

**Investigation of the effect of early intracoronary autologous bone
marrow cell infusion in the management and treatment of acute
myocardial infarction**

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Abstract

Cardiovascular disease (CVD) is a complex combination of multiple conditions. The majority of deaths within CVD include heart attacks and strokes caused by atherosclerotic disease. The pathophysiological process for atherosclerotic disease occurs within the endothelial lining of the vessels of the body. This prolonged process occurs when cholesterol deposits form irregularity in luminal flow resulting in decreased blood flow and ischaemia. This unstable cholesterol plaque can rupture resulting in clot formation and artery occlusion.

Within this thesis I aim to show background to the relevant pathophysiology of ischaemic heart disease (IHD) with the main emphasis on acute myocardial infarction (AMI), the history of its therapy to current therapy. I will discuss the theorised role of stem cell therapy within animal models and previous clinical trials within regenerative medicine and AMI. I will describe and discuss the method and the results of the REGENERATE-AMI trial (Clintrial.gov: NCT00765453), which will include the safety and efficiency of the therapy, and the possible cytokine mechanism by which this therapy may exert its effect. Additionally I will describe the potential for assessing myocardial oedema using 3-slice T2-STIR short axis stack imaging post AMI compared to the conventional 10-slice T2-STIR technique to assess its feasibility and clinical similarity to assess its use as a tool in translational research.

I hope this thesis, which describes the rapid use of autologous bone marrow progenitor cells to salvage myocardium in patients with anterior AMI will help our understanding of the outcomes in patients who have these cells rapidly infused after AMI.

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Abbreviations

AAR	Area at risk
ACEi	Angiotensin-converting-enzyme inhibitor
ACS	Acute coronary syndrome
ACT	Activated clotting time
ADP	Adenosine diphosphate
AHA	American heart association
AIDS	Acquired immune deficiency syndrome`
AMI	Acute myocardial infarction
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARB	Angiotensin-receptor-blocker
ASC	Adult stem cells
BAMI	Bone marrow in acute myocardial infarction
BARI	Bypass Angioplasty Revascularization Investigation
BCIS	British Cardiovascular Intervention Society
BMA	Bone marrow aspiration
BMI	Body mass index
BMNC	Bone marrow derived mononuclear cells
BNP	B-type natriuretic peptide
BSE	British society of echocardiography

CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CCS	Canadian Cardiovascular Society
CHF	Chronic heart failure
CMR	Cardiac magnetic resonance imaging
CPC	Cell processing centre
CRP	C-reactive protein
CRT	Cardiac resynchronization therapy
CVD	Cardiovascular disease
DES	Drug eluting stent
EAS	European Atherosclerosis Society
ECG	Electrocardiograph
EDTA	Ethylenediaminetetraacetic acid
EPC	Endothelial progenitor cell
ESA	Endothelial surface area
ESC	European society of cardiology
FSC	Forward scatter
G-CSF	Granulocyte-colony stimulating factor
GCP	Good Clinical Practice
GFP	Green fluorescent protein
GGT	Gamma-glutamyl transferase
HF	Heart Failure
HGF	Hepatocyte growth factor

HIV	Human immunodeficiency virus
ICD	Implantable cardioverter-defibrillator
IGF	Insulin-like growth factor
IHD	Ischeamic heart disease
INR	International normalised ratio
IQR	Interquartile range
LAD	Left anterior descending artery
LGE	Late gadolinium enhancement
LVEDV	Left ventricular end diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end systolic volume
LVOT	Left ventricular outflow tract
LVSV	Left ventricular stroke volume
MACE	Major adverse cardiac events
MCP	Membrane cofactor protein
MMP	Matrix metalloproteinases
MRI	Magnetic resonance imaging
MSC	Mesenchymal stem cells
MSI	Myocardial salvage index
NHS	National health service
NICE	National Institute for Health and Care Excellence
NNT	Numbers needed to treat
NRES	National Research Ethics Service

NYHA	New York Heart Association
PCI	Percutaneous coronary intervention
PET	Positron emission tomography
PIS	Patient information sheet
PLAX	Parasternal long axis
Primary PCI	Primary percutaneous coronary intervention
PSAX	Parasternal short axis
QLV	Quantitative left ventriculography
RAAS	Renin-angiotensin-aldosterone system
RAO	Right anterior oblique
RCA	Right coronary artery
SAX	Short axis
SEM	Standard error of the mean
SPECT	Single-photon emission computed tomograph
SPSS	Statistical Package for the Social Sciences
SSC	Side scatter
SSFP	Single steady-state free precession
STEMI	ST elevation myocardial infarction
STIR	Short tau inversion recovery
TIMI	Thrombolysis In Myocardial Infarction
TNF	Tumor necrosis factors
TTE	Transthoracic echocardiogram
VAS	Visual analogue scale

VEGF	Vascular endothelial growth factor
VLA	Vertical long-axis
WHO	World health organization
WMSI	Wall motion score index

Publications

Choudry F, Hamshere S, Saunders N, et al. A randomised double-blind control study of early intra-coronary autologous bone marrow cell infusion in acute myocardial infarction: the REGENERATE-AMI clinical trial. *European heart journal*. Sep 23 2015.

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Chapter 1

Introduction

1.1. Cardiovascular Disease

CVD is the leading cause of death in the developed world and is likely to remain so until at least 2030 (WHO). An estimated 17.3 million people died from CVD in 2008, representing 30% of all global deaths, with this predicted to increase to 25 million by 2030. Of these 17.3 million, 7.3 million were due to coronary artery disease (CAD). In middle-income countries these figures are more disproportionately affected with over 80% of deaths occurring due to CVD, with an equal distribution between sexes. Figure 1.1 (WHO 2011). Therefore CVD has become the worlds leading health problem in terms of mortality and will remain the leading cause of global mortality for the foreseeable future.

Within the UK there are over 150,000 deaths per year secondary to CVD, this mainly compromises of stroke and coronary artery disease (British Heart Foundation 2011). In 2004 the UK economy spent £29.1 billion on CVD of which £8.5 billion is spent on coronary artery disease. This was mainly due to direct cost of health care (60% of total), and then followed by financial losses secondary to mortality and morbidity (23%) (Luengo-Fernandez R 2006).

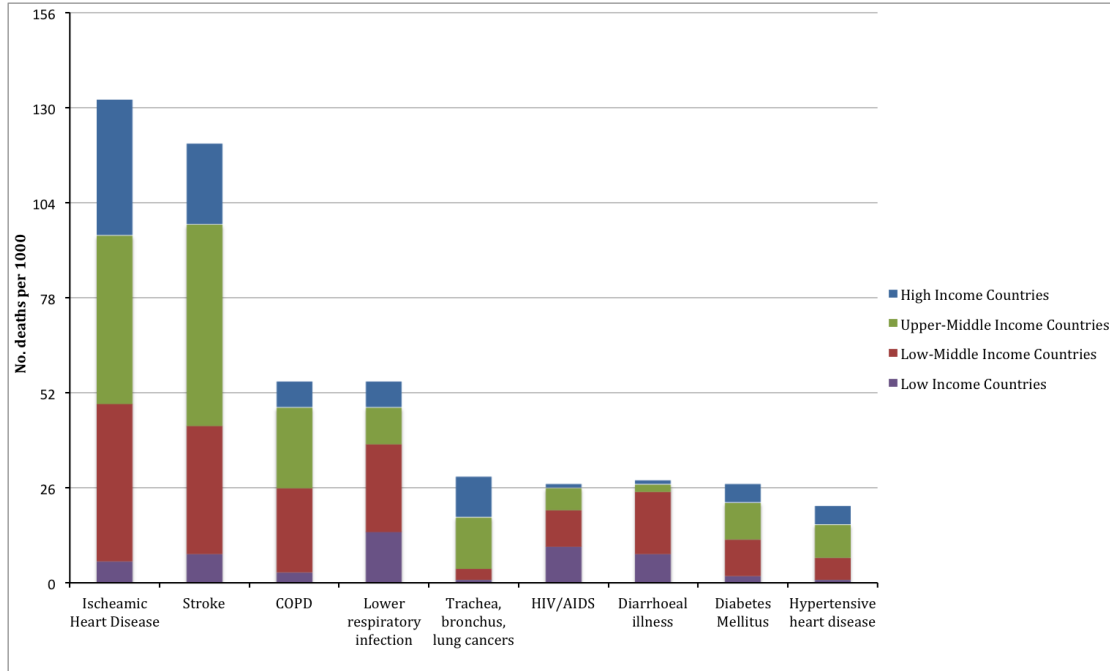


Figure 1.1. Mortality rates per 1000 from high income to low income countries adapted from the 2008 WHO statistics

1.2. Pathophysiology of Cardiovascular Disease

It is the formation of atheroma, lipid rich deposits, within the epicardial coronary arteries that cause CAD. The most common risk factors for atherosclerosis and their main pathobiological processes are shown on Table 1.1. Previous autopsy studies have shown that atherosclerotic plaques develop slowly over many years and in patients who die without morphological evidence of catastrophic atherosclerosis there are still significant plaque formations (Solberg and Strong 1983). The reduction in arterial diameter may lead to a slow but progressive increase in symptoms or an atheromatous plaque may rupture suddenly leading to thrombotic

occlusion of the coronary artery and the most notable manifestation of ischaemic heart disease (IHD), acute myocardial infarction (AMI) (Skyschally, Schulz et al. 2008, Rognoni, Cavallino et al. 2014).

Table 1.1. Risk Factors for IHD

Modifiable risk factors	Pathogenic Process
Dietary (high in saturated fat and cholesterol)	High blood cholesterol with increased oxidative stress
Increased BMI	Increased insulin resistance
Decreased activity	Poor perfusion with adverse lipid profile
Smoking	Increased oxidative stress
Hypertension	Increased oxidative stress and enhanced vasoconstriction
Alcohol intake	Increased oxidative stress
Serum lipids	Increased oxidative stress
Related conditions	Pathogenic Process
Familial hypercholesterolemia	Thrombogenesis
Diabetes mellitus	Increased oxidative stress and inflammatory process
Renal impairment	Increased oxidative stress
Non-modifiable risk factors	
Age	
Male sex	
Family history of heart disease (genetics)	
Cultural identity	
Socioeconomic status	

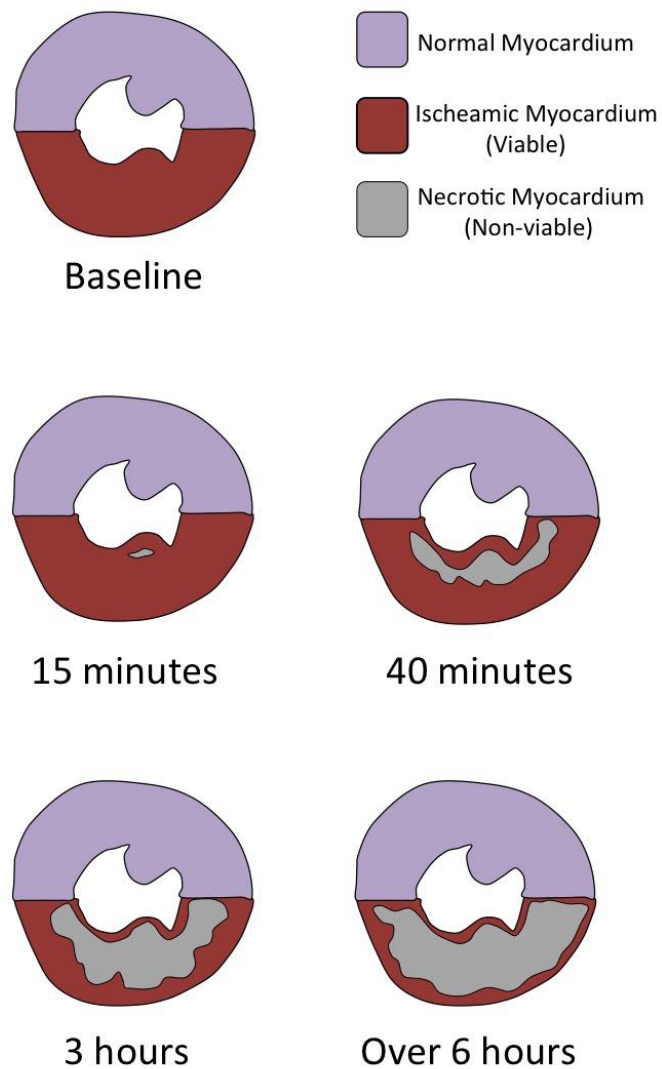
1.2.1. Pathophysiology of Acute Myocardial Infarction

There are around 175,000 AMI in the United Kingdom each year, with just over 60% affecting the male population (Townsend N 2012). AMI typically occurs when a plaque ruptures within a coronary artery leads to a sudden and total or near-total occlusion of that vessel. The region affected by the occlusion occurs along the distal distribution of the occluded vessel resulting in myocardial infarction. In the 1970s the concept of the ‘wavefront phenomenon’ was proposed by Reimer and Jennings

(Reimer and Jennings 1979). The wavefront phenomenon describes the increase of infarct size from the endocardium to the epicardium and is dependent on the duration of coronary obstruction and with increased severity of ischaemia (Figure 1.2)

Figure 1.2. Wavefront Phenomenon

Cross section of a left ventricle showing the development of necrosis in an infarcted region



The pathological process involved in the loss of myocardium after an ischaemic event results in migration of macrophages, monocytes, and neutrophils into the

infarcted area. This initiates a neurohormonal activation and intracellular signaling that focuses the inflammatory response. The 2 phases of myocardial remodeling post AMI are divided into early and late phases. The early phase remodeling occurs within the first 72 hours after AMI, and involves the expansion of the infarcted area, that can result in ventricular rupture or aneurysm formation (Erlebacher, Weiss et al. 1984). The late remodeling occurs after 72 hours and results in global LV dilatation and distortion. These stresses exerted on the LV lead to progressive dilatation, the formation of scar along the infarcted border zone and decreased contractility (White, Norris et al. 1987).

1.2.2. Acute myocardial mortality and management

Treatment for AMI has evolved over the last 20 years from thrombolytic therapy to primary percutaneous coronary intervention (primary PCI), which is now the gold standard treatment for patients suffering an ST-elevation myocardial infarction (STEMI). Nearly all cases of AMI are the result of a ruptured atherosclerotic plaque causing sudden occlusion of a coronary artery (Davies, Woolf et al. 1976). Reperfusion by primary PCI is a mechanical technique which restores coronary blood flow and includes the use of a guide wire with coronary ballooning or stenting. Multiple randomised controlled trials and meta-analyses have shown that primary PCI is superior in patients with STEMI compared to fibrinolysis (Keeley, Boura et al. 2003). In 2008 the final report of the National Infarct Angioplasty Project recommended the use of primary PCI within the UK as was feasible and cost effective

(Weaver, Simes et al. 1997). In addition the use of thrombus extraction was widely used during primary PCI, however recent evidence has shown this not to be of clinical benefit (Sirker, Mamas et al. 2016). The rate of primary PCI has risen to 374 people per million in the UK in 2012 (BCIS 2014). The current guidelines for primary PCI have been introduced to reduce myocardial damage from ischaemia and reduce the time from diagnosis to reperfusion (Brown 2014).

The outcome of patients suffering from AMI has significantly improved since the introduction of primary PCI, however despite this coupled with optimal medical management there remains a significant risk of mortality at 30 days (7%) (Widimsky, Wijns et al. 2010). The increasing numbers of survivors post AMI, has resulted in an associated increase in the incidences of heart failure (HF) (15-20%) in patients who still develop significant myocardial damage (Velagaleti, Pencina et al. 2008). Additionally to the damage caused by the period of ischaemia, reperfusion itself, although essential for myocardial salvage, actually results in further damage to cardiomyocytes that were alive at the time of reperfusion, so called reperfusion injury. Therefore although reperfusion with primary PCI is the gold standard for treating an occluded coronary vessel, reperfusion injury may account for up to 50% of the final infarct size (Yellon and Hausenloy 2007).

The modern management of AMI has been built on large-scale trials over the past four decades, and the transition of these trials to clinical practice has been the leading cause for the decrease in mortality associated with AMI. The current

combination of antithrombotic therapy with early reperfusion with primary PCI has been the mainstay of this therapy. The most commonly used anti-platelet medication in these patients is aspirin, the salicylate drug blocks the cyclooxygenase enzyme inhibiting the synthesis of thromboxane A₂. The initial benefit from aspirin seen in the first 24 hours following AMI was shown in the ISIS-2 trial where the combination of aspirin and streptokinase reduced five week vascular mortality in comparison to single agent therapy (Group. 1988). Current acute coronary syndrome (ACS) guidelines recommend a combination of dual anti-platelet medication of aspirin plus an additional P₂Y₁₂ receptor blocker. Clopidogrel is a P₂Y₁₂ receptor blocker that has been shown in combination with aspirin in the STEMI population to significantly reduce cardiovascular death, myocardial infarction (MI), or stroke (Mehta, Yusuf et al. 2001). More recent advances in P₂Y₁₂ receptor blocker such as Prasugrel and Ticagrelor have shown improvement in clinical outcomes in patients who suffer from ACS in comparison to the combination of aspirin and clopidogrel. Ticagrelor has a more rapid onset of action to that of clopidogrel, and differs from both prasugrel and clopidogrel by reversibly binding to the P₂Y₁₂ platelet receptor. In the PLATO trial, patients treated with ticagrelor had lower rates of MI, stroke, or cardiovascular death compared to clopidogrel (Husted, James et al. 2014) and it has now become the recommended antiplatelet therapy in addition to aspirin in patients who suffer with AMI. Additional anti-platelet medication used during primary PCI has been addressed with the use of glycoprotein IIb/IIIa inhibitors and bivalirudin. Due to the relatively limited antiplatelet potency of aspirin the addition of glycoprotein IIb/IIIa inhibitors in the context of high intracoronary (IC) thrombus

burden or inadequate P2Y12 antagonist loading is suggested in the guidelines (Carville, Harker et al. 2013, O'Gara, Kushner et al. 2013). An overview of current recommendation for antiplatelet medication during primary PCI is shown below on Table 1.2.

Table 1.2. Clinical trials and recommendations for antiplatelet medication

Recommendation	Class/Level	Trial
IV or oral Aspirin	I/B	(Group. 1988)
ADP-receptor blockers	I/A	
- Clopidogrel	I/C	(Leon, Baim et al. 1998)
- Ticagrelor	I/B	(Wallentin, Becker et al. 2009)
- Prasugrel	I/B	(Montalescot, Wiviott et al. 2009)
Routine use of GP IIb/IIIa	IIb/B	(De Luca, Suryapranata et al. 2005)
- Abciximab	A	(De Luca, Suryapranata et al. 2005)
- Eptifibatide	B	(Zeymer, Margenet et al. 2010)
- Tirofiban	B	(Valgimigli, Biondi-Zoccai et al. 2010)

For medical therapy the level of evidence assigned is based on the type of clinical trial and the strength of evidence shown below on Table 1.3.

Table 1.3. Classification and Level of Recommendations

Class I	Conditions for which there is evidence or general agreement that a given procedure or treatment is useful and effective.
Class II	Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a procedure or treatment. - IIa. Weight of evidence/opinion is in favour of usefulness/efficacy - IIb. Usefulness/efficacy is less well established by evidence/opinion
Class III	Conditions for which there is evidence and/or general agreement that the procedure/treatment is not useful/effective, and in some cases may be harmful
Level A	Data derived from multiple randomized clinical trials
Level B	Data derived from a single randomized trial, or non-randomized studies
Level C	Consensus opinion of experts

1.3. Heart Failure

These advances in the management of AMI together with population ageing have contributed to a growing burden of HF. Although there have been significant improvement in the management of patients who suffer from an AMI with decreases in mortality, there remains a large proportion of patients who still suffer from significant HF and subsequent symptoms (Velagaleti, Pencina et al. 2008). Despite the advances in both the acute and chronic setting, the prognosis for patients who are admitted to hospital with HF remains poor, with a 10-year survival of around 10% (Mosterd, Cost et al. 2001). Although primary PCI is now seen as the optimal therapy for a STEMI many studies have shown that the proportion of the patients that re-present with HF remains high (Bolognese, Neskovic et al. 2002). Within the HORIZON-AMI trial of the 2,946 patients enrolled without a history of chronic heart failure (CHF) 5.2% patients developed symptomatic CHF at 2 year post STEMI (Kelly, Gershlick et al. 2011). HF remains a major economic and public health issue, within the US it has been estimated that in 2010 that \$39.2 billion of the total health care budget was devoted to HF with a similar proportional allocation within European countries (Lloyd-Jones, Adams et al. 2010). On an individual patient basis, the diagnosis and treatment of HF has been associated with a cost of \$8500 annually (Stewart, Jenkins et al. 2002). The majority of the costs incurred for the treatment of HF occurs due to the hospitalisation of patients, and current therapy is aimed at the reduction of hospitalisation events. The economic impact of HF therapy will increase over the coming decades due to the growing number of elderly patients and the

improving mortality rates for with HF. Current therapy for HF is aimed at both prognostic and symptomatic therapy and in this next section there is an overview of HF pathophysiology and for the reasoning of current therapies.

1.3.1. Heart Failure Pathophysiology

HF (Left ventricular systolic dysfunction) is when there is impairment of left ventricular (LV) contraction and therefore ejection of blood. The leading cause for decreased systolic function is due to loss of functioning myocardium secondary to ischaemic injury (Bourassa, Gurne et al. 1993). The result of LV dysfunction leads to a decrease in cardiac output and therefore decreased global perfusion. The LV dysfunction results in increased volumes of blood in the ventricles, increasing end systolic and end diastolic volumes and elevated left atrial pressures. The increased left atrial pressure causes increased pressure in the pulmonary capillaries resulting in fluid leak producing pulmonary congestion and dyspnea.

The symptoms of HF directly result from inadequate cardiac output and venous return. Dyspnoea, cough and wheeze result from increased pressure in the pulmonary capillaries; fluid leak into the pulmonary alveoli decreases oxygenation and results in hypoxia and pulmonary oedema. The decrease in venous return to the right ventricle causes lower limb oedema and ascites. The result of decreased cardiac output can produce fatigue due to the poor vital organ perfusion.

1.3.2. Heart Failure Conventional Treatment

Current guidelines for the treatment of HF focus on both medical and lifestyle therapies (NICE 2010). The current life style advice for the management of HF echoes current advice for cardiovascular risk factor modification. Patients are encouraged to exercise, reduce weight as well as abstaining from smoking and alcohol use (Forouzanfar, Moran et al. 2012).

Current medical therapy can be divided into pharmacological and device-based therapy. The pharmacological management of HF aims to increase symptomatic relief as well as reducing the effects of the LV dysfunction and improve survival. In patients with IHD maximal revascularisation either with coronary artery bypass grafting (CABG) or PCI is suggested.

1.3.2.1. Heart Failure: Medical Therapy

A large number of trials have shown the benefits of pharmacological therapy in patients with all stages of symptomatic LV systolic HF.

1.3.2.1.1. Angiotensin-converting enzyme inhibitors

Angiotensin-converting enzymes inhibitors (ACEi) have been shown to have benefit in the treatment of HF. In the late 1980s the CONSeNSUS trial showed that enalapril resulted in a significant improvement in mortality in patients with congestive cardiac failure (Swedberg and Kjekshus 1988). Subsequent meta-analysis have also shown that in HF patients ACEi treatment reduces the relative risk of mortality by over 23% (95% CI 67 to 88%; ARR 6.1%) and hospitalisation admissions reduced by 35% (95% CI 26 to 43%; ARR 10.2%) (Garg and Yusuf 1995). ACEi are now recommended in all patients who have significant LV systolic dysfunction (left ventricular ejection fraction (LVEF) < 40%) (Yancy, Jessup et al. 2013). Patients who are unable to tolerate ACEi due to well-documented side effects are suggested to start angiotensin receptor blockers (ARB). However a side effect of both ACEi and ARB medications due to the effect on the renin-angiotensin-aldosterone system (RAAS)-activity is renal dysfunction.

1.3.2.1.2. Beta-Blockers

Beta-blockers have shown benefit in patients with significant LV systolic dysfunction in combination with an ACEi or ARB (CIBIS-II 1999, Hjalmarson, Goldstein et al. 2000, Packer, Coats et al. 2001). The majority of randomly controlled trials have shown a total mortality reduction seen with the use of B-blockers in chronic HF.

Previous analysis of the 4 major B-blockade trials (BEST, CIBIS-II, MERIT-HF and COPERNICUS) has shown an all cause mortality reduction by 23% (95% CI 8% to 35%) (Domanski, Krause-Steinrauf et al. 2003). B-blockers are suggested first line therapy in combination with ACEi in the treatment in CHF.

1.3.2.1.3. Diuretics

Diuretics are used in the majority of patients with HF to reduce fluid retention for symptomatic relief. The most commonly used diuretic is a loop diuretic, frusemide that acts directly on the ascending loop of Henle. Previous meta-analysis shown diuretics have a reduction on mortality and improvement in exercise capacity (Faris, Flather et al. 2002). One of the largest issues regarding these trials is the relatively small number of patients recruited into them and the inconsistencies of the patient's baseline therapy. However although prognostic evidence supporting their use is limited, the symptomatic relief from diuretic therapy for the treatment of oedema and dyspnea is accepted. Previous HF studies have shown that near 90% of patients were on at least 1 class of diuretic therapy for both the management of acute and chronic HF.

1.3.2.1.4. Digoxin

Digoxin is a cardiac glycoside that is extracted from *Digitalis lanta* (Hollman 1996). Digoxin therapy does not have the mortality benefit seen in other medical therapies such as ACEi or Beta blockade. However previous reviews have shown that digoxin therapy has a 64% improvement in symptoms (OR=0.31, 95% CI 0.21% to 0.43%) and a significant reduction in rates of hospitalisation (Hood, Dans et al. 2014). However, the Cochrane review relied heavily on the DIG study, which although a large randomised controlled study B-blockers or aldosterone antagonists could have influenced the beneficial effect on mortality (Group 1997).

