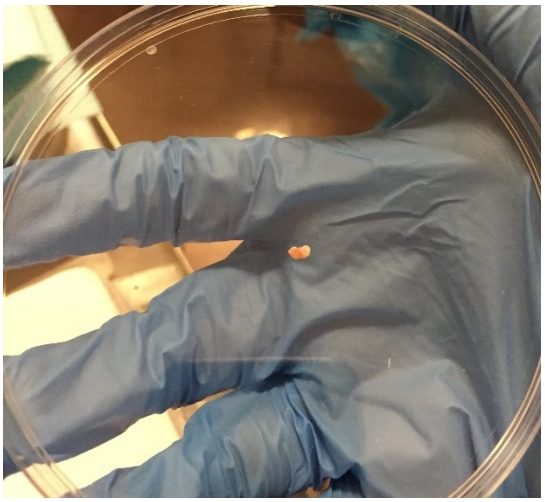
Three EM biopsy procedures were attempted in three patients. The procedure was not attempted in the remaining two patients, after early cessation of the trial. Vascular access via the right femoral artery was achieved in all cases using paediatric micropuncture access kits and without complication.

EM biopsy was performed with tissue extracted in two of the three patients. In patient 002, it was not possible to advance the biopsy catheter across the aortic valve and into the LV cavity despite multiple attempts and assistance from an experienced transcatheter aortic valve interventionist. This failure was due to abnormal aortic root anatomy and a prior porcine aortic valve prosthesis with cusp thickening and partial fusion. In this patient, the procedure was abandoned. In the remaining subjects, access to the LV cavity was obtained easily, and EM biopsy was performed. There were no procedural complications. Tissue of variable quality was extracted (see Table 5.6 and Figure 5.3, page 151). Tissue was prepared and immediately flash frozen in liquid nitrogen (see section 2.2, page 56).

Table 5.6: Record of endomyocardial biopsy procedures.			
	Patient number		
	002	**003**	**004**
Crossing aortic valve	Difficult; not achieved	Easy	Easy
Biopsy sites – number of specimens taken	No biopsy done	Lateral wall – 2 Other – 5	Lateral wall – 2 Mid/Basal inferior – 5
Visual assessment	No biopsy done	Intermediate quality; appearance of fibrous tissue	Some with visible cardiac muscle
Procedure complications	Unsuccessful	No tissue suitable for analysis	None

Figure 5.3: Visual inspection of biopsy specimen.

Macroscopic inspection of this specimen from subject 004 appears to show cardiac muscle within the biopsy.



5.3.5.3 Anticoagulation

Understanding and optimising anticoagulation, with a goal to minimise both the risk of thrombosis and risk of bleeding complications, was a key challenge of the study.

5.3.5.3.3 Adverse consequences of anticoagulation

There was no occurrence of major bleeding or pump thrombosis during the study. This included 8 occasions when anticoagulation was reduced to facilitate invasive vascular access (5 IMP infusions and 3 LV biopsy procedures), and 10 occasions when LVAD pump speed was reduced for low speed assessment. There were no episodes of significant uptrend in

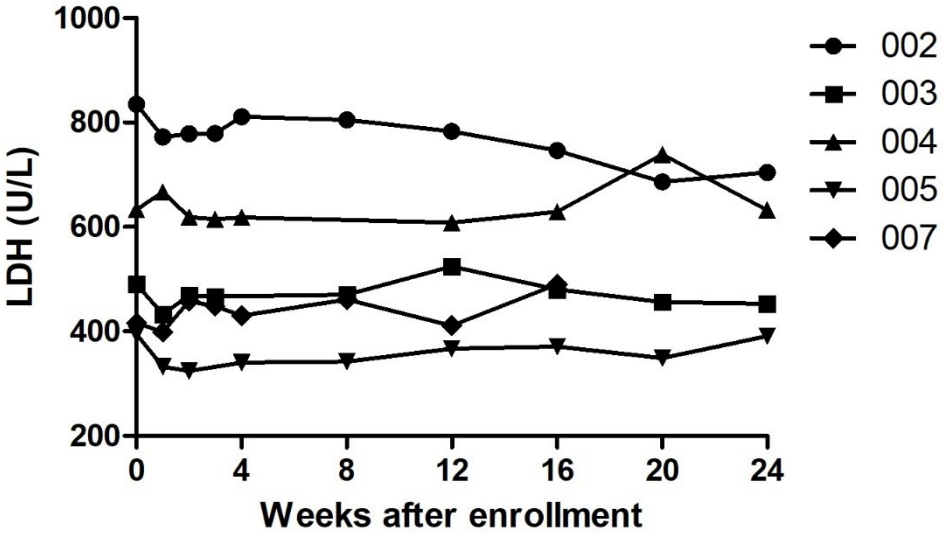
haemolysis markers (LDH, plasma Hb) after the invasive procedures or low speed echocardiograms, that might suggest thrombosis (see Table 5.7 and Figure 5.4).

Table 5.7: Serial changes in lactate dehydrogenase and plasma haemoglobin.

	ID	Baseline	Wk 1	Wk 2	Wk 3	M1	M2	M3	M4	M5	M6
Lactate Dehydrogenase (normal 100-500 U/L)	**2**	835	772	778	779	811	805	783	746	686	704
	3	490	433	469	467	-	470	524	480	456	453
	4	633	667	619	615	618	-	608	629	739	632
	5	395	332	324	-	340	342	367	371	349	391
	7	416	399	458	448	430	461	411	490	-	-
Plasma haemoglobin (normal <0.3 g/L)	**2**	.0	.0	-	-	.3	.0	.0	.3	.0	.0
	3	.2	.0	.0	.0	-	.0	-	.0	.0	.0
	4	.0	.0	.0	.3	.0	-	.0	.3	-	.3
	5	.4	.5	.4	.6	-	.0	.6	.3	.3	.3
	7	.0	.0	-	-	.0	-	.0	.3	-	-

Figure 5.4: Serial changes in lactate dehydrogenase.

The laboratory normal range is 100-600 U/L, though in practice the clinical interpretation is based on change from baseline in the individual patient.



One patient (subject 004) had an unscheduled hospital admission after a pump alarm associated with an increase in power demand. This was reported as an SAE (see section 5.3.9.1, page 159). This occurred 106 days after the baseline low speed echocardiogram, and 52 days (~7 weeks) after the IMP infusion procedure, prior to the midpoint assessments. Haemolysis markers were not elevated at the time of alarm, and there was no evidence of pump thrombosis on echocardiography. There was no conclusive evidence of pump thrombosis but he was treated empirically with intravenous heparin and observed, with normalisation of pump power trends. After this he remained well. It's unclear whether this represented an episode of pump thrombosis, or power spike due to another cause (e.g. dehydration and LV suction event). In this patient, there was a small and non-sustained increase in LDH but not plasma haemoglobin immediately after the IMP procedure.

None of the study participants suffered major vascular complication. One patient had a small superficial haematoma at the groin puncture site after the IMP procedure (see 5.3.5.1, page 149), which was managed conservatively.

5.3.5.3.4 Anticoagulation management during the study

Length of hospital stay for the infusion and biopsy procedures was determined primarily by time required to optimise anticoagulation pre- and post-procedure. The anticoagulation management underwent evolution and improvement during the study:

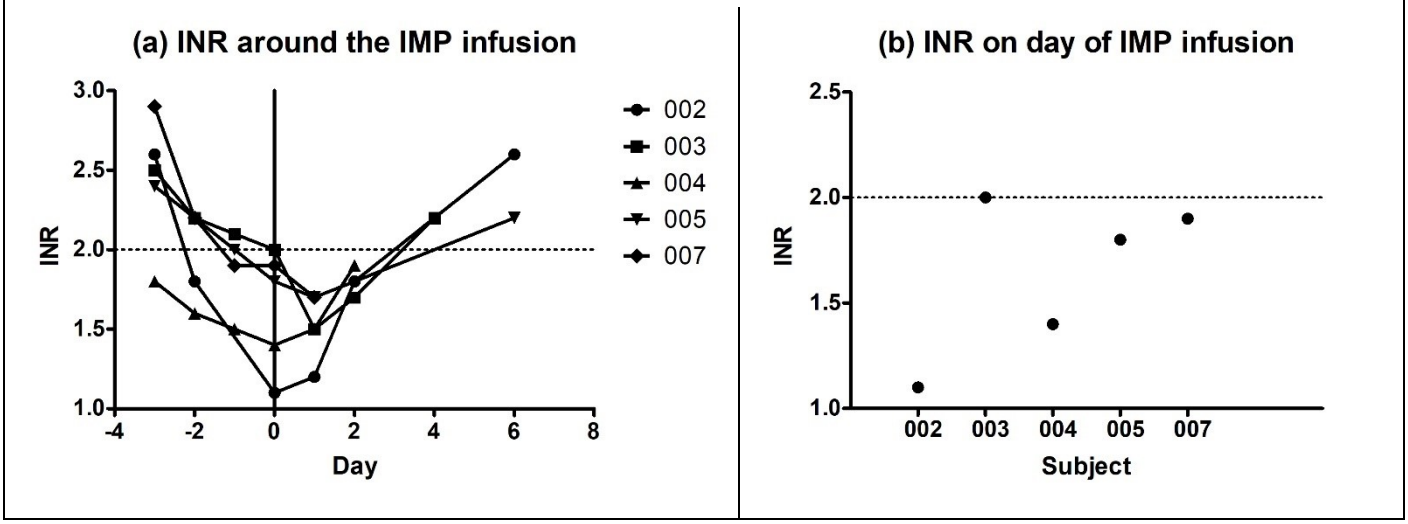
- Subject 002 was the first patient to undergo an invasive procedure during the study, and was managed with a highly conservative anticoagulation protocol. This subject completely stopped warfarin and was treated with intravenous unfractionated heparin (UFH) once INR fell below 2. The patient received UFH for 4 days while INR was subtherapeutic, with interruption of UFH during the infusion procedure itself. On day 3 post procedure the patient was transitioned to subcutaneous injections of low molecular weight heparin (LWMH) to facilitate discharge home, to be continued until INR had returned to >2.
- In all subsequent patients, LWMH was used for bridging anticoagulation in place of UFH.

- For subjects 004, 005 and 007, greater finesse was achieved using remote monitoring. INR monitoring and daily warfarin dosing was done daily for 5 days prior to the procedure, managed by the Clinical Nurse Specialist (CNS), to gradually reduce the INR to minimum therapeutic level. Remote monitoring was with the CoaguChek home INR monitor (Roche Diagnostics International Limited, Rotkreuz, Switzerland). Patients were trained to use the device in accordance with standard clinical protocol, and submitted daily INRs via text message to the CNS.

To evaluate the effect of refinements to the anticoagulation regime, the length of stay, duration of suboptimal anticoagulation (INR<2), and INR on the day of procedure were analysed. Median length of hospital inpatient stay was 5 nights (range 4-5 nights). The first three patients stayed 5 nights in hospital, while length of stay was reduced (4 nights) for the final two patients. The INR on the day of infusion procedure increased towards 2.0 during the trial, reducing the 'at risk' time of reduced anticoagulation. See Figure 5.5.

Figure 5.5: Refinements to anticoagulation management around the IMP infusion.

(IMP, investigational medical product; INR, international normalised ratio.)



5.3.6 Feasibility and safety of non-invasive testing procedures

5.3.6.1 Low speed echocardiography

Echocardiography with low speed assessment was scheduled to occur at 0, 3 and 6 months in trial follow-up, with a protocol for assessment at clinical LVAD operating speed and then at lowest safe speed for re-assessment of the loaded LV. Altogether fourteen echo scans were performed on the five trial participants. The fifteenth scheduled echo scan was not performed as the patient was critically ill awaiting cardiac transplantation.

The fourteen echocardiograms included ten low speed assessments. In each case the target speeds was reached (HeartMate II, 6,000rpm; HVAD, 1,800rpm; see Table 2.2, page 60). Four low speed assessments were omitted. Three of these were scheduled for timepoints after suspension of the trial, and these were curtailed as described above. The fourth (in subject 004 at month 3) was omitted due to a LVAD alarm and possible haemolysis episode occurring in the interval between 0 and 3 month assessments (see 5.3.5.3.3, page 151), and the theoretical risk of exacerbating or precipitating an LVAD thrombosis. This was reported as an SAE (see section 5.3.9.1, page 159).

Anticoagulation was reviewed prior to low speed assessment, and intravenous heparin administered as necessary, according to INR (see Table 2.2, page 60). During the ten completed low speed assessments, LVAD speed was reduced to the minimum speed. There were no adverse events. There was no evidence of increased blood haemolysis which could be indicative of thrombosis formation within the LVAD, with no significant change in plasma haemoglobin or lactate dehydrogenase (LDH) from baseline levels measured before the speed reduction.

There was a high rate of missing data points in the final echocardiography data set. This was expected, but the missing data impacts on feasibility of using this technique in assessing LVAD patients, where data collection is limited by obstruction of standard echocardiographic windows by the device's acoustic shadow. We were unable to obtain satisfactory apical views in three of the five patients, precluding measurement of atrial volumes, LV volumes and

LVEF in these patients. In all patients, adequate parasternal views were obtained for two-dimensional LV measurements.

5.3.6.2 Exercise testing

Cardiopulmonary exercise testing and 6 minute walk tests were completed as per study protocol and without complication. At month 6, subject 007 was severely symptomatic from HF and unable to complete both exercise tests, and in this case CPEX was prioritised.

5.3.7 Effectiveness of therapeutic gene delivery

A key determinant of success is the degree to which the gene product delivered is incorporated into cardiomyocytes within the recipient's heart after intracoronary infusion at the dose used. This had not been systematically studied prior to this trial. We collected two sources of human tissue for analysis, to address this question: firstly, tissue fragments collected at percutaneous EM biopsy; and secondly, tissue from explanted hearts at the time of cardiac transplantation. Alongside we analysed the systemic immune response to understand how this might influence transfection of the AAV1.SERCA2a product.

5.3.7.1 Tissue fragments from endomyocardial biopsy

Tissue was obtained from two patients after three attempted biopsy procedures. Safety and feasibility of the EM biopsy procedure is discussed in section 5.3.5.2 (page 150). Tissue specimens were flash frozen in liquid nitrogen and sent in bulk for analysis (see section 2.2, page 56).

AAV1/SERCA2a DNA was detectable at low levels in one tissue fragment from subject 004. This fragment was biopsied from the mid-basal inferior LV wall, and showed 38 ssDNA copy number per microgram human DNA. In the other fragments from subject 004, the levels of AAV1/SERCA2a were below the limit of detection. The fragments from subject 003 were very fibrotic, and minimal myocardium was sampled. In all samples from subject 003, the

levels of AAV1/SERCA2a were below the limit of detection. AAV1.SERCA2a transgene detection is detailed in Table 5.9.

5.3.7.2 Tissue collected from cardiac explant at transplantation

All enrolled patients were on the cardiac transplant waiting list at the time of enrolment, and were consented for tissue collection for specific trial analysis. To date, cardiac tissue has been recovered from two patients who have proceeded to cardiac transplantation (see Table 5.8). Detection levels of AAV1.SERCA2a transgene are detailed in Table 5.9. Levels of control targets were adequately expressed.

Table 5.8: Tissue collection at cardiac transplantation.

	Patient number				
	002	003	004	005	007
Transplant list at enrolment	Yes	Yes	Yes	Yes	Yes
Transplanted	No	No	Yes	No	Yes
Tissue obtained at transplant	n/a	n/a	Yes	n/a	Yes
Current status	Waiting list	Waiting list	Stable post-transplant	Waiting list	Died

Table 5.9: Detection of SERCA2a transgene in cardiac tissue.

(IW, inferior wall; LW, lateral wall; AW, anterior wall; AS, anteroseptum; PS, posteroseptum; PW, posterior wall; NSFA, not suitable for analysis; BLD, below limit of detection.)

Patient number	**Months after treatment**	**Source of tissue**	**Heart tissue**	**AAV1/SERCA2a copies DNA per µg of total DNA**
003	6	Biopsy	IW, LW	NSFA
004	6	Biopsy	IW(a)	38
			IW(b), LW	BLD
	22	Transplant	AW, AS, PS, PW	BLD
007	6	Transplant	AW	41
			AS	80
			PS	57
			PW	23

5.3.8 Immune response to AAV1 vector

5.3.8.1 Neutralising anti-AAV antibodies

The interaction of the AAV1 vector with the host immune system is an important parameter in understanding the success of therapy. On the one hand, previous work has suggested that pre-existing anti-AAV1 NAb might impair delivery to cardiomyocytes, while on the other, the stimulation of cytotoxic immune response carries risk of systemic inflammatory reaction. In the SERCA-LVAD trial, patients with and without pre-existing NAbs were included, and presence of anti-AAV1 NAbs and presence of AAV1-specific T cells were studied serially throughout the trial protocol.

Serial NAb titres are shown in Table 5.10. There are complete data for four patients. The one patient with negative titre for NAb at randomisation, who received placebo, remained NAb negative at the 6 month follow-up. The three other patients all received active AAV1.SERCA2a. Two of these were NAb negative at randomisation, and had seroconverted to become NAb positive with titre >1:64 after 6 months. The third was NAb positive at randomisation with a moderate titre (>1:16), and had developed a greater titre by 6 months (>1:64).

5.3.8.2 Cell-mediated immune response

Cell-mediated immune response to the AAV1 vector was studied using AAV1-specific ELISPOT to determine presence of specific anti-AAV1 T cells. Poor cell yield within the specimens precluded interpretable results from subject 002.

Available results showed a mixed pattern (see Table 5.10, page 159). Subject 003 was NAb positive at baseline and 6 months and had positive ELISPOT for AAV1-specific T cells at 3 months and 6 months. The presence of specific T cells may reflect the previous exposure, may reflect the response to the transfected gene product, or may reflect an existing specific T cell pool, amplified by the renewed exposure at transfection. Subject 004 was NAb negative at baseline but had seroconverted with positive NAb titre by 6 months. The ELISPOT is positive at 3 months but then negative at 6 months, suggesting transient presence of a specific

T cell pool during the period immediately following transfection. Subject 005 was NAb negative at baseline and, after receiving placebo product, remained NAb negative and ELISPOT negative throughout the follow-up period. Complete data was unavailable for subject 007 with regard to NAb status and ELISPOT (no 6 month result for either), but the ELISPOT remained negative at 3 months suggesting no specific T cell response was mounted.

Table 5.10: Serial titres of ant-AAV1 neutralising antibody and AAV-specific ELISPOT.
(NAb, anti-AAV1 neutralising antibodies; ELISPOT, AAV1- specific enzyme-linked ImmunoSpot test)

		Patient number				
		002	003	004	005	007
	Treatment Group	Active	Active	Active	Placebo	Active
Baseline	NAb status	Negative	Positive	Negative	Negative	Negative
	NAb titre	<1:2	>1:16	<1:2	<1:2	<1:2
3 months	ELISPOT	-	Positive	Positive	Negative	Negative
6 months	NAb status	Positive	Positive	Positive	Positive	-
	NAb titre	>1:64	>1:64	>1:64	<1:2	-
	ELISPOT	-	Positive	Negative	Negative	-

5.3.9 Safety of the gene product

5.3.9.1 Reported adverse events and adverse reactions

There was detailed monitoring of adverse events and adverse reactions during the study, in line with requirements by the regulatory bodies. Definitions and reporting procedures are included in the Methods (section 5.2.1.6, page 141). No SUSAR were reported during the study. There were 4 SAE all of which were classed as expected, and which were felt to be unrelated (n=1) or unlikely to be related to the IMP (n=3). These are set out in Table 5.11 (page 160). In addition to the 4 SAE, there were 12 adverse events reported during the 6 month study follow-up. These are listed in Table 5.12 (page 160). The most significant of these was a superficial haematoma that formed in subject 002 related to vascular access for the infusion procedure, and this was completely resolved without long term sequelae by the 3 month follow-up visit. Two AEs reported for subject 005 (recurrence of atrial flutter, and

mild troponin elevation) were thought at the time to be related to viral transfection and mild viral-induced myocarditis, but in fact this patient received placebo only.

Table 5.11: Serious adverse events during the SERCA-LVAD trial.

Patient ID	Onset time (Weeks after IMP)	Type	Severity	Relation to IMP
4	7	LVAD alarm, sustained increased power consumption, hospital admission	Mild	Unlikely
7	11	Worsening heart failure, hospital admission	Severe	Unlikely
7	24	Heart Transplantation	Severe	Unrelated
7	28	Death	Severe	Unlikely

Table 5.12: Adverse events during the SERCA-LVAD trial.

Patient ID	Onset time (Weeks after IMP)	Type	Severity	Relation to IMP
2	0	Haematoma at groin puncture site	Mild	Not applicable
2	15	Coryzal symptoms	Mild	Unrelated
3	2	Driveline infection	Mild	Unrelated
3	15	LVAD Battery Alarm	Mild	Unrelated
3	20	Skin lesion on forehead	Mild	Unrelated
4	2	Hyperkalaemia	Mild	Unlikely
4	8	Iron deficiency anaemia	Mild	Unrelated
4	15	Driveline bacterial swabs grew coagulase negative *Staphylococcus aureus*	Mild	Unlikely
4	19	Mild driveline exit site bleeding	Mild	Unlikely
5	1	Mild troponin rise; presumed mild viral-induced myocarditis	Mild	Possible
5	4	Recurrence of atrial flutter	Mild	Possible
7	-1	Nausea symptoms	Mild	Unrelated

5.3.9.2 Evidence of systemic and organ-specific adverse reactions

Previous gene therapy programmes have been abandoned due to severe systemic inflammatory responses and organ injury, in some cases causing death. During the study, there was monitoring for clinical evidence and blood markers that might suggest adverse reaction.