1.3.2.1.5. Aldosterone Antagonists

Aldosterone antagonists in the addition to ACEi have been shown to reduce all cause mortality by 30%-(RR 0.70, 95% CI 0.60% to 0.82%; $p<0.001$) (Pitt, Zannad et al. 1999). Patients who have suffered a recent AMI with significant LV impairment, were shown, in the EPHESUS study to have a 13% reduction in cardiovascular mortality and hospitalisation (95% CI 5% to 21%; $p=0.002$ ARR 3.3%; NNT=30) and a mortality rate from cardiovascular causes (Pitt, Remme et al. 2003).

1.3.2.1.6. Angiotensin Receptor-Neprilysin Inhibitors

Recently newer therapies have emerged which show potential for the treatment of HF. Neprilysin is a neutral endopeptidase that degrades several endogenous vasoactive peptides (Rademaker, Charles et al. 1996) that inhibits the neurohormonal over activation, that contributes to vasoconstriction and remodelling (Kuhn 2004). The publication of PARADIGM-HF has raised hope for the arrival of a new class of drug the angiotensin receptor-neprilysin inhibitors (ARNi). The known benefits of both ACEi such as enalapril, which has shown to reduce mortality and the use of a neprilysin inhibitor, which increase levels of natriuretic peptide and bradykinin have created the niche for these drug (Swedberg and Kjekshus 1988, Cruden, Fox et al. 2004). The PARADIGM-HF trials was able to demonstrate that the use of ARNi reduces the risk or morality and hospitalisations with heart failure compaired to monotherpay of enalapril (McMurray, Packer et al. 2014).

1.3.2.2. Heart Failure: Device Therapy

There have been significant advances in the development of device therapy for use in HF over the last 2 decades. Approximately half of HF patients who die unexpectedly are due to ventricular arrhythmias.

1.3.2.2.1. Implantable cardiac defibrillators

The rationale for the use of implantable cardioverter-defibrillator (ICD) in HF is to prevent sudden cardiac death due to ventricular arrhythmias, which are responsible for up to 50% of deaths in HF patients. Although pharmacological therapy can reduce mortality in patients with HF, there is a risk that some anti-arrhythmic drugs may increase the rates of dysthymias (Zipes, Camm et al. 2006). ICD therapy has been shown to be of benefit in patients who have haemodynamic compromise with ventricular arrhythmias, or in patients with impaired LV systolic function who suffer from symptomatic HF and is the optimal pharmacological treatment or therapy (Bardy, Lee et al. 2005).

1.3.2.2.2. Cardiac Resynchronisation Therapy

The role of resynchronisation therapy in patients with HF is a challenging issue. The initial outlay cost for the implantation of the device needs to be weighed against both the short and long term benefit (van Veldhuisen, Maass et al. 2009). The effect of the long term use of CRT have been evaluated extensively in large scale trials and recent meta-analysis (Bradley, Bradley et al. 2003). Two types of devices that have been studied have been CRT pacemakers or CRT-ICD devices. The two main trials that were powered to assess the effect of CRT on all causes of mortality were CARE-HF and COMPANION (Bristow, Saxon et al. 2004, Cleland, Daubert et al. 2005). These trials failed to demonstrate a superior CRT strategy, however, in CARE-HF the use of

CRT pacemakers showed a 36% reduction in all cause of death (Cleland, Daubert et al. 2006).

1.3.2.3. Heart Failure: Transplantation

Cardiac transplantation remains the optimal treatment choice in patients who are suffering from ESHF and remain symptomatic. Although the prevalence of HF has increased over the past decade the number of cardiac transplantations have decreased, in the United States 2692 transplants occurred in 2009.

1.3.2.3. Heart Failure: Ventricular Assist Devices

The use of mechanical circulatory support for the treatment of severe heart failure has grown in the last decade (Lampropulos, Kim et al. 2014). The development of reduced size and function has helped to improve both mortality and symptoms of heart failure (Ozalp, Bhagra et al. 2014). The main aim of the ventricular assist device (VAD) is to provide a bridge-to-transplant until a suitable organ can be acquired. The most recent guidelines for outpatient use of VADs have a level of evidence of B and C (Feldman, Pamboukian et al. 2013).

1.3.2.5. Heart Failure: Future Therapies

The use of stem cell therapy in chronic heart disease has been gaining momentum in the last decade. The two main areas of interest has been within chronic IHD and dilated cardiomyopathy (DCM). Although no large phase III trials have been performed in these fields to date multiple meta analyses have demonstrated the benefits of this therapy in chronic IHD (Fisher, Doree et al. 2013). Within DCM only a single randomized controlled study has been published, the REGENERATE-DCM study was able to demonstrate the improvement in both LVEF and symptoms with stem cell therapy (Hamshere, Arnous et al. 2015). Although not part of guidelines the use of stem cell therapy on compassionate grounds has become an option for these patients who have no other treatment options.

1.3.2.6. Heart Failure: Current Guidelines

Current guidelines both in Europe and USA suggest the initiation of ACEi or ARB with B-blockers in patients who have symptoms of HF as a Class 1 recommendation (Yancy, Jessup et al. 2013).

1.4 Need for new heart failure treatments

AMI results in the loss of cardiomyocytes with the formation of fibrosis and scar tissue that results in decreased LV function and arrhythmic potential. Despite the advances in ACS therapy there have been little progress in reducing cardiomyocyte loss other than the early mechanical intervention and newer pharmacological therapies. The realisation of a novel therapy that could either regenerate or replace the damaged myocardium, and/or improve myocardial remodeling during the early phases of healing has been the main goal. Potential therapies have been addressed including the use of pharmacological agents during primary PCI such as nitrite for the reduction of ischaemic reperfusion injury (Jones, Pellaton et al. 2015), the use of gene therapy for the targeting of specific pathways involved in the development of HF or biological agents such as stem cell therapy after AMI with their proposed role in regenerative medicine.

1.5 Regenerative Medicine

The Unitarian theory of haematopoiesis that stated that all cells develop from the same precursor cell, was first hypothesised by Alexander Maximow in the early part of the 20th century (Konstantinov 2000). The discovery of stem cell phenotypes has yet to deliver their full therapeutic potential (Friedenstein, Chailakhjan et al. 1970). William Haseltine first coined the term regenerative medicine in the late 20th

century. The introduction of cell-based therapy has become one of the most important fields in cardiovascular research in the last two decades. The transition of pre-clinical trials to the current boom in numbers of trials involving stem cells and progenitor cells aimed at repairing damaged myocardium has led to the possibility of improving outcomes for these patients with reductions in the incidence of HF.

1.6. The Heart and Regeneration

The heart has historically been considered a terminally differentiated organ with no ability to self repair (Chien and Olson 2002). However recent studies (Liao, Pfister et al. 2007, Blum and Benvenisty 2008, Clifford, Fisher et al. 2012) have implied that there may be a background level of cellular renewal by engraftment and differentiation of primitive cells. Limited regeneration could occur through circulating primitive bone marrow mononuclear cells (BMNC) that could engraft in the heart in response to injury (Orlic, Kajstura et al. 2001) or from a resident cardiac stem-cell population (Oh, Bradfute et al. 2003). This reparative potential is not sufficient to respond to the significant myocardial damage caused by AMI but the discovery of this endogenous repair process has provided further rationale for the use of cell therapy based to amplify this natural process.

The quintessential example of cardiac regeneration would be that of the Zebra fish. After approximately 20% surgical amputation of the total ventricular mass the Zebra fish is able to fully regenerate itself (Poss, Wilson et al. 2002). Further investigations

into the Zebra fish with gene mapping has shown that the regeneration of the myocardium is caused by pre-existing cardiomyocytes rather than undifferentiated progenitor cells that had been previously thought. The mammalian heart in comparison to that of the Zebra fish, is unable to regenerate itself (Porrello, Mahmoud et al. 2011).

In the mammalian heart regeneration studies have shown that very early post partum, there is a degree of myocardial regeneration. However after 7 days this ability for regeneration declines; this loss in regeneration may coincide with the reduction of the cell cycle activity (Porrello, Mahmoud et al. 2011). Again, gene mapping has shown that the regeneration is led by pre-existing cardiomyocytes, and this study demonstrates that there is a Zebra fish like regenerative mechanisms latent in mammalian hearts. Therefore the aim of stem cell therapy use in the post AMI heart would be the repair of damaged myocardium with the subsequent improvement in the patients' mortality and morbidity.

1.6.1. Overview

Stem cells have two defined characteristics, the ability for unlimited self-renewal and the potential to differentiate into all different cell lineages. The most potent cell type that can differentiate into all cell types is the zygote (Figure 1.3).

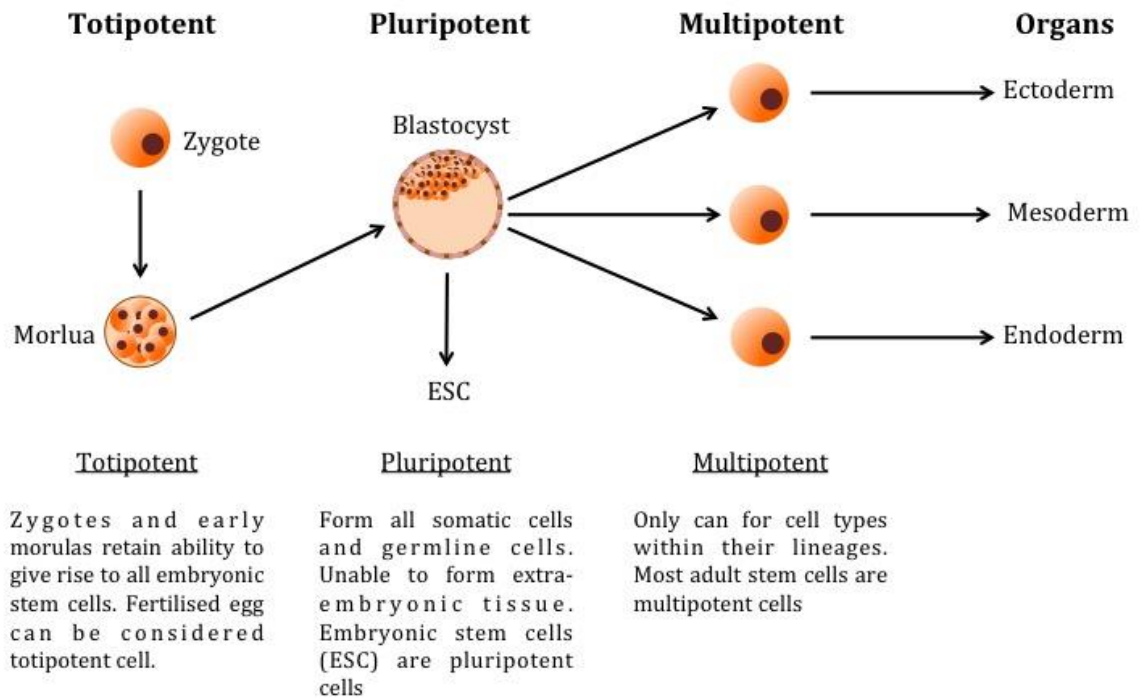


Figure 1.3. Stem Cell Classification

The zygote is the cell that results of the fusion of two haploid cells during fertilisation until the first cleavage, the further divisions of the zygote are therefore called an embryo. The ability for multi cell differentiation of the zygote is termed totipotent. The developed embryo contains embryonic stem cells (ESCs) that are pluripotent as they have the ability to differentiate into three germinal layers and therefore are able to produce functional organisms. Adult organisms have no ESCs, only adult stem cells (ASC) and progenitor cells. Like ESCs, ASCs share the ability of self-renewal and production of cell lineages, however ASCs have limited degree of daughter cells production in the organ that they reside.

1.6.2. Stem Cell Types

In theory the cell with the ability to form any new cell type and have unlimited replication ability would be ideal for cell-based therapy. The myriad of cells that have the potential to be used in regenerative medicine is wide, and to date no leading cell type has emerged. As seen in previous trials (Table 1.3) different stem cell types have been used giving rise to differing results, the most commonly used stem cells are discussed below.

1.6.2.1. Embryonic Stem Cells

As stated previously ESCs are able to generate multiple cell lineages and undergo self renewal during early fetal development Therefore the use of ESCs would be an obvious choice for myocardial regeneration. In 1998, two papers reported the isolation of ESCs from human embryos was possible (Thomson, Itskovitz-Eldor et al. 1998). The technological advances of these early trials, lead to excitement in the field of regenerative medicine and the possible application into chronic diseases. The observed potential for the use of ESCs to transform into fully functionally cardiomyocytes in vitro (Kehat, Kenyagin-Karsenti et al. 2001) and successful use of ESCs to treat MI in animal model (Behfar, Hodgson et al. 2005) has given rise to great interest.

There is however considerable development and ethical issues that need addressing before this promising technique is used in man. The technical issues of selecting ESCs as they start to differentiate so they can be delivered to a certain tissue at the right time. The transition of ESCs into specific cardiomyocytes would be the result of local host signaling, however due to the greater cell differentiation the result could be an abnormal amount of specialised cardiomyocytes such as Purkinje fibres resulting in possible arrhythmogenesis (Behfar, Hodgson et al. 2005). A major issue lies with the risk of tumour formation where ESC transdifferentiate into non-relevant cells or uncontrolled transdifferentiation, however this has been partly addressed by the pre-differentiation of ESC into cardiomyocytes phenotypes prior to implantation (Laflamme, Chen et al. 2007).

The ethical and moral consideration for the use of ESCs is highly controversial and has been linked to the debate on human abortion due to the destruction of human embryos. The complex debate is divided between the belief that life begins at conception or that the embryo becomes a person at a later stage of development (Daley, Ahrlund Richter et al. 2007).

1.6.2.2. Skeletal myoblasts

Skeletal myoblasts (SM) are derived from satellite cells, which are responsible for postnatal growth of skeletal muscle (Schultz 1996). The first description of SM were reported in frogs (Mauro and Adams 1961) and over a decade later isolated in mammalian muscle (Schultz 1976). The relative ease of extraction from muscle biopsies, expansion in vitro and skeletal muscles resistance to ischaemic conditions made skeletal stem cells the first assessed in pre-clinical and clinical trials (Chachques, Acar et al. 2004).

Skeletal satellite cells were considered to be monopotent, with the ability to give rise to a few cell of the myogenic lineage. By the end of the 20th Century evidence arose for the bi-directional differentiation potential between bone marrow and muscle (Ferrari, Cusella-De Angelis et al. 1998). Two studies have demonstrated bi-directional placidity of muscle and bone marrow, and the repopulation of muscle cells from lethally irradiated mice. These trials have demonstrated the ability for muscle derived cells to be able to repopulate major blood linages and muscle (Gussoni, Soneoka et al. 1999).

Within animal studies initial trials showed promise with the limitation of post infarction LVEF deterioration and the improvement in scar tissue through the colonisation of fibrosis by skeletal muscle stem cells (Ghostine, Carrion et al. 2002). In more recent studies, the benefit was less imposed on the skeletal stem cell

infusion but the activation of cardiac stem cells (CSC) secondary to the secretion of growth factors (Pouly, Hagege et al. 2004).

These encouraging preclinical results were quickly translated into clinical application. The only patients who were considered as suitable for skeletal myoblast transplantation were patients who suffered from CHF due to the period of time for cell expansion. The first application in man of skeletal myoblast by Menasche et al was on HF patients with significant LVEF impairment and poor exercise class as measured by the New York Heart Association (NYHA) functional class during CABG. In this trial a total of 800×10^6 cells, of which 65% were defined as myoblasts as CD56+ cells were injected in to the scarred region of the LV during CABG. This intervention was associated with a significant improvement in NYHA class and LV function, however these findings are difficult to interpret due to concurrent revascularisation and no true control group (Menasche, Hagege et al. 2001). Further small number trials resulted in improved LV function, LV remodeling and increased myoblast survival on histological examination in intramyocardial (IM) injections in patients (Steendijk, Smits et al. 2006)

After these promising initial small-scale trials, larger randomised trials have been set up. The randomised, double blinded placebo controlled trial; Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) included patients with severely impaired LV function with an indication for CABG. There was no significant difference between adverse advents or change in LV function, however there was a

significant decrease in LV end diastolic volume (LVEDV) and LV end systolic volume (LVESV) (Menasche, Alfieri et al. 2008). Prior to this trial, the issue was raised regarding possible increased arrhythmias with the use of SM, this was addressed within MAGIC with patients fitted with ICDs. There was no significant difference between the burden of arrhythmias. However further analysis showed that there was an earlier burden of arrhythmias in the skeletal myoblast group compared to the control group in the immediate post op period, with a more even distribution in the following 6 months.

To date although the smaller non-randomised trials gave hope for the use of skeletal myoblast transplantation the larger trials have failed to fulfill this promise.

1.6.2.3. Cardiac Stem Cells

It has been traditionally regarded that cardiomyocytes were a terminally differentiated cell that react to any disease process by hypertrophy (Kelly, Alonso et al. 1995). However ongoing research in the last decade have shown the potential ability for cardiomyocyte regeneration and cell division in adult hearts post AMI or in HF (Beltrami, Urbanek et al. 2001). In addition to this it has been found that the heart contains a large reservoir of stem cells, which are positive for a large number of stem cell phenotypes (Boyle, Schulman et al. 2006).

The thought of using CSC is an attractive option for cardiac regeneration, as their role is intended to repair the damaged heart. However, CSC are difficult to harvest and isolate in comparison to BMNC. At present the largest model for CSC is in large animal models post AMI, although CSC have been used in humans in chronic ischaemic cardiomyopathy (Lee, White et al. 2011). In the CADUCEUS trial, a prospective randomised trial of 25 patients of whom 17 underwent endomyocardial biopsy and autologous stem cell expansion with IC infusion at 3 months post AMI. This trial showed the safety of cardiac stem cell infusion and also showed a significant reduction of scar size and increase in viable heart mass. However it failed to show significant improvement in LV function (Makkar, Smith et al. 2012).

1.6.2.4. Bone Marrow Derived Stem Cells

Bone marrow derived stem cells are the most common used cell type within regenerative medicine and heart disease. The first suggestion of their use came when in animal models it was demonstrated that circulating endothelial cells from bone marrow origin could contribute to neovascularisation (Takahashi, Kalka et al. 1999). Early preclinical animal models of wild type mice, showed that marked green fluorescent protein (GFP) positive bone marrow, contributed to cardiomyocytes and endothelial formation after an induced myocardial infarction (Jackson, Majka et al. 2001). There have been mixed results within animal models showing the differentiation of progenitor bone marrow derived cells to cardiomyocytes (Marchetti, Gimond et al. 2002, Min, Yang et al. 2002). Adult bone marrow derived

stem cells include three populations of stem cells. These include hematopoietic stem cells (HSCs) that give rise to all blood lineages, mesenchymal stem cells (MSCs) that can differentiate into different cell lineages (Pittenger, Mackay et al. 1999, Kondo, Wagers et al. 2003) and endothelial progenitor cells (EPC).

1.6.2.4.1. Endothelial Progenitor Cells

Damage to the endothelium has been linked to atherosclerosis and hypertension, and it is the balance between injury and endothelial recovery that results in the reduction of cardiovascular events (Endemann and Schiffrin 2004). Mature endothelial cells have limited capacity for regeneration therefore the focus has centered on circulating EPC and their role in maintenance of endothelial integrity and neovascularisation (Rafii and Lyden 2003).

Bone marrow is a reservoir of specific stem and progenitor cells, including endothelial progenitor cells. There is an increase in circulating endothelial progenitor cells following endothelial injury (Majka, Jackson et al. 2003). Endothelial progenitor cells and circulating endothelial progenitor cells express CD133⁺ phenotype, and cells that express CD133⁺VEGFR2⁺ can proliferate in vitro into endothelial cells (Peichev, Naiyer et al. 2000). The recruitment of endothelial progenitor cells to regions of injury has been extensively studied (Zhang, Zhang et al. 2002, Massa, Rosti et al. 2005). Post AMI there is an increase in circulating endothelial progenitor cells that is mirrored by an increase in vascular endothelial

growth factor A (Rabbany, Heissig et al. 2003). In pre-clinical animal models the infusion of endothelial progenitor cells has shown improvement in vascular supply and improvement in cardiac function (Kocher, Schuster et al. 2001). The transplantation of endothelial progenitor cells has been shown in multiple trials in both CABG patients and IC infusion (Hamano, Nishida et al. 2001, Assmus, Rolf et al. 2010).

1.6.2.4.2. Mesenchymal Stem Cells

Friedenstein et al demonstrated that bone marrow cell population contained hematopoietic stem cells and a rare population of plastic-adherent stromal cells at a ratio of 1 in 10,000 nucleated cells (Friedenstein, Chailakhjan et al. 1970). After these new plastic-adherent cells were expanded and the formation of round colonies of cells that resembled fibroblastic cells Friedenstein identified the importance of these cells in the control of the haematopoietic niche (Friedenstein, Chailakhyan et al. 1974). These cells were initially referred to as stromal cells but have now commonly been known as mesenchymal stem cells (MSCs). MSCs express CD29 and CD105 with the lack of expression of CD34 and CD45 surface markers (Dominici, Le Blanc et al. 2006).

MSCs have been shown to differentiate into osteoblasts and adipocytes, with evidence that cartilage turnover was mediated by MSC differentiation (Caplan 1991). In the early 21st century both in-vivo and in-vitro studies have shown that MSCs can

transdifferentiate into cardiomyocytes (Xu, Zhang et al. 2004, Sato, Araki et al. 2005). The transition to large animal model studies has shown that MSC administration in ischaemic models have demonstrated the ability for MSCs to engraft and improve cardiac function (Shake, Gruber et al. 2002). The use of cardiac magnetic resonance (CMR) imaging has further shown the regeneration of viable tissue with improved contractile function in a fibrotic region (Amado, Schuleri et al. 2006).

The initial trials of MSCs have shown safety and improved left ventricular function following the IC infusion of MSCs after MI (Chen, Fang et al. 2004) however the range of improvement and clinical outcomes has varied within trials and more data is required.

1.6.3. Proposed mechanism of action

The potential mechanism of action of stem cell therapy remains hotly debated with a unified hypothesis yet to be agreed. Further information is emerging all the time from the large numbers of ongoing regenerative medicine clinical trials. The 4 most likely mechanism of action of stem cell therapy will now be discussed (Figure 1.4).

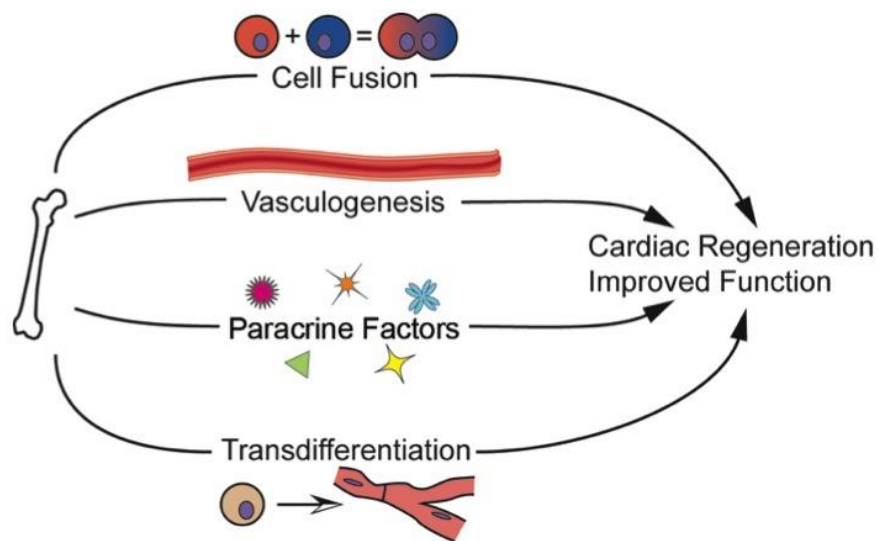


Figure 1.4. Proposed mechanism of action of cell therapy (Lovell and Mathur 2011)

1.6.3.1. Paracrine Effect

Recent research has provided supporting evidence that the introduction of aASC play an important role in the paracrine pathway. It has been shown that the introduction of progenitor cells in the infarcted heart results in the secretion of a variety of cytokines, chemokines and growth factors that results in cardiac repair (Caplan and Dennis 2006).

Patients post AMI undergoing stem cell therapy have higher protein tissue concentrations of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF)-I (Nagaya, Kangawa et al. 2005).

The increasing evidence shows that progenitor cells secrete growth factors, chemokine and cytokines that regulate their own cellular biology in an autocrine or paracrine manner with interaction with their surrounding environment (Majka, Janowska-Wieczorek et al. 2001). It has been shown that the infusion of activated cells from bone marrow or mobilised peripherally from the blood results in secretion of these factors (Sahoo, Klychko et al. 2011), and therefore all stem cell types have the potential in regenerative medicine to exert a paracrine force.

These paracrine factors may be able to inhibit apoptosis, stimulate neovascularisation and proliferation (Tse, Siu et al. 2007). There are a wide range of factors secreted from progenitor cells, however the most important include VEGF, HGF, IGF-1 and necrosis factor alpha (NF-A). It has also been shown that in times of hypoxic stress states there is an increase in several of these paracrine factors (Kinnaird, Stabile et al. 2004).

It is believed that that secretion of paracrine factors varies with the stem cell types, and that paracrine factors have an important role in the regeneration of damaged tissue (Schweitzer, Johnstone et al. 2011). BMNC expressing various growth factors,

chemokine and cytokines by enzyme linked immunosorbent assay have further supported this (Janowska-Wieczorek, Majka et al. 2001).

The role of paracrine factors and their influence on their environment, their role in myocardial protection and neovascularisation have been extensively examined. The immediate paracrine effect after stem cell infusion in an ischaemic environment is the release of molecules that improve cardiomyocyte survival. It has been shown that the hypoxic MSCs can reduce necrosis in isolated cardiomyocytes that were exposed to low tension of oxygen (Gnecchi, He et al. 2005).