5.3.9.2.5 Markers of liver injury

In previous gene therapy studies, hepatitis has been observed resulting from direct viral infection after systemic administration, or as an immune phenomenon related to systemic inflammation. Therefore, patients underwent monitoring of liver enzymes as evidence of liver injury.

Abnormal results were limited to data from patients 2 and 7 (Table 5.13 and Figure 5.6 on pages 163 and 165 respectively). Patient 2 had persistent elevated levels of transaminases (ALT and AST) and to a lesser extent ALP that was present at enrolment and remained stable throughout the study. These had been present, in fact, for several years and been investigated in the clinic with no cause found and specifically no suggestion of significant right sided HF. Patient 7 had a pattern suggestive of hepatic congestion related to heart failure (raised total bilirubin which was predominantly unconjugated, alongside high ALP). Intravascular haemolysis could be another explantation for the hyperbilirubinaemia, but the non-variation of plasma Hb and LDH mitigates against this (Figure 5.4, page 152).

5.3.9.2.6 Markers of systemic inflammation

Total white cell count remained within normal range (4.0-10.5 $x10^9$/L) for all patients except subject 7 in whom there was a gradual rise peaking at 11.1 $x10^9$/L at 3 months and then declining. This was accounted for by a mild neutrophilia developing in patient 7 at this time point (rising to 9.0 $x10^9$/L; normal range 1.8-7.8 $x10^9$/L); neutrophil counts were normal in other patients at all timepoints. Lymphocyte count remained within the normal range (0.7-4.5 $x10^9$/L) for all patients except patient 3 who was borderline lymphopenic at enrolment (0.7 $x10^9$/L) and dropped to 0.6 $x10^9$/L at week 3 before recovering and re-entering normal range. No consistent patterns were evident between active versus placebo treated patients. There

was no suggestion of significant early leucopenia or leucocytosis in response to viral transfection. See Table 5.14 and Figure 5.7 (pages 164 and 166 respectively).

There were no significant rises in CRP (normal <10mg/L) during the study, except in patient 7 at the 6 month timepoint related to hospital admission with worsening heart failure (Table 5.14 and Figure 5.7). There were small rises in patients 4 and 5, not associated with clinical adverse events and unlikely to be clinically significant. This further mitigates against presence of any significant systemic inflammatory syndrome.

5.3.9.2.7 Markers of myocardial or skeletal muscle injury

We hypothesised that AAV1 transfection to cardiomyocytes could generate a viral-induced or immune mediated myocarditis due to local cell-mediated immune response within the myocardium. We further hypothesised that off-target transfection to skeletal muscle could generate a similar effect within skeletal muscle. To assess these hypotheses there was serial monitoring of cardiac-specific troponin I and of creatine kinase (CK).

Serial CK measurements were within normal limits for all patients throughout the study, mitigating against significant myositis (Table 5.14 and Figure 5.7). Patient 2 had CK measurements higher than other patients, but stable and within normal limits. Troponin measurements were normal at all timepoints for patients 2, 3 and 4. Patient 5 had a raised troponin from week 1 post-infusion through to month 5. During the blinded phase, we postulated that this was mild viral-induced myocarditis, and reported an adverse event; however, at unblinding it transpired patient 5 received placebo, excluding a viral effect. Patient 7 had significantly elevated troponin throughout the study, probably reflecting that patient's advanced disease state.

Table 5.13: Serial changes in liver injury markers.

		Baseline	Wk 1	Wk 2	Wk 3	M1	M2	M3	M4	M5	M6
Alkaline Phosphatase (normal 25-150 U/L)	**2**	157	140	140	140	148	155	140	134	124	127
	3	56	63	61	67	83	52	57	62	52	-
	4	56	66	62	56	60	-	69	70	65	64
	5	52	46	47	51	49	45	54	46	46	62
	7	127	130	114	122	117	117	119	172	148	200
Alanine Transaminase (normal <55 U/L)	**2**	111	71	84	81	94	80	85	63	83	53
	3	22	27	22	20	30	17	15	21	15	-
	4	23	36	28	21	21	-	15	19	19	18
	5	16	26	14	18	12	13	16	26	25	20
	7	18	12	13	17	20	17	18	13	20	-
Aspartate Aminotransferase (normal <40 U/L)	**2**	-	66	64	63	66	66	55	59	-	-
	3	-	27	30	33	29	26	-	25	-	-
	4	38	39	32	33	-	-	31	31	28	38
	5	29	18	23	-	20	23		24	24	29
	7	-	30	30	35	-	-	-	-	-	-
Total Bilirubin (normal <21 µmol/L)	**2**	6	6	6	6	5	5	5	5	6	4
	3	12	9	11	11	9	11	10	10	12	-
	4	8	6	5	8	6	-	5	7	8	8
	5	7	4	5	3	3	4	5	6	7	7
	7	24	21	20	23	27	26	30	33	17	-
Indirect Bilirubin (Unconjugated) (normal <10 µmol/L)	**2**	6	6	6	6	5	5	5	5	6	4
	3	12	9	11	11	9	11	10	10	12	-
	4	8	6	5	8	6	-	5	7	8	8
	5	7	4	5	3	3	4	5	6	7	7
	7	24	21	20	23	27	26	30	33	17	-

Table 5.14: Serial changes in leucocyte counts, C-reactive protein, troponin-I and creatine kinase.

(WBC, total white blood cell count; CRP, C-reactive protein)

		Baseline	Wk 1	Wk 2	Wk 3	M1	M2	M3	M4	M5	M6
Total WBC (normal 4.0-10.5 x10^9/L)	**2**	4.6	5.1	3.9	5.0	4.1	3.9	4.4	4.5	3.9	5.0
	3	5.3	5.5	5.6	4.7	4.9	4.8	5.4	6.6	5.2	6.0
	4	5.8	5.0	5.7	5.5	6.1	-	6.3	5.5	6.6	5.6
	5	6.3	5.9	6.6	6.8	7.2	6.6	5.5	6.0	6.4	5.9
	7	9.7	8.1	9.1	9.6	9.3	9.9	11.1	7.7	8.1	-
Lymphocyte (normal 0.7-4.5 x10^9/L)	**2**	1.2	1.1	.9	1.1	1.0	.8	.9	.9	1.0	1.0
	3	.7	.8	.7	.6	.7	.7	.8	.9	.7	1.0
	4	1.2	1.2	1.3	1.0	1.3	-	1.0	1.0	1.1	1.2
	5	1.4	1.4	1.5	1.4	1.7	1.4	1.4	1.4	1.3	1.3
	7	1.2	1.1	1.3	1.2	1.0	1.2	.9	1.0	1.2	-
Neutrophil (normal 1.8-7.8 x10^9/L)	**2**	2.5	3.1	2.3	3.0	2.4	2.4	2.6	2.7	2.0	3.3
	3	3.8	3.8	3.9	3.3	3.4	3.3	3.7	4.8	3.5	4.1
	4	3.7	2.9	3.4	3.6	3.7	-	4.3	3.5	4.2	3.6
	5	3.8	3.7	4.2	4.5	4.5	4.0	3.4	3.2	4.2	3.5
	7	7.1	5.9	6.8	7.2	7.1	7.6	9.0	5.9	5.8	-
CRP (normal <10 mg/L)	**2**	3	8	5	3	2	3	3	3	3	3
	3	1	0	1	2	3	1	1	2	1	1
	4	1	2	2	-	2	-	5	2	11	3
	5	6	6	7	5	5	7	4	15	6	7
	7	6	7	5	4	4	5	6	7	4	113
Troponin I (normal <0.04 mcg/L)	**2**	.027	.000	.025	.038	.021	.025	.031	.000	.000	.031
	3	.000	.000	.000	.000	-	.000	-	.000	.000	-
	4	.000	.000	.000	.000	.000	-	.040	.000	.000	.000
	5	.027	.048	.058	.072	.059	.049	.042	-	.046	.033
	7	.130	.129	.097	.109	.108	.125	.128	-	-	-
Creatine Kinase (normal 25-600 U/L)	**2**	340	238	288	274	295	357	315	315	284	378
	3	129	103	102	129	144	125	121	128	111	-
	4	168	142	157	157	136	-	180	161	137	163
	5	132	153	116	142	131	162	116	-	97	97
	7	105	87	103	107	113	124	98	-	-	-

Figure 5.6: Serial changes in liver injury markers.

Graphs showing serial measurements of (a) Alkaline phosphatase (ALP), (b) Alanine transaminase (ALT), (c) Aspartate aminotransferase (AST), and (d) Total Bilirubin. See data in Table 5.13.

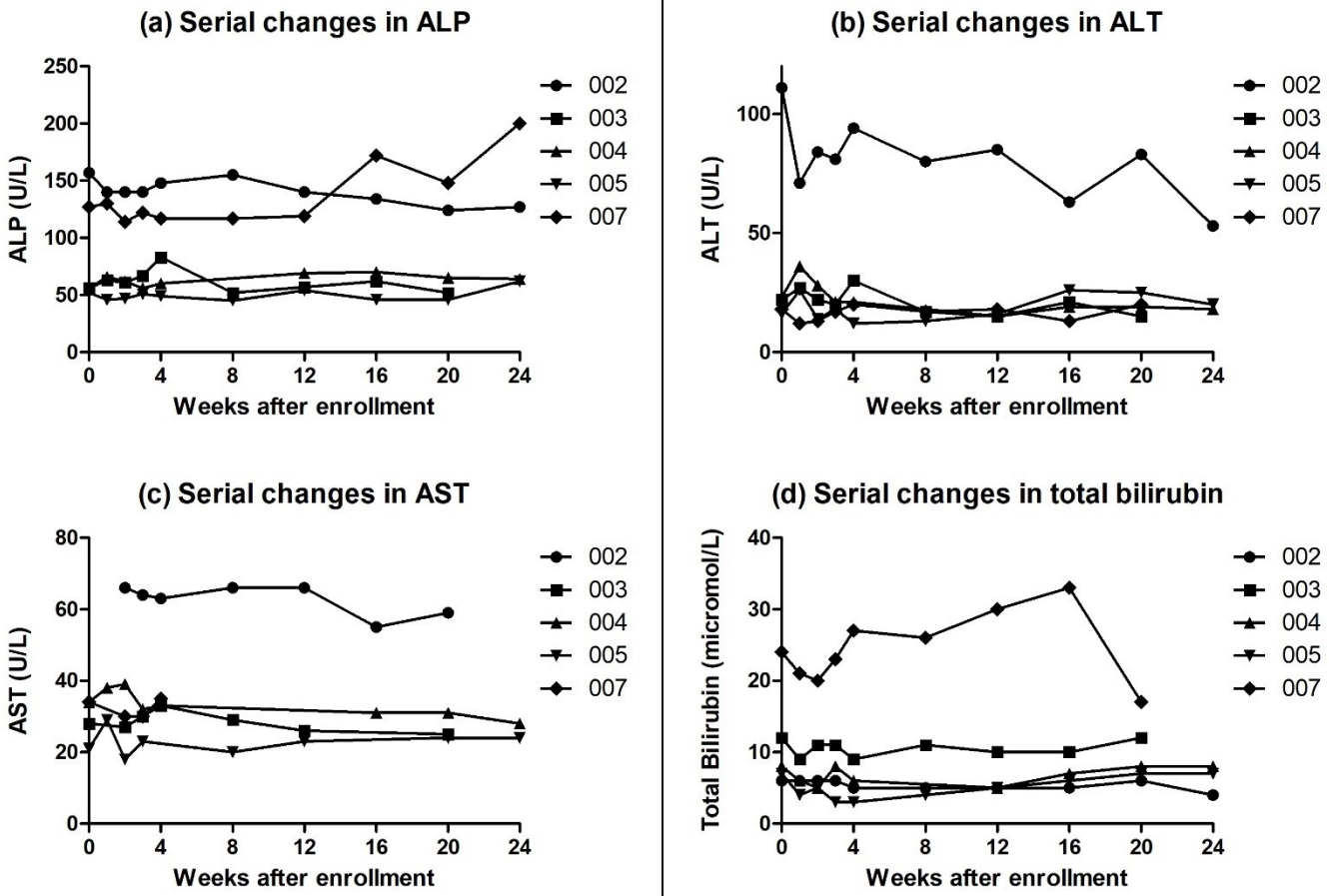
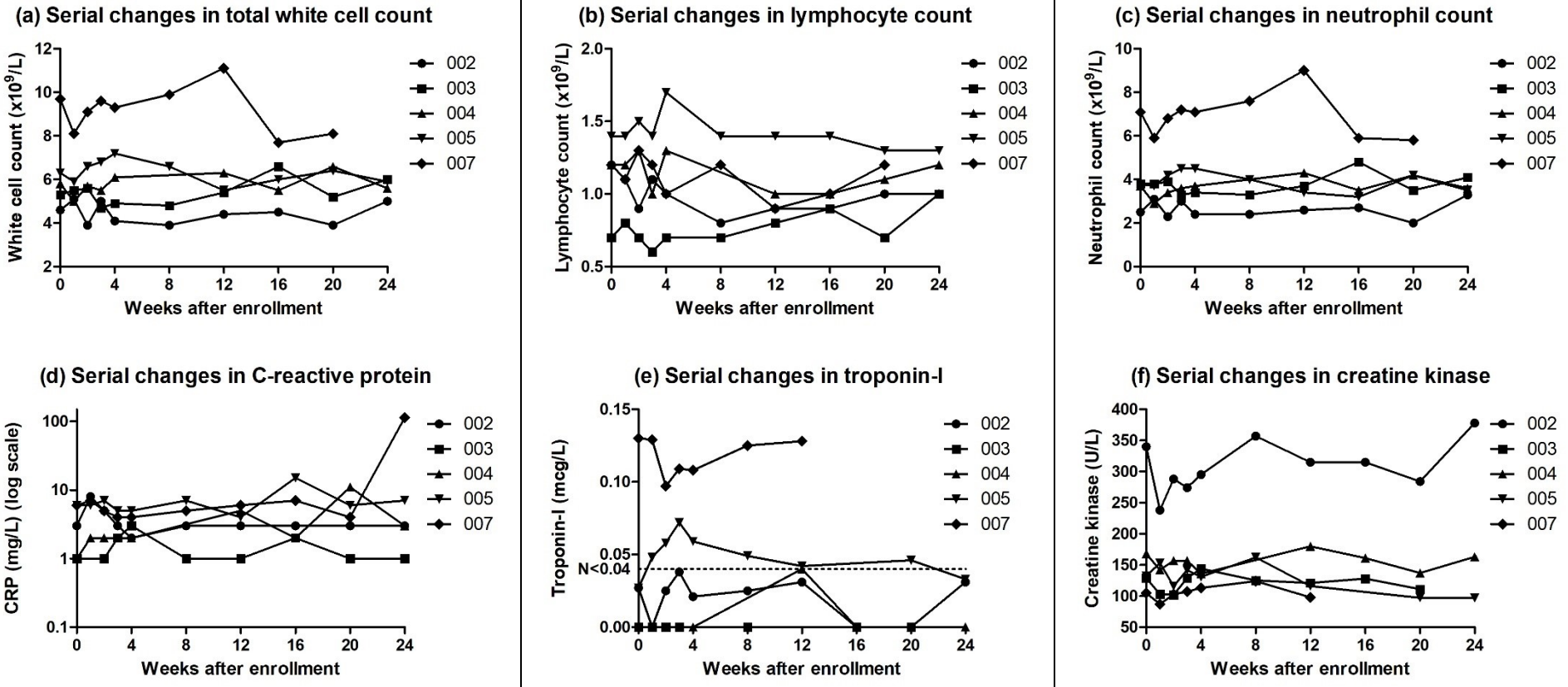


Figure 5.7: Serial changes in leucocyte counts, C-reactive protein, troponin-I and creatine kinase.

Graphs showing serial measurements of (a) Total white cell count, (b) Lymphocyte count, (c) Neutrophil count, (e) Troponin-I, and (f) Creatine kinase, all shown on standard linear scales; and (d) C-reactive protein (CRP), shown on a logarithmic scale. Normal range (N) for troponin-I on this assay is <0.04mcg/L. See data in Table 5.14. (WBC, total white blood cell count)



5.4 Discussion

The SERCA-LVAD trial was designed to evaluate use of the AAV1.SERCA2a gene product in patients with advanced HF and long term LVAD, with a primary endpoint of feasibility and safety. This Chapter reports the results from the SERCA-LVAD study related to recruitment, trial course, feasibility and safety. Results related to clinical efficacy of the gene product are reported in Chapter 6.

We hypothesised that (1) AAV1.SERCA2a gene therapy was safe and feasible in patients with advanced HF and LVAD; (2) intracoronary delivery of the AAV1.SERCA2a gene product would yield detectable viral transgene in myocardial tissue from treated subjects; and (3) pre-existing NAb would not prevent successful transfection in treated subjects. The key conclusions from data presented in this Chapter support the first two hypotheses. Specifically, the data show that (1) delivery of the AAV1.SERCA2a gene product by a single intracoronary infusion at the dose of 1x 10^{13} DNase resistant particles is feasible and safe in patients with advanced HF with an LVAD; (2) the procedure to deliver the gene product was safe in this patient group, and the gene product delivered cause no discernible complications; (3) the presence of pre-existing NAb does not cause concerning immune reaction; (4) with the current dose of AAV1.SERCA2a there is evidence of transgene expression in treated myocardium, but at low levels of expression; (5) EM biopsy is feasible in most patients and is safe, but carries higher risks than expected, and may not yield sufficient data to justify these risks; (6) recruitment is challenging in the LVAD population due to limited patient numbers, pre-existing co-morbidities or complications, and younger age of the patients; and (7) low speed echocardiography was safe in this cohort.

5.4.1 Administration of the gene therapy

All previous studies with the AAV1.SERCA2a product at the current dose have demonstrated that the product is safe and its administration as a single intracoronary infusion was feasible. Results from this study concur with these previous findings.

We have found no evidence of systemic or organ specific adverse responses to the gene product. We undertook serial monitoring with ELISPOT testing monitoring for AAV1-specific T cell responses. There was a mixed outcome from these tests, with no relation between ELISPOT positivity and adverse clinical or laboratory markers. There was one participant in whom transient elevation of cardiac troponin and recurrence of atrial flutter in the weeks immediately following the infusion raised concern for viral-induced myocarditis. At the time, there were safety discussions and careful clinical monitoring, but after unblinding it transpired that the participant in question had received placebo treatment only. This highlights the importance of studying new approaches or therapies within the context of a properly controlled and blinded trial, including sham procedures, lessons learnt in the recent past from studies of renal denervation for hypertension.

The IMP infusion was completed to protocol in the 5 patients in whom it was attempted. There were no SAE, and one AE related to vascular access.

The study protocol specified use of continuous intravenous GTN as adjunctive therapy to improve myocardial transfection. We developed a protocol for dose escalation and following this we encountered no untoward effects. However, the haemodynamic effects of GTN are likely to differ in LVAD patients from the effects seen in unsupported HF patients, and further work could characterise these effects in more detail and study whether the GTN alters the transfection efficiency in LVAD patients. We do not believe the difference in GTN rates influenced efficacy of delivery.

One outstanding question is safety after cardiac transplantation in the immunosuppressed patient. It is possible that latent viral infection with exogenous AAV could reactivate in the context of T cell suppression. There has not been adverse consequence reported from CUPID programme participants to date. In this study, long term safety follow-up for subject 004, and any other participants who undergo transplant in future, will be informative.

5.4.2 Vascular access and anticoagulation

A key novelty of the SERCA-LVAD trial was assessing the process of intracoronary delivery of gene therapy in LVAD patients. We identified vascular access and anticoagulation management as particular challenges in this patient group.

The process of coronary angiography and intracoronary infusion requires placement of an arterial access sheath, which is inherently complex in LVAD patients due to anticoagulation. There is a subtle balance between minimising the time of subtherapeutic anticoagulation (which would precipitate device thrombosis) and minimising the risk of bleeding complications from the access site. All arterial access was via the femoral artery, accentuating the importance of safe vascular access due to risk of catastrophic retroperitoneal haemorrhage.

During the study, we adopted various approaches to strike this balance. All arterial access was gained by an experienced consultant operator using real time ultrasound guidance. Regarding anticoagulation, the initial approach was conservative, stopping warfarin completely and bridging with intravenous heparin during the period when INR < 2. This approach was adjusted with subsequent cases, using home monitoring and clinical expertise from the LVAD nursing team to drop the INR to just below 2 for the day of the procedure, and cover with subcutaneous low molecular weight heparin until INR had returned to >2. The data show that the period of subtherapeutic anticoagulation and length of stay both shortened with this approach. Furthermore, the arterial sheath was placed with a micropuncture kit intended for paediatric patients, assisting operator accuracy. Overall there was one AE related to vascular access, a haematoma sustained by subject 002 during the first IMP infusion case, corresponding to 1 out of 8 (12.5%) instances of arterial access during the trial. There were no further complications related to vascular access.

The experience in SERCA-LVAD supports the hypothesis that access via the femoral artery for intracoronary delivery is safe and feasible in LVAD patients. To further refine the procedure and minimise risks, the next step would be to move to access via the radial artery. Radial access for coronary angiography and percutaneous intervention is associated with

reduced bleeding risk and reduced length of hospital stay, and in the current setting would facilitate greater freedom with anticoagulation and potentially a marked reduction in length of stay, perhaps making it a day or overnight case.

5.4.3 Pre-existing NAb

Previous clinical studies with AAV vectors have suggested that pre-existing anti-AAV neutralising antibodies may prevent successful viral transduction into target tissues. Consequently, clinical studies with AAV1.SERCA2a to date had a pre-screening stage to quantify the titre of pre-existing NAb and exclude all patients with titre >1:2 who were classed as NAb positive. A key hypothesis in SERCA-LVAD was that presence of NAb would not reduce viral transduction, and both NAb positive and NAb negative patients were recruited to the study, with each arm (placebo versus active) stratified 50:50 positive to negative.

Four out of five patients were NAb negative at enrolment. This in itself is unexpected, as previous data has suggested around 50-70% of patients are normally NAb positive[166], and within recruitment for CUPID-2 in the UK, this figure was greater. This may be random effects in a small group, but a potential scientific explanation relates to the age of this cohort. AAV is a naturally occurring, non-pathogenic virus and studies have suggested that increasing age is associated with greater likelihood of being NAb positive[166], probably due to increased likelihood of environmental exposure with increasing age. The patients recruited to SERCA-LVAD were younger than those enrolled in the CUPID programme, so it may be that our group, being younger, were more likely to be naïve to AAV. The one patient who was NAb positive at enrolment was the oldest patient enrolled (age 69 years, 20 years older than the next oldest).

All patients tested who received active AAV1.SERCA2a either seroconverted to become NAb positive at the end of the trial (subjects 002 and 004), or increased their NAb titre after re-exposure (subject 003). There is no 6 month NAb titre for subject 007. Subject 003 was NAb positive at enrolment and received active treatment. There was no suggestion that prior exposure to AAV and pre-existing NAb caused any uncontrolled immune reaction or other

safety concern, as has been previously been postulated. Unfortunately, the EM biopsy in this patient yielded only fibrotic material and no myocardial tissue for qPCR analysis for viral transgene, so the question of whether NAb blocked successful transduction remains unanswered.

5.4.4 Tissue expression and endomyocardial LV biopsy

The EM biopsy was a key aspect of the SERCA-LVAD trial that promised novel data to complement concurrent trials using AAV1.SERCA2a such as the CUPID-2 trial. Systematic collection of LV biopsy specimens, coupled with tissue collected from explanted hearts at cardiac transplantation, would allow systematic quantification of transgene expression. To date, expression data had only been ascertained opportunistically, typically in patients from the CUPID trials who died, underwent LVAD implantation or underwent cardiac transplantation. These patients are a skewed group, as death, LVAD and transplant were defined as clinical endpoints in the CUPID programme and could be seen to define non-response to the gene therapy.

EM biopsy was attempted in 3 patients, from whom LV tissue suitable for analysis was obtained from 1 patient. One patient had previously undergone aortic valve replacement, and in this patient several consultant operators, including an experienced transcatheter AV interventionist, were unable to cross the AV to gain access to the LV, and no tissue was obtained. Tissue samples were obtained from the third patient, but were predominantly fibrotic scar tissue without sufficient myocardial tissue for analysis. This is characteristic of the failing heart, but the difficulty obtaining useable myocardial tissue had been underestimated. The tissue obtained from subject 004 did show expression of transgene, but only in one of the samples and at very low levels. This pattern was observed in tissue collected at cardiac transplantation in subjects 004 and 007. Copy number of viral genomes confirms viral transduction has occurred, but presence of viral genome does not necessarily equate to increase in sarcoplasmic reticulum ATPase activity. This and the implications for clinical efficacy are discussed further in Chapter 6.

There were no AEs reported related to the biopsy procedure and, apart from the failure to collect tissue, the procedures went as planned. However, the procedures were more challenging and posed greater risks than had been anticipated. Biopsies in transplant recipients are typically done via the right internal jugular vein and sampling the RV endocardium, and this procedure including use of the biopsy catheter and bioptome is well practised. The LV was preferred in this study as the LV myocardium was the protocoled target for the gene product, and the thicker LV wall would theoretically reduce the risk of cardiac tamponade. Previous clinical studies at Harefield involved LV EM biopsy and the operator drew on this experience. However, those studies were done in patients with older LVADs, specifically the HeartMate I or II. Four of the five patients recruited were implanted with Heartware HVAD devices, and a key characteristic of these is the placement of the device directly at the LV apex, and the consequently very short inflow cannula. Precise control of the biopsy catheter was essential in these patients, as misplacement by just a few centimetres could place the catheter within the pump causing stoppage and likely pump failure, potentially catastrophic.

In summary, the EM biopsy procedure was feasible and was safe in this small group, but posed greater challenges than anticipated and had poor yield of data. Consequently, a key conclusion is that EM LV biopsy is probably not a useful investigation to include in future clinical studies to assess viral genome expression after delivery of gene therapy. An alternative approach could be substitution with RV septal biopsy performed via the right internal jugular vein. Ultimately, analysis of the whole heart after explant for cardiac transplantation remains the most reliable tool.

5.4.5 Recruitment

The LVAD population make a good study group as they have well characterised HF, tend to be engaged patients used to visiting hospital, have a secure backup in the case of adverse myocardial responses to new therapies (e.g. in the context of an acute severe viral-induced myocarditis related to gene therapy), and, in most cases, they are on the waiting list for cardiac transplantation with the possibility of obtaining the explanted heart for research purposes. Furthermore, they allow direct assessment of hypotheses that adding an adjunctive, disease modifying therapy to an environment of optimised myocardial loading conditions,

could facilitate additional beneficial reverse remodelling. However, in addition to these undoubted benefits, progress with SERCA-LVAD identified a number of challenges.

The main challenge is the limited numbers of LVAD patients, and in turn the limited number of stable LVAD patients, without complications of therapy. Another key factor is the age of the group, typically younger than a standard HF cohort, and often less able to commit to hospital visits due to personal commitments at home or work. At Harefield when the study opened there were 82 patients receiving long term circulatory support all of whom were screened to participate. Quickly the numbers shrank, due to unsuitable devices (e.g. patients with total artificial heart), ongoing treatment for complication (e.g. chronic driveline infection), or high risk of LVAD thrombus (e.g. specific device types). At the time of halting recruitment, 18 patients had been approached, of whom 6 had agreed to participate, one of whom later withdrew for personal reasons. Reasons given for not participating included (1) inability of patients to commit the time to multiple visits, reflecting the fact that many LVAD patients are in full time work or education, in turn reflecting the younger age of this cohort of patients; (2) inability of the research team to reassure reproductively active patients about potential risks of vertical viral transmission to future children; (3) geography, in that patients would travel in some cases hundreds of miles to Harefield from their home, and this impacted on their decision to commit to the study visits.

Strategies to overcome these difficulties were developed during the study, and these would need to be key considerations planning any future study. Firstly, increasing the pool of potential patients requires collaboration between centres. As well as numbers, the multi-centre approach makes the science more robust. We were collaborating with Papworth Hospital, although they hadn't initiated recruitment before the trial was halted. Secondly, we tried to streamline the hospital visits to convenience patients and keep bed occupancy in the Transplant Unit to a minimum. For the baseline, 3 and 6 month visits there were too many investigations to achieve in one day, and patients were accommodated in the patient residential block on the hospital site for one night. This increased costs but significantly helped to facilitate the trial.

In summary, there are strong practical and scientific reasons to study LVAD patients, but there are drawbacks that must be borne in mind and planned for when planning future interventional studies.

5.4.6 Low speed and deformation echocardiography

Finally, the trial sought to affirm the safety of low speed echocardiography in the Heartware HVAD, and assess feasibility of deformation echocardiography as an outcome parameter in this patient group.

Ten echocardiograms were performed that included low speed assessments, without evidence of complication and specifically without clinical or biochemical evidence of secondary LVAD thrombosis. Low speed testing using the Harefield protocol has previously been reported in HeartMate II patients[125], and this cohort now helps to confirm safety of this approach in the HVAD. This could facilitate greater use of low speed testing for clinical assessment within the transplant programme.

Strain parameters were recorded in all patients at clinical operating speed and where possible at low speed, to address the hypothesis was that strain parameters, recorded at full speed, may provide a method for assessing underlying LV function without needing to perform a low speed assessment. This is addressed in Chapter 6. In the first instance, the difficulty obtaining complete datasets erodes its feasibility and reproducibility. Apical placement of the LVAD makes any assessment from apical windows very challenging or impossible. In this cohort of 5 patients, apical images of borderline quality could be obtained from 2 patients, with no apical windows at all in the remaining 3 patients. Those apical images that were obtained were off axis, missing apical segments, and with poor endocardial definition, and making it difficult to draw data which is truly comparable patient to patient. In contrast, parasternal images were more reliably obtained, and a full dataset of strain data was analysed, though undoubtedly some of these were borderline quality images.

In summary, in this cohort the low speed echo assessments were safe and the collection of myocardial strain data was feasible in part. Future studies in LVAD populations need to consider the role of echo given its significant limitations and difficulty in obtaining comprehensive and comparable data.

5.5 Conclusions

Intracoronary delivery of AAV1.SERCA2a gene therapy was safe and feasible in patients with advanced HF and LVAD, yielding viral genome detectable in low levels in myocardial tissue from two of the five treated subjects without any significant safety concerns. There are important lessons learnt which can be applied to future interventional studies planned in the LVAD patient population. Presence of NAb is not a contraindication to gene therapy with AAV, though it remains undetermined what effect they might have on successful transduction. Clinical efficacy outcomes from the SERCA-LVAD trial are discussed in Chapter 6.

6: The SERCA-LVAD Trial – Clinical efficacy

6.1 Introduction

6.1.1 Background and Rationale

This has been discussed in Chapter 1 (section 1.5.3.4, page 47) and Chapter 5.

6.1.2 Study questions

Discussion of SERCA-LVAD outcomes is split between two Chapters, divided according to the three study questions as outlined in the introduction to Chapter 5 (section 5.1.2, page 136). The focus in this Chapter is on the third question:

3. Does AAV1.SERCA2a delivered at this dose by intracoronary infusion result in clinical benefit for patients with HF?

6.1.3 Hypotheses

We hypothesised that a single intracoronary infusion of the AAV1.SERCA2a gene product at the dose of 1x 10^{13} DNase resistant particles in patients with advanced HF with long term MCS from an LVAD would yield evidence of beneficial reverse remodelling as evidenced by functional, structural and neurohormonal measures.

We also hypothesised that echocardiographic strain would (1) provide a novel, low risk method to determine and monitor LV function during ongoing LVAD support; and (2) form a tool to predict clinical response to gene therapy.

6.2 Methods

6.2.1 Aims

The aims of the study were:

- with regard to clinical efficacy, to establish whether AAV1.SERCA2a gene therapy delivered as a single intracoronary infusion to patients with advanced HF and an LVAD was clinically efficacious, improving functional, structural and neurohormonal outcomes;
- with regard to strain imaging, to evaluate its use as a diagnostic and prognostic biomarker in the context of AAV1.SERCA2a gene therapy.

6.2.2 Study design

This has been discussed in Chapter 5 (section 5.2, page 137).

6.2.3 Efficacy outcomes

The co-primary efficacy outcomes were change in peak VO_2, BNP and LVEF at 6 months. The secondary efficacy outcomes were change in VE/VCO_2 slope, change in 6 minute walk distance, change in LV dimensions on echocardiography, and change in global strain on echocardiography, all at 6 months versus baseline.

6.2.4 Statistical analysis and data reporting

The small number of study participants makes detailed statistical analysis inappropriate in reporting data. Most data are presented on an individual patient basis, with non-parametric summary methods used where appropriate. For correlation testing, the Spearman coefficient was used.

6.3 Results

6.3.1 Baseline characteristics, NAb status and randomisation

These data have been reported in Chapter 5 and will be cross-referenced in this Chapter. Key details are summarised in Table 6.1.

Table 6.1: Trial subject key baseline characteristics.

See Table 5.3 and Table 5.4 for full information. (DCM, dilated cardiomyopathy; peak VO_2, peak oxygen consumption; BNP, B-type natriuretic peptide; NAb, anti-AAV1 neutralising antibody.)

	Patient number				
	002	**003**	**004**	**005**	**007**
Core details					
Age (years)	36	69	29	49	30
Gender	Male	Male	Male	Male	Female
HF aetiology	Valvular	Idiopathic DCM	Familial DCM	Idiopathic DCM	Familial DCM
Type of LVAD	Heartware HVAD	Heartware HVAD	Thoratec HeartMate II	Heartware HVAD	Heartware HVAD
LVAD implant to study enrolment (months)	24	153	60	48	18
Baseline HF severity parameters					
Peak VO_2 (ml/kg/min)	28.3	19.6	20.3	13.5	15.6
6 minute walk distance (metres)	623	627	480	563	397
BNP (ng/L)	58	314	37	155	359
Anti-AAV NAb status and randomisation					
NAb status	Negative	Positive	Negative	Negative	Negative
Randomisation	Active	Active	Active	Placebo	Active

6.3.2 Primary endpoint

The co-primary efficacy outcomes were change in peak oxygen consumption, BNP and LVEF at 6 months. The small number of study participants (placebo n=1, and active AAV1.SERCA2a n=4) mean the study is underpowered and preclude any definitive

conclusions regarding efficacy. Furthermore, there is missing data for 2 participants at the 6 month timepoint.

Based on the data available, there does not appear to be a consistent pattern of change in the co-primary efficacy outcomes. Serial measurements and relative changes are shown in Table 6.2 (page 181) and illustrated in Figure 6.1 (page 182). There was heterogeneity amongst participants regarding baseline peak VO_2. Subjects 002, 003 and 004 all received active therapy but showed no clinically meaningful change at 3 or 6 months. Subject 005 (placebo group) showed a 22% increase in peak VO_2 to 3 months (13.5 to 16.4ml/kg/min), which was not sustained at 6 months. Subject 007 (in the active group) showed marked worsening in peak VO_2 at 3 months (15.6 to 9.2ml/kg/min, drop of 41%) which was reflected in worsening clinical condition. At the time of the investigation, the patient was on the national urgent waiting list for cardiac transplantation and remained an inpatient on inotropic therapy.

There was no improvement in BNP during the study period (Table 6.2 and Figure 6.1b, pages 181 and 182 respectively). Subjects 003 and 007 had generally higher BNPs than other participants. BNP increased significantly in subject 007 (359 to 477ng/L, 33% increase) at 3 months. This had decreased to 344ng/L at 6 months, but this probably reflects inotropic therapy rather than improvements in native heart function.

The lack of consistent apical view on echocardiography precludes complete assessment of effect of AAV1.SERCA2a on LVEF. There were acceptable apical views in 2 patients, one receiving placebo and one active treatment. In these patients, there were small changes in LVEF which are unlikely to be clinically significant.

6.3.3 Secondary endpoints

VE/VCO2 slope, 6 minute walk distance, LV end diastolic dimension and LV global circumferential strain were evaluated as secondary efficacy endpoints. The echocardiographic parameters were evaluated at full and low LVAD speeds.

Again, the data were heterogeneous and the study was underpowered to draw definitive conclusions. The data from echocardiography, in particular from low speed assessment, are limited due to small patient numbers and due to low speed assessments being omitted after study suspension. Based on the data available, there was no evidence of meaningful and/or consistent change in any of VE/VCO2 slope, 6 minute walk distance, LV dimensions at normal or low speed, or global circumferential strain at full or low speed. Data are shown in Table 6.3, Figure 6.1d-e, Table 6.4, Figure 6.2 and Figure 6.3 (pages 181 to 185).

Table 6.2: Co-primary endpoints – serial measurements and change at 6 months.

Delta change at 6 months is from baseline (B), shown as absolute and relative change.

Subject	Peak VO_2 (ml/kg/min)					B-type natriuretic peptide (ng/L)					Full speed LV ejection fraction (%)				
	Serial			Delta change at M6		Serial			Delta change at M6		Serial			Delta change at M6	
	B	M3	M6	Absolute	Relative (%)	B	M3	M6	Absolute	Relative (%)	B	M3	M6	Absolute	Relative (%)
002	28.3	26.9	26.6	-1.7	-6	58	101	77	+19	+33	-	-	-	-	-
003	19.6	19.9	-	-	-	314	343	325	+11	+4	-	-	-	-	-
004	20.3	19.4	19.6	-0.7	-3	37	76	47	+10	+27	45	40	42	-3	-7
005	13.5	16.4	13.9	+0.4	+3	155	88	128	-27	-17	25	22	26	+1	+4
007	15.6	9.2	-	-	-	359	477	344	-15	-4	-	-	-	-	-

Table 6.3: Secondary endpoints – serial measurements and change at 6 months.

Delta change at 6 months is from baseline (B), shown as absolute and relative change.

Subject	VE/VCO_2 slope					6MWD (metres)				
	Serial			Delta change at M6		Serial			Delta change at M6	
	B	M3	M6	Absolute	Relative (%)	B	M3	M6	Absolute	Relative (%)
002	25	25	27	+2	+8	623	643	648	+25	+4
003	45	46	-	-	-	627	311	627	0	0
004	30	29	29	-1	-3	480	508	528	+48	+10
005	33	35	34	+1	+3	563	577	525	-38	-7
007	11	46	-	-	-	397	-	-	-	-

Figure 6.1: Co-primary and secondary efficacy endpoints in the SERCA-LVAD trial.

Co-primary endpoints were (a) peak VO_2 on cardiopulmonary exercise testing, (b) B-type natriuretic peptide (BNP), (c) LV ejection fraction at full LVAD speed, (d) VE/VCO2 slope, and (e) 6 minute walk distance. Patients receiving AAV1.SERCA2a shown with continuous lines (subjects 002, 003, 004 and 007). Patient receiving placebo shown with dashed line (subject 005).

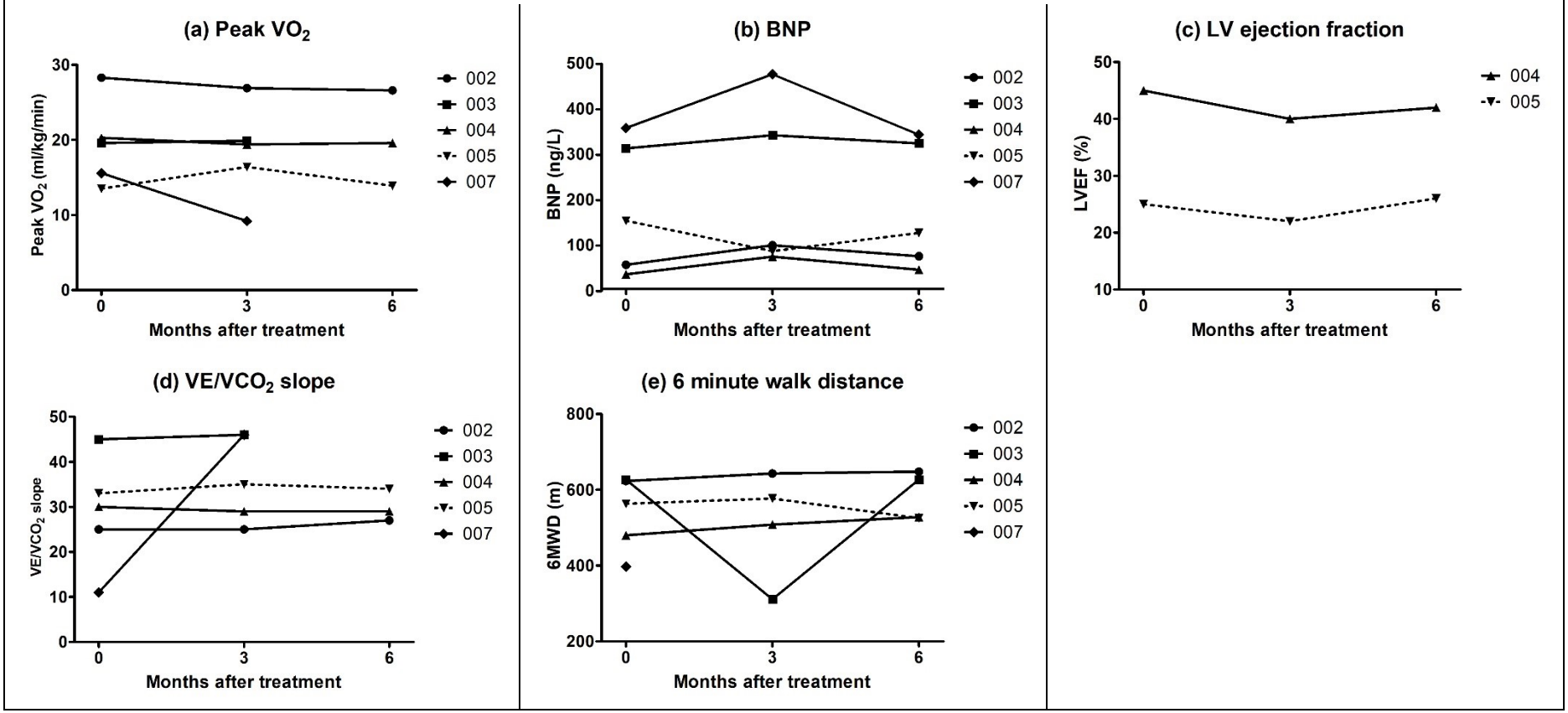


Table 6.4: Echocardiographic measurements in the SERCA-LVAD trial.