There is therefore increasing evidence that paracrine mechanisms may be important in explaining how progenitor cells result in myocardial regeneration post AMI. Any future study assessing the use of stem cell therapy should measure levels of specific cytokines to investigate whether the effects of early IC infusion of BMNC in patients with AMI may be in part mediated by an increase in beneficial paracrine factors.

1.6.3.1.1. Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor (VEGF) plays a role in the proliferation and recruitment of endothelial cells and ischaemic damage. The expression of VEGF is up regulated during an AMI and studies have shown correlation between serum VEGF levels and size of infarction (Hojo, Ikeda et al. 2000). Previous animal models have demonstrated the direct IM injection of stem cells results in localised enhancement

of VEGF levels in post infarction myocardium (Mathison, Gersch et al. 2012). In addition to this VEGF also mediates the phosphorylation of endothelial nitric-oxide synthase, which plays a major role in the regulation of angioblasts and endothelial cell proliferation (Gentile, Muise-Helmericks et al. 2013).

1.6.3.1.2. Tumour necrosis factor- α

Tumour necrosis factor- α (TNF- α) was originally recognised to exert antiproliferative and cytotoxic effects on tumours and was indicated in the role of septic shock (Tracey, Vlassara et al. 1989). However recently TNF- α has been shown to have actions on the differentiation and proliferation of different cell types and is considered to play an integral part in the signal transduction cascade that regulates the inflammatory process (Vilcek and Lee 1991). Multiple studies have shown that TNF- α has an effect on many cardiac pathologies ranging from viral myocarditis (Matsumori, Yamada et al. 1994) to the progression of chronic HF (Torre-Amione, Kapadia et al. 1996). TNF- α has shown to be released from ischaemic myocardium and is involved in myocardial dysfunction (Nakano, Knowlton et al. 1998). Within stem cell therapy, levels of TNF- α increase after an injection of stem cells in post AMI animal models (Takahashi, Li et al. 2006). We hypothesize that serum level of TNF- α after AMI will be increased in both groups of patients with a greater serum level seen in patients who have stem cell therapy that is maintained throughout follow up with greater levels in patients who had a significant improvement in LVEF from baseline to 12 months.

1.6.3.1.3. Interleukin 6 (IL-6)

Interleukin 6 (IL-6) is a pleiotropic cytokine that has a wide range of cellular immune effects (Van Snick 1990). IL-6 is pivotal in the mediation of the acute phase response and is produced in response to factors such as infection and IL-1 (Heinrich, Castell et al. 1990, Ng, Tan et al. 1994). The evidence of serum IL-6 levels being raised has been shown in inflammatory conditions and its secretion from vascular endothelial and smooth muscle cells from arteries (Loppnow and Libby 1989, Papanicolaou, Wilder et al. 1998). Further research has shown that IL-6 is increased in patients after AMI and this correlates with other inflammatory markers such as C reactive protein (Miyao, Yasue et al. 1993). It is believed that IL-6 plays a role in the regulation of collagen formation in ventricular remodeling post AMI (Puhakka, Magga et al. 2003). LV dysfunction and symptomatic HF are associated with increased serum levels of IL-6 however it is not known if levels of IL-6 play a detrimental effect of LV function after stem cell therapy.

1.6.3.1.4. Granulocyte-colony stimulating factor

Meta-analyses have shown that using BMNC after AMI in the last decade have shown a beneficial physiological response (Clifford, Fisher et al. 2012). However controlled trials using granulocyte-colony stimulation factor (G-CSF) post AMI have been inconclusive with conflicting results. The use of subcutaneous G-CSF post AMI has

been shown to be safe (Ince, Petzsch et al. 2005). The use of G-CSF has shown to promote the migration of progenitor cells and can also act directly promote cardiomyocytes survival in ischaemic conditions (Harada, Qin et al. 2005). Meta analysis has shown that G-CSF therapy in patients with AMI is safe however has failed to show any overall benefit (Abdel-Latif, Bolli et al. 2008). It is not known if intrinsic G-CSF has an effect on LV function post BMNC therapy delivered after AMI.

1.6.3.1.5. Matrix metalloproteinases (MMP-9)

Matrix metalloproteinases (MMPs) play a major pathophysiological regulation role within the extracellular matrix. MMP are structurally related to endopeptidases and are able to degrade components of the extracellular matrix. The classification of MMP is in accordance to their substrate specificity. The MMP family has a wide range of actions and have been shown to be involved in LV remodeling following AMI and are involved in the development of LV failure (Creemers, Cleutjens et al. 2001). As shown previously MMP-9 is unregulated post AMI and plays an important part in LV remodeling as well as potentially playing a predictive role (Lu, Gunja-Smith et al. 2000).

1.6.3.2. Cell Fusion

There have been multiple pre-clinical trials looking at the natural process for stem cells fusing with several cell types after cell injury. Cell fusion is a natural process throughout all stages of our lives, from the first cell fusion where a spermatozoa fuses with an ovum to infection and the immune response, cell fusion plays a pivotal role. Cell fusion has been shown to have a role within tissue regeneration. In the last decade the discovery that BMNC can fuse with several cell types has promoted new ways that cell therapy can help in regeneration.

The basic concept of cell fusion was first proposed by Schwann's microscopic observations of pig embryos (Schwann 1839). Most current work on cell fusion has been derived from study of fusion of viruses with cells (Hernandez, Hoffman et al. 1996). In stem cell therapy, stem cells and a non-haematopoietic cell type fuse together forming a multi-nuclei cell. These cells known as heterokaryon are a mixture of two cell type lineages that result in the original stem cell type expressing the phenotype of the fusion cell (Hardeman, Chiu et al. 1986).

1.6.3.3. Neovascularisation

Multiple trials have shown that progenitor cells have a role in neovascularisation in ischaemic tissue as well as in tumour tissue (Furuya, Nishiyama et al. 2005). The

process of neovascularisation is essential to the development and differentiation of tissue throughout our life cycle from embryo development to wound repair.

Trials have demonstrated that the use of BMNC has resulted in increased recovery of vascular supply to a region of tissue damage; this has been shown to improve the overall function and neovascularisation (Berger, Bergers et al. 2005).

1.6.3.4. Transdifferentiation

There is still debate regarding if cell fusion or cell transdifferentiation play a role in cardiomyocyte regeneration post AMI. In essence transdifferentiation is the reprogramming of cell lineages into another type without reversion to a pluripotent cell. Several studies have shown, using time-lapse microscopy, the colonisation of endothelial cells from ESC that could express certain cell antigens (Eilken, Nishikawa et al. 2009).

Eglitis et al have proposed that ASCs could switch lineages, they were able to show that bone marrow from mice gave rise to neuronal astrocytes (Eglitis and Mezey 1997). Orlic et al showed that new cardiomyocytes cells were formed with the improvement in cardiac function after bone marrow derived stem cells were injected in to infarcted myocardium (Orlic, Kajstura et al. 2001), however since this early data, more recent studies have demonstrated conflicting results in regard to transdifferentiation of cells post implantation (Murry, Soonpaa et al. 2004).

1.6.4. Systems of delivery

The major aim of stem cell delivery is to transfer a large enough quantity of cells into the area of damaged myocardium. As seen in previous trials different methods of delivery has given rise to differing results, the most commonly used approaches to deliver stem cells are discussed below.

1.6.4.1. Peripheral stimulation of progenitor cell release

G-CSF, a hematopoietic cytokine produced by monocytes and endothelial cells is effective in stimulating the mobilisation of BMNC into the peripheral blood. Animal studies have previously shown the beneficial effect of G-CSF mobilisation of stem cells on LVEF after an AMI (Kawamoto, Gwon et al. 2001, Minatoguchi, Takemura et al. 2004). The mechanistic action of G-CSF on the infarcted myocardium is thought to prevent cardiac remodeling after AMI by inhibiting the apoptosis of cardiac myocytes (Harada, Qin et al. 2005).

In human clinical trials, these beneficial pre-clinical effects have not been reproduced with meta-analyses of G-CSF use post AMI have failed to show improvement in LVEF (Zohlhofer, Dibra et al. 2008).

1.6.4.2. Intravenous cell delivery

The intravenous infusion of stem cell therapy has been used in some clinical trials. The risk of post primary PCI arrhythmias in patients post AMI remains high and the infusion of products directly into the myocardium is similarly high (Mattioli, Lonardi et al. 2012). The idea of intravenous infusion post primary PCI relies on the concept of stem cell homing to the region of myocardial injury, but as with peripheral stimulation the results have not been favorable (Price, Chou et al. 2006).

1.6.4.3. Intracoronary delivery of cell therapy

The majority of the trials within AMI patients use the IC infusion of stem cells for their mode of administration. The most commonly used protocol of progenitor cell infusion was developed by Professor Zeiher et al (Schachinger, Erbs et al. 2006). This involved a 0.014-inch guide wire introduced into the infarcted artery and a 0.5mm oversized “over-the-wire” balloon catheter positioned within the stented segment. The balloon is inflated to low pressure-causing occlusion of the artery, and infusion of the stem cells occurs over three minute intervals with deflation for a further three minutes prior to the next occlusion and infusion in order to avoid extensive ischaemia (Figure 1.5). It is thought that this method aims to allow for adhesion and potential transmigration of the infused cells through the endothelium (Assmus, Schachinger et al. 2002).

In 2001 the first clinical use of unfractionated autologous mononuclear bone marrow stem cells were injected IC for the first time after AMI for poor LVEF in a 46-year-old male (Strauer, Brehm et al. 2001).

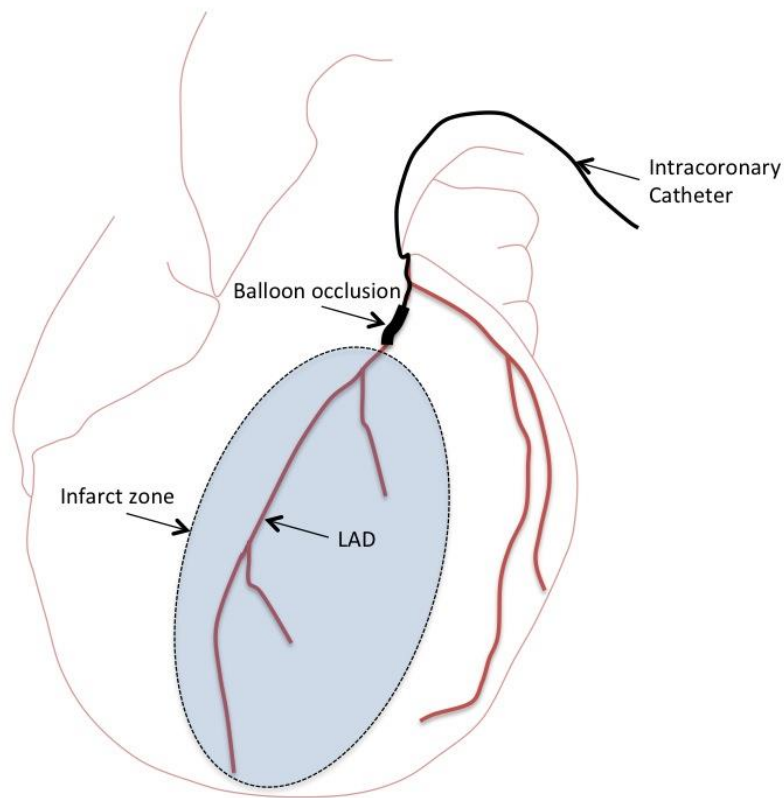


Figure 1.5. Intracoronary Infusion

1.6.4.4. Intramyocardial cell delivery

As previously stated stem cells can be injected directly into the ventricular wall, although this is with the increased risk of ventricular arrhythmia post AMI. The direct injection of stem cells during CABG or the transendocardial approach and IM electrophysiological mapping of scarred or damaged myocardium plus infusion of

stem cells with devices such as the NOGA™ device have been previously used (Banovic, Ostojic et al. 2011) (Figure 1.6).

In July 2001 the first clinical use of CD133⁺ bone marrow derived stem cells were injected via the IM route in a 64-year-old patient with significantly impaired LVEF during CABG (Stamm, Westphal et al. 2003).

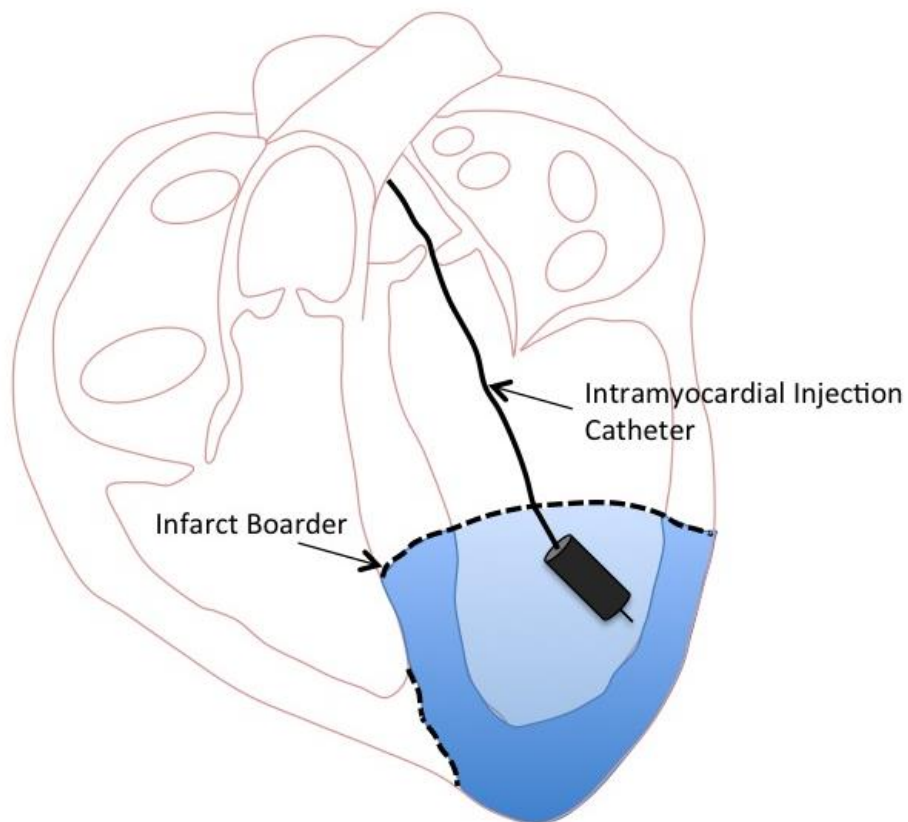


Figure 1.6. Intramyocardial Injections

1.6.4.5. Best delivery method

Questions still remain regarding the optimum delivery method for stem cell therapy in patients post AMI, and to date there has been no consensus reached. Peripheral stimulation with G-CSF although an ideal low risk route has failed to show any benefit post AMI and therefore is not being tested in ongoing studies (Engelmann, Theiss et al. 2006).

The issue with both invasive delivery routes is the potential for fatal risks to the patients undergoing the procedure. Very few trials have used IM infusion of stem cells in patients after an AMI due to the proposed high risk of arrhythmia (Rodrigo, van Ramshorst et al. 2012). The route of choice that has been used widely in previous trials has been the IC infusion of stem cells due to its safety and reduced risk of arrhythmias in comparison to IM. Table 1.3 shows the all the major stem cell trials in which IC infusion route was used.

1.7. Research Trials into the use of Stem Cells and Acute Myocardial Infarction

The transition from preclinical studies to human clinical trials has been rapid over the last decade. There are currently over 100 actively recruiting clinical trials into heart disease being run worldwide as of December 2015.

1.7.1. Animal Models

The method for inducing AMI in animal models is achieved in many ways. The majority of preclinical trials aim to narrow or block the coronary artery that results in myocardial ischaemia this can be achieved by surgical ligation or drug intervention

The initial work by Orlic et al studied the IM injection of lineage-negative (Lin⁻) bone marrow cells from transgenic mice after left anterior coronary artery ligation, Lin⁻ c-*kit*⁺ cells were injected in the contracting wall bordering the infarct. They found that 68% new cardiomyocytes were formed of the infarcted portion of the ventricle 9 days post procedure (Orlic, Kajstura et al. 2001). These findings suggested that an injection of hematopoietic stem cells generate *de novo* myocardium. Further studies by Jackson et al. has shown that the infusion of highly enriched hematopoietic stem cells into the myocardium of ligated and reperfused lethally irradiated mice myocardium resulted in the demonstration of cardiomyogenic process at the borders of the infarcted region (Jackson, Majka et al. 2001).

The evidence that human stem cells had the potential to regenerate the myocardium in rodents has further helped the transition to human clinical trials. Kocher et al. showed that the infusion of human CD34⁺ stem cells into the infarcted myocardium of rats resulted in neovascularisation and the proliferation of preexisting of vasculature (Kocher, Schuster et al. 2001).

1.6.7. Clinical Trials

The first randomised control trial assessing cell therapy post AMI was TOPCARE-AMI, published in October 2004 (Schachinger, Assmus et al. 2004). The primary focus of the trial was to show the safety of IC cell infusion post AMI. The 3-minute balloon inflation technique used for cell infusion was well tolerated and showed no procedural complications (Schachinger, Erbs et al. 2006). Secondary imaging end points showed improvement in LVEF from $51.6 \pm 9.6\%$ at baseline to $60.1 \pm 8.6\%$ at 4 months in the cell therapy arm vs. $51.0 \pm 10\%$ at baseline to $53.5 \pm 7.9\%$ in the control arm (Assmus, Schachinger et al. 2002). The largest randomised controlled trial addressing cell therapy in AMI has been REPAIR-AMI, which looked at 204 patients recruited from April 2004 to June 2005. REPAIR-AMI showed a significant improvement in LVEF ($5.5 \pm 7.3\%$ vs. $3.0 \pm 6.5\%$, $p=0.01$) and mortality in the cell based therapy treated patients (Schachinger, Erbs et al. 2006).

Contrary to these results a number of other clinical trials over the last decade have failed to replicate the results these early trials have shown. The LEUVEN-AMI trial failed to show a significant change in LVEF after cell therapy although of note did show an improvement in infarct size (Janssens, Dubois et al. 2006), and further trials have pushed the timings of the cell therapy infusion beyond the standard 3-5 days post infarction. In the TIME (Traverse, Henry et al. 2012) and LATE-TIME (Traverse, Henry et al. 2011) the time points were pushed to 1 week and 2-3 weeks post AMI

respectively, with both these trials failing to show improvement in LVEF with late infusions.

Meta-analyses of all randomised controlled stem cell trials have identified 24 trials, with a total of 1,624 patients who received stem cell or placebo after successful primary PCI treating AMI. These results have showed that progenitor cells treated patients had significant improvement in LV function at 6 months (2.23% (95% CI 1.00 to 3.47); $p < 0.001$) and at 12 months this trend continued in 11 studies (3.91% (95% CI 2.56 to 5.27); $p < 0.001$) (Delewi, Andriessen et al. 2013). A further Cochrane review of 33 randomised control trials showed stem cell therapy resulted in a significant improvement in LV ejection fraction of 3.75% at 1 year compared to patients treated with conventional therapy (Clifford, Fisher et al. 2012, Fisher, Doree et al. 2013).

Table 1.4. Stem Cell Clinical Trial: Stem cell dose and Timing of delivery

Study	Year of Publication	Cell Type	Mode of Delivery	Time of Delivery post AMI	Result	Ref.
Janssens et al	2005	MC-MNC 11-90 x 10 ⁶	IC	1 day	Increase scar size but no improvement in LVEF at 4 months	(Janssens, Dubois et al. 2006)
TOPCARE-AMI	2002	BMNC 2.4 x10 ⁸ CPC 1.3 x10 ⁷	IC	3-7 days	Improved LVFE and reduced infarct size at 4-12 months	(Assmus, Schachinger et al. 2002)
TIME	2012	BM-NMC 150x10 ⁶	IC	3 + 7 days	No difference in LVEF at 6 months	(Traverse, Henry et al. 2012)
REPAIR AMI	2006	BMNC 2.4 x 10 ⁸	IC	4 days	Improved LVEF and reduced infarct size at 4 months	(Schachinger, Erbs et al. 2006)
ASTAMI	2006	BMNC 8.7 x 10 ⁸	IC	5-8 days	No difference in LVEF at 6 months	(Lunde, Solheim et al. 2006)
HEBE	2011	BMNC 296 x 10 ⁸ P-MNC 287 x 10 ⁸	IC	3-8 days	No difference in LVEF at 4 months	(Hirsch, Nijveldt et al. 2011)
BOOST	2004	BMNC 24x10 ⁹	IC	6 days	Improved EF at 6 months, increased regional contractility	(Wollert, Meyer et al. 2004)
SWISS-AMI	2010	BMNC 153 x10 ⁶	IC	5-7 days 3-4 weeks	No difference in LVEF at 4 months	(Surder, Schwitter et al. 2010)
LATE-TIME	2011	BMNC 150x10 ⁶	IC	2-3 weeks	No difference at 6 months	(Traverse, Henry et al. 2011)

1.6.7.1. Limitations of current trials

Despite the increasing clinical experience of stem cell therapy post AMI, there remain unanswered questions, these include optimal delivery time and route of administration of cells. A key question that still needed to be assessed is whether any meaningful results can be achieved by the delivery of cell therapy within a normal clinical length of hospitalisation post AMI.

1.6.7.1.1. Timing of stem cell transplantation

The ideal timing for stem cell infusion in patients post AMI has been much debated. The balancing of the proposed benefit against the risk of cell infusion has been a question that has been addressed in multiple trials. To date as seen in Table 1.3 the trials have focused their time points 24 hours post primary PCI. The issue regarding the best time point for infusion is in part related to the underlying physiological process of myocardial healing. Within the first 24 hours after AMI the ischaemic cardiomyocytes undergo necrosis and cell swelling, by day 3-4 granulation tissue forms with new capillary formation. During the following weeks the granulation tissue migrates inwards toward the centre of the infarct maturing with the increase in connective tissue. The proposed improvement in cardiac function post AMI with stem cell infusions will therefore be related to the stage of physiological healing process the myocardium is undergoing.

1.6.7.1.2. Preferred imaging modality for the assessment of intermediate end points

Within regenerative medicine imaging of the heart is essential in the evaluation of cardiac repair. For the majority of stem cell trials the measurable end points have included LVEF, infarct size and myocardial contractility. Early trials have used LV angiography or echocardiography. With the development of advanced cardiac imaging techniques such as CMR and Positron emission tomography-computed tomography (PET CT) later trials have utilised these imaging techniques. The benefit of the later techniques have included further quantification of myocardial damage and viability as well as better reproducibility.

1.7. Research Trials into the use of Cardiac Magnetic Resonance

Imaging in Acute Myocardial Infarction

The use of magnetic resonance imaging (MRI) has been used within clinical medicine for over 20 years and has played an important role in the diagnosis and management in the fields of musculoskeletal medicine and neurology. The improvements in the last decade in resolution and reduced scan timings have resulted in the ability to perform dynamic scans. Since the first description of the behaviour of magnetic imaging after AMI in dogs the interest has grown within cardiology (Higgins, Herfkens et al. 1983).

The ability for high reproducibility and accuracy of images including the function and tissue characterisation of the myocardial in multiple views has made CMR the gold standard in cardiac imaging post AMI. However, the use of CMR should also be regarded as part of a multitude of imaging modalities within cardiology. The ability for a rapid scan in acute circumstances render the use of CMR difficult so methods such as transthoracic echocardiography will remain the method of choice in these cases for the coming years. Another weakness of CMR is the inability to image certain cardiac patients with pacemakers and ICD, although some modern devices are CMR compatible, although they do produce image artefacts.

As previously mentioned CMR has advanced to allow for detailed characterisation of cardiovascular structural and functional phenotypes of HF. This versatility and power of CMR has been able to address key issues in the evaluation of new novel therapy within many CVD.

1.7.1. Cardiac Imaging: Angiographic Area at Risk and Myocardial Salvage

CMR has become the gold standard in the quantification of ventricular volumes, function and tissue characterisation (Lorenz, Walker et al. 1999). Myocardial oedema can be one of the early manifestations of ischaemia and can occur without irreversible myocardial damage. Myocardial oedema has recently been used to quantify the area at risk (AAR) after AMI. The size of the AAR plays a

major determination in the final infarct size (Reimer and Jennings 1979). T2 short tau inversion recovery (T2-STIR) imaging is very sensitive in myocardial AAR after AMI (Aletras, Tilak et al. 2006). In combination with delayed gadolinium enhancement it can differentiate between irreversible and reversible damage after reperfusion to measure myocardial salvage index (MSI) (Friedrich, Abdel-Aty et al. 2008).

1.7.2. Cardiac Imaging: Stem Cell Trials

The assessment of LV function is an important factor in patients' management and outcomes (White, Norris et al. 1987). Within cardiac phase II trials several diagnostic methods have been used to evaluate LV function these include 2-dimensional echocardiography, left ventriculography, cardiac computed tomography and CMR imaging.

Each of these techniques have been used within cardiovascular stem cell research although each technique does have its benefits and limitations. Echocardiography is often used in acute trials as it offers fast inexpensive functional imaging of the LV, however due to the need to have optimal acoustic windows, the quality is dependent on the operator skills and patients chest wall anatomy. Early acute trials used left ventriculography after PCI was performed; this method is an invasive imaging technique (see chapter 2.7.3.4.1) however this method does have the drawbacks that include 2D image acquisition that suffers the same LV geometrical assumptions that echocardiography provides. Cardiac

CT had been shown to be reliably accurate in assessing the coronary arteries, as well as that of LV function (Budoff, Dowe et al. 2008, Wu, Tadamura et al. 2008). CMR is now considered to be the method of choice for the assessment of global and regional LV function (Bellenger, Burgess et al. 2000). In addition to volume measurement, CMR is able to give structural assessment that these other images techniques are unable to provide such as infarct size and AAR.

All of the early cardiovascular stem cells trials have used LV function as its surrogate primary end point marker. The range of imaging sequences that CMR provides has given further ability to assess the effect of regenerative medicine.