Serial measurements of LV ejection fraction, LV end diastolic dimension and LV global circumferential strain, and their change at 6 months, at high and low LVAD speeds. Delta change at 6 months is from baseline (B), shown as absolute and relative change. Missing data is shown with a dash (-).

		LV ejection fraction (%)					**LV end diastolic dimension (mm)**					**Global circumferential strain (%)**				
		Serial			Delta change at M6		Serial			Delta change at M6		Serial			Delta change at M6	
	Subject	B	M3	M6	Absolute	Relative (%)	B	M3	M6	Absolute	Relative (%)	B	M3	M6	Absolute	Relative (%)
Full speed	**002**	-	-	-	-	-	48	54	47	-1	-2	-18.4	-14.5	-15.0	+3.4	+19
	003	-	-	-	-	-	60	61	64	+4	+6	-6.7	-6.1	-9.7	-3.0	-40
	004	45	40	42	-3	-7	58	59	61	+3	+5	+2.3	-0.2	-2.6	-4.9	-210
	005	25	22	26	+1	+4	61	56	65	+4	+7	-4.3	-4.8	-5.7	-1.4	-30
	007	-	-	-	-	-	58	55	-	-	-	-8.0	-	-	-	-
		LV ejection fraction (%)					**LV end diastolic dimension (mm)**					**Global circumferential strain (%)**				
		Serial			Delta change at M6		Serial			Delta change at M6		Serial			Delta change at M6	
	Subject	B	M3	M6	Absolute	Relative (%)	B	M3	M6	Absolute	Relative (%)	B	M3	M6	Absolute	Relative (%)
Low speed	**002**	-	-	-	-	-	50	51	54	+5	+9	-19.2	-17.2	-18.0	+1.2	+6
	003	-	-	-	-	-	65	58	66	+1	+2	-9.5	-10.0	-8.6	+0.9	+10
	004	42	-	-	-	-	64	-	-	-	-	-11.2	-	-	-	-
	005	33	28	-	-	-	69	63	-	-	-	-7.3	-4.2	-	-	-
	007	-	-	-	-	-	63	-	-	-	-	-9.7	-	-	-	-

Figure 6.2: Echocardiographic measurements in the SERCA-LVAD trial.

LV ejection fraction at (a) full and (b) low speed. 2D LV end diastolic dimension at (c) full and (d) low speed. LV global circumferential strain at (e) full and (f) low speed. Patients receiving AAV1.SERCA2a shown with continuous lines (subjects 002, 003, 004 and 007). Patient receiving placebo shown with dashed line (subject 005).

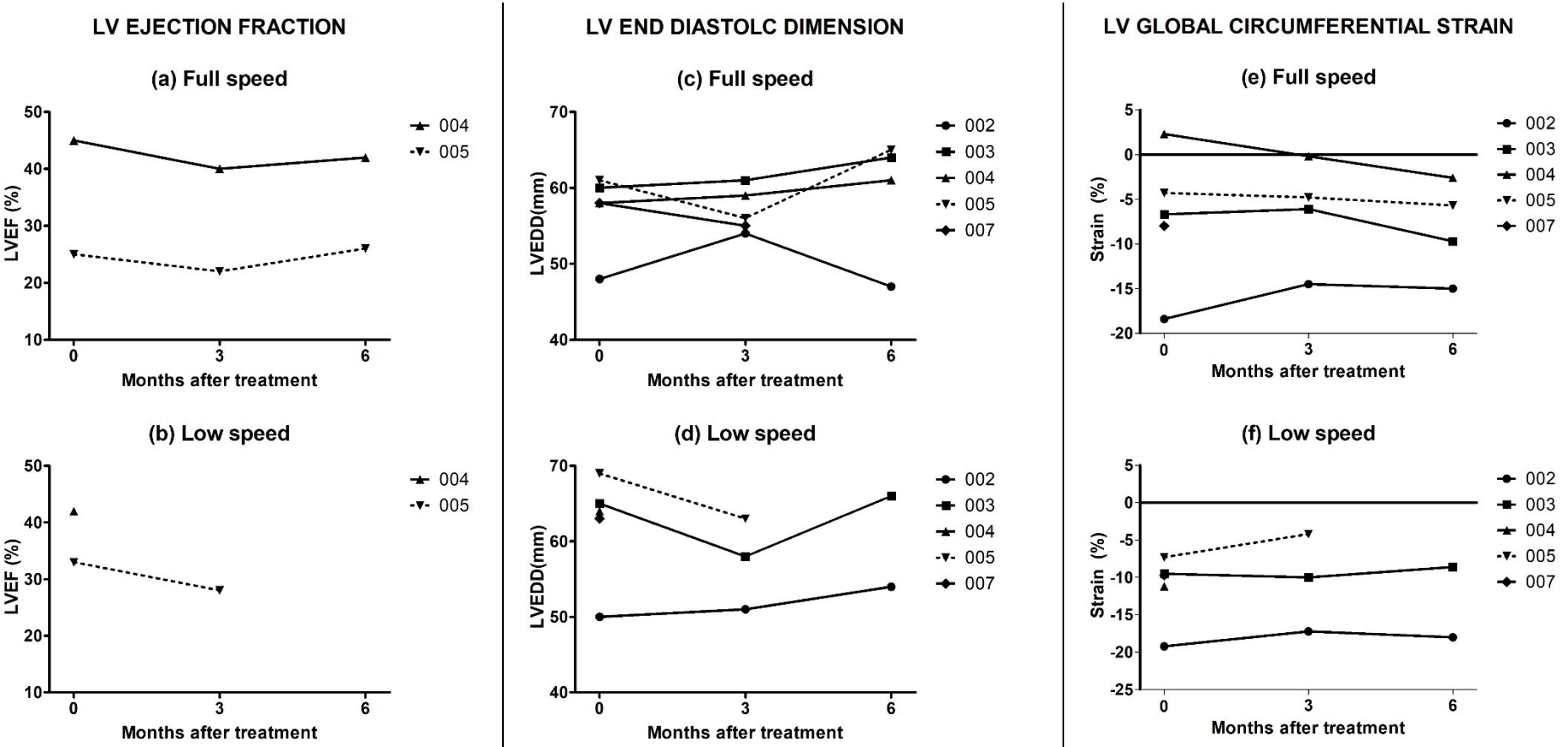
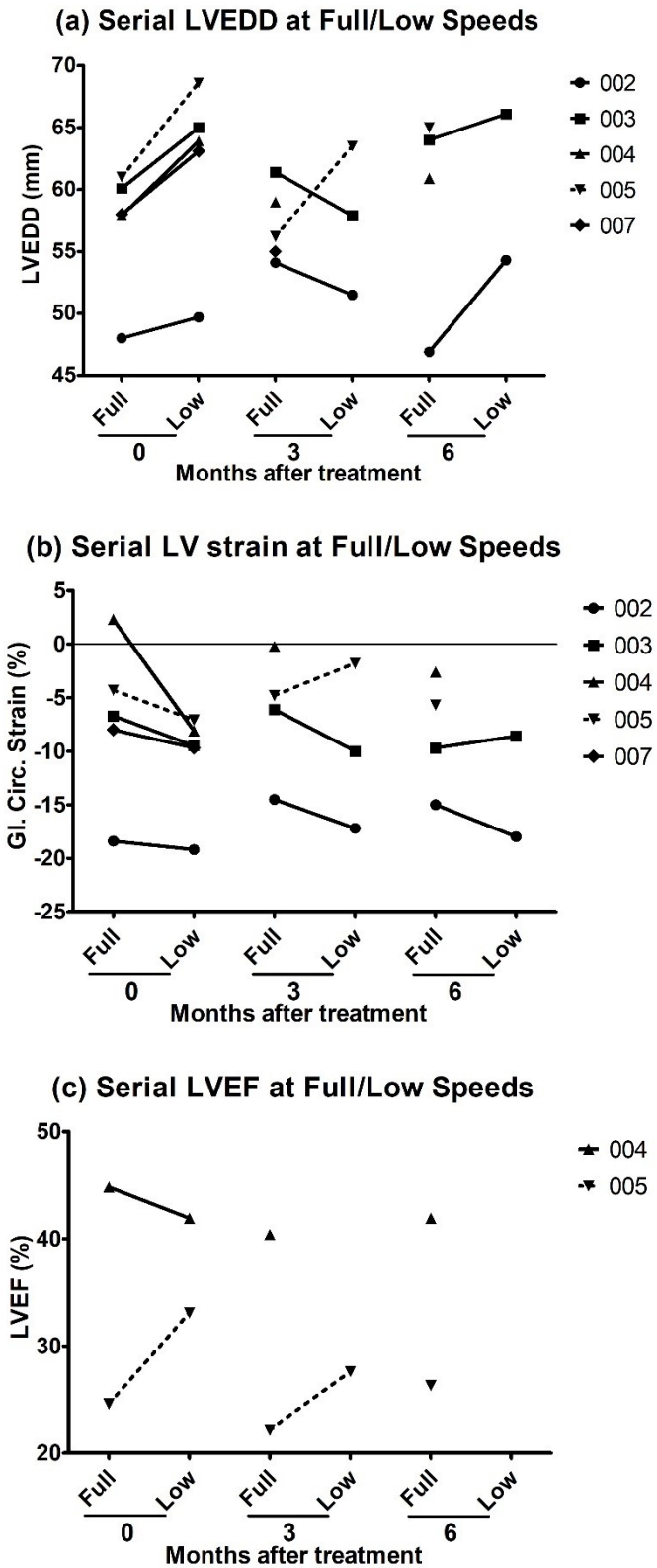


Figure 6.3: Variation in echocardiographic parameters with LVAD speed.

Comparison of parameters at full and low LVAD speeds throughout the trial period. (a) LV end diastolic dimension (LVEDD), (b) LV global circumferential strain (LV strain), and (c) LV ejection fraction (LVEF).



6.3.4 Echocardiography results

Further to the core efficacy analysis, there was further consideration of outcome parameters from echocardiography.

6.3.4.1 Evidence of increased LV loading at low LVAD speeds

Ten of the echocardiograms included a low speed assessment in addition to recording parameters at full speed. After 10 minutes at low LVAD speed, there was increase in LV end diastolic dimension (LVEDD) and increase in global circumferential strain. Considering data from all 10 echo studies regardless of study time point, LVEDD increased significantly from 58mm (53, 61) to 63mm (54, 65; p=0.04), reflecting volume loading of the LV. Similarly, global circumferential strain also increased with a trend towards significance (-6.1% (-12.1, -3.5) to -9.6% (-17.4, -7.9), p=0.07), reflecting increased ventricular performance at higher workloads (Figure 6.3, page 185). LVEF increased with loading in 2 studies in subject 005, while it decreased in subject 004, but small numbers make data interpretation difficult.

6.3.4.2 Strain as a biomarker of native LV function

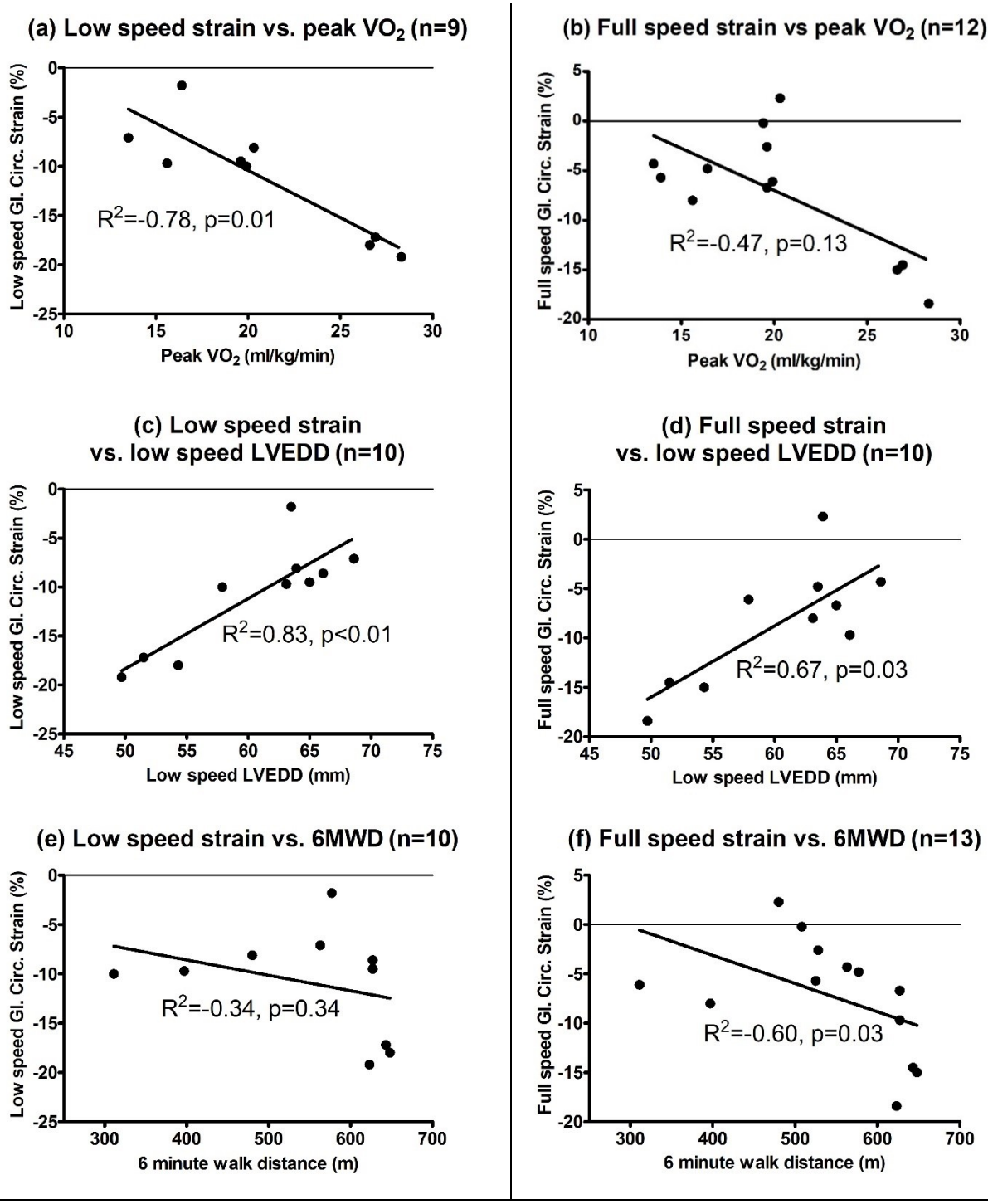
Separate to the main efficacy outcomes, a related study aim was to validate strain imaging as a tool for assessment of underlying function in ventricles supported by LVAD. Based on negative data for the primary and secondary endpoints, this analysis was done assuming no effect from the treatment group allocation. Data were available from 13 studies with strain data, of which 10 included a low speed assessments. PSAX images were analysed in all 13 cases and these were processed for global circumferential strain (section 2.3.2.2, page 61).

At low LVAD speed, global circumferential strain shows strong correlation with peak VO_2 (R=-0.78, p=0.01), and similarly correlated with LVEDD at low speed (R=0.83, p<0.01; Figure 6.4, page 187). Global circumferential strain recorded at full LVAD speed shows weaker correlations with 6 minute walk distance (R=-0.6, p=0.03) and with LVEDD at full speed (R=0.67, p=0.03). There was no relation at full or low speed of global circumferential strain with VE/VCO2 or LVEDD at full speed. The changes in global circumferential strain with different LVAD speeds (Figure 6.3b, page 185) illustrate that strain is a load-dependent

marker of LV function, like LVEF, and will be susceptible to changes in LV loading conditions brought about by the LVAD.

Figure 6.4: Global circumferential strain correlates with key clinical markers.

Correlation using Spearman rank method. (Gl. Circ. Strain, global circumferential strain; peak VO_2, peak oxygen consumption; LVEDD, left ventricular end diastolic dimension; 6MWD, 6 minute walk distance).



6.3.4.3 Strain for predicting response to therapy

The intended analysis would have required splitting the study cohort by clinical response to qualify the biomarker as a prognostic tool. Due to the negative primary and secondary outcome variables, this analysis has not been attempted.

6.4 Discussion

While the SERCA-LVAD trial was designed and powered primarily to study feasibility and safety of the AAV1.SERCA2a gene product in patients with LVADs (as outlined in Chapter 5), it also sought to examine clinical efficacy outcomes within the study cohort. These data have been presented in this Chapter.

We hypothesised that a single intracoronary infusion of the AAV1.SERCA2a gene product at the dose of 1x 10^{13} DNase resistant particles in patients with advanced HF and an LVAD would yield evidence of beneficial reverse remodelling as evidenced by functional, structural and neurohormonal measures. Further, we hypothesised that echocardiographic strain would (1) provide a novel, low risk method to determine and monitor LV function during ongoing LVAD support; and (2) form a tool to predict clinical response to gene therapy. The data presented in this Chapter are insufficient to support or reject these hypotheses, due to small patient numbers and consequent underpowering. Specifically, (1) the efficacy data show no clinically meaningful or consistent changes in any of the primary or secondary endpoint parameters; (2) the echocardiography analysis is limited due to small patient numbers, but global circumferential strain at low speed correlates with peak VO_2 and may provide a non-exercise technique for characterising cardiac functional capacity. Addressing the hypothesis that strain imaging might form a prognostic tool was not possible due to the negative efficacy outcomes.

6.4.1 Clinical efficacy of the AAV1.SERCA2a gene product

The SERCA-LVAD trial marked the first delivery of a gene therapy product to LVAD patients anywhere in the world. The trial was coordinated with the CUPID programme, beginning enrolment after the CUPID-1 trial had shown safety and possible efficacy of

SERCA2a gene therapy in clinical studies in chronic HF [111,116], and during the final stages of the CUPID-2 trial. As explored in Chapter 1, patients receiving the 1x 10^{13} DRP dose in CUPID-1 had shown signs of clinical benefit, and the hope in SERCA-LVAD was that the dual therapy with optimised haemodynamics, alongside the molecular therapy, would yield an additive clinical benefit to LVAD patients.

However, the positive results from CUPID-1 were not replicated in the CUPID-2 trial[123], and once this trial had reported its preliminary conclusions the SERCA-LVAD study (and similarly the AGENT-HF study, another study of AAV1.SERCA2a running in parallel[124]) halted recruitment, and later closed early. Consequently, while there is enough experience to analyse feasibility and safety in general terms, as discussed in Chapter 5, there is little hard clinical outcome data on which to base any clinical efficacy conclusions. The data presented in this Chapter are heterogeneous, in small numbers, and showing no meaningful or consistent patterns. It is likely that they represent random variation in following a small group of LVAD patients over 6 months, though equally there could be genuine clinical difference which the trial is underpowered to detect. For example, there is a possible signal of clinical benefit seen in subject 004 who was NAb negative and received active AAV1.SERCA2a. By 6 months there was a 10% increase in 6 minute walk distance (480m to 528m) which could be clinically significant, or equally could be confounding due to trial participation (Hawthorne effect) or simple random variation. There was small reduction in VE/VCO_2 slope, though no meaningful change in peak VO_2, BNP, or echo parameters at full or low speed.

The detailed findings from the CUPID-2 trial are reviewed in Chapter 1. In the context of these data it would not be surprising, even if we had a full dataset for SERCA-LVAD, for the trial to be negative for clinical efficacy, unless there was an amplified response in more severe disease or the LVAD did serve to facilitate the clinical effect. Moreover, the AGENT-HF study has now reported negative efficacy data, specifically no appreciable improvement in LV volumes or other functional endpoints, in the small numbers recruited[124]. The key question, therefore, is what information the SERCA-LVAD trial can give us to understand why the AAV1.SERCA2a approach is efficacious in animal models, isolated human cardiomyocytes, and possibly in CUPID-1 – but not in CUPID-2, and seemingly not in the

current study. The key factors to consider include (1) the efficiency of SERCA2a transgene transduction into cardiomyocytes, in particular considering the choice of AAV1 as the delivery vector, the dose used, and the mode of administration; and (2) the choice of SERCA2a transgene as a target[167].

There is converging evidence from the CUPID-2 trial, and now from the SERCA-LVAD trial, that the magnitude of transduction to cardiomyocytes in vivo is significantly less than observed in previous animal models and in vitro studies. Tissue analysis in animal studies has the myocardial transgene expression around a hundred-fold greater than in human studies to date (see Table 6.5, page 191). The systematic tissue analysis data from SERCA-LVAD corroborates experience from sporadic collection within the CUPID programme. Patients for whom tissue is available from the CUPID studies were patients who deteriorated and needed LVAD or transplant, so by definition these were patients who did not show clinical response to the gene therapy and perhaps they do not reflect transduction efficiency across the whole cohort. SERCA-LVAD patients were all at similar HF severity at enrolment, though it's clear subject 007 – from whom most of our tissue expression data is available – deteriorated significantly and could fit this category as well. In any case, evident from the 18 patients from whom tissue expression data is now available is that the efficiency of viral transduction was low.

6.4.1.1 Efficiency of transduction: vector choice, dosing and mode of administration

One explanation for lack of efficacy could be that insufficient quantity of SERCA2a transgene is reaching an insufficient number of cardiomyocytes to appreciably alter myocyte calcium cycling and then whole heart function. What remains unclear is whether this is due to inadequate number of viral particles reaching cardiomyocytes, or poor transduction of the transgene by the viral vector; in other words, whether the rAAV1 vector was inefficient delivering transgene, that an inadequate dose of AAV1.SERCA2a was used, or that the mode of administration was suboptimal, or all three.