The majority of trials to date have used only a single imaging technique to assess the end points, however it would be beneficial when designing a future study to include several different imaging modalities to help answer which is the best imaging modality to be used in cardiac regenerative medicine; in addition to provide comparison to previous trials.

1.7.3. REGENERATE-AMI Trial

The REGENERATE-AMI is the first trial of autologous bone marrow derived cells delivered to patients less than 24 hours after presentation with AMI. This trial will demonstrate whether such early delivery is achievable and safe as well as completing the time course for cell administration that has previously been investigated (Hamshere, Choudhury et al. 2014). This time point is the closest to that used in the preclinical models, which demonstrated a significant benefit in restoration of cardiac function. In addition patients underwent CMR, TTE and LV angiography to help provide further mechanistic information using the latest advanced imaging techniques.

MD Res Hypothesis and Aims

Hypothesis

The administration of autologous bone marrow derived progenitor cells (in addition to standard medical therapy) within 24 hours of primary PCI for anterior AMI leads to improvement in LVEF compared to standard medical therapy alone.

Specific Aims

- 1). To demonstrate that the use of the IC infusion of autologous bone marrow derived progenitor cells within 24 hours of primary PCI following an anterior AMI is safe and feasible.
- 2). To demonstrate whether the administration of autologous bone marrow derived progenitor cells (in addition to standard medical therapy) leads to improvement in LVEF.
- 3). To assess whether the mechanisms underlying changes in LV function, dimension and scar burden in patients treated with early IC infusion of BMNC is related to changes in circulating cytokines.
- 4). To assess the feasibility of 3 slice T2-STIR short axis stack imaging for assessing myocardial oedema and the correlation to AAR compared to other assessment measures in patients who suffer a AMI.

Chapter 2

General Methods

2.1. Study Design

The design of the REGENERATE-AMI trial followed the early small clinical trials that had translated the seminal work of Orlic et al in 2001 that showed the therapeutic benefit of allogeneic bone marrow derived cells in a mouse model of AMI (Orlic, Kajstura et al. 2001). The time point used in the REGENERATE-AMI trial was the closest used to Orlic et al, in addition the inclusion of only anterior AMIs targeted the patient population most likely to benefit from therapy (Richardson, Bertaso et al. 2013)

The study performed employed a double-blind, randomised, multi-centre, placebo controlled trial to determine if the early use of IC infusion of BMNC improved LVEF after an anterior AMI (REGENERATE-AMI). The REGENERATE-AMI trial was designed to establish for the first time whether the early infusion of BMNC (within 24 hours of primary PCI) is safe, beneficial and feasible when used as an adjunct to current 'gold standard' therapy for an anterior AMI. The recruiting centres that participated were The London Chest Hospital (London, UK), Royal Free Hospital (London, UK), Heart Hospital (London, UK), Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland) and Rigshospitalet (Copenhagen, Denmark).

2.2. Ethics and Trial Registration

The REGENERATE-AMI trial was performed in accordance with the Declaration of Helsinki (1993) and the principles of the International Conference of Harmonization– Good Clinical Practice (ICH-GCP) guidelines, and was approved by a local ethics committee (NRES Committee London West London: 07/Q0603/76). The trial was registered with a public registry <http://clinicaltrials.gov> (NCT00765453). The trial protocol design was published in 2014 (Hamshere, Choudhury et al. 2014).

2.2.1. Patient selection

Consecutive patients presenting to each of the recruiting centres were approached up to 18 hours post successful primary PCI for an anterior AMI. Patients who attend the recruiting centres out of normal working hours (outside weekday 9am to 5pm and weekends) were approached in normal working hours if within the 18 hour time period. Potentially eligible patients were identified by the trial teams at each of the recruiting centres and were approached either prior to successful primary PCI or afterwards on the ward. They had the trial explained to them and were given a summary patient information sheet (PIS) (APPENDIX I) and a full version PIS (APPENDIX II), after which patients were left to read the PIS. If patients agreed to participate, they were asked to provide written consent (APPENDIX III). In addition to this patients were consented for viral blood screening (APPENDIX IV) in accordance to the research protocol. In addition to

written consent for the trial patients provided written consent for bone marrow aspiration (BMA) and infusion procedure.

2.2.2. Inclusion and Exclusion criteria

2.2.2.1. Inclusion criteria:

- Patients aged between 18 to 80 years
- Acute anterior myocardial infarction (ST elevation in at least 2 contiguous anterior leads ≥ 0.2 mV) and treated with acute primary PCI with stent implantation within 24 hours after symptom onset
- Acute primary PCI / stent implantation has been successful (residual stenosis visually $< 30\%$ and TIMI flow ≥ 2)
- At the time of inclusion patient no longer requires i.v. catecholamines or mechanical hemodynamic support (aortic balloon pump)
- Significant regional wall motion abnormality on LV angiogram in left anterior descending (LAD) territory at the time of acute primary PCI
- Written informed consent

2.2.2.2. Exclusion criteria:

- Regional wall motion abnormality not consistent with culprit vessel
- Need to revascularise additional vessels, outside the infarct artery as a planned procedure (these vessels can be treated at baseline)
- Arteriovenous malformations or aneurysms
- Active infection, or fever or diarrhoea within last 4 weeks
- Chronic inflammatory disease
- Known human immunodeficiency virus (HIV) infection or active hepatitis
- Neoplastic disease without documented remission within the past 5 years
- Cerebrovascular insult within 3 months
- Impaired renal function (creatinine > 200mmol) at the time of cell therapy
- Significant liver disease (GGT > 2x upper limit) or spontaneous INR > 1,5)
- Anemia (haemoglobin < 8.5 mg/dl)
- Platelet count < 100.000/ μ l
- Hypersplenism
- Known allergy or intolerance to clopidogrel, heparin or abciximab
- History of bleeding disorder
- Gastrointestinal bleeding within 3 months
- Major surgical procedure or trauma within 2 months
- Uncontrolled hypertension
- Pregnancy

- Mental retardation leading to inability to obtain informed consent
- Previously performed stem / progenitor cell therapy
- Participation in another clinical trial within the last 30 days

2.2.3. Data collection and Statistical analysis

To calculate the target sample size for the trial, an absolute increase in ejection fraction of 6% compared to baseline as measured by CMR were considered significant and consistent with previous studies that used advanced imaging to measure end-points (Wollert, Meyer et al. 2004). The published standard deviation for these observations is approximately 8% and in keeping with what has been observed by Janssens et al (Janssens, Dubois et al. 2006). For a statistical power of 90% and a probability of a Type I error of 0.05 using a two-sided t-test, with an estimated 25% drop-out at 1 year we will recruit a total of 100 patients in this study. Previous trials have shown a 2% improvement in patients who underwent primary PCI alone (Petronio, Rovai et al. 2003). Therefore using this information we will perform a post hoc analysis to compare the two groups, which will have 80% power to detect a 4% difference.

The analysis of the data was based on the intention to treat principle. Baseline demographic and clinical variables are summarised for each randomised arm of the study. Continuous variables will be presented as mean (SD) or median (IQR). Categorical data are presented by frequencies and percentages.

The statistical comparisons within the treatment arms with respect to the primary endpoint will be performed using the independent-samples t-test as the principal analytic tool. Further statistical comparison between treatment arms with respect to the primary endpoint, will be performed using the analysis of covariance (ANCOVA), including LVEF values at 12 months as dependent variables, the associated baseline values and the factor treatment as independent variables. Estimates of the treatment effect are presented together with the 95% confidence interval. Comparisons will be between the stem cell-treated and placebo control-treated group for the primary and secondary outcomes. Estimates of the treatment effects will be presented together with 95% confidence intervals. All p-values are 2-sided (Surder, Schwitter et al. 2010).

For clinical outcomes such as the incidence of MACE (predefined clinical scenarios; death, myocardial infarction, readmission for HF or revascularisation), Kaplan-Meier curves displaying the pattern of events over the 6 and 12-month follow-up period are drawn. Statistical significance and 95% confidence intervals are calculated using Cox's proportional hazards model. All statistical analyses were performed using SPSS version 19 (IBM Corp. Armonk, NY, USA) and graphs produced using Graphpad Prism version 5.0 (GraphPad Software, San Diego, CA).

2.2.4. Randomisation

Patients who consented for the REGENERATE-AMI trial were randomised using dedicated trial software IHD CLINICAL™ (Bishops Stortford, UK). IHD CLINICAL™

is a web-based password secured and encrypted data management system that has been designed specifically for clinical trials. All members of the REGENERATE-AMI trial teams at each recruiting centre had an individual password and any change was recorded. After registering patients on IHD CLINICAL, patients would undergo a 1:1 block randomisation allocation to receive either the IC infusion of autologous BMNC or the IC infusion of placebo. The placebo infusate had a colour characteristic similar to the IC stem cell suspension making them indistinguishable from each other. All study personnel and patients were blinded to treatment allocation until the study had been completed and analyses performed.

2.3. Primary Percutaneous Coronary Intervention (PCI)

The PCI procedure was performed as per operator preference with no restriction of procedural route or interventional technique (pre-dilatation or direct stenting). Bystander PCI was allowed at time of index event, however staged PCI procedure at a later date was not permitted.

2.3.1. LV ventriculography

LV ventriculography was performed after primary PCI in the 30-degree right anterior oblique (RAO) position and recorded at a minimum of 15-30 frames per second. Contrast was injected through a standard pigtail catheter over 10 seconds, with at least 2 well opacified sinus beats that were not post-premature

beats. All LV ventriculograms had simultaneous electrocardiogram (ECG) recording during injection as well as pressure measurement within the aorta and LV prior and after contrast administration.

2.4. Bone marrow aspiration and Processing

2.4.1. Bone Marrow Aspiration

All patients who were randomised into the REGENERATE-AMI trial underwent a BMA procedure. A fully trained member of the research team performed this BMA. The patient was placed on their side and landmarks for the posterior superior iliac spine were assessed. This region was then cleaned (in accordance with the recruiting centres local policy on antibacterial skin preparation) and draped with a sterile drape. Local anaesthetic was administered from the epidermis to the posterior superior iliac spine. A heparinised BMA needle was used to aspirate 100mls of bone marrow into 20 luer-lock syringes and placed into a sterile specimen bag for transport to the recruiting centres local stem cell processing unit. (Figure 2.1 and Figure 2.2)



Figure 2.1. Sterile trolley prepared for bone marrow aspiration

A - Sterile swabs, B - Chlorhexidine, C - Sterile transfer bags, D - 10mls of 1% Lidocaine in sterile syringe, E - Sterile IV bungs, F - Bone marrow Jamshidi needle, G - Sterile drape, H - 10mls luer-lock syringe with 1 ml of 1:1000 heparin, I - Sterile gauze

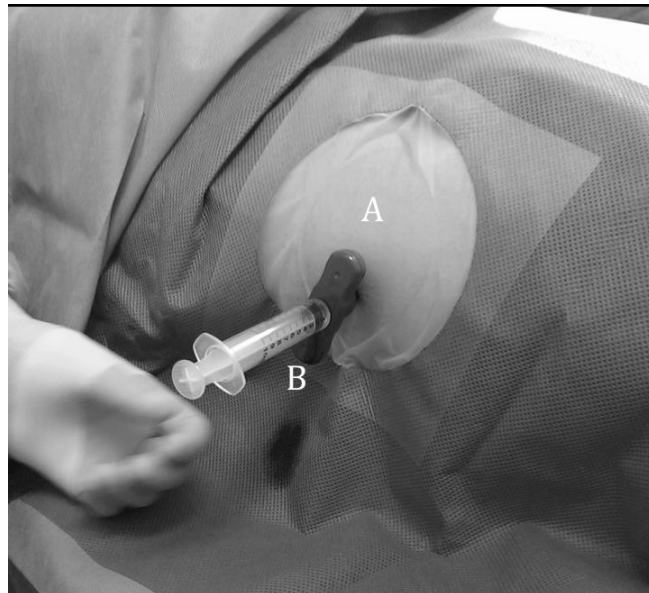


Figure 2.2. Bone marrow aspiration procedure

A - Sterile and covered patient, B - Inserted BMA needle with attached aspiration syringe

2.4.2. Bone marrow processing

The procedure of bone marrow processing was carried out in the recruiting centres cell culture laboratory using a standardised protocol. The three London based recruiting centres (The London Chest Hospital, Royal Free Hospital, Heart Hospital) used the same stem cell laboratory (Stem Cell Laboratory, Barts Health NHS Trust and Blizzard Institute). The bone marrow processing method was based on previous work by Schächinger et al (Schachinger, Assmus et al. 2004) to GCP standard.

2.4.3. Intracoronary Stem Cell preparation

Progenitor cell infusate preparation was performed in a laminar flow cabinet. 100mls of bone marrow was mixed in a transfer bag using sterile luer locks syringes connected to the bags coupling site. The 100mls of bone marrow was diluted by adding 100mls of 0.9% saline via the cleaned coupling site and mixed thoroughly. A platelet transfer set was connected to the coupling site, a 3 way tap connected to the end of the platelet transfer set and 1.5mls of bone marrow harvest was aspirated from the transfer bag. This 1.5 mls of bone marrow harvest was then transferred equally into 1x2ml cryovial and two 'Bact Alert' culture bottles. Patient identified 50ml conical tubes were placed into a sterile tube rack with their lids removed and 15mls of Ficoll solution was placed in each conical tube. The harvest was drawn into a 50ml syringe that was placed at the end of the platelet transfer set with a 200µm filter. The harvest was placed on top of the Ficoll solution so no mixing occurred in the 50ml conical tubes (Figure 2.3). To ensure all the bone marrow aspirate was utilised the bag and filter were

washed with 0.9% saline and placed onto the bone aspiration sample in the 50ml conical tube. Once all bone marrow has been layered onto the Ficoll, the lids were tightly screwed onto the tube and placed in a centrifuge at 1900rpm for 30 minutes.

During centrifugation the laminar flow cabinet was cleaned and a further 4-8 conical tubes were labeled. On removal of the centrifuged conical tubes the small band of mononuclear cells (Figure 2.3) were transferred to the empty 50ml tubes, with the remainder of the liquid discarded. The new 50ml conical tubes containing mononuclear cells were centrifuged at 2500rpm for 10 minutes, after which the supernatant was discarded. To the 50ml conical tubes 5ml of re-suspend was added and all 50ml conical tubes solutions were combined to a single tube. This single conical tube was centrifuged for 2500rpm for 10 minutes after which the supernatant was discarded. The tube was then filled up to 25ml with re-suspend and repeat centrifugation at 2500rpm for 10 minutes with supernatant discarding at the end. The reconstitute was then passed through a BD Falcon Cell trainer 100 μ m and rinsed with 1ml of 0.9% saline. Samples were sent as before for analysis and sterility checking, the remainder of the cell suspension was made up to 10mls with 0.9% saline and placed in a sterile labeled luer-lock syringe (infusate).

Placebo was created by the combination of serum and 0.9% saline in a sterile labeled luer-lock syringe. The colour of the placebo was such so it matched the infusate in the stem cell prepared syringe.

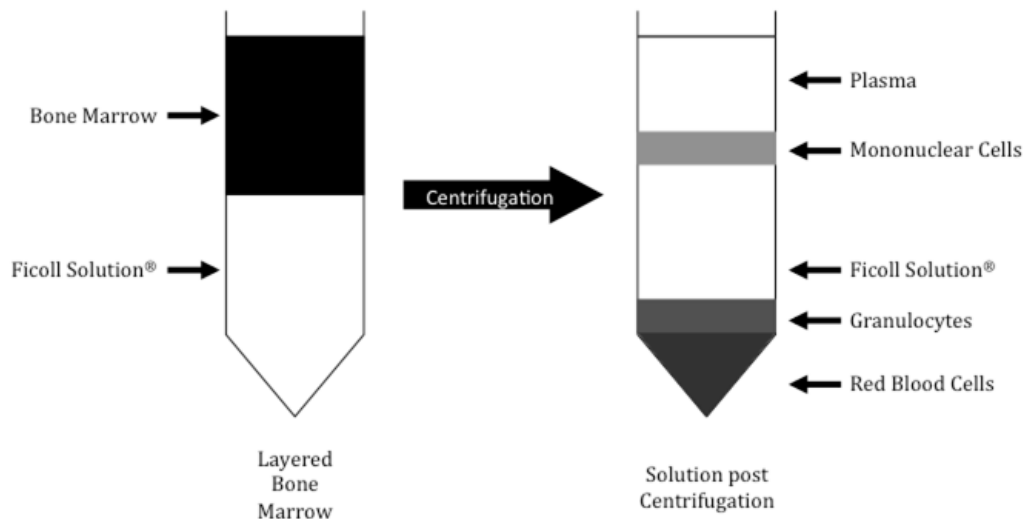


Figure 2.3. Density Gradient Separation

2.5. Intracoronary infusion

Patients underwent the IC infusion of either BMNC or placebo within 24 hours of primary PCI, ideally within 6-8 hours when possible. The processed infusate was returned to the recruiting centres catheterisation laboratory in a sterile sealed specimen bag. The infusion procedure was based on a protocol used in previous positive clinical trials (Schachinger, Erbs et al. 2006).

Patients underwent the reinfusion procedure from either the radial or femoral access route, a bolus of body weight adjusted dose of heparin was given so that the ACT remained < 200 throughout the procedure. A guide catheter was used to intubate the left main coronary ostium and diagnostic pictures were acquired, with estimation of coronary blood flow and the integrity of the treated vessel documented and assessed. A 0.014-inch guide wire was passed to the distal part of the left anterior descending artery and a 10mm “over-the-wire” balloon was placed within the stented segment of the left anterior descending artery. The

“over-the-wire” balloon’s diameter was ± 0.5 mm the size of the implanted stent, so to achieve complete occlusion of the vessel at nominal pressure; with angiographic occlusion assessed prior to infusion. A total of 10ml of the infusate was infused in three portions (each 3.3ml) during three minutes occlusion time for each portion. After each infusion the “over-the-wire” balloon was deflated for three further minutes, prior to the next occlusion in order to avoid extensive ischaemia. This method has been used in previous trials with the aim to allow for adhesion and potential transmigration of the infused cells through the endothelium (Assmus, Schachinger et al. 2002).

Patients returned to the ward after the infusion underwent standard ward based care including cardiac monitoring for a minimum of 24 hours post procedure. Any complications during or after the IC infusion were documented. The patients’ clinical care was carried out by the clinical team with length of stay and medical therapy at the discretion of the treating physician.

2.6. Study Endpoints

2.6.1. Primary Endpoint

The primary efficacy endpoint was change in global LVEF assessed by advanced cardiac imaging (in patients who were clinically unable to undergo CMR cardiac CT was performed as an alternative) at 12 months compared to baseline. The choice of change in global LV function was decided as to allow comparison previous studies (Mills and Rao 2007, Surder, Schwitter et al. 2010).

2.6.2. Secondary Endpoints

2.6.2.1. Secondary Imaging Endpoint

Additional advanced cardiac imaging related secondary endpoints were change in left ventricular volumes, myocardial salvage and infarct size at 3 months and 1 year. LV ventriculography secondary endpoint was change in global LVEF at 6 months. Echocardiographical secondary endpoint was change in LVEF as calculated by Simpson's rule and change in wall motion scoring at 3 days, 6 months and 12 months.

2.6.2.2. Secondary Safety Endpoint

Safety endpoints included the acute safety and tolerability of IC stem cell infusion following AMI (haemodynamics and in-patient major adverse events (MACE) occurring after stem cell infusion, including Death {independent of cause and sudden death}, myocardial infarction {Q-wave and Non-Q-wave}, vascular complications, bleeding, and any ventricular rhythm disturbance during infusion). Additional safety endpoints included assessment of MACE at 3 months, 6 months and 12 months.

2.6.2.3. Secondary Clinical Endpoint

Clinical secondary endpoints included change in HF status as measured by NYHA (Table 2.1), angina symptoms as measured by Canadian Cardiovascular Society (CCS) (Table 2.1), evidence of HF (defined as physical signs of pulmonary crackles/rales, peripheral oedema, jugular venous distension, S3 gallop, radiological evidence of pulmonary oedema), number of HF admissions and changes in medications.

Table 2.1. New York Heart Association and Canadian Cardiovascular Society classification and functional status

NYHA Class	Functional Capacity
I	No limitation of everyday physical activity
II	Slight limitation of everyday physical activity
III	Marked limitation of everyday physical activity
IV	Unable to carry out any physical activity / bedbound

CCS Class	Functional Capacity
I	Angina only on strenuous or prolonged activity
II	Slight limitation with angina during vigorous activity
III	Moderate limitation with symptoms on everyday physical activity
IV	Severe limitation with angina on limited physical activity or at rest

2.7. Data Collection

2.7.1. Blood sampling

All patients underwent routine clinical blood tests prior to primary PCI. These bloods included routine full blood count, renal function, clotting, glucose, liver function, creatine kinase (CK), serum cholesterol levels, and troponin T. The attending clinical team responded to abnormal routine blood results at baseline. Consented patients had further blood test at the time of BMA for virology testing (HIV, Hepatitis B and C, HTLV and Syphilis). At the London Chest recruiting centre only, additional blood was taken in a citrate bottle, EDTA bottle, and 2 serum bottles. These were left to stand for 10 minutes after which they were spun in a centrifuge at 1000g for 10 minutes. The serum was then pipetted into patient unique identifier number and time stamped cryovials. Three 500ul samples from the citrate, EDTA and 500ul from the serum sample were stored in a -80°C freezer, the final serum tube sample was sent to a central reference

laboratory for analysis for N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) within UK sites.

2.7.2. Quality of life questionnaires

To assess patient quality of life, patients were given an EQ5D, MacNew health questionnaire and SF36 questionnaire during their admission (Brazier, Roberts et al. 2004, Hofer, Lim et al. 2004). This standardised questionnaire was a health outcome measurement tool and will be used throughout follow up.

2.7.3. Advanced Cardiac Imaging

2.7.3.1. Cardiac MR (CMR)

CMR is the gold standard for the assessment of LV volumes and tissue characterisation (Lorenz, Walker et al. 1999, Schelbert, Hsu et al. 2010). CMR imaging was performed on a 1.5 Tesla (T) scanner with a cardiac 32-channel phased array coil. Each examination used cine-CMR for ventricular volumes and function, and delayed enhancement (DE)-CMR for infarct size assessment and evaluation of microvascular obstruction (MVO). Myocardial oedema was assessed at all time points using 3 slice T2-weighted triple inversion turbo spin echo STIR imaging. The CMR scan protocol commenced with acquisition of a stack of localizer images in the transaxial plane with a half-fourier acquisition single-shot turbo spin echo (HASTE) sequence during free breathing. From these images, a

vertical long-axis (VLA) cine image was obtained using a balanced SSFP sequence with breath-holding in end-expiration. Five single steady-state free precession (SSFP) short-axis (SAX) scout images were acquired from the VLA image, covering the base to mid-ventricle. Breath-hold SSFP cine images in 2-, 3- and 4-chamber views were piloted from the short-axis scout and VLA images. Finally, the 4-chamber and 2-chamber cine images at end-diastole were used to plan a stack of short-axis SSFP cine images. Contiguous short-axis slices were then acquired at 10mm intervals (7mm slice thickness, 3mm gap) from base to apex, ensuring complete ventricular coverage. For cine imaging, 25 frames or phases were acquired per cardiac cycle with an approximate temporal resolution of 30-40ms. Images were routinely obtained with retrospective electrocardiographic gating, to allow the entire cardiac cycle to be acquired; typical sequence parameters for the SSFP cines were TE 1.6ms, TR 3.2 ms, in plane pixel size 2.1 x 1.3mm, and flip angle 60°.

T2 STIR imaging occurred prior in injection of gadolinium with a short repetition time of 1800-2200ms and an inversion time of 120-130ms. The inversion time was used to null any fat signal, and breath-hold 4- and 2-chamber STIR views as well as 3 slice short-axis STIR images were acquired. Gadolinium enhanced imaging was performed by a peripheral injection of gadolinium (0.2 mmol/kg of body weight). A three-dimensional inversion-recovery T1-weighted sequence was used with a repetition time of 3.5 to 4.2 ms, an echo time of 1.2 ms, flip angle of 15° and patient image dependent inversion time of 200 to 300ms. For early gadolinium enhancement patients had images taken within the first 5 minutes of injection, for delayed gadolinium enhancement images were acquired at 15 to 20

minutes post infusion. Both early and delayed enhanced sequences included 2-, 3- and 4-chamber views as well as a 10-slice short axis stack.

Images were anonymised, batched and analyzed in blinded fashion by two experienced operators (Dr Stephen Hamshere and Dr Danielle Longchamp). LV volumes and LVEF were calculated by manually drawing endocardial and epicardial contours of the SAX cine (Figure 2.4). Scar (infarct size) and oedema (AAR) volumes were calculated by manually drawing endocardial and epicardial contours followed by semi-automated selection of normal remote myocardium per slice (Figure 2.5). Myocardial oedema was described as $>2SD$ in signal intensity from remote normal myocardium. Infarct and AAR endocardial surface area (ESA) was calculated as the percentage of the endocardial enhancement against the total endocardial area. Where cases of discordance were found between operators, a independent blinded review by a level III accredited CMR reader was performed (Dr Mark Westwood). Analysis was performed using a dedicated software system (CVI⁴², Circle Cardiovascular Imaging Inc, Calgary, Alberta, Canada). Interobserver variability was calculated. CVI⁴² is a commercially available software that has been widely used and validated for the assessment of LV volumes (Childs, Ma et al. 2011), infarct size and myocardial fibrosis (Vermes, Childs et al. 2013) and myocardial oedema (D, Ridgway et al. 2012).