Table 6.5: Detection of SERCA2a transgene in cardiac tissue in clinical trials.

(IW, inferior wall; LW, lateral wall; AW, anterior wall; AS, anteroseptum; PS, posteroseptum; PW, posterior wall; NSFA, not suitable for analysis; BLD, below limit of detection.)

Clinical Trial	Patient ID	Dose	Months post-infusion	Source	Heart tissue	AAV1/SERCA2a copies DNA per µg of total DNA
CUPID-1	031001	1.4x 10^{11}	8	Tx	AS, PS, AW, PLW	BLD
	011002	3x 10^{12}	1 21	LVAD Tx	LVAC AS, PS, AW, PLW	BLD BLD
	051002	3x 10^{12}	5	Tx	AS, PS, AW, and PLW	BLD
	081006	3x 10^{12}	10	Tx	AS, PS, AW, and PLW	BLD
	151003	3x 10^{12}	11	LVAD	LVAC	BLD
	091006	1x 10^{13}	18	Biopsy	PLW	BLD
	091007	1x 10^{13}	11 23	LVAD Tx	LVAC AS PS AW, PLW LVAC RVAC	>20 to <200 561 365 >20 to <200 230 250
	011005	1x 10^{13}	31	Unspec	LVAC	>20 to <200
	011010	1x 10^{13}	22	Tx	PLW AS, PS, AW	223 >20 to <200
CUPID-2	021020	1x 10^{13}	12	LVAD	LVAC	14-62
	161039	1x 10^{13}	13	Tx	AS PS AW PLW	80 71 36 77
	501024	1x 10^{13}	1.5	Tx	AS PS AW PLW LVAC RVAC	26 37 40 134 <10 <10
	231028	1x 10^{13}	8	Tx	AW Heart	115 72-123
	251008	1x 10^{13}	14	Tx	AS PS AW PLW	27 84 62 102
	501002	1x 10^{13}	10	Tx	AS PS AW PLW LVAC	43 33 53 37 36
	021009	1x 10^{13}	29	Tx	Heart	192
SERCA-LVAD	003	1x 10^{13}	6	Biopsy	IW, LW	NSFA
	004	1x 10^{13}	6 22	Biopsy Tx	IW(a) IW(b), LW AW, AS, PS, PW	38 BLD BLD
	007	1x 10^{13}	6	Tx	AW AS PS PW	41 80 57 23

The rAAV1 was adopted as vector for strong reasons outlined earlier, with the main disadvantage being the high occurrence of NAbs in the population[106]. Certainly, data from the CUPID and SERCA-LVAD studies to date have confirmed its safety, and its low immunogenicity reflected by the absence of inflammatory sequelae seen with other vectors such as adenovirus[105,116,122]. However, it remains possible that the AAV1's ability to transduce human cardiomyocytes is reduced in vivo. Further serotypes of AAV have been discovered or engineered since the CUPID programme initiated, and in particular the AAV9 serotype may show greater efficiency of transduction and greater tropism for cardiac muscle allowing larger doses to be used more efficienctly[168]. A new generation of so called bioengineered nanoparticles (BNPs) may come to fruition, allowing further cardiac specificity and conferring other advantages such as enlarged genome size and antigenic profile nontypical for AAV (helping to avoid cross-reactive NAbs)[169]. The dose used in SERCA-LVAD and in CUPID-2 was the highest of the doses tested in CUPID-1 ($1x10^{13}$ DNase-resistant particles)[119], and is equivalent or exceeding the dose used in other AAV studies[115], but in the absence of safety concerns at this dose there could be rationale to increase this (indeed there was a study planned testing $2.5x10^{13}$ DNase-resistant particles which has now terminated; see section 1.5.3.4, page 47).

It may be that the immunological effects have been underestimated. The CUPID-2 study is said to have only recruited patients "negative" for NAb, but in fact this was defined as patients with titres <1:2. The study could have included patients with previous exposure, subsets of AAV specific memory T cells, and low levels of circulating NAb, and perhaps in these patients there was immune-mediated destruction of large numbers of viral capsids prior to transduction to cardiomyocytes. Indeed the exact composition of the infusion solution has also come under scrutiny, after the observation that the solution used in CUPID-1 differed from that in CUPID-2 and SERCA-LVAD with regard to presence of empty, inactive capsids[122]. While both preparations contained $1x10^{13}$ DRP, the total capsid load was higher in CUPID-1 then subsequent studies ($7.7x10^{13}$ versus $2.3x10^{13}$). There is new evidence that co-administering empty capsids with those containing transgene improves efficiency of transduction in a dose-response manner, perhaps by absorbing the effect of NAbs[170]. In CUPID-1 the empty capsids were 85% of the total infused, but in SERCA-LVAD/CUPID-2 this figure was 25%, so if the effect Mingozzi and colleagues identify is relevant, this could

pose a significant variation, perhaps accounting for the lesser efficacy seen in CUPID-2 compared to patients in CUPID-1.

The mode of vector delivery should also be reviewed. The SERCA-LVAD trial used a single anterograde intracoronary infusion, but there are different modes of delivery have their advantages and disadvantages[104]. With a rAAV, the possible routes could include: (a) peripheral intravenous infusion, beneficial for the patient and relying on the rAAV's cell tropism to select cardiomyocytes, but suffering from the dilutional effect of the large circulating volume; (b) infusion into the coronary arteries, exposing cardiomyocytes to a higher concentration of viral particles on first pass and reducing infection of other organs; (c) retrograde infusion via the coronary sinus, which may allow controlled dwell times and prolonged exposure, and has been tested with good result in a large animal model[171]; (d) closed loop recirculation, with the vector infused into the coronary artery and then drawn from the coronary sinus back to the coronary artery catheter via a cardiopulmonary bypass system for oxygenation, thereby increasing exposure time[172]; (e) direct intramyocardial injection (such as was used in the recent STOP-HF trial[173]) which can yield sustained transgene expression but may be only in a limited cell number around the injection site; or (f) injection to the pericardial space. Efficacy of transduction by different routes was examined in animal studies, and while the anterograde coronary route showed initial modest results, subsequent studies with modifications (e.g. greater virus concentration, adjustments to catheter delivery systems, increased virus exposure time) yielded improvements and detailed toxicity and dosing assessments[117]. Further efforts have included anterograde infusion with venous occlusion, ischaemic preconditioning aiming to increase vascular permeability, and concurrent GTN infusion aiming to prevent coronary vasospasm and achieve coronary vasodilation[120], though only the latter was adopted in the CUPID and associated studies, and it's unclear what benefit this has in LVAD patients with unique systemic and coronary flow patterns.

In practice, there are multiple interactive factors related to the vector, the method and route of delivery at optimal dose, and its interaction with the host. Part of the problem going forward is that each clinical trial has varied several factors at the same time, making it difficult to understand which combination of approaches is best.

6.4.1.2 Is SERCA2a the correct target?

The SERCA2a enzyme is the final common pathway of a complex molecular network, and its activity is subject to control by other factors[95-98]. While data from animal studies and ex vivo human cardiomyocytes supports direct transduction of human SERCA2a gene into cardiomyocytes as a therapeutic approach[99,101,102], it's possible that, if the cellular milieu is already downregulating SERCA2a activity, the exogenous SERCA2a may be subject to the same processes. This could explain the lack of effect seen. Indeed, the dosing could be important here: perhaps supraphysiological levels of SERCA2a mRNA are required to overwhelm the cardiomyocyte's regulatory mechanisms and allow the change in ATPase activity to come apparent.

Phospholamban (PLN) is one such SERCA2a modulator. PLN complexes with SERCA2a and inhibits its activity, most potently when in its dephosphorylated form. In turn PLN phosphorylation state is determined by the balance of kinase activity (particularly protein kinase A, whose activity is upregulated via β-adrenergic activity) and phosphatase activity (particularly protein phosphatase-1 [PPI-1], whose activity is downregulated by the modulator molecule inhibitor-1c (I-1c)). In HF, the SERCA2a:PLN ratio is reduced with relatively higher preponderance of dephosphorylated PLN, I-1c activity is decreased, and β-receptor signalling is desensitised. Conversely, signs of therapeutic benefit have been seen in studies that block PLN with RNA interference a rat HF model[174], increase I-1c activity using AAV9- or BNP-mediated I-1c gene delivery in a pig HF model[169], deliver the G-protein inhibitory peptide βARKct via AAV6 in a pig HF model[175], and cause overexpression of adenyl cyclase-6 in HF mice[176]. Some of these approaches are already in clinical programme development. Notably, development of direct PLN inhibitors for clinical use has been slowed by observation of a severe HF phenotype in humans with a sporadic loss of function mutation in PLN[177].

Separately the molecules SUMO-1 (small ubiquitin-like modifier-1) and S100A1 have roles regulating SERCA2a activity. SUMOylation by SUMO-1 at specific and conserved sites causes post-transcriptional modification of the SERCA2a enzyme to increase its activity and stability. Levels of SUMOylated SERCA2a are reduced in HF, and SUMO-1 gene delivery

using AAV9 restores function in a pressure overload model in mice[97]. The small calcium-binding protein S100A1 is down-regulated in HF and regulates activity of SERCA2a as well as RyRs in the SR membrane. S100A1 gene transfer using an AAV6 vector has shown signs of efficacy in small and large animal models[171].

All these factors – and many others – could have impinged on the success of direct SERCA2a gene transfer in the SERCA-LVAD and CUPID-2 studies, and equally could form targets for indirect modulation of SERCA2a activity. But none can be taken completely separately from the next: they form a complex network of interactions, and will all be vulnerable to reciprocal regulation that could blunt clinical efficacy. However, for the time being SERCA2a remains an attractive target for clinical gene therapy for HF.

6.4.2 Measuring myocardial responses in LVAD patients

One of the challenges of clinical intervention studies in LVAD patients is the lack of effective tools for monitoring heart function separately to function of the heart-LVAD continuum, a physiological relation that is constantly changing but depends principally on preload, afterload and pump speed. In this Chapter, we hypothesised that strain imaging could provide an alternative method allowing non-invasive assessment of LV function. To date strain has been only sporadically studied in LVAD patients during low speed studies.

The small numbers of patients recruited, and the lack of clinical efficacy to split the cohort as an outcome variable, has prevented any rigorous testing of this hypothesis. The data available suggest that global circumferential strain correlates strongly with peak VO_2 on CPEX, and as such could form an imaging based biomarker for cardiac functional capacity. This could be favourable in terms of reproducibility, eliminating variation day-to-day that is seem with exercise testing. However, global circumferential strain is seen to vary with loading status, mitigating against the hypothesis that it is a load-independent parameter. Ultimately no definitive conclusions are possible due to small sample size and underpowering.

Future work will be needed to elucidate this hypothesis further. As demonstrated, relying on interventional studies requires the intervention to show benefit for a predictive biomarker to be elucidated successfully. A preferable approach to pilot strain as a prognostic marker might be a retrospective study of an existing cohort, identifying clinical endpoints such as worsening HF, requirement for urgent transplant listing, or death and studying strain parameters at baseline, though this would carry the problems of retrospective association studies and the echocardiographic images may not be suitable for strain analysis. Feasibility of the technique is discussed in Chapter 5 (see section 5.4.6, page 174), but of note here is whether echo is a useful research tool in LVAD studies at all, due to the frequently poor echo windows and high proportion of missing data. If precise quantification of LV volumes is the priority then it might be that 3D echocardiography, or contrast enhanced cardiac CT, is preferable. The latter was the primary outcome variable in AGENT-HF and the authors of that study have not highlighted problems with CT artefact as a significant concern[124]. However, if imaging modalities are poorly equipped to assess underlying LV function in LVAD patients, then perhaps molecular biomarkers, including novel circulating microRNAs, might be a preferable approach. This is discussed in Chapter 7.

6.5 Conclusions

Despite being feasible and safe as discussed in Chapter 5, the SERCA-LVAD trial does not provide evidence that intracoronary delivery of AAV1.SERCA2a gene therapy to patients with advanced HF and an LVAD provides any clinical benefit. However, the study is underpowered owing to early cessation of recruitment, and definitive conclusions are not possible based on the current data. Nonetheless neutral findings do concur with allied clinical trials including the CUPID-2 trial, and SERCA-LVAD provides further evidence that this might be related to poor efficiency of viral transduction to cardiomyocytes. Regarding strain echocardiography, this warrants further investigation as a tool for assessment of myocardial function during LVAD support, but again study power is limited. Alternative biomarkers of underlying LV function might include novel circulating microRNA, discussed in Chapter 7.

7: Circulating microRNA as a novel biomarker in heart failure

7.1 Introduction

7.1.1 Background and rationale

This was set out in Chapter 1 (section 1.6, page 49).

7.1.2 Hypotheses

We hypothesised that (1) the circulating miRome shows plasticity in patients with LVADs, and this plasticity can be quantified as a novel biomarker; (2) individual miRs or patterns within the miRome can act as novel biomarkers to monitor native LV function in patients with LVADs, and predict which patients respond to LVAD therapy; (3) plasticity in the circulating miRome reflects plasticity of expression within the ventricular myocardium.

7.1.3 Previous publication

Parts of the work in this chapter have been published[178] as Morley-Smith AC, et al. Circulating microRNAs for predicting and monitoring response to mechanical circulatory support from a left ventricular assist device. Eur J Heart Fail. 2014 Aug;16(8):871-9.

7.2 Methods

7.2.1 Study design

The study was conceived with three stages using paired plasma and tissue samples from LVAD patients. Firstly, I used a non-biased screening microarray to identify and validate candidate miRs which showed plasticity with LVAD therapy. Secondly, I quantified these candidate miRs in plasma samples from a cohort of LVAD patients collected serially during

LVAD support. Finally, I analysed the same candidate miRs in paired samples from ventricular myocardium.

The miR expression data was combined with available clinical data. The parameter for which there was most systematic data available was NT-proBNP, and change in NT-proBNP was adopted as primary outcome for clinical response. There was greater occurrence of missing data points for other heart function and HF prognostic markers, such as peak VO_2 or LVEF. If reported consistently these might form a more robust endpoint parameter, but the absence of consistent reporting favoured using NT-proBNP.

Human ventricular myocardial and plasma samples were obtained from patients who underwent implantation of a Thoratec HeartMate II LVAD at University Hospitals Leuven (UHL), Belgium between November 2009 and September 2011. All samples were collected with informed consent and ethical approval as part of a previous research project (Study number S-52-659; Ethics approval number ML-6832).

Plasma samples were collected prior to LVAD implantation and at 3 month intervals after LVAD support until time of cardiac transplantation. Myocardial samples were collected at the time of LVAD implantation and subsequent cardiac transplantation. Samples were obtained and processed as set out in section 2.1, page 54. Clinical data were obtained contemporaneously with sample collection. Assays of NT-proBNP and estimation of LVEF were performed respectively by the clinical and core echocardiography laboratories at UHL.

7.2.2 Laboratory work and governance

I performed all bench work at the Institute for Molecular and Translational Therapeutic Strategies in Hannover Medical School, Hannover, Germany under supervision by Professor Thomas Thum. RNA benchwork is challenging principally due to instability of the molecule and problems with environmental RNA contaminants, despite miRs' greater stability compared to other forms of RNA (for example, miRs can resist temperature or mechanical degradation). All experimental work was done in Professor Thum's RNA laboratory with

attention to sterile technique and equipment, and use of RNAse solutions. Professor Thum is an expert in the field and it was invaluable having his expertise and collaboration.

Transfer and use of clinical specimens and confidential clinical data from UHL was done under a Material Transfer Agreement agreed by UHL and RBHT dated January 2014.

7.2.3 General approach to miR quantification

The steps in miR quantification are: (1) isolation of total RNA from sample of interest, and total RNA quantification by ultraviolet spectroscopy where indicated; (2) a series of reverse transcription (RT) reaction to transform RNA into copy DNA (cDNA), which can be done using RT promoters to include all miRs (poly(A) approach using SYBR-Green reagents) or specific miRs (stem-loop approach using TaqMan reagents); and (3) a series of real-time quantitative polymerase chain reaction (RT-qPCR) experiments to quantify the amount of specific miRs present. These steps can be applied to the screening microarray and to the validation steps of my methods.

7.2.3.1 Quantitative polymerase chain reaction techniques

MiRs are identified and quantified using RT-qPCR experiments. PCR uses the DNA polymerase enzyme and sequential heat activation cycles to double the product yield at each step eventually yielding exponential growth until the PCR reagents are consumed. RT-qPCR couples the PCR reaction with a detectable marker that changes according to the quantity of product yield. The number of cycles taken to reach exponential expansion is described as the threshold (Ct), and this provides quantification, either actual (in comparison to a control with a defined amount of input DNA) or relative (in comparison to expression across the sample cohort). Change in expression is expressed as 'fold change', calculated as $2^{(Ct_a-Ct_b)}$.

There are two general methods for RT-qPCR, and both were used here in different sections.

- Firstly, the poly(A) method, using SYBR Green reagents, was used for the RT-qPCR microarray step. In this approach, there is polyadenylation of the 3' terminal of all mature

miRs present in the sample, and this poly(A) tail forms the target for a non-specific RT step using a universal oligo-d(T) RT primer. The result is RT to cDNA of all miRs present in the sample yielding a heterogeneous cDNA sample from which specific miR types are identified using miR-specific primers at the PCR stage. The quantification uses SYBR green dye which fluoresces when bound to double stranded DNA (dsDNA). The main advantage of this approach is the ability to run multiple RT-qPCR experiments from one RT step, with consequent preservation of clinical samples and cost saving. The disadvantages are related to poor specificity, resulting from the universal RT step, possibility of cross-reactivity between primers for different miRs with conserved sequences, and SYBR fluorescence to any dsDNA rather than primer-based recognition.

- Secondly, the stem-loop primer method, using TaqMan reagents, was used for the validation and cohort quantification steps. This approach uses an miR-specific stem loop primer that binds to the 3' terminal of the miR and primes the RT reaction, yielding only cDNA for specific miRs of interest. An miR-specific TaqMan probe is included in the RT-qPCR reaction, configured such that whilst intact the fluorescence from a reporter dye on the 3' end is quenched by a quencher on the 5' end. The probe is cleaved on each extension cycle, unquenching the reporter fluorescence relative to the quantity of miR-specific DNA manufactured. The key advantage of TaqMan methodology is the enhanced specificity for individual miRs in comparison to the poly(A) method. The key disadvantages are higher cost, more time consuming and higher use of clinical samples (though this latter point can be offset by running TaqMan RT reactions in parallel with several primers).

7.2.3.2 Data normalisation

The process of normalisation aims to control for variations in experimental parameters between runs, for example controlling for efficiency of RNA purification and controlling for run-to-run variability in the qPCR. In tissue RNA analysis, the standard is to use a 'reporter' as an internal control – an RNA known to be expressed in that tissue, that can be assumed to be present in the total RNA sample, and thereafter reverse transcribed and amplified in the qPCR reaction. In analysis data are then compared to the expression data for the internal control.

However, in studies of circulating miRs the RNA expression levels are very low and there are no reliable internal controls. Various methods have been proposed to circumvent this problem[179,180]. The current standard is use of a spike-in RNA, typically *Caenorhabditis elegans* miR-39 (cel-miR-39). This exogenous RNA is added at the point in RNA extraction after lysis and before RNA purification, and thus it can control for variations in RNA purification and in variations in qPCR reactions. This process is known as 'relative normalisation'. When spiked-in a known quantity of RNA it can be possible to precisely calculate the quantity of target miR present in the sample. An alternative approach is 'global normalisation', where the mean Ct expression of all miRs studied in a single sample is taken and used as normalising reference[179]. Both techniques were used in the work presented here.

7.2.4 Total RNA isolation

Total RNA isolation was performed using the Qiagen miReasy Mini Kit and 96 Kit (Qiagen, Venlo, Netherlands) with QIAzol Lysis Reagent used for cell lysis. This uses a silica membrane for RNA purification, with RNA binding to the membrane, and during a series of wash and centrifugation steps the lysis reagents and contaminants are washed away before RNA is eluted in a small volume of sterile water.

For plasma samples a fixed volume (200µl) was used and the homogenate spiked with RNA from *C.elegans-39* (5µl of 1 fmol/µl RNA) as external control. For myocardial tissue samples the concentration of total RNA in the final eluate was determined using ultraviolet spectroscopy, and samples prepared each containing a fixed quantity of total RNA. RNU48 was used as endogenous control for tissue samples.

7.2.5 Candidate identification by microarray

Microarray profiling is a robust method for an unbiased screen of candidate miRs showing plasticity between two groups of samples. Samples A and B are selected between which it is hypothesised there will be a difference in expression. A large panel of targets can be analysed by running multiple experiments in parallel in A and B, with comparison of target expression

by relative quantification to identify key differences. Here, a RT-qPCR microarray was used, based on poly(A)/SYBR Green methodology.

RNA was pooled into 4 pairs of samples for miR expression profiling. At the screening stage this facilitates inclusion of samples from a larger pool of patients without creating prohibitively high cost or time requirements, though suffers from a loss of individual-patient comparison and some loss of specificity. cDNA was prepared for real time-quantitative polymerase chain reaction (RT-qPCR) from these pooled samples using QuantiMir (BioCat GmbH, Heidelberg, Germany). The QuantiMir kit uses a three stage RT reaction (Table 7.1).

Table 7.1: Reactions in the QuantiMir Microarray Kit.