Figure 2.4. LV function analysis in short axis images using long axis views as a cross-reference
 * - 4 chamber long axis view with cross sectional (green) reference levels shown

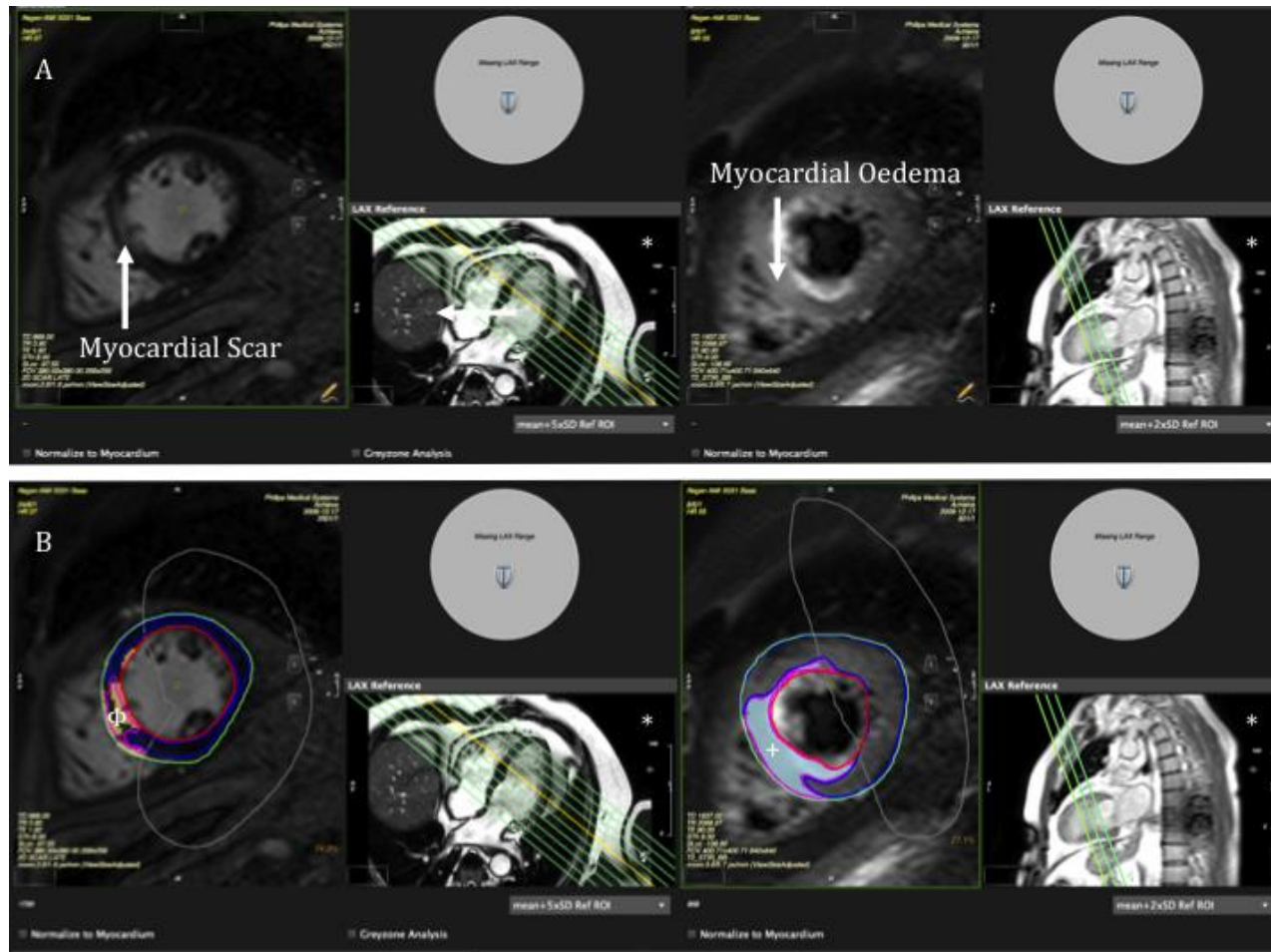


Figure 2.5. Screenshot of left ventricular scar and oedema analysis

A = without computer generated borders showing myocardial scar and myocardial oedema

B = Left view screen shows myocardial scar assessment; right view screen shown myocardial oedema assessment

Green contour – epicardial contour, Red contour – endocardial contour, Grey contour – excluded myocardium, Blue contour – Normal myocardium, Pink contour – abnormal myocardium, + – blue zone shows area of myocardial oedema, Φ – gold zone shown area of myocardial scar

2.7.3.1.1. Myocardial salvage index

When assessing the efficacy of a reperfusion treatment strategy, it is essential to express myocardial infarct size as a percentage of the AAR. In this study, the AAR was quantified using both coronary angiography (modified Bypass Angioplasty Revascularisation Investigation (BARI) (Rippel, Ghanbari et al.) (Alderman and Stadius, 1992) and the modified Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease jeopardy score (APPROACH) (Ortiz-Perez et al., 2007) and the acute (2 day) CMR scan (Myocardial oedema-T2 imaging). Myocardial infarct size as previously described. MSI was calculated as the ratio of the CMR determined infarct size and the AAR (Bøtker, Kaltoft et al. 2012).

2.7.3.2. Cardiac CT

A small number of patients who were unable to tolerate, or had a contraindication to CMR underwent Cardiac CT imaging. Studies were performed on an ECG-gated 64-slice spiral CT (Sensation 64, Siemens, Forchheim, Germany) scanner. Cardiac CT has provided accurate and reproducible assessment of both LV and RV volumes in comparison to CMR (Maffei, Messalli et al. 2012), and is a reliable alternative in patients who are unable to under CMR imaging.

Intravenous B-Blockers were used in patients to achieve optimal pulse rate prior to initiation of the scan, all patients were on cardiac monitors during administration. All scans were performed in the craniocaudal direction. An

initial scouting x-ray (topogram) was performed to ensure correct alignment of the patient for the remainder of the scan. The scan was initiated when contrast arrives within the ascending aorta, this can be performed manually after test bolus or using an automated CT triggering once the Hounsfield unit crosses a threshold in a set region of the aorta. A total of 50 – 100 mls of contrast was used for the scan with a duration of 10 – 15 minutes for the scan. Reconstruction algorithms were used to convert the raw data into interpretable images, which were then analyzed on specialist software. The data was reconstructed throughout the cardiac cycle and cine movie images are collected in 10 phases along the cardiac cycle and 10 levels. Analysis of LV volumes and function was performed using dedicated PC-based software Siemens (Forchheim, Germany) to calculate epicardial and endocardial borders in both systole and diastole (Keenan, Pugliese et al. 2014) (Figure 2.6 and Figure 2.7) by two experienced operators (Dr Stephen Hamshere and Dr Tawfiq Choudhury). Where cases of discordance were found between operators, an independent blinded review by a third operator was performed (Dr Ceri Davies). The Cardiac CT scans were performed at the same time points as the CMR scans.

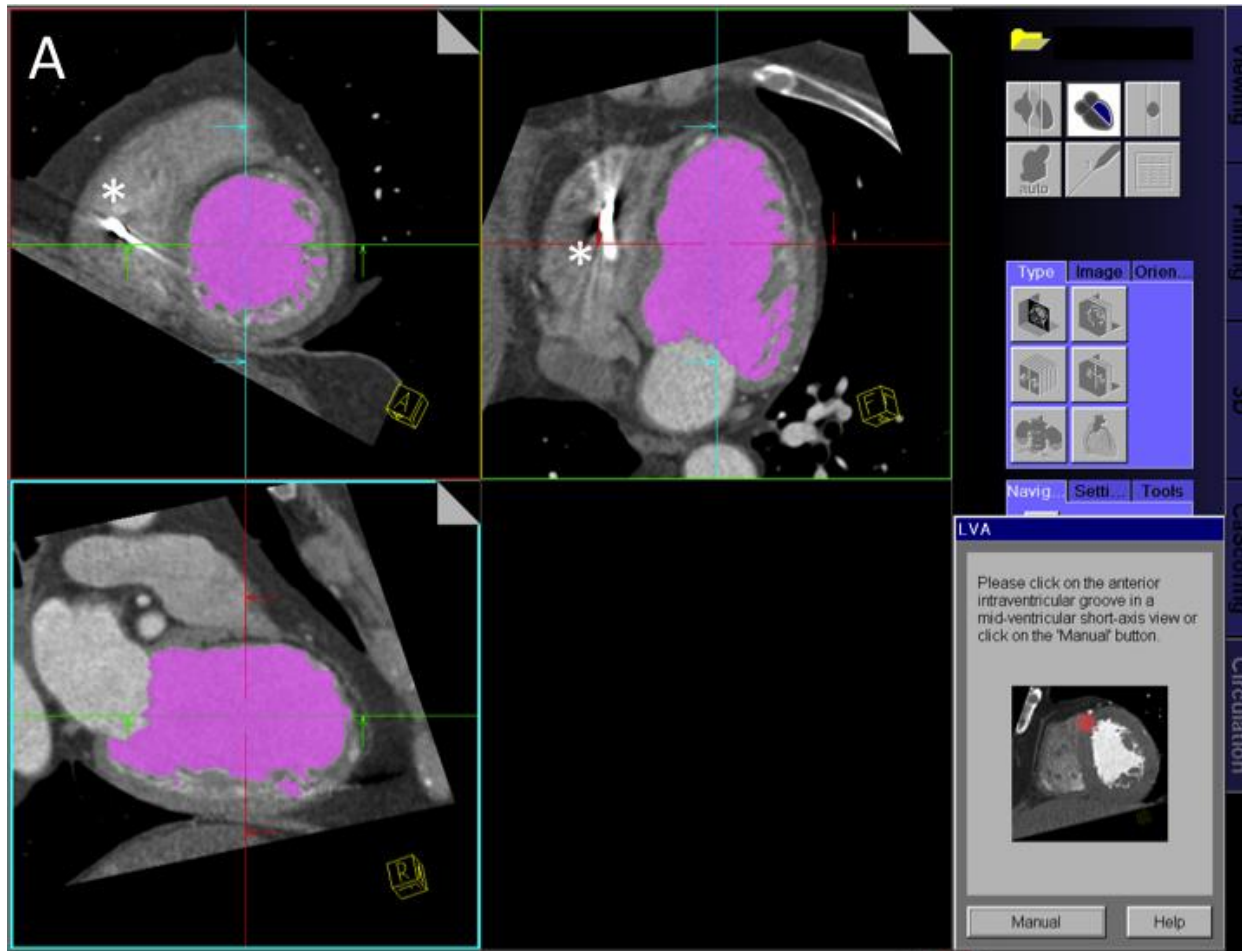


Figure2.6. Screenshot of analysis method left ventricular ejection fraction on cardiac CT

Top left is basal short axis view of RV and LV. Top right is 4-chamber view. Bottom left is 2 chamber view. Auto generated LV volumes in purple, * RV pacing lead

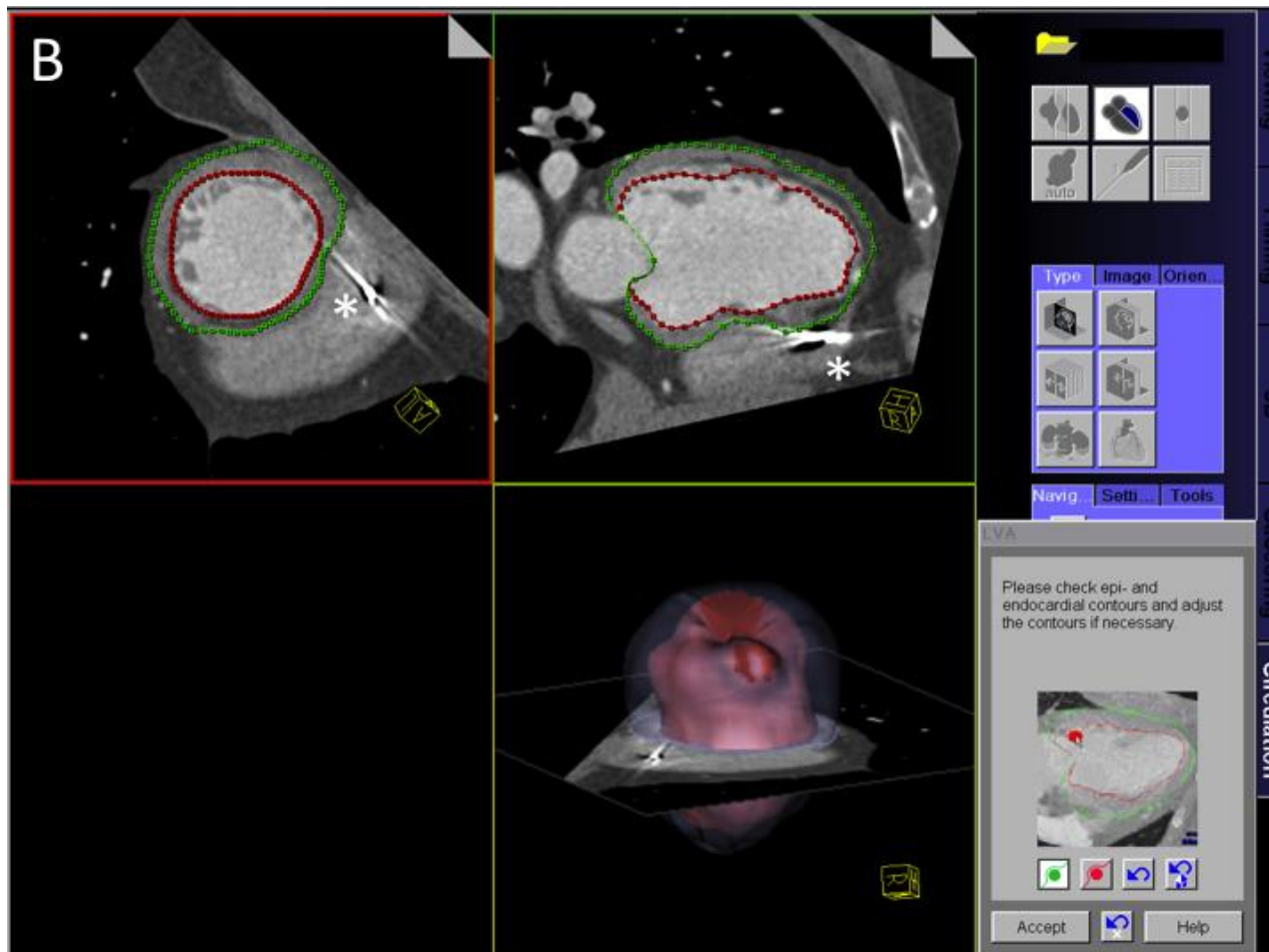


Figure 2.7. Screenshot of left ventricular ejection fraction on cardiac CT

B = Manual epicardial (Red contour) and endocardial (Green contour), * RV pacing lead

Top left is basal short axis view of RV and LV. Top right is 2 chamber view, bottom right is 3D LV map

2.7.3.3. Echocardiography

The imaging modality of echocardiography was chosen due to the availability directly after an AMI and the ease of getting repeated measurements. Patients underwent standard views as defined by the British Society of Echocardiography (Wharton, Steeds et al. 2012). These images were parasternal long axis (PLAX), parasternal short axis (PSAX), apical four chamber (A4C), apical two chamber (A2C), apical five chamber (A5C) and apical three chamber (A3C) (Wharton, Steeds et al. 2012). Echocardiograms were performed 3 days, 6 and 12 months after primary PCI. The scans were anonymised, batched and analysed by an experienced operator (Dr Stephen Hamshere and Dr Denis Pellerin). LVEF was calculated using the Simpson's Biplane method and regional wall was assessed using wall motion index.

2.7.3.4. Angiographic analysis

2.7.3.4.1. LV ventriculography

LV ventriculograms were performed prior to consent and directly after primary PCI and at 6 months. All images were analysed using a dedicated system (CMS version 6.0, Medis, Leiden, Netherlands) by tracing the endocardial border (Figure 2.8) by two-experienced operators (Dr Stephen Hamshere and Dr Tawfiq Choudhury)

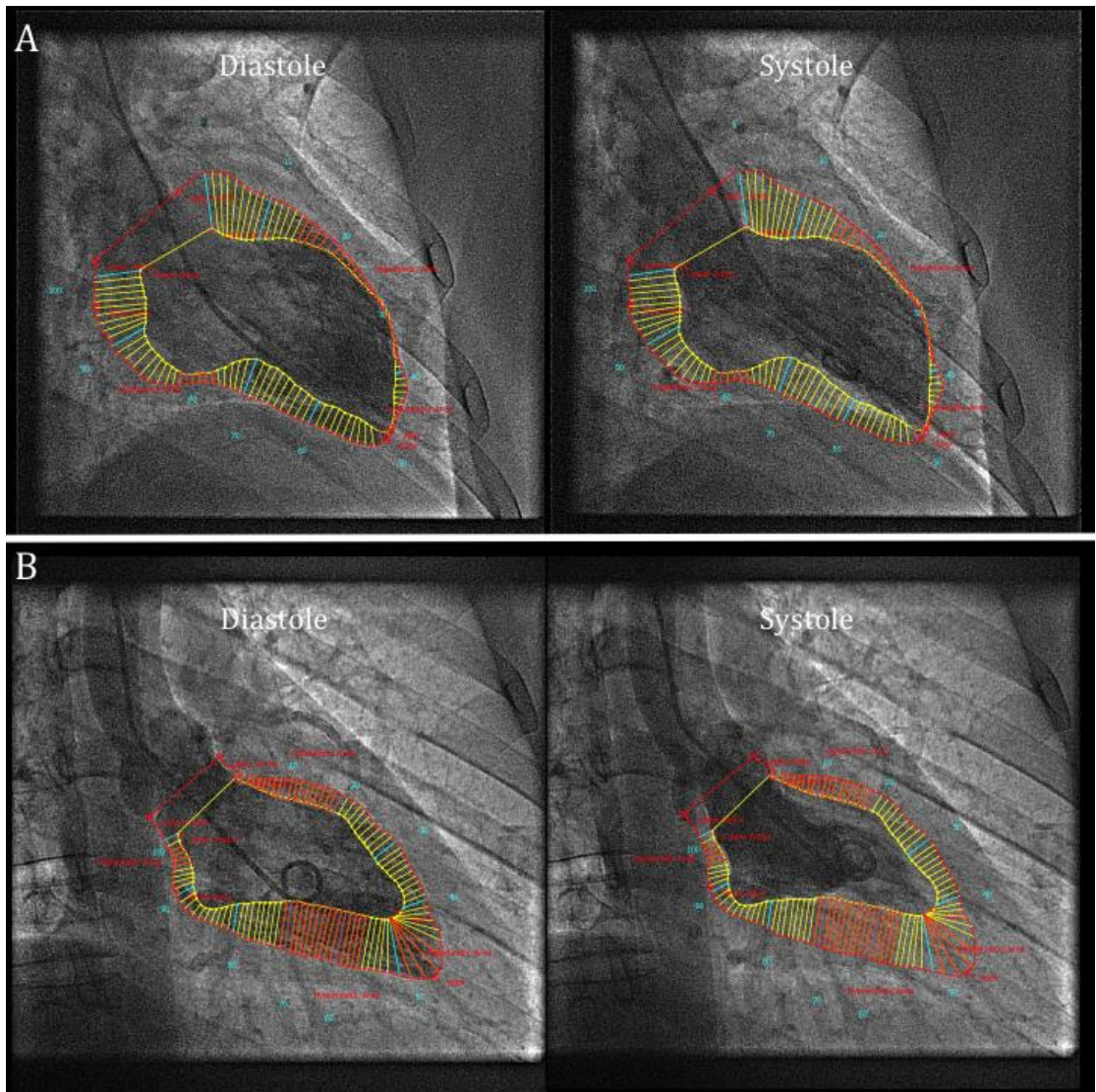


Figure 2.8. Quantitative Left Ventricular Analysis: Centreline method

A: Admission Left ventriculograms with diastolic and systolic endocardial mapping and centerline motion indexing

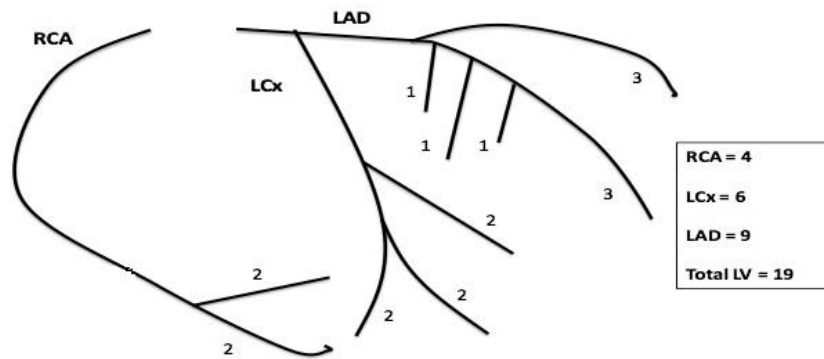
B: 6 month Left ventriculograms with diastolic and systolic endocardial mapping and centerline motion indexing

2.7.3.4.2. Angiographic risk scores (Area at risk)

Patients underwent coronary angiography prior and after primary PCI. All angiograms were reviewed by 2 experienced blinded observers and were assessed for the angiographic AAR using both the modified BARI and modified APPROACH jeopardy scoring systems. (Figure 2.9 and Figure 2.10).

Culprit lesion Location	Infarct related artery Side branches		Size		
			Diagonal (LAD)	Posterolateral (All others)	
			Small	Medium	Large
LAD (Right dominance or Left dominance)		Distal	13.75	14.8	15.9
		Mid	27.5	29.7	31.8
		Proximal	41.25	44.5	47.75
Proximal LCx (Right dominance)	Obtuse marginal	Small	9.25	12.5	15.75
		Medium	15.25	18.5	21.75
		Large	21.25	24.5	27.75
Proximal LCx (Left dominance)	Posterior descending artery (PDA)	Small	23.5	28	32.5
		Medium	29.5	34	38.5
		Large	35.5	40	44.5
RCA (Right dominance) Mid LCx (Left dominance)	Posterior descending artery (PDA)	Small	9.25	12.5	15.75
		Medium	15.25	18.5	21.75
		Large	21.25	24.5	27.75
Mid LCx (Right dominance)			3.25	6.5	9.75

Figure 2.9. The modified- Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease (APPROACH) angiographic myocardial risk score (Ortiz-Perez, Meyers et al. 2007)



RCA- right coronary artery, LCx- left circumflex artery, LAD- left anterior descending, LV- left ventricle.

Figure 2.10. Diagram to show the BARI angiographic myocardial risk score (Alderman and Stadius 1992)

2.8. Patient discharge

Patients' clinical care was under their attending clinical physician, their medical therapy was optimised as far as possible prior to discharge. All patients were on dual antiplatelet therapy for a minimum of 1 year, life long statin therapy if tolerated, as well as ACEi and B-blockade if indicated and tolerated.

Follow up

Patient follow up is shown in Table 2.2.

Table 2.2. Follow up investigations and measurements timeline

Procedures	Admission	< 24 hours	Day 3	3 months	6 months	12 months
Screening	X					
Physical examination	X		X		X	X
Consent	X	X				
Randomisation	X					
Bloods	X		X	X	X	X
ECG	X	X	X		X	X
primary PCI	X					
Echocardiogram		X	X		X	X
BMA		X				
Reinfusion		X				
QoL Assessment			X		X	X
Advanced Cardiac Imaging			X	X		X
Left Ventriculogram		X			X	

2.9.1. 3 month follow up

At 3 months follow up patients underwent a CMR and routine blood testing

2.9.2. 6 month follow up

At 6 months follow up patients underwent clinical examinations, repeat echocardiogram, routine bloods including NT-proBNP, left ventriculography and quality of life questionnaires.

2.9.3. 12 months follow up

At 12 months follow up patients underwent clinical examinations, CMR imaging, echocardiogram, quality of life questionnaire and routine bloods including NT-proBNP.

2.10. Chemokine analysis methods

Patients who were admitted to the London Chest Hospital who were enrolled in the REGENERATE-AMI trial consented to have additional blood tests taken at baseline, 3, 6 and 12 months. Patients who were recruited at other centres were not entered into the analysis.

2.10.1 Blood collection and storage

Peripheral blood samples were taken using the BD Vacutainer® system, blood samples were taken in EDTA tubes, serum and heparinised containers in

accordance to the manufactures specifications. All patients from the London Chest Hospital had bloods taken at baseline (directly after primary PCI and consenting for the trial and prior to stem cell infusion), patients were asked to consent for additional bloods at 3, 6 and 12 months. Blood samples were centrifuged for 10 minutes at 3000 revolutions per minute at room temperature. The serum was aliquoted and placed in labeled cryovials and stored at -80°C until analysis.

2.10.2. Serum defrosting

Samples were transferred for analysis in -80°C transport container. Samples were removed from the -80°C containers and were allowed to acclimatize to room temperature for 10 minutes. Each cryovial was placed in a 30-37°C sterile water bath, a constant thermostatic temperature measurement was maintained to reduce the risk of excessive temperature fluctuations and possible degradation of the samples. The cryovials were regularly gently shaken until completely thawed then removed to analyze.

2.10.3. Preparation of samples for FlowCytomix analysis

25µl of each standard, blank assay buffer solution and defrosted serum were transferred into the flow cytometry tubes, 25µl of bead mixture and 50µl of biotin-conjugate-mix was added to these samples and incubated for 2 hours at room temperature. After incubation the mixture was washed twice with 1ml of

buffer. 50µl of Streptavidin-Phycoerythrin solution was added to the samples with a further hour of incubation at room temperature followed by two washes and the addition of 400µl assay buffer was added to each tube.

For the analysis of MMP-9 the serum samples had to be diluted 1:1250 with milli-Q water. The processed samples were analyzed using a BD LSR Fortessa Cell Analyser ("BD LSRFortessa | Cell Analyzer | BD Biosciences, 2014).

2.10.4. FlowCytomix System

The serum cytokine concentrations were assessed using FlowCytomix kits (Bender Medsystems Inc. Austria), the FlowCytomix kit allows for multiple measurements of multiple cytokines. Flow cytometry is an effective technique that allows for the identification and analysis of various cytokines using specific fluorescence-labeled antibodies.

Within the FlowCytomix system a single file of cells pass through a hydrodynamic layer with a laser beam focused on this region. The fluorescence that is emitted is focused using a lens system and converted to electrical pulses that is translated into digital data.

The FlowCytomix system is a fluorescent bead immunoassay system where beads are coated with antibodies reacting to a specific analyte. The beads are incubated with the analyzing sample serum and then biotin-conjugated second antibody is

added. The beads are internally dyed with the addition of Streptavidin-Phycoerythrin (PE), which binds to the biotin conjugate, emitting fluorescent signals. Within the FlowCytomix there are two size of beads that makes it possible to distinguish 20 sets of single fluorescence channels and the simultaneous quantification of multiple analytes.

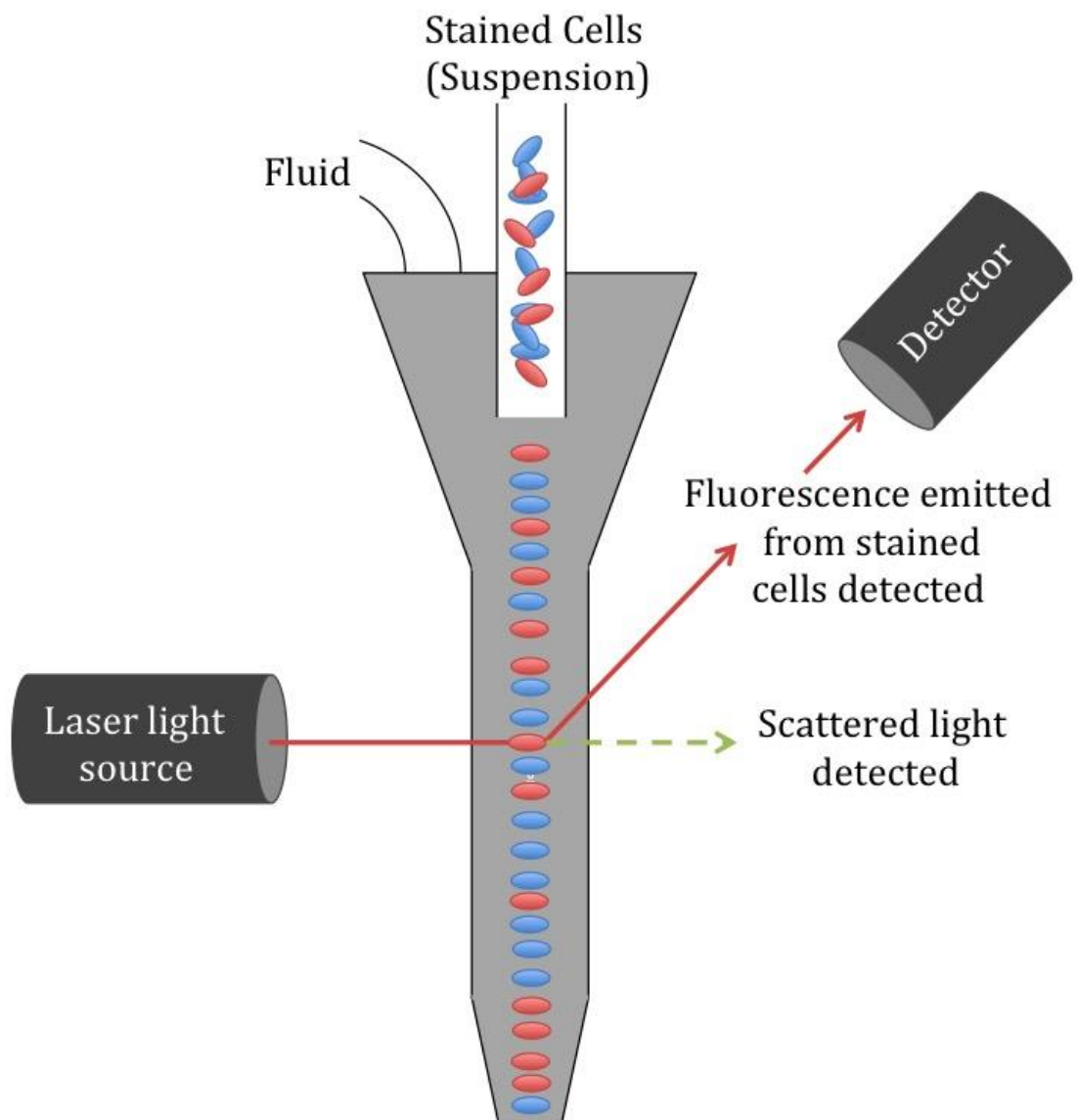


Figure 2.11. Schematic of Flow Cytometry

Fluorochromes employed to detect target proteins emit fluorescence when excited by a laser with the correspondent excitation wavelength. Each stained particle or cell is detected individually and the data analysed through sensors called photo multiplying tubes (PMTs) detecting fluorescent light filtered at a specific wavelength. PMTs are able to convert the energy into an electronic signal. As each fluorescing cell passes the laser beam a pulse of voltage, described as an event. Each event is displayed digitally.

Light from the laser results in a scattered patterned after coming in contact with the beads. Light that is scattered in a forward direction generates a forward scatter (FSC) parameter whilst light that is scattered elsewhere generates a side scatter (SSC) parameter. Each particle has a unique combination of FSC and SSC therefore allowing identification of multiple particles.

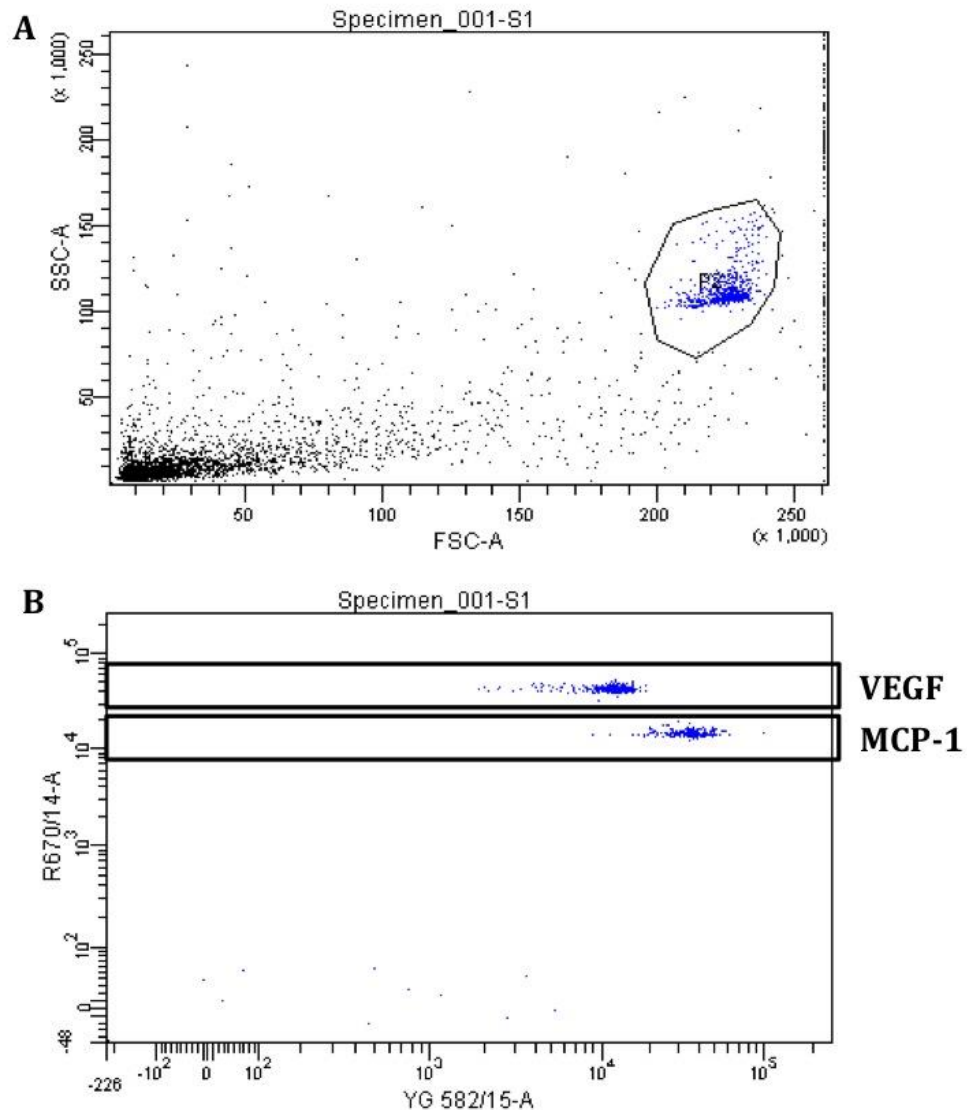


Figure 2.12. Flowcytomix Flow Cytometry

The antibody bead population was gated according to the FSC/SSC and the variable signal from the 700nm excitation probe (R670/14-A). This allows differentiation between MCP-1 and VEGF (B). The amount of cytokines for numerous data-points vary in the amount of reporter fluorescence PE. This allows standard curves to be generated from the median fluorescence intensity of the standards. Test sample MFI values are then read off the standard curves.

Analysis was performed by Dr Stephen Hamshere and a BMedSci student Mr Salem Shawaf, supervised by Dr Daniel Jones PhD. The detection limit of the assay and inter-assay and intra-assay coefficients are shown below (Table 2.3).

Table 2.3. Flowcytomix assay detection limit and inter- and intra assay coefficient of variance

Assay	Detection Limit	Inter-assay coefficient of variance	Intra-assay coefficient of variance
IL-6	7.2pg/ml	<10%	<10%
TNF- α	3.2pg/ml	8.9%	12.0%
VEGF	7.9g/ml	4.3%	6.2%
G-CSF	6.35pg/ml	7.6%	8.1%
MMP-9	0.05ng/ml	10.2%	7.3%

Chapter 3

REGENERATE-AMI Results

3.1. Introduction

As discussed in chapter 1, despite the advances in the acute management of AMI with the introduction of primary PCI centres, 30-day mortality is still estimated at approximately 7% despite optimal medical management (Widimsky, Wijns et al. 2010). Although prompt PCI results in decreased ischaemic injury it also can result in further damage associated with ischaemia-reperfusion injury, which can account for at least 50% of final infarct size (Yellon and Hausenloy 2007, Jones, Andiapen et al. 2013). The final infarcted myocardium undergoes a process of remodeling that is characterised by LV cavity dilation, dysfunction and subsequent HF (Pfeffer and Braunwald 1990).

The optimal time for the delivery of progenitor cells has been a topic of debate and remains unknown. The first trials used time points different to the first animal studies to reduce the risk of harm to the patients and increase the change of survival of the transplanted progenitor cells. The timeline for the infusion can now be placed and future clinical trials can compare the unanswered early and late stem cell therapy time points following primary PCI (Figure 3.1).

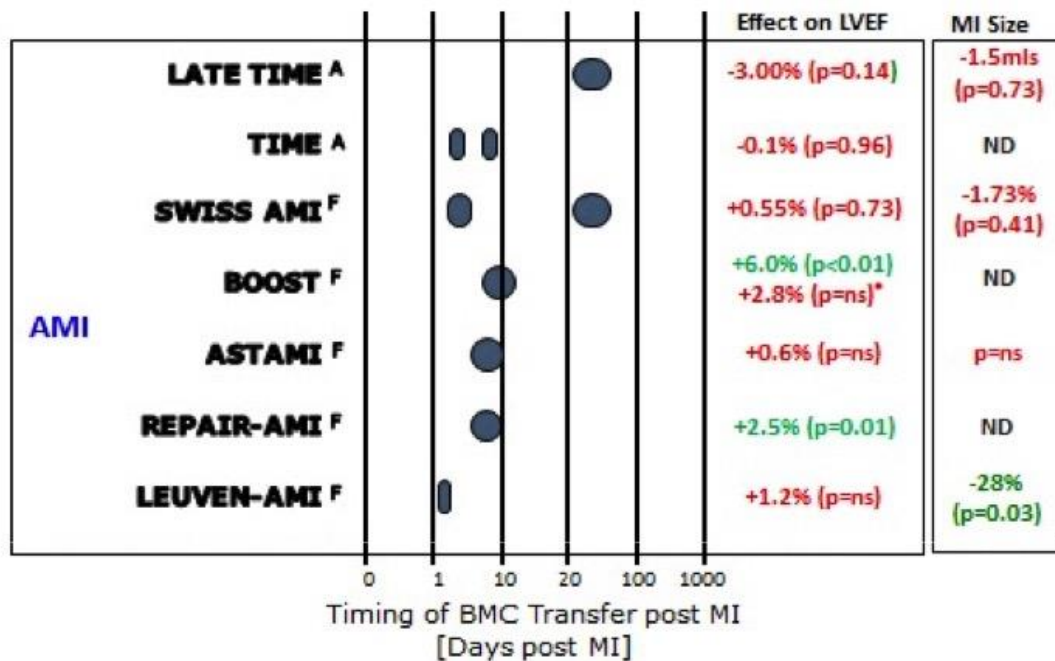


Figure 3.1. Cell delivery timeline of selected randomised control trials of bone marrow derived cell therapy in acute myocardial infarction (Choudhury 2014)

The relationship between time to delivery of progenitor cells after AMI and the change in LVEF and infarct size.

Green text donates trials with positive improvements in cardiac function whilst red text reflects trials in which the results did not reach statistical significance.

(ND-Not done/reported; ns-not significant; BMMC - Bone marrow-derived mononuclear cells: F-manual Ficoll cell preparation, A-Automated cell preparation of BMMC; *-18-months follow-up)

REGENERATE AMI was designed to test whether following primary angioplasty early infusion of autologous bone marrow derived cells is safe and would lead to an improvement in cardiac function.

3.2. Results

3.2.1. General characteristics of Study population

Between 19th March 2008 and 5th March 2013, 984 patients were screened after hospitalisation for AMI at 5 recruiting centres (The London Chest Hospital (London, UK), Royal Free Hospital (London, UK), Heart Hospital (London, UK), Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland) and Rigshospitalet (Copenhagen, Denmark)). 840 patients were evaluated and excluded for the following reasons: culprit vessels that were not the LAD (n=506), normal LV function (n=78), delayed presentation (n=59), intubated patients or on inotropes (n=61), Age < 18 or > 80 (n=49), Left ventriculography not performed (n=39), not suitable for IC infusion (n=28), participating in other trials (n=20). This left 144 suitable patients, of which 44 patients declined to participate in the study. The data is thus presented for the 100 patients (45 in control group and 55 in BMNC group, Figure 3.2).

The mean age for the total population was 56.50 ± 10.51 months and 87% were male. Patients who were randomised to BMNC had a longer ischaemia time than those who received placebo, however it was not significant (233.0 ± 38.15 vs 193.0 ± 26.52 minutes, $p=0.2257$). All baseline characteristics were similar between the treatment groups (Table 3.1). The two study groups were similar with respect to TIMI flow, medication use pre-PCI and stent type used.

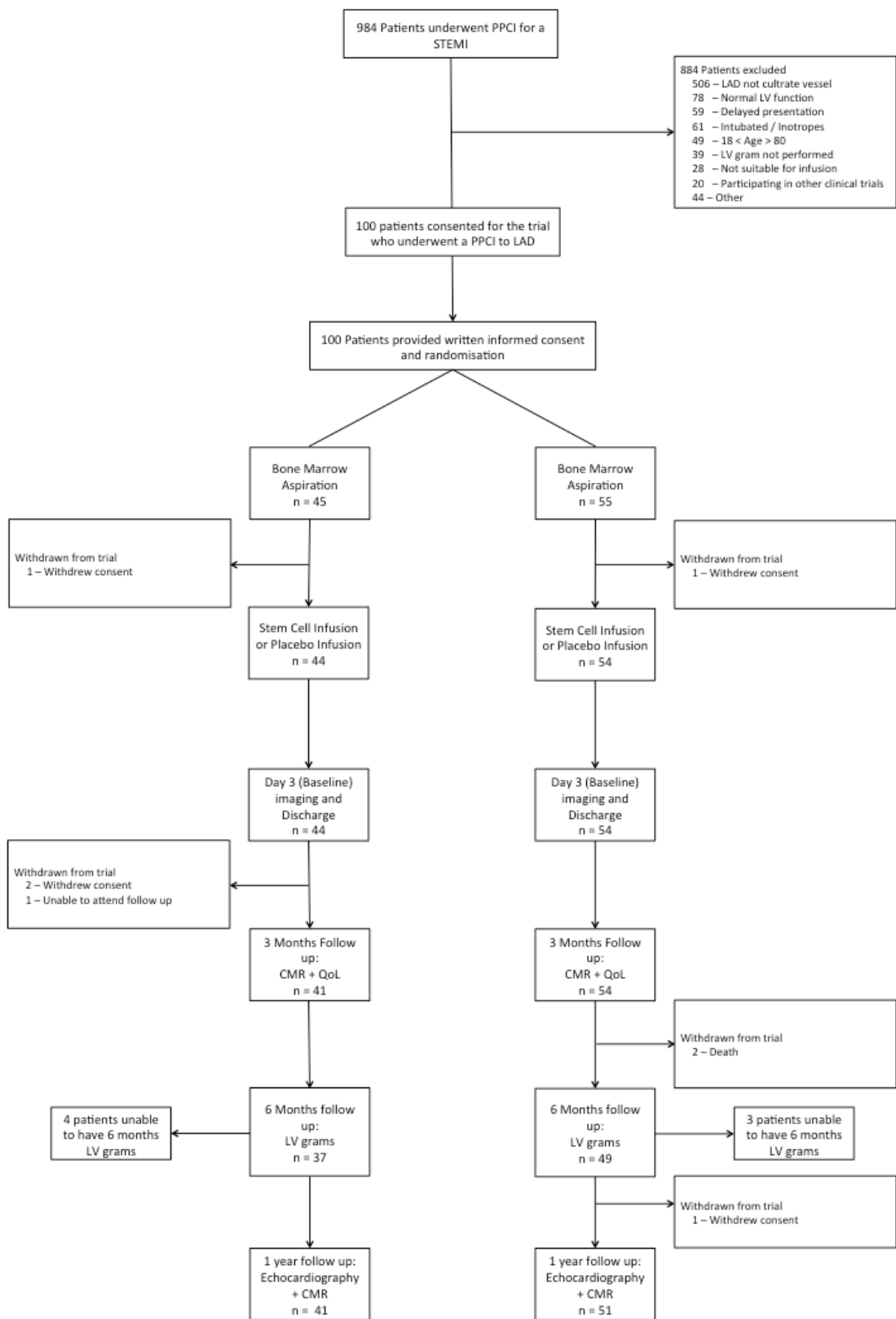


Figure 3.2. Consort Diagram for REGENERATE AMI

Table 3.1. Baseline characteristics of the study population

	Control (n=45)	BMNC (n = 55)	p-value
Age (yr), mean ± SEM	56.67 ± 1.60	56.36 ± 1.41	0.8868
Sex (M/F), (No.)	41/4	46/9	
Ethnicity (Caucasian) (No. (%))	36 (80%)	47 (85.5%)	
Medical History prior to infarct:			
Hypertension (No. (%))	12 (26.7%)	24 (43.6%)	
Hypercholesterolemia (No. (%))	10 (22.2%)	19 (34.5%)	
Diabetes mellitus (No. (%))	4 (8.8%)	6 (10.9%)	
Active smoker (No. (%))	24 (53.3%)	27 (49.0%)	
Previous MI (No. (%))	1 (2.2%)	1 (1.8%)	
Previous PCI (No. (%))	0 (0%)	1 (1.8%)	
Family history (No. (%))	13 (28.8%)	17 (30.9%)	
Medical Therapy prior to primary PCI:			
Aspirin (No. (%))	45 (100%)	55 (100%)	
Clopidogrel (No. (%))	39 (86.7%)	50 (90.9%)	
Prasugrel (No. (%))	4 (8.9%)	3 (5.5%)	
Ticagrelor (No. (%))	2 (4.4%)	2 (3.6%)	
Heparin (No. (%))	40 (88.9%)	50 (90.9%)	
Bivalirudin (No. (%))	5 (11.1%)	5 (9.1%)	
GP iib/iiia inhibitors (No. (%))	33 (73%)	44 (80%)	
DES Stent used (No. (%))	32 (71%)	36 (65%)	
Concomitant PCI performed (No. (%))	1 (2.2%)	3 (5.5%)	
Baseline Observations:			
Blood Pressure (Diastolic/Systolic), mean	138.6 / 85.64	138.0 / 83.91	
Pulse (bpm), mean ± SEM	84.45 ± 4.68	80.25 ± 3.14	
BMI (kg/m ²), mean ± SEM	27.11 ± 0.68	26.69 ± 0.42	0.5801
CCS > 1 (No. (%))	1 (2.2%)	5 (9.1%)	
NYHA > I (No. (%))	3 (6.7%)	4 (7.3%)	
Timings:			
Chest Pain to primary PCI (min), median (IQR)	193.0 (145.5 – 320.5)	233 (155.0 – 348)	0.2257
Door to primary PCI time (min), median (IQR)	36.0 (26.0 – 55.5)	40.0 (32.0 – 58.0)	0.2243
primary PCI to BM aspiration time (min), median (IQR)	230.0 (112.0 – 966.0)	172.0 (105.0 – 976.0)	0.6060
primary PCI to reinfusion (min), median (IQR)	583.0 (458.0 – 1276)	532.0 (403.0 – 1312)	0.4090
BM aspiration to infusion (min), median (IQR)	313.0 (287.0 – 374.0)	323.0 (290.0 – 370.0)	0.7414

Data are presented as No. (%) unless otherwise specified. BMI - body mass index, G-CSF - granulocyte-colony stimulating factor, ACE - angiotensin-converting enzyme inhibitor, ARB - Angiotensin receptor blocker, BM – bone marrow

3.3. Safety

3.3.1. Bone Marrow Aspiration

All patients underwent successful BMA. The most common symptom experienced by patients during the procedures was bone pain at site of aspiration, with no patient experiencing more than mild pain and did not require any sedation or opiates. There were three events within the control (bone marrow site bleeding n=2 and bone marrow site infection n=1) compared to two events in the BMNC (bone marrow site infection n=2) (Table 3.2). Both bone marrow site bleeding events required compression bandaging to the puncture site and did not require blood transfusion, all site infections required short course of oral antibiotics.

3.3.2. Stem cell infusion

Two patients in the control group did not undergo the reinfusion procedure. There was a total of 23 events that occurred during the reinfusion procedure. There was no significant difference seen between groups in events including arrhythmias, bleeding, no reflow and repeat PCI (Table 3.2).

There were three events within the trial of infusion-induced arrhythmias (control n=1 and BMNC n=2), two patients had non-sustained ventricular tachycardia not requiring cardioversion or antiarrhythmic agents and one patient had second-

degree atrioventricular block during balloon inflation that resolved after balloon deflation. All 10 episodes of ST change occurred during coronary artery balloon occlusion, which resolved after balloon deflation. Ischemic pre-conditioning was seen during the three occlusions with improvement in ST changes over time. Two patients underwent PCI during infusion, this was not related to balloon inflation but distal stent dissection seen on initial angiogram prior to infusion.

Table 3.2. Bone marrow aspiration complications and re-infusion complications across the study population

	Control (n=45)	BMNC (n = 55)
Events During Bone Marrow Aspiration:		
Bone Marrow Bleeding	2 (4.4 %)	0 (0 %)
Bone Marrow Infection	1 (2.2 %)	2 (3.6 %)
Events During Intracoronary Infusion:		
Infusion Arrhythmia	1 (2.2 %)	2 (3.6 %)
Infusion Bleeding/Heamatoma	3 (6.7 %)	3 (5.5 %)
Infusion no-reflow	2 (4.4 %)	0 (0 %)
Infusion PCI	1 (2.2 %)	1 (1.8 %)
Infusion ST Changes with CP	2 (4.4 %)	2 (3.6 %)
Infusion ST Changes without CP	4 (8.9 %)	2 (3.6 %)

Values are No. (%)

3.3.3. Renal function and cardiac markers

Blood analysis for Creatine, Troponin T and Creatine Kinase (CK) were performed on admission, discharge, 6 months and 12 month months for each patient. At baseline both the groups had similar concentrations (Table 3.3).

Table 3.3. Concentrations of routine bloods at baseline

	Control n = 45	BMNC n = 55	p-value
Creatine (umol/L)	82.16 (76.62 – 87.69)	78.72 (74.28 – 83.17)	0.3276
Creatine Kinase (IU/L)	788.2 (360.5 – 1216)	565.1 (291.1 – 838.3)	0.3566
Troponin (ng/L)	1283 (463.7 – 2103)	647.9 (227.2 – 1069)	0.1390

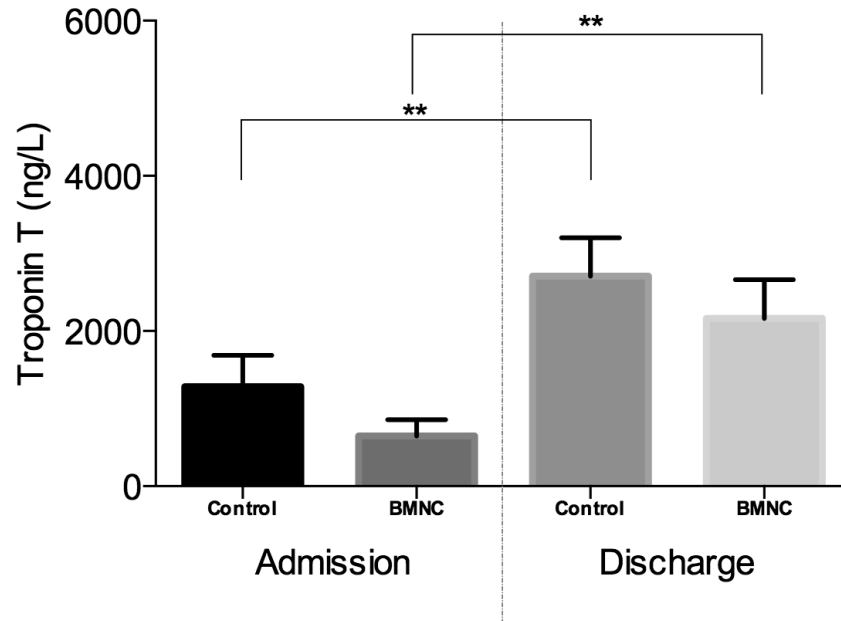
Values are mean (95% CI)

Creatine concentrations significantly increased in the BMNC from admission to 12 months by 8.52 umol/L from 77.56 ± 16.55 umol/L at admission to 86.06 ± 21.58 umol/L at 12 months (p<0.0001), that was not seen in the control (3.67%, 95% CI, -0.8117 to 8.145%; p=0.1057). CK peaked at admission and decreased significantly over time (-455.6IU/L, 95% CI, -772.8 to -139.0IU/L; p=0.0058) in the BMNC and (-340.0IU/L, 95% CI, -639.0 to -40.95IU/L; p=0.0273) the control.