			µl
Step 1 Poly(A) tail	Combine:	Total RNA	5
		5X PolyA Buffer	2
		25mM MnCl2	1
		5mM ATP	1.5
		PolyA Polymerase	0.5
		Total	10
		↓	
		Incubate for 30mins at 37ºC	
		↓	
Step 2 Anneal anchor dT adapter	Add:	Oligo dT Adapter	0.5
		↓	
		Heat for 5mins at 60ºC & cool 2mins	
		↓	
Step 3 Synthesis of cDNA	Add:	5X RT Buffer	4
		dNTP mix	2
		0.1M DTT	1.5
		Nuclease free H20	1.5
		Reverse transcriptase	1
		Total	10
		↓	
		Incubate for 60mins at 42ºC	
		Heat for 10mins at 95ºC	

cDNA samples were stored chilled at -20ºC until ready for RT-qPCR microarray analysis. This was performed with the Biocat Human Genome Wide microRNA 384-well RT-qPCR Array (BioCat GmbH, Heidelberg, Germany) using the BioRad CFX384 Touch RT PCR Detection System (BioRad, Hemel Hempstead, UK). This array uses SYBR Green reagents and detects 1,113 known miRs identified in the Sanger miRBase Version 15. The plates were

customised to include primers for *C.elegans-39* as the exogenous control, and primers were resuspended with 20µl sterile water before use.

This microarray kit has 3 plates of 384 wells to accommodate the 1,113 miRs, so for 4 pooled pairs this required 24 RT-qPCR runs to complete the microarray. For each run, a Mastermix (with quantities customised from the product literature to allow for loss from robotic pipette) was prepared and 5µl aliquoted to each well. The Mastermix preparation and the RT-qPCR protocol for the microarray are shown in Table 7.2. For all RT-qPCR runs the threshold for detection was set by personnel blinded to the study groups.

Table 7.2: Mastermix and RT-qPCR Protocol for the QuantiMir microarray.

(a) Preparation of the Mastermix. (b) Protocol for the RT-qPCR.

(a)

		µl
For entire microarray plate	2X SYBR Green qPCR Mastermix	1375
	Universal Reverse Primer	55
	Prepared cDNA	5.5
	Nuclease-free water	855.8
	Total	2291.3

(b)

1	50°C 2 min	
2	95°C 10 min	
3	95°C 15 sec	50 Cycles of Step 3-4
4	60°C 1 min	
5	melting curve	
6	15°C hold	

(data read at Step 4)

7.2.6 Selection of candidate miRs

Data from the microarray was analysed in steps. Firstly, the data was normalised by relative (to *C.elegans-39*) and global normalisation methods to ensure control across the 24 RT-qPCR runs (see earlier section). Secondly, miRs were excluded unless they showed robust expression in all the test samples. Any miRs not expressed in one of the test samples were excluded automatically. Thirdly, the fold change differences were calculated for each remaining miR within the microarray, to identify miRs which showed significant plasticity between baseline and the 6-month timepoint. Fourthly, the differences were tested

statistically to assist prioritisation using paired t-tests. Finally, all the data were reviewed to identify candidates to take forward for validation.

7.2.7 Validation of candidate miRs using TaqMan

After identification of candidate miRs these data were validated using the more specific TaqMan method. cDNA transcripts were prepared using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, California, USA), and real-time qPCR was done using the ViiA 7 Real-Time PCR System (Applied Biosystems, California, USA) using Absolute Blue reagents. All TaqMan reactions were performed in duplicate. For the validation experiment, the same 4 pairs of RNA pools were used. For the validation RT reactions, a Mastermix was prepared (Table 7.3a). Each reaction was prepared with 5µl of Mastermix with 2.5µl RNA. Two TaqMan primers were included in each Mastermix preparation to generate cDNA for 2 miRs in parallel. The protocol for RT is shown in Table 7.3b. cDNA samples were diluted and stored chilled at -20ºC until ready for RT-qPCR.

RT-qPCR was performed for each of the candidate miRs. Because each cDNA sample contained 2 specific cDNA types, each cDNA was included separately in 2 separate RT-qPCR reactions. The Mastermix for RT-qPCR was prepared (see Table 7.3c), and 8µl of Mastermix was pipetted to each well and mixed with 2µl cDNA template solution and the RT-qPCR experiment run as per Table 7.3d. For all experiments, the threshold for detection was set by personnel blinded to the study groups.

Table 7.3: Mastermix and Heating Protocols for the TaqMan reverse transcription and real-time quantitative polymerase chain reaction experiments.

(a) Preparation of the Mastermix and (b) heating protocol for reverse transcription. (c) Preparation of the Mastermix and (d) heating protocol for RT-qPCR

(a)	µl
Nuclease free water	0.58
10x RT buffer	0.75
5x miRNA specific RT primer **A**	1.5
5x miRNA specific RT primer **B**	1.5
Multiscribe RT enzyme (50 U/µl)	0.5
dNTP mix (100 mM total)	0.075
RNase inhibitor (20 U/µl)	0.095
Total volume	**5**

(b)		
1	16°C	30 min
2	42°C	30 min
3	85°C	5 min
4	8°C	hold

(c)	µl
Absolute Blue qPCR Mix 2x	5
ROX Reference Dye (diluted 1:10)	0.05
Nuclease free water	2.45
Specific TaqMan probe	0.5
Total volume	**8**

(d)			
1	95°C	15 min	
2	95°C	15 sec	50 Cycles of Step 3-4
3	60°C	1 min	
4	15°C	hold	
	(data read at Step 3)		

7.2.8 Quantification of candidate expression

After validation of candidate miRs the next stage was to quantify their expression in all samples within the cohort. Fresh extracts of total RNA were prepared from the clinical samples (plasma and myocardial tissue), and TaqMan RT and RT-qPCR were performed using methods as above on all samples. For all runs the threshold for detection was set by personnel blinded to the study groups.

7.2.9 Statistical analysis

The primary outcome was change in miR expression at 6 months. For assessment of predictive biomarkers, we classified the clinical response to LVAD therapy based on their change in NT-proBNP at 3 months, and divided the cohort at the 50th centile for a binary outcome. Patients whose change in NT-proBNP was above the 50th percentile (i.e. smaller change) were identified as poor responders and patients whose change in NT-proBNP was below the 50th centile (i.e. larger change) were identified as good responders. Descriptive statistics are shown as median (interquartile range). Final data showed non-normal distribution and non-parametric tests were used for outcome analysis. I performed all data analysis using IBM SPSS Statistics (Versions 21, 22 and 23) with log-transformed data. Results with $p<0.05$ were considered statistically significant.

7.3 Results

7.3.1 Patient characteristics and clinical response to LVAD therapy

There were 53 serial plasma and 20 ventricular myocardial samples from 19 patients with severe advanced heart failure who underwent LVAD implantation. Patients' baseline characteristics are summarised in Table 7.4 (page 207) and reflect the advanced nature of their disease, with 80% in INTERMACS profiles 1-3, NT-proBNP concentration 4724ng/L (1526-20890) and LVEF 15% (15-25). These values are similar to parameters reported in previous clinical trials, confirming this sample is a representative LVAD population[32,36]. All patients were implanted with a Thoratec HeartMate II LVAD at baseline. NT-proBNP levels show inverse correlation with duration of LVAD support (r=-0.508, p=0.0001), with NT-proBNP concentration falling to 924ng/L (653-2792) and 674ng/L (524-1421) by 3- and 6-months respectively. During the study period, 15 patients (79%) underwent cardiac transplantation after 200 days (133-299), and at the time of transplantation NT-proBNP was 1116ng/L (828-2130).

7.3.2 Identification of candidate biomarkers by microarray

To identify candidate miR biomarkers, we studied a screening cohort for whom there were paired plasma samples from baseline and after 6 months LVAD support (n=8). After total RNA isolation and cDNA synthesis, the RT-qPCR microarray compared differential expression of 1,113 plasma miRs in 4 pairs of pooled samples (see section 7.2.5, page 201). In one of the sample pools, there was failure of the *C. elegans* spike-in to amplify, alongside high rate of amplification failure for other target miRs, probably related to the presence of heparin or PCR inhibitors. These data were excluded from the analysis, making a final n=6 (baseline n=3 pools, 6 months n=3 pools) in the screening cohort.

One hundred and thirteen miRs met the criteria for consideration, of which 12 miRs showed statistically significant variation. These are shown in Table 7.5 (page 208). Expression fold change data was normalised using relative and global normalisation methods, with comparable results.

Table 7.4: Patient characteristics at the time of LVAD implantation.

Data is shown for the whole cohort and for the good- and poor-responder subgroups. These subgroups are defined in section 7.3.5, page 213. The p-value refers to comparison of the subgroups. IQR, inter-quartile range. IV, intravenous. (ACE, angiotensin converting enzyme. ARB, angiotensin receptor blocker. MRA, mineralocorticoid receptor antagonist. INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support. NT-proBNP, N-terminal pro-hormone of brain natriuretic peptide. LVAD, left ventricular assist device.)

	Whole cohort (n=19)	**Good responders (n=7)**	**Poor responders (n=6)**	**p-value**
Gender, n (%)				
Female	4 (21)	2 (29)	1 (17)	0.612
Male	15 (79)	5 (71)	5 (83)	
Age in years, median (IQR)	52 (30-61)	40 (14-62)	55 (36-60)	0.731
Aetiology, n (%)				
Ischaemic	10 (53)	2 (29)	3 (50)	0.429
Non-ischaemic	9 (47)	5 (71)	3 (50)	
Body mass index (kg/m^2), median (IQR)	23.4 (22.5-26.6)	22.1 (20.9-25.4)	23.3 (22.8-27.8)	0.181
Current or previous smoker, n (%)	7 (37)	1 (14)	4 (67)	0.053
Diabetes mellitus, n (%)	1 (5)	1 (14)	0 (0)	0.335
Chronic renal failure, n (%)	0 (0)	0 (0)	0 (0)	-
Medications, n (%)				
IV inotrope	10 (53)	4 (57)	3 (50)	0.797
ACE inhibitor	12 (63)	5 (71)	4 (67)	0.853
ARB	1 (5)	0 (0)	1 (17)	0.261
β-blocker	11 (58)	6 (86)	3 (50)	0.164
MRA	6 (32)	0 (0)	4 (67)	0.009
Digoxin	3 (16)	3 (43)	0 (0)	0.067
Diuretic	14 (74)	5 (71)	5 (83)	0.612
Platelet inhibitor	6 (32)	2 (29)	1 (17)	0.612
Anticoagulant	0 (0)	0 (0)	0 (0)	-
INTERMACS profiles 1-3, n (%)	15 (80)	6 (86)	4 (67)	0.416
NT-proBNP (ng/l), median (IQR)	4724 (1526-20890)	17499 (4724-30212)	1443 (1306-3858)	0.008
Change in NT-proBNP after 3 months LVAD support	0.23 (0.07-0.62)	0.08 (0.04-0.13)	0.62 (0.54-1.14)	0.001
Left ventricular ejection fraction (%), median (IQR)	15 (15-25)	20 (10-30)	15 (13-23)	0.662

Table 7.5: Microarray data based on pooled RNA samples from 6 patients, showing fold change in miR expression between baseline and 6 months.

P-values refer to paired t-tests on absolute Ct values from baseline vs. 6 months, normalised relative to cel-miR-39 expression. Note on nomenclature: ΔCt indicates fold change (Δ) in cycle threshold (Ct), and ΔΔCt refers to ΔCt normalised by the method specified.

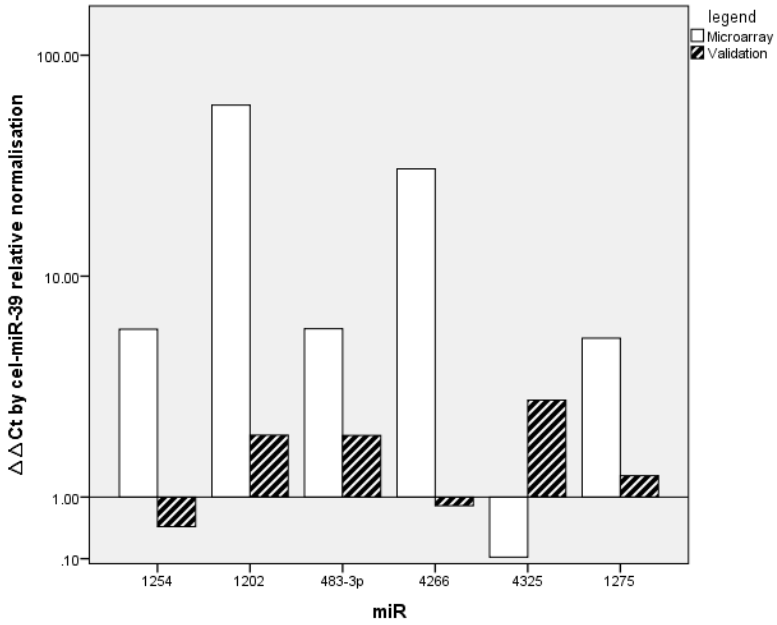
miR	ΔΔCt by cel-miR-39 relative normalisation	ΔΔCt by global normalisation	p-value
1254	5.75	5.18	<0.01
33a	118.25	117.50	<0.01
219-1-3p	4.15	4.14	<0.01
548l	0.16	0.12	0.01
1250	11.12	11.68	0.02
483-3p	5.78	4.46	0.03
4266	30.57	10.25	0.03
4325	0.13	0.05	0.03
938	121.43	93.75	0.03
1202	59.50	92.39	0.03
557	20.00	12.35	0.05
1275	5.24	6.05	0.05

7.3.3 Validation of candidate miRs using TaqMan

For greater specificity, these findings were validated using TaqMan RT-qPCR in the individual samples used during the screening microarray (n=14; see Figure 7.1, page 209). miR-1202 and miR-483-3p were detected robustly (mean Ct values across all samples were 19.365±1.090 and 31.497±1.342 cycles respectively) and showed change in expression consistent with the microarray but with a lesser amplitude (fold change 1.91, p=0.10; and fold change 1.90, p=0.11 respectively). These lesser degrees of statistical confidence were accepted for candidate selection at this stage, given the limitations of the small sample size. The remaining miRs were false positives, showing either robust expression but no significant difference (miRs-4266, -4325 and -1275), difference which was discordant to microarray findings (miR-1254), or unreliable expression data (e.g. Ct > 35 cycles, high standard deviation between duplicates; miRs-1250, -557, -938, -219-1-3p, -33a and -548L). Thus, the screening yielded miR-1202 and miR-483-3p as candidate miR biomarkers that show significant change during 6 months of LVAD support, and these were taken forward for further assessment.

Figure 7.1: Fold changes of 6 microRNAs with reliable expression in validation.

Validation of microarray data using the more specific TaqMan primers. Results are displayed as fold change from baseline to 6 months of LVAD support, using PCR data normalised using spike-in C.elegans-39. Data from the microarray are shown in white bars, with corresponding validation findings in hatched bars.



7.3.4 After LVAD implantation, miR-483-3p is upregulated in plasma and in ventricular myocardium

Having identified two candidate biomarkers, we characterised how their expression varied after LVAD implantation in plasma and ventricular myocardium. We used a test cohort including all available myocardial and plasma samples from all 19 patients. Fresh total RNA isolation was performed, followed by quantification of miR-1202 and miR-483-3p in all plasma and myocardial samples using TaqMan RT-qPCR (n=73). Of the 53 plasma samples, there was poor amplification in 2, and these were excluded. In the remaining plasma samples (n=51), both miRs were expressed robustly (Ct values 19.64 (19.25-19.87) and 31.85 (31.25-32.44) cycles for miR-1202 and miR-483-3p respectively). Both miRs were strongly expressed in the ventricular myocardium (n=20; Ct values 21.19 cycles (21.05-21.32) and 25.28 cycles (24.83-25.92) for miR-1202 and miR-483-3p respectively).

For temporal assessment of circulating miR expression, duration of LVAD support was categorised as 0 months (n=14; before LVAD implantation), 3 months (n=9; 91 days (83-91)

after implant), 6 months (n=8; 187 days (181-193)), 9 months (n=7; 271 days (265-272)) and 12 months (n=6; 371 days (361-392)). For comparison of plasma and myocardial expression we studied paired samples of ventricular myocardium at baseline and after LVAD support at the time of transplantation, and compared this to corresponding plasma expression (n=5 pairs). Circulating miR-483-3p showed early and sustained upregulation with LVAD support, with fold changes from baseline of 2.17 (1.43-2.62; p=0.011), 2.27 (1.12-2.42; p=0.036), 1.87 (1.64-4.36; p=0.028) and 2.82 (0.70-10.62; p=0.249) at 3, 6, 9 and 12 months respectively (see Table 7.6 below and Figure 7.2, page 212). These results mirrored the reduction in NT-proBNP levels (e.g. at 6 months, fold change 0.30 (0.08-0.43; p=0.004). Myocardial expression of miR-483-3p also showed upregulation, with absolute expression increased from 1.153 A.U. (1.093-1.171) at the time of LVAD implantation to 1.180 A.U. (1.167-1.191) at the time of transplantation, equating to fold change of 1.80 (0.72-4.72; p=0.169; Figure 7.3, page 213).

Table 7.6: Temporal changes in plasma levels of miR-483-3p and plasma concentration of NT-proBNP after LVAD implantation.

Exp, plasma expression in arbitrary units (A.U.). FC, fold change in miR expression from 0 months, RC, relative change in NT-proBNP as proportion of NT-proBNP concentration at 0 months. p, p-value for comparison versus 0 months.

Duration of LVAD support (months)	microRNAs							NT-proBNP			
	n	miR-483-3p			miR-1202			n			
		Exp., A.U.	FC from 0m	p	Exp., A.U.	FC from 0m	p		Conc., ng/mL	RC from 0m	p
0	14	0.884 (0.857-0.957)	-	-	1.308 (1.296-1.313)	-	-	17	4724 (1526-20890)	-	-
3	9	0.915 (0.906-0.941)	2.17 (1.43-2.62)	0.011	1.308 (1.306-1.319)	1.04 (0.85-1.57)	0.515	13	924 (653-2792)	0.23 (0.07-0.62)	0.006
6	8	0.907 (0.891-0.923)	2.27 (1.12-2.42)	0.036	1.307 (1.304-1.326)	0.94 (0.79-1.51)	1.000	11	674 (524-1421)	0.30 (0.08-0.43)	0.004
9	7	0.922 (0.881-0.942)	1.87 (1.64-4.36)	0.028	1.314 (1.304-1.323)	1.07 (0.71-1.71)	0.612	9	873 (420-1300)	0.14 (0.05-0.42)	0.008
12	6	0.897 (0.851-1.011)	2.82 (0.70-10.62)	0.249	1.313 (1.290-1.320)	1.14 (0.54-1.50)	0.917	7	1190 (534-1367)	0.26 (0.10-0.64)	0.028

In contrast, detection of circulating miR-1202 showed only small changes from baseline at all timepoints in the test cohort, and the change in miR-1202 expression between baseline and 6 months that was observed in the screening cohort was not replicated (fold change 0.94 (0.79-1.51; p=1.000)). Tissue expression of miR-1202 also showed no change with LVAD support (fold change 0.99 (0.81-1.07); p=0.575; see Table 7.6 and Figure 7.3, pages 210 and 213 respectively).

Considering all plasma samples (n=51), there was trend towards correlation between NT-proBNP levels and plasma expression of miR-1202 (r=-0.270, p=0.055), but no association of NT-proBNP with miR-483-3p (p=0.406) and no correlation between duration of LVAD support in days and fold change in plasma expression of miR-483-3p (p=0.498) or miR-1202 (p=0.759). Despite the relation between plasma and myocardial expression for each miR, we found no direct correlation between plasma and myocardial expression in the limited number of patients for whom we had paired plasma-myocardial samples (n=5). Furthermore, there was no difference in plasma expression of either miR (miR-483-3p, miR-1202) by gender (p=0.733, p=0.839) or heart failure aetiology (p=0.282, p=0.228).

Figure 7.2: Temporal changes in plasma levels of miR-483-3p and plasma concentration of NT-proBNP after LVAD implantation.

*There is significant early and sustained upregulation of plasma miR-483-3p after LVAD implantation (A), which mirrors the reduction in levels of NT-proBNP (B). Each line represents one patient. For miR-483-3p, data is shown for n=14, n=9, n=8, n=7 and n=6 for 0, 3, 6, 9 and 12 months respectively. For NT-proBNP, data is shown for n=14, n=13, n=11, n=9 and n=7 for 0, 3, 6, 9 and 12 months respectively. *=p<0.05; **=p<0.01. Reproduced with permission[178].*

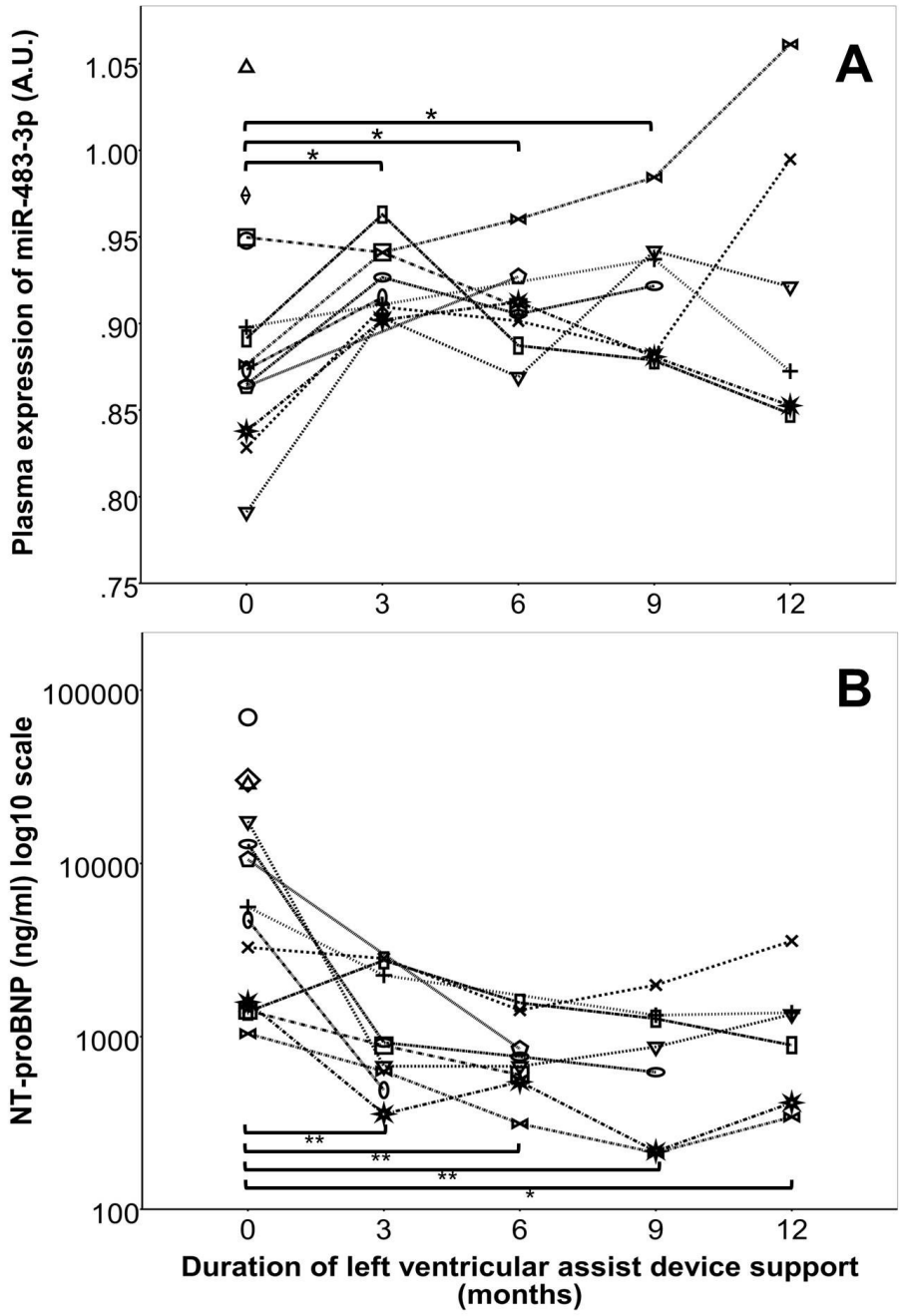
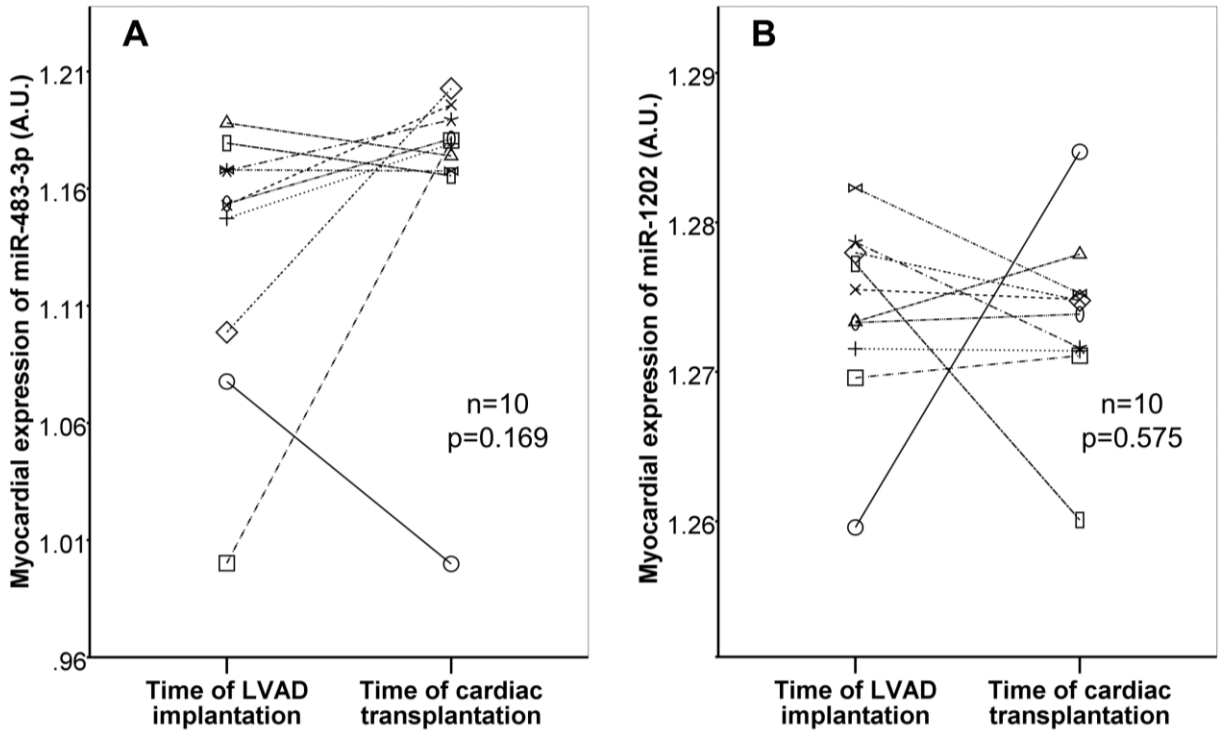


Figure 7.3: Changes in myocardial expression of miR-483-3p and miR-1202 between the time of LVAD implantation and time of subsequent cardiac transplantation.

There were paired samples from the time of LVAD implantation and time of cardiac transplantation from 5 patients. The median time to transplantation was 200 days (133-299). After LVAD support, there was upregulation of myocardial miR-483-3p expression (A, median fold change 1.799 (0.717-4.719; p=0.169)), but no significant change in myocardial miR-1202 expression (B, median fold change 0.988 (0.808-1.070; p=0.575)). Each line represents one patient. Reproduced with permission[178].

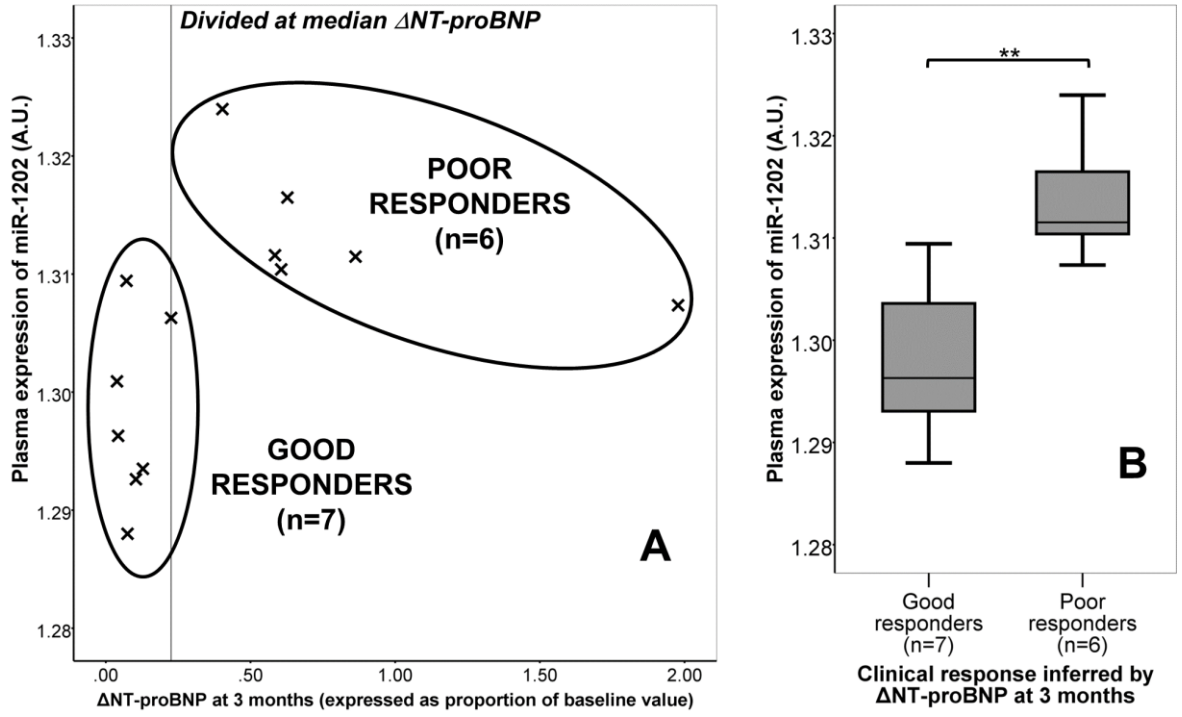


7.3.5 miR-1202 predicting response to LVAD support

We hypothesised that baseline measurement of the candidate miRs might provide novel biomarkers that predict individual patients' response to LVAD support. We classified patients' clinical response to LVAD therapy based on their change in NT-proBNP at 3 months (n=13). There was a strong correlation between baseline miR-1202 expression and change in NT-proBNP at 3 months (r=0.604, p=0.029). We divided the cohort at the 50th centile for change in NT-proBNP at 3 months to identify good responders (n=7) and poor responders (n=6; see Figure 7.4, page 214). Expression of circulating miR-1202 varied significantly between these groups (1.296 A.U. (1.293-1.306) vs. 1.311 A.U. (1.310-1.318); p=0.004). Finally, receiver operator characteristic (ROC) curve analysis to compare baseline circulating miR-1202, baseline NT-proBNP and pre-operative INTERMACS profile

identifies baseline miR-1202 as the best predictor of change in NT-proBNP at 3 months (n=13; AUC 0.976 (0.904-1.000), 0.714 (0.397-1.000) and 0.071 (0.000-0.210) for pre-implant miR-1202, INTERMACS profile and NT-proBNP respectively, p=0.04).

Figure 7.4: miR-1202 identifies patients at high risk of inadequate response to LVAD support.
We used change in change in NT-proBNP after 3 months of LVAD support as a marker of clinical response to LVAD support (n=13). Dividing the cohort at the median identified good (n=7) and poor (n=6) responders. Scatterplot (A) and boxplot (B) showing plasma miR-1202 expression before LVAD implantation identifying good versus poor responders. Expression of circulating miR-1202 varied significantly between these groups (good 1.296 A.U. (1.293-1.306) vs. poor 1.311 A.U. (1.310-1.318); p=0.004). Reproduced with permission[178].



7.4 Discussion

This Chapter is concerned with circulating miRs and their potential use as novel biomarkers. It builds on existing work in patients with HF and seeks a role for these biomarkers in patients with LVADs. Specifically, we hypothesised that (1) the circulating miRome shows plasticity in patients with LVADs, and this plasticity can be quantified as a novel biomarker; (2) individual miRs or patterns within the miRome can act as novel biomarkers that to monitor native LV function in patients with LVADs, and predict which patients respond to

LVAD therapy; (3) plasticity in the circulating miRome reflects plasticity of expression within the ventricular myocardium. The data presented support the first and second hypotheses, and provide some support for the third. Specifically, we have (1) demonstrated that isolation and quantification of circulating miRs is feasible in patients with LVADs, and through a large scale, non-biased screening microarray have identified miR-483-3p and miR-1202 as two candidate miRs that show plasticity with LVAD support; (2) shown that there is plasticity of miR-483-3p expression in plasma and in ventricular myocardium in response to LVAD support; (3) shown potential for circulating miRs to act as biomarkers for predicting (miR-1202) and monitoring (miR-483-3p) individual patients' response to LVAD therapy.

7.4.1 The need for new biomarkers

There is a paucity of non-invasive biomarkers that assist patient selection and serial monitoring of LVAD patients. Contemporary risk stratification relies on an experienced clinician integrating multiple prognostic parameters including clinical situation, maximal oxygen uptake, levels of natriuretic peptides, results from invasive right heart catheterisation, and risk scores such as the Seattle Heart Failure Model[25,181-183]. As discussed in earlier Chapters, there are inadequate tools for assessing changes in LV function after LVAD implantation: cardiac catheterisation is informative but invasive, echocardiography studies the unloaded LV and is limited by obstruction of apical views, and others such as exercise testing and natriuretic peptides give information about overall circulatory function yielded by the heart and pump together. Use of the turn down study, where the LVAD speed is temporarily reduced to challenge the ventricle with volume loading, partially overcomes this to allow functional, haemodynamic or echocardiographic assessments, but is time consuming and carries a theoretical risk of LVAD thrombosis due to transiently low pump speeds[125]. Within my PhD research, I sought to examine two biomarkers in LVAD patients: strain echocardiography (see section 6.4.2, page 195), and circulating miRs, discussed here.

7.4.2 Monitoring response to LVAD therapy

This cohort of 19 patients had severely advanced heart failure at enrolment, with 80% in INTERMACS profiles 1-3, median NT-proBNP 4724ng/l and median LVEF 15%. All were implanted with a Thoratec HeartMate II with varying degrees of response, with median NT-

proBNP falling to 674ng/l by 6 months. Circulating miR-483-3p shows a significant upregulation with LVAD support that mirrors the suppression of NT-proBNP levels (Figure 7.2). miR-483-3p in the ventricular myocardium is also upregulated with LVAD support in some patients (Figure 7.3). These changes in miR-483-3p expression could provide a more specific assessment of LV function that complements the changes in systemic neuroendocrine milieu recorded by serial measurement of natriuretic peptides. This hypothesis requires further assessment in a larger cohort to determine how changes in miR-483-3p expression correspond to changes in functional and haemodynamic indices, and long term outcomes.

7.4.3 Identifying the likely non-responder

The heterogeneity of clinical response to LVAD support can be challenging, and better tools for identifying patients unlikely to benefit from this invasive and expensive therapy could significantly reduce morbidity, mortality and treatment cost. Our data identifies miR-1202 as a biomarker that predicts early response to LVAD support. Baseline plasma miR-1202 levels correlate with change in NT-proBNP at 3 months, and stratify the cohort into poor versus good responders with greater accuracy than either baseline NT-proBNP or preoperative INTERMACS profile, parameters which are crucial aids to decision making around the time of implant (Figure 7.4). Clinically this would be valuable for judging the likelihood of good response to LVAD support, such that patients identified a priori as poor LVAD responders could be put forward for an alternative therapy such as urgent cardiac transplantation. However, this analysis is limited by the cohort size, and by the use of change in NT-proBNP as a surrogate endpoint for clinical response. In the long term, miR-1202 will need to be validated in a larger, prospective trial with long term clinical outcome and survival data.

7.4.4 Strengths and limitations of the current study

The key advantage of circulating miR is that it is a non-invasive test and as such has the capacity to improve management for this patient cohort. This relies in turn on miRs' stability in the circulation and in ex-vivo storage, and their ready isolation and quantification. However, levels of circulating miRs are dependent on multiple factors, many independent of the cardiac disease itself. We noted two points of relevance here. Firstly, the presence of heparin interferes with miR quantification[184,185], and one of the samples was probably

affected by this, though this is mitigated in chronic LVAD patients because they are normally anticoagulated with warfarin. Secondly, antiplatelet medications can affect the circulating miR profile and, in some patients, are commenced after LVAD implantation, though our candidates show no overlap with the platelet responsive targets identified recently[186]. Nonetheless, these factors and their implications for RNA extraction efficiency may explain our observation that miR-1202 expression showed significant variation at 6 months in the microarray and validation steps, but not in the test cohort after re-isolation of RNA.

It is interesting that the microarray has not identified miRs previously characterised in the heart failure or LVAD populations[135-137,139,141]. Moreover, the chosen approach of screening to identify novel miR candidates pertaining to this precise clinical scenario, rather than simply miRs previously identified and associated with heart failure, would be criticised by some authors. There are several reasons for the approach taken here. Firstly, this cohort represents the extreme end-stage of the heart failure spectrum, in comparison to others studies with largely NYHA II-III patients. Secondly, we have studied a larger panel of miRs than previous studies, using rigorous candidate selection. Finally, our microarray was designed to compare differences between baseline and post-LVAD therapy where others studies have compared differences between healthy controls and heart failure, and miRome plasticity differs between these scenarios, so this is studying a fundamentally different hypothesis. The counterargument is that miRs have already been proposed that may play an important role as biomarkers or inter-cellular signallers, and validation of these in this different scenario could produce important confirmatory data.

There are important limitations of the conclusions set out in this Chapter. The conclusions rely on a retrospective analysis of a small, heterogeneous clinical sample, and the necessity, in the absence of superior alternatives, to use change in NT-proBNP as a surrogate marker for treatment response. Whilst this is not a standard outcome measure in heart failure trials, there is well established evidence of natriuretic peptides' prognostic role, and evidence that changes in BNP after LVAD implantation are accompanied by changes in cellular markers of heart failure. Alternative outcome measures for response which would be more robust might include haemodynamic assessment at right heart catheterisation, or echocardiography, both with a low speed assessment and assessed prospectively.

7.4.5 Future work

7.4.5.1 Validation in other LVAD cohorts

This data presents preliminary conclusions about potential biomarkers for monitoring (miR-483-3p) and predicting (miR-1202) response to an LVAD in patients with advanced HF. To progress these ideas into clinical practice large, prospective studies will be required to test the hypothesis that these biomarkers are consistent and contribute additional clinical information to current approaches. My intention had been to test this prospectively in plasma samples collected during the PAVE-UP (Chapter 3) and SERCA-LVAD (Chapters 5-6) trials, but neither trial yielded sufficient data to make these analyses worthwhile. Next steps could include systematic collection linked with a transplant programme, or linking with large scale clinical trials to integrate miR as a variable.

7.4.5.2 Potential biological correlates

The finding of plasticity in miR-483-3p and miR-1202 makes them of interest as biomarkers, but a key question that arises is what is their biological role. The linked plasticity of miR-483-3p in ventricular myocardium and circulating plasma suggests that the plasma component may be reflective of release from myocardial tissue (whether as intercellular messenger, marker of cell damage akin to cardiac troponin, or physiological release) and could be informative about underlying physiology. A defined role for either miR in the pathophysiology of HF would provide biological plausibility for their use as biomarkers.

A first step is to confirm whether either or both miRs are released from ventricular myocardium. With this in mind, we had been granted ethical permission to collect paired right atrial and aortic samples as part of the protocol for further evaluation of the C-Pulse extra-aortic balloon pump, though unfortunately this did not come to fruition due to withdrawal of the device (Chapter 3). These paired samples would have allowed comparison of expression between venous and arterial samples, and any increase across the heart would support the hypothesis that the miRs were released from the myocardium.

Another approach is to pursue existing reports and in silico testing to identify putative functional roles and DNA targets. There are several avenues of evidence related to miR-483-3p. Several studies in cancer cells have shown that this miR may play a role in key intracellular processes related to apoptosis. In the heart, miR-483-3p is upregulated in cardiomyocytes of mice subjected to toxic hyperglycaemia by repressing insulin growth factor-1 activity[187], and upregulated in human ventricular myocardium after myocardial ischaemia[188]. MiR-483-3p also has a functional role in regulation of the renin-angiotensin system, working as an effector molecule for Angiotensin II within vascular smooth muscle cells[189], which may be relevant in the context of a continuous flow LVAD and non-pulsatile flow and potential for shear stress to affect miR expression[190]. Existing literature for miR-1202 biology is less consistent.

Together there is sufficient suggestion that miR-483-3p may have a biological role in the failing heart to justify these pursuing these systems and cellular approaches in tandem to further elucidate the underlying pathophysiology. Ultimately the goal would be to harness the pathophysiology to therapeutic end, for example with miR analogue or anti-miR technology.

7.4.6 General progress with circulating miR biomarkers

The field of circulating miR biomarkers exploded in around 2010 when they were first identified as being robust and reproducible within circulating plasma. Since then there have been large numbers of original research papers reporting novel findings in the field. However, at the time of writing no circulating miR biomarker is in clinical practice.

Data to date are principally from small, exploratory studies with little consistency between findings in different studies. Within HF there is not yet consensus on which of the various proposed markers should be taken forward for evaluation. Different laboratories use different methodologies, and sometimes it's not clear from articles exactly how the miRs were selected or how the qPCR calculations were performed. Details of data manipulation and assumptions are crucial, because the low levels of expression within plasma (typically <10ng from a standard 0.1ml sample, compared to >1000ng from fresh tissue[191,192]) and the chance of false positive results. For example, in this study any miRs with Ct>40 were judged undetectable

and excluded, but in some studies this is represented in analysis as a 'zero' expression and any increase compared to zero. This might be a valid alternative approach, despite the chance of detecting clinically insignificant background variation at these lower limits of sensitivity, but methods need to be absolutely clear for proper interpretation[193]. Finally, miRs are thought to be highly stable, but most of the articles to date report data obtained from existing plasma samples. These may not have been collected with strict sterile procedures as necessary for miR analysis, and may have been stored for many years with unknown numbers of freeze-thaw cycles. Both effects might have deleterious effect on sample quality, and excluding these confounders in prospective studies may remove any biomarker associations seen.