Troponin T concentrations significantly increased from admission to discharge in both groups (Figure 3.3). There was a significant increase in troponin T in the BMNC group during admission from 282.1 ± 319.2ng/L at baseline to 2165 ± 1933ng/L at discharge (p=0.0020) and within the control from 1193 ± 2477ng/L

at baseline to 2709 ± 2091 ng/L at discharge ($p=0.0082$). The change in Troponin did not differ between both groups at any time point.

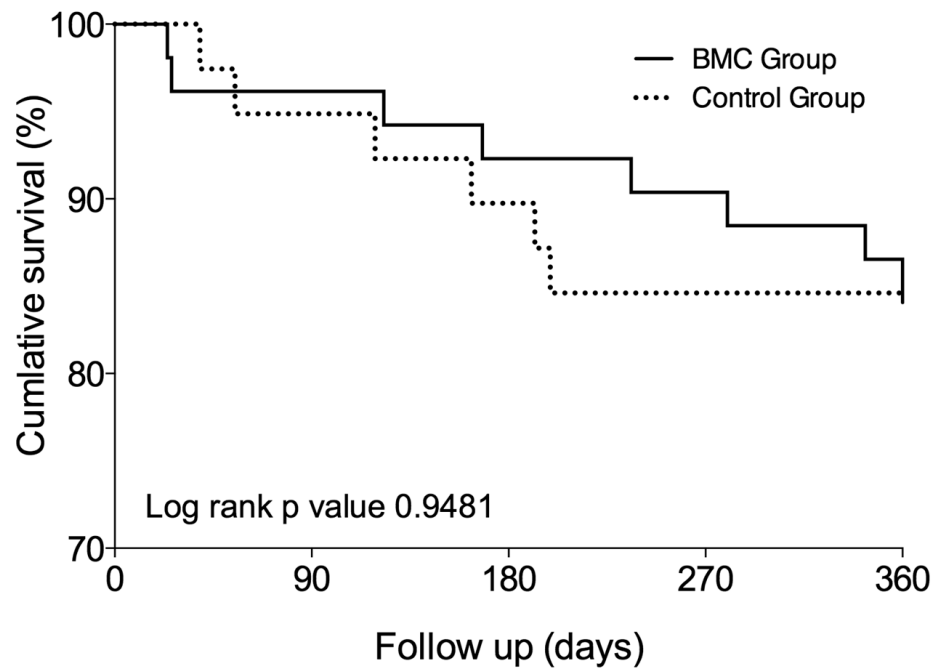
Figure 3.3. Change in Troponin T concentrations from admission to discharge



Bar chart (mean and SEM) of change in Troponin T concentrations at admission and discharge

3.3.4. Major Adverse Cardiac Events

12 months after MI, 15.4% of patients in the control suffered MACE compared to 15.9% of patients in the BMNC ($p=0.9481$) (Figure 3.4).



Number at Risk		Follow up (days)				
	0	90	180	270	360	
Control	45	43	40	38	38	
BMNC	55	53	51	49	46	

Figure 3.4. Kaplan Meier curves showing cumulative probability of major adverse cardiac events (MACE) after primary PCI according to treatment group

3.4. Left Ventricular Ejection Fraction assessed by advanced imaging (Primary Endpoint)

Baseline scans were performed at discharge on day 3-4 after primary PCI, and of the 100 patients recruited, 93 patients underwent advanced cardiac imaging at 3 months and 91 completed the primary endpoint for advanced cardiac imaging at 12 months (n=51 in BMNC group and n=41 in control group). Ninety-six patients underwent CMR assessment and two underwent CT assessment of cardiac function, due to the small number of patients who underwent CT assessment all cardiac function data was pooled and analysed.

Baseline measurement of LVEF did not differ significantly between the treatment groups (Table 3.4).

Global LVEF increased in the BMNC by 5.06% from $47.51 \pm 9.18\%$ at baseline to $52.57 \pm 10.45\%$ at 12 months ($p < 0.0001$) and in the control by 2.82% from $49.15 \pm 9.59\%$ at baseline to $51.96 \pm 9.13\%$ at 12 months ($p = 0.0019$) (Figure 3.5). There was a greater trend for improvement seen in the BMNC group compared to control at 12 months, with the mean improvement of $5.06 \pm 7.27\%$ in the BMNC versus $2.89 \pm 5.05\%$ in the control (2.17%; 95% CI, -0.576 to 4.91; $p = 0.1200$) (Figure 3.5).

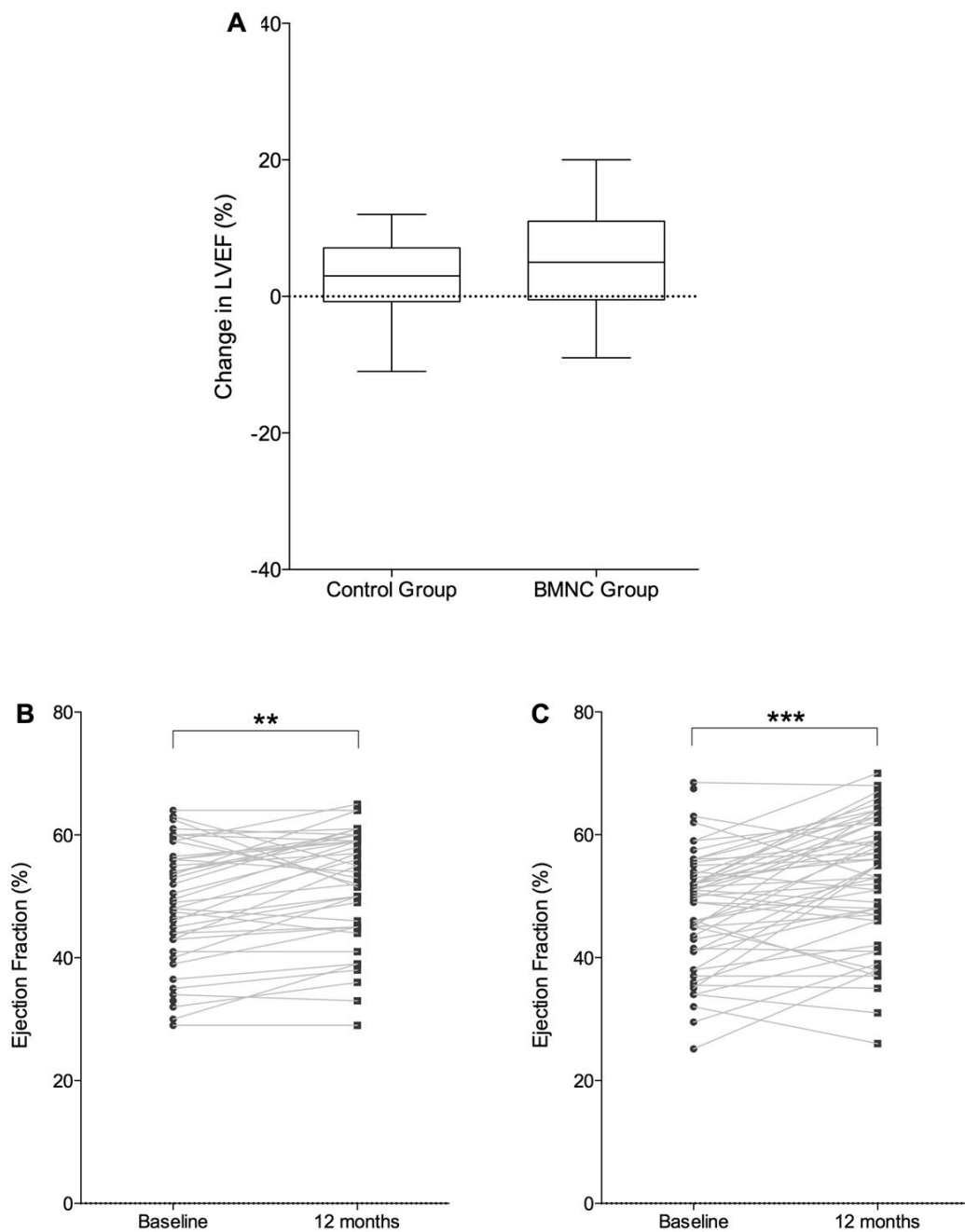


Figure 3.5. Change in LVEF between groups and change in LVEF from baseline to 12 months assessed by advanced cardiac imaging

Panel A: Box and whisker plot (median and range) in change in LVEF at 12 months between groups in the REGENERATE AMI trial (primary end-point). Panel B and C: Dot plots showing LVEF measured at baseline and 12 months after delivery of either placebo (B) or intra-coronary BMNC (C).

Further assessment of change in Global LVEF was assessed at 3 months. There was a greater trend for improvement seen in the BMNC group compared to control at 3 months. The mean improvement in the BMNC group was $5.71 \pm 12.50\%$ versus $1.59 \pm 10.30\%$ in the control (4.13 %; 95% CI, -0.528 to 8.79; $p=0.0816$) (Table 3.4 & Figure 3.6).

Table 3.4. LVEF data by advanced cardiac imaging for study population

	Control	BMNC	Between Group P value
3 months paired samples	(n = 40)	(n= 53)	
12 months paired samples	(n = 41)	(n= 51)	
Primary Endpoint			
Baseline LVEF (%)	49.15 (46.12 – 52.17)	47.51 (44.93 – 50.10)	0.5553
3 months LVEF (%)	49.90 (46.90 – 46.90)	53.26 (50.38 – 56.15)	
12 months LVEF (%)	51.96 (49.08 – 54.84)	52.57 (49.63 – 55.51)	
In group p value (3 months)	0.3358	0.0048	
In group p value (12 months)	0.0019	<0.0001	
ANOVA of LVEF (p value)	0.3490	0.0094	
Difference in LVEF (%)			
3 months	1.588 (-1.707 – 4.882)	5.717 (2.645 – 8.789)	0.0816
12 months	2.888 (1.141 – 4.634)	5.056 (3.011 – 7.101)	0.1200

Values are mean (95% CI).

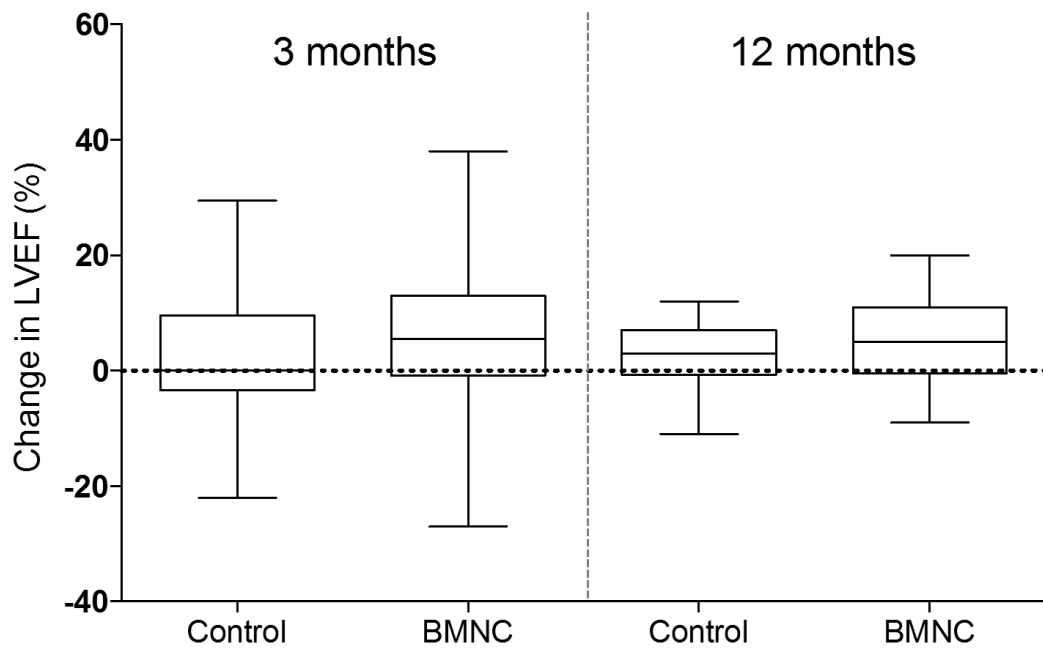


Figure 3.6. Change in LVEF at 3 months and 12 months

Box and whisker (median and range) of change in LVEF at 3 months and 12 months. BMNC administration did not result in a significant improvement in LVEF as measured by advanced cardiac imaging compared to placebo administration at 3 and 12 months.

Analysis of serial changes in LVEF over time was performed by repeated-measures ANOVA. Global LVEF increased statistically significantly between time points ($p = 0.0094$). This trend was not seen in the control across the 1-year time course ($p=0.3490$) (Figure 3.7).

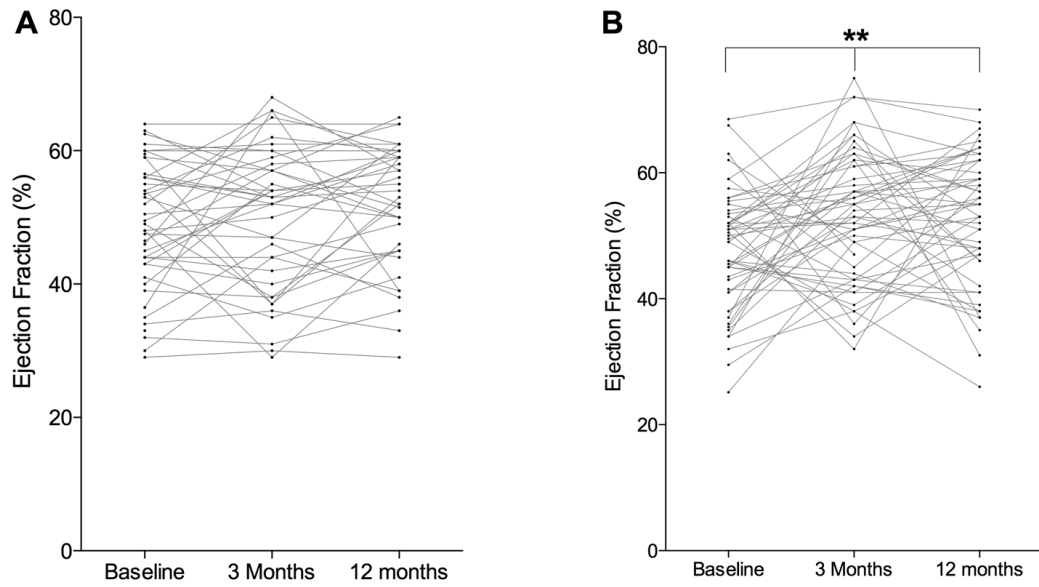


Figure 3.7. Assessment of LVEF function over time

LVEF assessment was performed at baseline, 3 and 12 months after IC delivery of either BMNC or placebo in all patients. Each line representing the difference between baseline, 3 months and 12 month LVEF values are shown for each patient in the control in panel A and BMNC in panel B.

**p<0.001 using a one-way ANOVA

3.5. Effects of Bone Marrow Progenitor cells on Geometry

3.5.1. LV volumes

LVESV, LVEDV and LVSV were assessed at baseline, 3 months and 12 months. There was no evidence of a difference seen between treatment groups in LVESV, LVEDV or LVSV at baseline, 3 months or 12 months.

There was trend for greater improvement in LV volumes in the BMNC group compared to control. There was a trend for an increase in LVEDV in both groups with greater improvement in BMNC group (3.65mls; 95% CI, -8.60 to 15.89; $p=0.5556$). There was a greater trend for increase in LVEDV from baseline to 12 months in the BMNC group 9.92mls (155.2 ± 32.05 mls to 164.2 ± 41.19 mls; $p=0.0561$) and in the control by 7.27mls (159.5 ± 33.41 mls to 164.8 ± 35.64 mls; $p=0.1553$). There was similar decrease in LVESV in both groups with no significant difference between the 2 treatment groups (5.53mls; 95% CI, -2.066 to 13.12; $p=0.1516$) There was a significant increase in LVSV from baseline to 12 months in both groups, in the BMNC the LVSV increased by 11.61mls (73.39 ± 17.01 mls to 85.0 ± 20.41 mls; $p<0.0001$) and in the control by 7.27mls (78.32 ± 23.20 mls to 85.59 ± 22.11 mls; $p=0.0015$), with no significant difference between the 2 treatment groups (5.53mls; 95% CI, -2.066 to 13.12; $p=0.1516$) (Table 3.5).

Table 3.5a. LV dimensions by advanced cardiac imaging for study population

	Control (n = 40)	BMNC (n= 53)	Between Group p value
3 months paired samples	(n = 40)	(n= 53)	
12 months paired samples	(n = 41)	(n= 51)	
<u>LV End diastolic volume</u>			
Baseline LVEDV (mls)	159.5 (148.9 – 170.0)	155.2 (146.2 – 164.2)	0.4430
3 months LVEDV (mls)	166.7 (149.9 – 170.2)	162.6 (151.3 – 173.9)	
12 months LVEDV (mls)	164.8 (153.6 – 176.1)	164.2 (152.6 – 175.7)	
In group p value (3 months)	0.1477	0.1987	
In group p value (12 months)	0.1553	0.0561	
Difference in LVEDV (mls)			
3 months	6.625 (-2.447 – 15.70)	7.076 (-2.341 – 16.49)	0.9487
12 months	5.275 (-2.440 – 12.99)	8.922 (-0.2431 – 18.09)	0.5556
<u>LV End systolic volume</u>			
Baseline LVESV (mls)	80.67 (73.33 – 88.01)	82.49 (75.26 – 89.72)	0.9271
3 months LVESV (mls)	84.33 (75.03 – 89.22)	78.19 (69.28 – 87.09)	
12 months LVESV (mls)	79.23 (71.18 – 87.28)	79.98 (70.47 – 89.49)	
In group p value (3 months)	0.5799	0.4360	
In group p value (12 months)	0.5994	0.4764	
Difference in LVESV (mls)			
3 months	2.200 (-5.773 – 10.17)	-4.508 (-12.73 – 3.710)	0.2753
12 months	-1.550 (-7.182 – 4.082)	-2.510 (-9.535 – 4.516)	0.8372
<u>LV Stroke Volume</u>			
Baseline LVSV (mls)	78.32 (70.99 – 85.64)	73.39 (68.61 – 78.18)	0.2660
3 months LVSV (mls)	81.33 (74.56 – 88.09)	84.89 (79.47 – 90.31)	
12 months LVSV (mls)	85.59 (78.61 – 92.56)	85.00 (79.26 – 90.74)	
In group p value (3 months)	0.2369	0.0007	
In group p value (12 months)	0.0015	<0.0001	
Difference in LVSV (mls)			
3 months	3.838 (-2.624 – 10.30)	11.48 (6.303 – 16.65)	0.0688
12 months	7.275 (2.839 – 11.71)	12.80 (6.951 – 18.66)	0.1516

Values are mean (95% CI). LVEF - left ventricular ejection fraction, LVEDV - left ventricular end diastolic volume, LVESV - left ventricular end systolic volume, LVSV - left ventricular stroke volume

Table 3.5b. BSi LV dimensions by advanced cardiac imaging for study population

	Control (n = 40)	BMNC (n= 53)	Between Group p value
3 months paired samples	(n = 40)	(n= 53)	
12 months paired samples	(n = 41)	(n= 51)	
<u>LVEDVi</u>			
Baseline LVEDVi	82.56 (77.42 – 87.69)	80.04 (76.01 – 84.07)	0.4328
3 months LVEDVi	86.87 (80.46 – 93.27)	84.59 (79.37 – 89.81)	
12 months LVEDVi	84.85 (79.52 – 90.18)	85.06 (79.60 – 90.53)	
In group p value (3 months)	0.0972	0.1950	
In group p value (12 months)	0.1978	0.0375	
Difference in LVEDVi			
3 months	3.87 (-0.736 – 8.477)	3.850 (-2.034 – 9.733)	0.9958
12 months	2.582 (-1.403 – 6.586)	4.718 (0.283 – 9.152)	0.4831
<u>LVESVi</u>			
Baseline LVESVi	42.35 (38.23 – 46.27)	42.32 (38.98 – 45.69)	0.9789
3 months LVESVi	44.06 (38.80 – 49.31)	40.49 (36.20 – 44.78)	
12 months LVESVi	40.90 (36.71 – 45.09)	41.31 (36.68 – 45.93)	
In group p value (3 months)	0.5151	0.4140	
In group p value (12 months)	0.5357	0.4418	
Difference in LVESVi			
3 months	1.327 (-2.759 – 5.413)	-2.098 (-7.211 – 3.015)	0.3192
12 months	-0.884 (-3.742 – 1.975)	-1.327 (-4.766 – 2.111)	0.8469
<u>LVSVi</u>			
Baseline LVSVi	40.30 (37.19 – 43.42)	37.72 (35.42 – 40.01)	0.1730
3 months LVSVi	42.81 (39.53 – 46.09)	44.10 (41.39 – 46.81)	
12 months LVSVi	43.95 (40.79 – 47.11)	42.75 (40.75 – 46.77)	
In group p value (3 months)	0.1109	0.0006	
In group p value (12 months)	0.0099	<0.0001	
Difference in LVSVi			
3 months	2.544 (-0.610 – 5.698)	5.948 (2.677 – 9.218)	0.1448
12 months	3.466 (0.879 – 6.053)	6.045 (3.539 – 8.551)	0.1563

Values are mean (95% CI). LVEDVi – indexed left ventricular end diastolic volume, LVESVi – indexed left ventricular end systolic volume, LVSVi – indexed left ventricular stroke volume

3.5.2. Infarct Size

Measurement of infarct size was performed 2-3 days post IC infusion of BMNC or placebo. At baseline infarct size was significantly reduced in the BMNC compared to the control (-4.44%; 95% CI, -8.35 to -0.53; $p=0.0264$) (Figure 3.8).

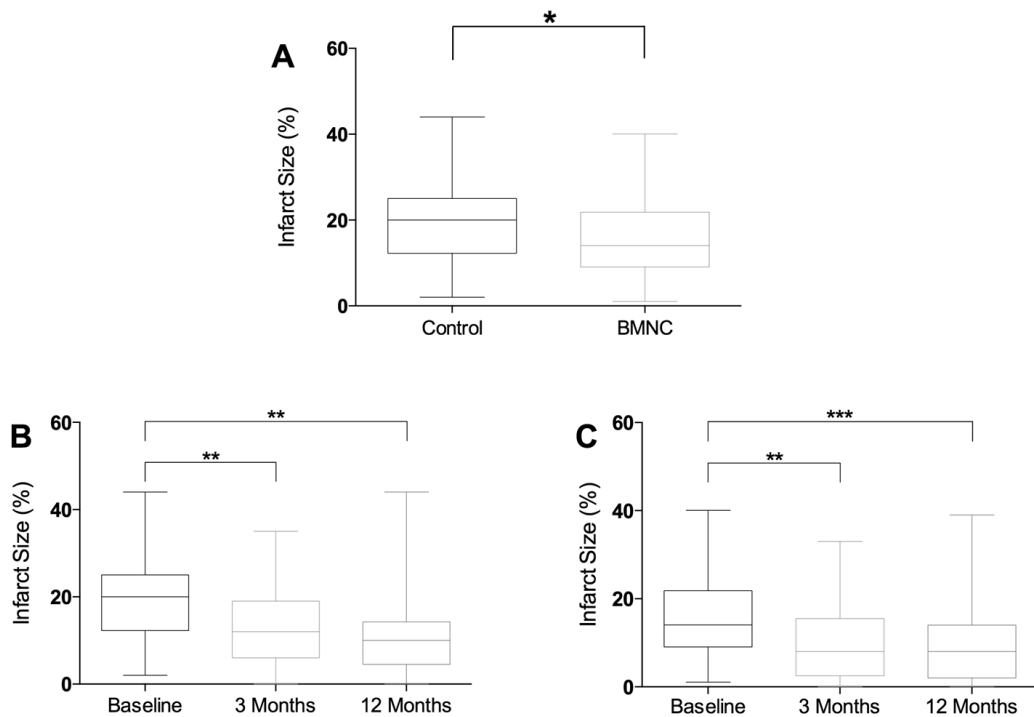


Figure 3.8. Infarct size assessment by CMR

Panel A - Box and whisker (median and range) of baseline infarct size (%), $*p<0.005$ using unpaired t-test. Panel B + C - Box and whisker (median and range) of infarct size (%) at baseline, 3 and 12 months after delivery of either intra-coronary placebo (panel B) or BMNC (panel C). $*p<0.005$, $**p<0.001$, $***p<0.0001$ using paired t-test.

Infarct size decreased significantly in both groups over time. In the BMNC infarct size decreased by 4.68% (14.72 ± 7.69 to 10.07 ± 10.13 ; $p=0.0003$) with a greatest decrease seen in the control of 8.91% ($20.14 \pm 10.57\%$ to $11.38 \pm 9.14\%$; $p<0.0001$) (Table 3.6). At 12 months there were similar infarct sizes in both groups (-0.69% ; 95% CI, -4.89 to -3.50 ; $p=0.7428$).