Once there is consistent laboratory data from a prospective study, a further key challenge in translating any miR biomarker approach will be the practicality of RT-qPCR as a clinical test. The isolation of RNA and subsequent RT and RT-qPCR is technically challenging and time consuming, and rapid access as a point of care or standard clinical laboratory test will require significant streamlining before it is practical. This will be a key hurdle before widespread adoption.

7.5 Conclusions

This work has provided preliminary data that shows the feasibility of using circulating miRs as biomarkers in patients with LVADs, and identified candidates that show potential for use as clinical biomarkers in the future. However, there are several limitations of the current study, and future work is proposed to develop my hypotheses further.

8: Discussion

Mechanical circulatory support remains a fundamental tool in the management of advanced HF, in the bridge to transplant setting and increasingly as long term destination therapy. However, many patients with HF are poorly served by existing technology, and high device-related morbidity limits its use to the most severely ill. The phenomenon of cardiac recovery has been much sought after, but identifying and harnessing the potential to recover are still poorly understood.

In this thesis, I have considered a trio of new approaches that might improve our biological understanding of these complex topics and enable greater clinical provision. Firstly, I have evaluated partial LV support as a novel mode of long term MCS, studied evidence for reverse remodelling of ventricular function in patients receiving this mode of support, and investigated acquired haematological abnormalities and their relation to pump dysfunction (Chapters 3-4). Secondly, I have studied the use of AAV1.SERCA2a gene therapy as an adjunctive therapy for patients with LVADs that might promote reverse remodelling, and reported the first use of this gene therapy in LVAD patients in the SERCA-LVAD trial (Chapters 5-6). Finally, I have evaluated strain echocardiography and circulating miRs as novel biomarkers in LVAD patients, and made the first report describing the latter in this context (Chapter 7). There is discussion of each of these in relevant chapters throughout the thesis. This section will aim to distil out key conclusions and lessons, and reflect on future approaches to clinical management and research in the field of long term MCS.

8.1 Partial LV support

Both assist devices under scrutiny in this thesis are no longer on the market, both having been withdrawn after fundamental flaws were identified causing too great a risk to patient safety to permit further new implants. The first device, the CircuLite Synergy LVAD, was a rotary blood pump with a novel circuit, such that blood was drawn from the LA and discharged to the right subclavian artery and sternotomy was avoided during implantation[62,63]. The second, the Sunshine Heart C-Pulse, was an extra-aortic cuff for chronic counterpulsation, implanted

off-pump and with potential for reductions in haematological complications associated with intravascular blood trauma[69]. The Synergy was withdrawn due to recurrent episodes of inflow cannula fracture, and high rates of pump thrombosis and exchange. The C-Pulse study was terminated due to problems with device infection. Owing to cessation of new implants of both devices, the prospective studies we had planned were both impossible to pursue. The data presented in Chapter 3 provide a retrospective, observational analysis of experiences at Harefield and at University Hospitals Leuven, the centre with largest experience with the Synergy LVAD.

8.1.1 Key findings

Therapy with the Synergy LVAD was associated with consistent haemodynamic benefits including increased cardiac output, reduction in LA pressures and consequently LV preload, and reduction in PASP. Nine of the 25 patients had been bridged to transplant by the time of closing the database. The haemodynamic improvements were accompanied by improvements in neurohormonal markers of HF, though without consistent improvements in exercise capacity and in fact some evidence of adverse remodelling in worsening LVEF. These benefits were offset by significant risks, including a 44% risk of pump thrombosis and 71% risk of major non-surgical bleeding, and significant numbers of patients required upgrade of their Synergy to a full support device. Comment on the C-Pulse is limited to experience in one patient, and consequently general conclusions are difficult to draw. Our patient did not experience significant device-related morbidity. However, despite some signs in this patient of improvement evidence by increasing walk distance and reductions in natriuretic peptides, overall his HF followed a deteriorating trajectory after device implantation, and there is little objective evidence of clinical benefit.

8.1.2 The end for partial support?

Both devices were marketed as solutions for patients in INTERMACS≥4 whose HF syndromes were poorly controlled with maximal medical and device therapy. I would argue that both devices failed in this regard. To justify clinical use, the device needs to improve symptoms reliably and significantly. It is striking when a shocked patient arrives on the ICU unable to walk a step, and then after full support LVAD implantation and a few months'

rehabilitation, she is back in full time work. The functional improvement is dramatic, objectively documented on exercise testing and fairly consistent across patient cohorts. With the C-Pulse, the benefits were subtle – perhaps a few extra metres on a 6 minute walk, or a subjective NYHA class improvement[69,152]. With the Synergy, some patients drew great benefit and for others it was simply insufficient to interrupt the cycle of the progressive HF syndrome. With these variable benefits, the risks associated with both these modes of partial support as currently delivered, in device implantation and during ongoing support, are currently too great to justify their routine use in patients with INTERMACS≥4 HF syndromes.

8.1.3 Future synergies

On this basis, one could make an argument that partial support devices are ill-equipped for and have no role in management of HF. With partial support bringing equivalent or greater risks than full support devices, why would any surgeon opt for the partial support device that delivers less incremental benefit? Absolutely, specific challenges have limited the success of partial support in its infancy – but similar challenges prevailed when previous generations of LVADs were introduced. These challenges were addressed then, and can be addressed again now.

The concept of partial support with LA to arterial bypass has been established by the Synergy LVAD, and while the device in its current form will not be implanted again, the mode of support and its potential benefits should not be abandoned. Firstly, a key issue is patient selection. Undoubtedly there were patients who received the Synergy LVAD whose HF syndrome was too advanced for the degree of support the Synergy could provide, in our cohort and in other centres. In some of these patients, the device seemed initially to be beneficial, but later native function worsened and the level of support needed increased; and in other patients, with hindsight, the HF was probably too far advanced at the outset. Patient selection for partial support is particularly an issue in an exclusive BTT programme, such as in the UK, as most true BTT candidates will be in INTERMACS 1-3 (Figure 3.12, page 98). At Harefield, there was greater freedom in patient selection when we were participating in the initial Synergy safety study, when CircuLite were funding the devices. Once we relied on NHS England funding, the BTT criteria kicked in, and the pool of potential candidates

became much smaller. The larger number of INTERMACS≥4 in the Leuven patients underlines this. We tried to address this at Harefield with an agreed set of guidelines for patient selection (Table 3.12, page 107), but implants were suspended before this had a proper road test. Secondly, it's essential that we understand and address the precipitants for pump thrombosis and high rates of bleeding in the Synergy patients. I have evaluated patient factors such as LA pressure (section 3.3.2.5, page 89) and haematological factors (Chapter 4), and engineers at CircuLite (subsumed to Heartware and now Medtronic) are considering the mechanical factors as outlined in section 3.4.2.1, page 98. Adjustments to the surgical implant procedure may help to reduce risks of surgical bleeding, and ameliorate the chance of inflow cannula fracture as observed prior to withdrawal[154]. Thirdly, the nature of the Synergy device has potential for other modes of support such as RV support or MCS in congenital heart disease[194]. The large delta pressure across full support pumps precludes their use in low flow settings (such as supporting a failing single ventricle circulation) or in low pressure chambers (such as the RV). Like the INTERMACS≥4 group, these patients are poorly served by current technology (Figure 1.2, page 28).

Together, I would hope that by optimising patient selection and addressing current problems with the Synergy LVAD it might be possible to present a device that delivers partial support in a low risk intervention with reduced morbidity compared to full support pumps, and with potential for use in a broad range of situations not currently served well. In contrast, the degree of evolution required for chronic counterpulsation to find a niche in HF management seems much greater. Here the clinical benefit derived, in our patient and in published data, seems minimal, and consequently the morbidity burden of the procedure would need to be dramatically reduced to make it viable. Particular aspects carrying high morbidity are the sternotomy and the large driveline for pneumatic control of the cuff.

In summary, I would argue that the concept of partial support is strong, and the next stage is refinement of the engineering and clinical approach to ensure that we select the patients most likely to benefit from this mode of support, whilst keeping the incumbent risks to the minimum level.

8.2 Gene therapy with AAV1.SERCA2a

While MCS facilitates symptomatic and functional improvements in parallel with the heart, another approach to the symptomatic HF patient is to directly influence cardiomyocyte and hence ventricular performance to reach the same end. One strategy is gene therapy using the AAV1 to introduce the SERCA2a transgene to cardiomyocytes and recover the dysfunctional calcium cycling that is characteristic of the failing cardiomyocyte (section 1.5.2, page 35). The combination of optimised ventricular loading conditions with direct modulation of cellular calcium dynamics might produce an additive beneficial effect. This forms the focus of Chapters 5-6.

8.2.1 Key findings

The SERCA-LVAD trial was the first study worldwide to deliver AAV1.SERCA2a gene therapy to patients undergoing long term MCS. Owing to neutral results from the related CUPID-2 study, the SERCA-LVAD trial closed early without completing recruitment, but nonetheless the data has shown that the approach of intracoronary delivery of the AAV1.SERCA2a product at the dose of $1x10^{13}$ DNA resistant particles is feasible and safe in this group of 5 patients. Even at target recruitment the efficacy outcomes in the SERCA-LVAD trial were planned to be exploratory only, and with these small numbers recruited there was no apparent benefit – though whether this reflects underpowering or truly no effect remains difficult to determine. We can draw crucial lessons about study design. The endomyocardial biopsy was safe in this group, but in the era of intrapericardial devices with short inflow cannulae the procedure was riskier than anticipated at the outset. Use of the femoral artery for vascular access was again safe in this cohort, but in future trials safety would be enhanced by using radial access, both through reducing bleeding risk and through removing the need to hold anticoagulation. Interventional studies in LVAD patients should be embarked upon in collaboration with multiple centres, both for improving the scientific merit and for helping to make recruitment targets achievable.

Some study questions remain unanswered. Just one of our patients had pre-existing circulating anti-AAV NAb. He suffered no immunological ill-consequence, providing reassurance about the possibility about extreme immune response to the viral challenge, with

the caveat that n=1. But the key question of whether the NAb blocks viral transduction remains open, as his biopsy specimens yielded no analysable material and he has not yet undergone cardiac transplantation. Furthermore, the interaction between ventricular loading and improvements in calcium physiology has not been addressed completely (discussed further in section 8.3, page 227).

8.2.2 What is next in the clinical pipeline?

The lack of benefit derived from AAV1.SERCA2a in the CUPID-2, SERCA-LVAD and AGENT-AF trials has stimulated a great deal of reflection about why the real-world experience departs from previous laboratory and clinical results. This is discussed in detail in section 6.4.1, page 188. While the community continues this reflection, there are several alternative gene therapy paradigms in preparation for clinical evaluation. Results from the initial AC6 trial were published last year, demonstrating safety and some suggestion of benefit, though with many patients excluded from efficacy analysis due to incomplete data and improvements in some parameters not sustained[195]. Trials of inhibitor-1c and S100A1 trials are promised. A few general challenges should be borne in mind. Firstly, it will be important to ensure clarity of scientific effect with new trials: already the next two trials planned are changing several variables at once (e.g. the S100A1 trial, which will use a different vector, different transgene and different delivery route compared to the AC6, SDF-1 and CUPID trials). While the novel approach may be valuable, the risk of this is that understanding and integrating the outcome data with other trials could become more challenging. Secondly, there remains the challenge of pre-existing NAbs. In the case of AAV1 this seems to occur in around 60% of patients, and is particularly problematic due to cross-reactivity of antibodies to AAVs of varying serotypes. Currently the paradigm is to exclude these patients from trials, which could significantly impede widespread clinical uptake. Various approaches to combat NAbs are being studied, such as plasmapheresis and bioengineering the AAV capsid to alter its antigenic regions as with the BNP AAV2i8 proposed for the I1-c study[106,196], and these warrant further investigation. Finally, the importance of investor funding for these innovative programmes remains crucial.

8.3 Cardiac recovery

The theme of reverse remodelling runs throughout the thesis, with focus both on how to stimulate it in the failing ventricle and how to assess it. At the extreme of reverse remodelling lies the phenomenon of cardiac recovery, when there is sufficient improvement in ventricular performance to allow cessation of MCS and explant of the support device. Partial support (with the Synergy LVAD and C-Pulse device) and AAV1.SERCA2a gene therapy were both hypothesised as treatments that individually or in concert might facilitate reverse remodelling.

There were no consistent signs of reverse remodelling across the cohorts studied in this thesis. A subgroup of Synergy recipients showed beneficial changes in functional parameters, and two patients improved sufficiently to explant the device, though others showed deterioration in LVEF and went on to need full support upgrades. The C-Pulse patient showed no signs of reverse remodelling. Within the small group who received AAV1.SERCA2a, there were no signs of beneficial effects on ventricular performance, nor evidence of reverse remodelling. On this basis, the story for cardiac recovery with these techniques sounds bleak. However, the data from the Synergy and C-Pulse patients is all retrospective, and largely obtained in the setting of a safety study where extreme caution to maintain device integrity was the priority. Similarly, there may be biological reasons why AAV1.SERCA2a at the current dose may not yield benefit, but equally within the SERCA-LVAD trial there was no protocol-specified procedure for optimising ventricular unloading. Previous studies have shown that cardiac recovery is seen most when trial protocols or clinical programmes are specifically designed to look for it[57] and when LVAD settings and medical therapy are rigorously managed to optimise unloading[54,55,58]. This includes the preservation of ventricular ejection as evidenced by aortic valve opening, something that partial support with the Synergy can maintain. Studies aiming to elicit reverse remodelling from whatever intervention need to be designed with these points in mind.

Finally, the context of cardiac recovery in HF is changing with the evolution of cardiac genetics and genomics. These may assist our understanding of mechanisms of reverse remodelling and could act as biomarkers of disease or recovery potential[197]. There is

increasing understanding of genetic predeterminants of HF such as the titin truncating mutation[198]. Recent data has shown that patients with titin truncating variants are amenable to cardiac recovery[199], and this welcomes the spectre that in future personalised medicine may facilitate individualised gene therapy to address specific deficits in individual patients.

8.4 Biomarkers in LVAD patients

One challenge in studies of reverse remodelling is how to identify and assess it in the LVAD-supported patient, and we sought to evaluate strain as an echocardiographic parameter (Chapter 6) and circulating miR (Chapter 7) as new biomarkers.

8.4.1 Challenges of echocardiography

Echocardiography is at the same time essential and exceptionally challenging in LVAD patients. Its clinical role is focussed on detection of valve disease such as acquired aortic regurgitation, assessment of the RV for progressive dysfunction, and assessment of inflow/outflow graft flow looking for evidence of reduced flow which may indicate thrombosis. Much of this is qualitative assessment, and probably the only reproducible parameters are LV dimensions from the parasternal views. In this context, it's not surprising that one of the key limitations in the dataset presented here is the frequent occurrence of missing data limiting the analysis (sections 5.4.6, page 174 and 6.4.2, page 195). Apical views were obtainable from only 2 of 5 patients recruited to the SERCA-LVAD trial, and parasternal views, though obtained and interpreted, were suboptimal in several patients. We have demonstrated that low speed assessments are safe in HVAD patients using our low speed protocol. Further, we have demonstrated that it's possible to measure strain systematically across a cohort, and when measured at low speed could act as a surrogate for peak VO_2 in patients unable to exercise. These findings show promise but need further evaluation in prospective studies. There is some evidence that strain rate may have greater load-independence than strain, and future studies could evaluate circumferential strain rate as a biomarker in LVAD patients.

8.4.2 Pros and cons of miR biomarkers

The other biomarker studied in detail was circulating miR. The hypothesis that miR plasticity may relate directly to changes in the myocardial miRome and myocardial biology was borne from studies of LVAD unloading in humans[136,137], and with the finding that circulating miRs were stable biomarkers with importance in HF[139], we sought to exploit this. In an exciting three-way collaboration, I took plasma samples from the LVAD programme in Leuven and, working in Thomas Thum's laboratory in Hannover, generated the first report of circulating miR plasticity in response to LVAD support. We identified miR-483-3p and miR-1202 as candidate miRs showing significant plasticity within plasma, and quantified these across the cohort of 19 patients. MiR-483-3p is upregulated in plasma and ventricular myocardium with LVAD unloading, and may be important as a biomarker of native ventricular function. MiR-1202 was able to stratify patients based on their clinical response to LVAD support at 3 months. The dual ability to monitor LV function (miR-483-3p) and add to the tools for assessing patients prior to LVAD implant (miR-1202) is an exciting offer to VAD physicians.

However, in addition to specific limitations in this study, there is not yet a coalescence of science around a specific group of biomarkers that should be taken forward in HF studies (discussed in section 7.4, page 214). Whether this is due to failure of the biological hypothesis or variable methodology remains unclear. In the former scenario, it remains possible that a series of small, preliminary studies are reporting variable results due to random variation. However, the latter scenario is illustrated by a recent study of miR in LVAD patients. The study has not reproduced our findings in their cohort, but their methodology precluded this[200]. Rather than a screening approach, they adopted a specific miR-targeting approach, but omitting the miR-483-3p and miR-1202 that we had identified. Similar variation in approach and in clinical cohorts, have hampered efforts to develop the field of circulating miR in HF onto the larger prospective studies that would constitute the next step towards clinical use.

Ultimately, this may be superseded by changes in methodology (deep RNA sequencing allows greater coverage of non-coding RNA) and expanding horizons to other targets (other long non-coding RNAs, already characterised in HF in several settings including the LVAD supported myocardium[201,202]).

8.5 Challenges of clinical trials in MCS

The numbers of patients suitable for, funded for, or already receiving MCS is small, and this makes clinical research in MCS challenging. Random variation within clinical cohorts requires large sample sizes for sufficient statistical power to demonstrate change related to an intervention. This combined with the high attrition rate of new technologies, as illustrated in my experiences with the Synergy and C-Pulse devices, makes research in MCS a challenging enterprise. Consequently, there is a tendency to report and rely on case report data rather than large scale prospective trials, and even the proper clinical trials have frequent weaknesses. The largest trials in MCS are many fold smaller than similar trials in new pharmaceuticals. Many trials are designed as single arm studies. A recent major trial of the HVAD comparing the device to standard care in fact drew on comparative registry data, rather than prospectively recruited and randomised subjects[36]. While it would be challenging in practice, the importance of sham treatment is identified in the SERCA-LVAD trial, where one patient had increasing troponin raising suspicion for viral-induced myocarditis, but in fact had received placebo, and there are recent lessons from studies in renal denervation. The pressure to use new and promising devices needs to be counterbalanced by an attention to proper consent and patient safety. These factors highlight problems with the clinical evidence within MCS, and highlight the importance of collaboration and multicentre approaches.

8.6 Final Conclusions

There are many complexities to research in MCS, brought about by the challenging clinical setting, heterogeneity within patient populations, limited pool of recruitment, high risk nature of clinical intervention and difficulty assessing the impact of new treatments in controlled studies. Key conclusions from the work presented here are: (1) partial LV support is a plausible concept, but significant challenges will have to be met to justify its adoption into routine clinical practice; (2) AAV1.SERCA2a gene therapy is safe and feasible in patients with long term LVADs, but shows no signs of clinical efficacy at the current dose; and (3) specific circulating miRs show potential as a biomarker for use in the long term MCS setting and warrant further investigation.

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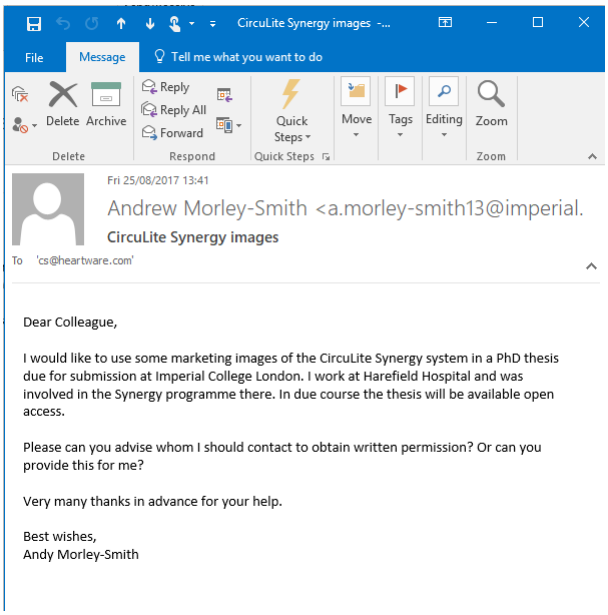

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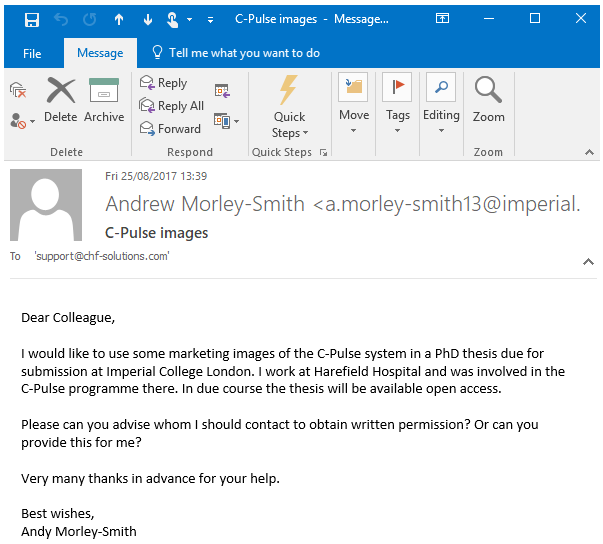

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