Table 3.6. Infarct size for study population

	Control	BMNC	Between Group P value
12 months paired samples	(n = 37)	(n= 45)	
<u>LV Infarct Size (%)</u>			
Baseline IS (%)	20.14 (16.61 – 23.66)	14.72 (12.43 – 17.00)	0.0264
3 months IS (%)	13.37 (10.16 – 16.58)	10.42 (7.736 – 13.10)	
12 months IS (%)	11.38 (8.33 – 14.43)	10.07 (7.057 – 13.07)	
In group p value (3 months)	0.0004	0.0006	
In group p value (12 months)	<0.0001	0.0003	
<u>Difference in Infarct size (%)</u>			
3 months	-6.611 (-9.995 – -3.227)	-4.835 (-6.985 – -2.685)	0.3531
12 months	-8.910 (-12.07 – -5.755)	-4.681 (-7.057 – -2.305)	0.0301

Values are mean (95% CI). IS – infarct size

3.5.3. Area at Risk

Measurement of AAR was assessed by STIR imaging technique by CMR at baseline, 3 and 12 months. AAR was greatest at baseline ($34.34\pm 14.06\%$ in the control versus 32.80 ± 9.634 in the BMNC) with significant reductions over time at 3 months ($4.10\pm 5.69\%$ in the control versus $4.42\pm 7.17\%$ in the BMNC) with the lowest values at 12 months ($1.09\pm 3.50\%$ in the control versus $0.27\pm 1.03\%$ in the BMNC) (Figure 3.9).

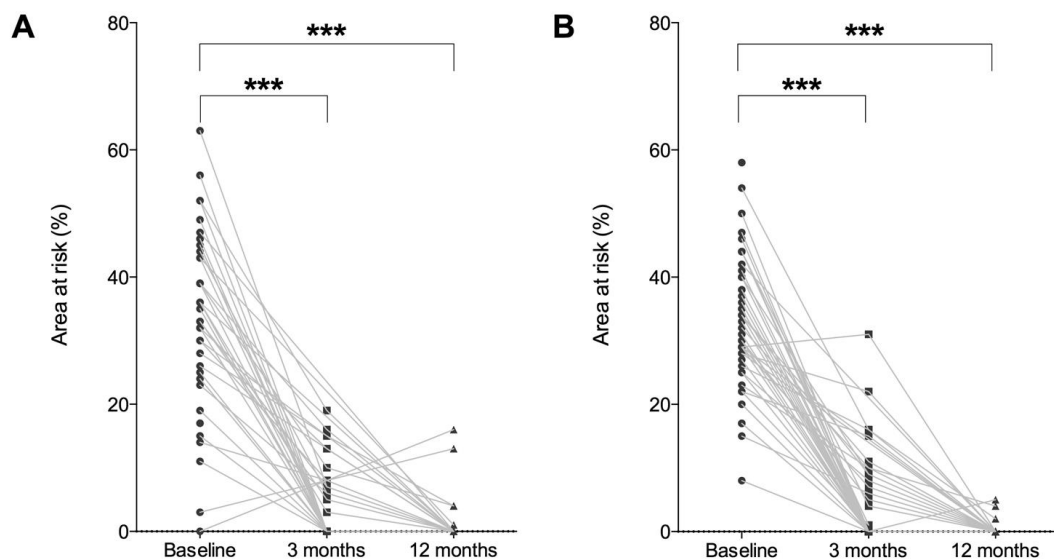


Figure 3.9. Area at risk assessment by CMR

Dot plots showing AAR measured at baseline, 3 and 12 months after delivery of either intra-coronary BMNC or placebo in all patients reaching primary end point. Each line representing the difference between baseline to 3 months to 12 month, AAR values are shown for each patient in the control in panel A and BMNC in panel B. *** $p < 0.0001$ using paired t-test.

There was no significant difference between the 2 treatment groups at both 3 months (3.57%; 95% CI, -2.215 to 9.357; $p = 0.2231$) and 12 months (0.66%; 95% CI, -5.406 to 6.723; $p = 0.8292$).

3.5.4. Myocardial Salvage index

Acute myocardial salvage was calculated as by (Baseline AAR – Baseline Infarct Size) / Baseline AAR. There was a significantly greater myocardial salvage index seen in the BMNC compared to the control at baseline (0.1; 95% CI, 0.0008 to 0.2025; p=0.0483) (Figure 3.10).

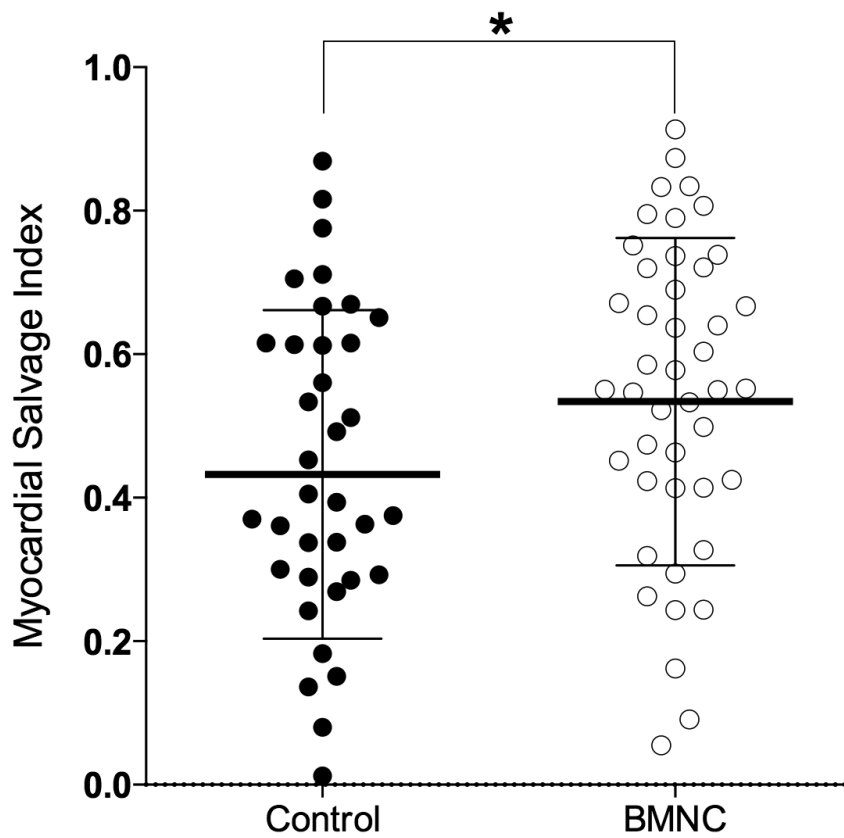


Figure 3.10. Assessment of myocardial salvage index on CMR

The mean index as assessed by CMR was significantly larger in the BMNC group compared to the control (P=0.0483). Significance evaluated using unpaired t test and data shown as mean ± SEM

At 12 months myocardial salvage was calculated by:

$$\text{(Baseline AAR – 12 month Infarct Size) / Baseline AAR}$$

The trend that was seen at baseline was not present at 12 months (-0.074; 95% CI, -0.1813 to 0.034; p=0.1784)

3.5.5. Sub-analysis of BMNC based on baseline ejection fraction

Based on previous studies a sub analysis of patients within the BMNC group with a LVEF < 45% was performed to assess if the improvement in LVEF was greater than patients who had a baseline LVEF > 45% (Dill, Schachinger et al. 2009). Both sub-groups showed improvement in LVEF at 12 months with the greatest increase seen in the subgroups of LVEF < 45%. There was a mean improvement of $6.62 \pm 8.31\%$ in the subgroup with LVEF less than 45% versus $3.79 \pm 6.71\%$ in the BMNC subgroup with LVEF greater than 45% ($p=0.1853$) (Figure 3.11).

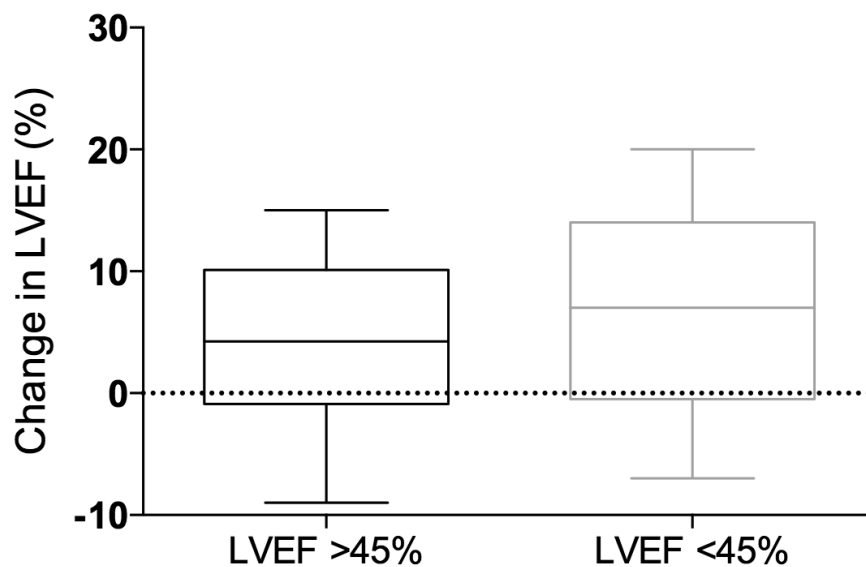


Figure 3.11. Sub-analysis of BMNC with LVEF less or greater than 45% at baseline

Box and whisker (median and range) of change in LVEF at 12 months between sub-group analysis, Patients with a lower LVEF at baseline after BMNC administration did not results in a significant improvement in LVEF as measured by advanced cardiac imaging compared to patients who LVEF was greater than 45%

3.5.6. Microvascular Obstruction

MVO was assessed was performed in 68 baseline studies. There was a significantly lower number of patients with MVO in the BMNC group 36.8% vs. 60.6% in the control arm. However there is no difference in the amount of MVO between the two groups (Table 3.7).

Table 3.7. MVO assessment in study population

	Control (n = 33)	BMNC (n= 38)	p-value (between group)
<u>MVO</u>			
MVO Present (n)	20	14	0.0456
MVO (g)	1.0 (0.0–5.0)	0.0 (0.0–2.0)	0.0721
MVO (% of LV mass)	2.2 (0.0-7.3)	0.0 (0.0–3.0)	0.0777

Values shown as Median (IQR). MVO, microvascular obstruction

4.5.6. Transthoracic Echocardiography

Echocardiography was performed on all patients after PCI prior to stem cell infusion, discharge, 6 months and 12 months as per protocol. At admission all of the 100 patients recruited underwent echocardiography, 98 patients at discharge, 89 patient at 6 months and 93 patients underwent echocardiography at 12 months (n=52 in BMNC group and n=41 in control group).

3.5.6.1. Left Ventricular Ejection Fraction by Echocardiography

Global LVEF at admission did not differ significantly between groups (-2.03%; 95% CI, -5.687 to 1.622; p=0.2724) (Table 3.7). In the BMNC the LVEF increased significantly at 6 months by 1.90% (47.26 ± 9.01% to 49.16 ± 8.74%; p=0.0308) and at 12 months by 3.077% (47.98 ± 9.30% to 51.06 ± 9.82%; p=0.0088). There was a significant improvement in LVEF seen at 12 months in the control by 1.90% (50.41 ± 9.63% to 52.32 ± 9.09%; p=0.0167), which was not seen at 6 months (0.41% (50.15 ± 9.75% to 50.56 ± 9.84%; p=0.5214)) (Figure 3.12).

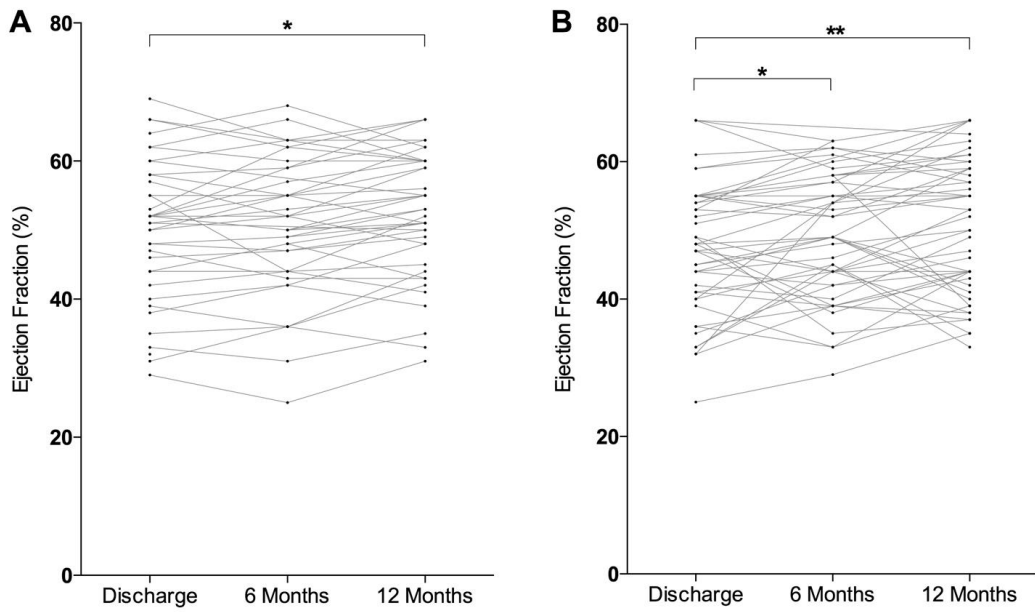


Figure 3.12. LVEF at baseline, 6 months and 12 months assessed by echocardiography

Dot plots showing LVEF measured at baseline, 6 months and 12 months after delivery of either IC BMNC or placebo in all patients. Each line representing the difference between the 3 time points. Values are shown for each patient in the control in panel A and BMNC in panel B.

** $p < 0.001$, *** $p < 0.0001$ using paired t-test.

Table 3.8. LVEF and WMSI measured by echocardiography

	Control	BMNC	Between Group P value
6 months paired samples	(n = 39)	(n=50)	
12 months paired samples	(n = 41)	(n=52)	
Admission LVEF (%)	48.78 (45.92 – 51.63)	46.75 (44.34 – 49.16)	0.2724
Discharge LVEF (%)	50.41 (47.38 – 52.45)	46.98 (45/39 – 50.57)	
6 months LVEF (%)	50.56 (47.37 – 53.75)	49.16 (46.67 – 51.65)	
12 months LVEF (%)	52.32 (49.45 – 55.19)	51.06 (48.33 – 53.79)	
In group p value (6 months)	0.5214	0.0308	
In group p value (12 months)	0.0167	0.0088	
Difference from baseline LVEF (%)			
6 months	0.41 (-0.87 – 1.69)	1.90 (0.18 – 3.62)	0.1863
12 months	1.90 (0.35 – 3.44)	3.08 (0.81 – 5.35)	0.4176
Admission WMSI (%)	13.42 (10.76 – 16.09)	14.39 (12.32 – 16.46)	0.5604
Discharge WMSI (%)	12.20 (10.59 – 15.80)	15.63 (13.44 – 17.81)	
6 months WMSI (%)	13.31 (10.39 – 16.22)	15.14 (12.60 – 17.68)	
12 months WMSI (%)	12.29 (9.27 – 15.32)	12.82 (10.38 – 15.27)	
In group p value (6 months)	0.9704	0.1703	
In group p value (12 months)	0.3024	0.0002	
Difference from baseline WMSI (%)			
6 months	0.51 (-1.31 – 1.41)	-0.78 (-1.97 – 0.41)	0.3561
12 months	-0.85 (-2.56 – 0.86)	-2.79 (-4.11 – -1.47)	0.0696

Values are mean (95% CI). LVEF - left ventricular ejection fraction, WMSI - Wall score motion index

There was no evidence of difference in LVEF between the BMNC and Controls at both 6 and 12 months. There was trend for improvement in both groups with the greatest improvement seen in the BMNC group of $1.90 \pm 6.04\%$ in the BMNC versus $0.41 \pm 3.96\%$ in the control at 6 months ($p=0.1863$), and $3.08 \pm 8.15\%$ in the BMNC versus $1.90 \pm 4.88\%$ in the control at 12 months ($p=0.4176$) (Figure 3.13).

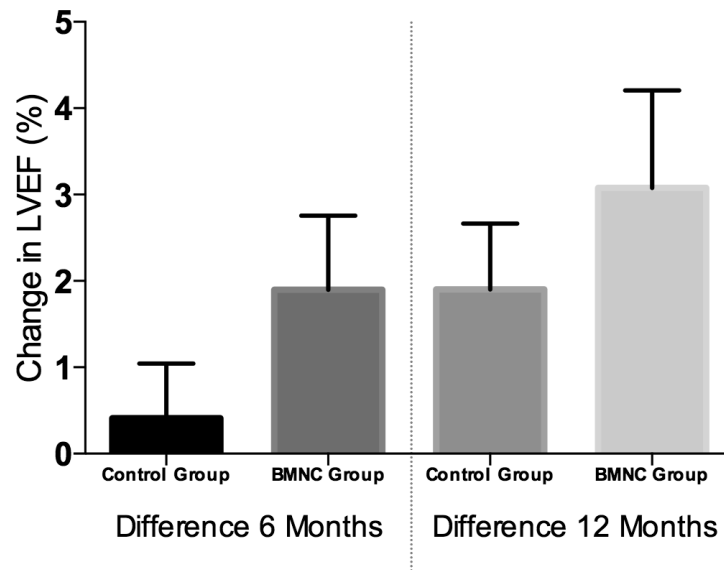


Figure 3.13. Change in LVEF between group at 6 months and 12 months
 Bar graph (mean and SEM) of change in LVEF at 6 months and 12 months between groups.

3.5.6.2. Wall Motion Score Index

WMSI was assessed in all patients and did not differ significantly between groups on admission or baseline (Table 3.7). In the BMNC WMSI decreased significantly at 12 months by -2.80% ($15.63 \pm 7.77\%$ to $12.82 \pm 8.69\%$; $p=0.0002$), no significant changes in WMSI was seen in the control (Figure 3.14). The change in WMSI over time between groups was at the border of conventional statistical significance (-1.94%; 95% CI, -4.028 to 0.1580; $p=0.0696$) (Figure 3.15).

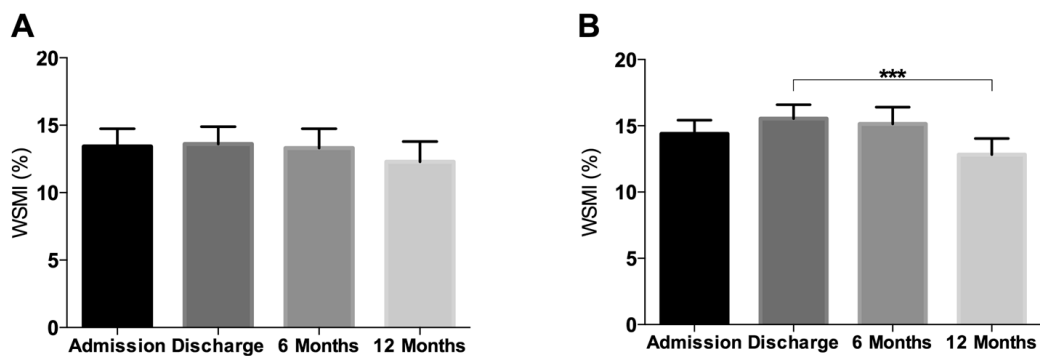


Figure 3.14. WMSI at admission, baseline, 6 months and 12 months assessed by echocardiography
 Bar graph (mean and SEM) showing WMSI measured on admission, discharge, 6 months and 12 months after delivery of either IC BMNC or placebo in all patients in the control in panel A and BMNC in panel B. **p<0.001, ***p<0.0001 using paired t-test.

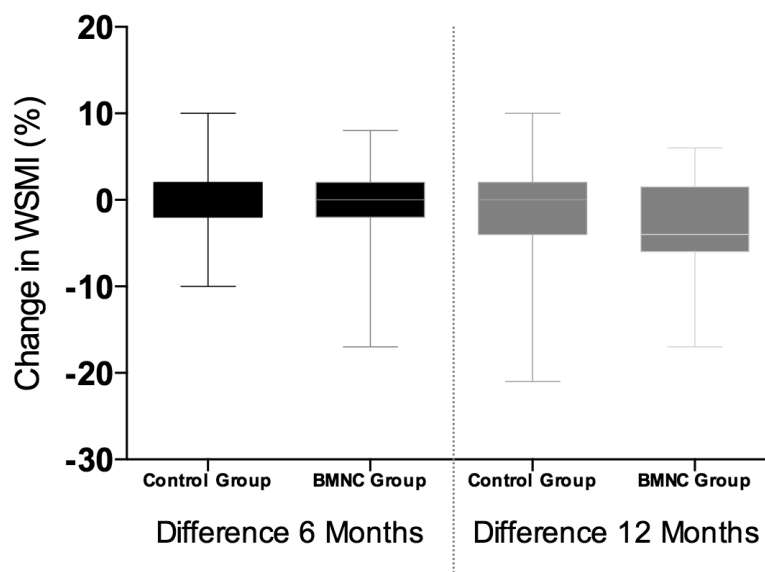


Figure 3.15. Change in WMSI between group at 6 months and 12 months
 Box and whisker (median and range) of change in WMSI at 6 months and 12 months between groups.

3.5.7. Quantitative Left Ventriculography

Quantitative Left Ventriculography (QLV) was performed on all patients at baseline after PCI as per protocol and at 6 months. Of the 100 patients recruited, 87 patients undertook 6-month angiography with QLV (n=48 in BMNC and n=39 in control). There was no difference in baseline LVEF between the treatment groups. Global LVEF increased significantly in the BMNC by 7.12% from $49.23 \pm 11.12\%$ at baseline to $56.35 \pm 14.92\%$ at 6 months ($p=0.0007$) and by 5.00% from $52.41 \pm 10.29\%$ at baseline to $57.42 \pm 12.14\%$ at 6 months ($p=0.0121$) in the control (Figure 3.14). There was a trend for a greater improvement in global LVEF in the BMNC group compared to the control at 6 months (2.12%, 95% CI, -3.401 to 7.632; $p=0.4478$).

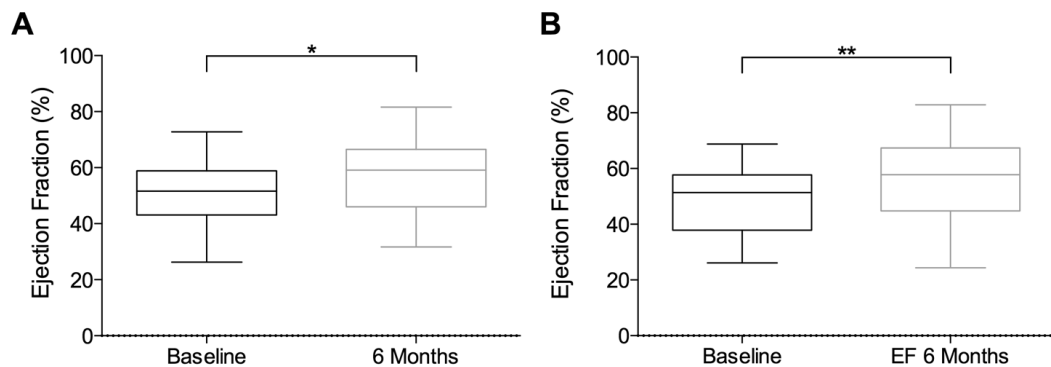


Figure 3.14. LVEF at baseline and 12 months assessed by quantitative left ventriculography Box and whisker (median and range) showing LVEF measured at baseline and 12 months after delivery of either control (panel A) or BMNC (panel B). * $p<0.005$, ** $p<0.001$ using paired t-test.

3.5.8. Comparison of imaging modalities

There was a weak correlation between LVEF assessed by QLV and CMR ($r=0.4598$, $p<0.0001$) and between QLV and echocardiography ($r=0.3480$, $p<0.0001$). Echocardiography showed a strong correlation with CMR ($r=0.8597$, $p<0.0001$) (Figure 3.17).

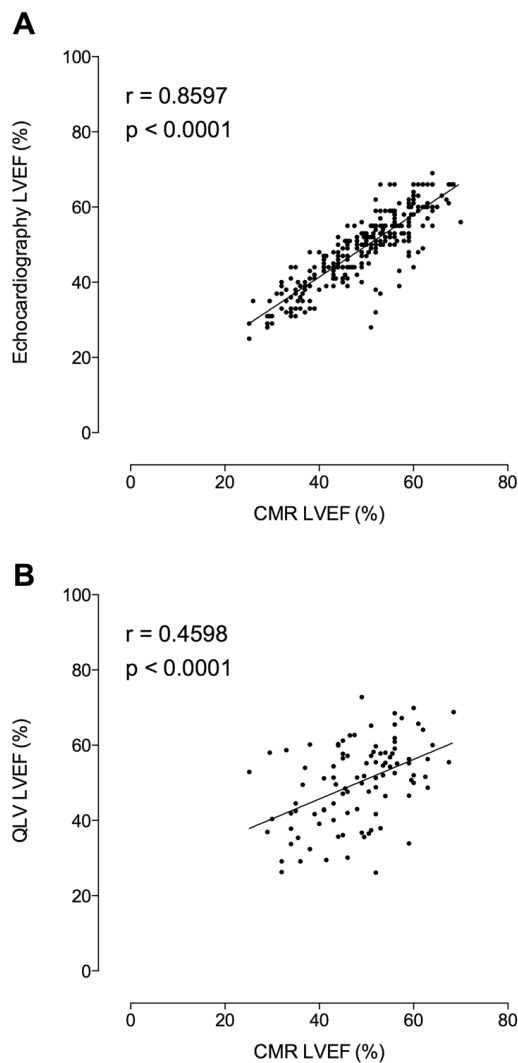


Figure 3.17. LVEF association between echocardiography and CMR and LV Angiography
There was a significant positive correlation between echocardiography LVEF measurements and CMR ($r=0.8597$) as shown in panel A. Panel B depicts the weak association between CMR and LV angiography ($r=0.4598$).

3.6. Effects of Bone Marrow Progenitor cells on the patient

3.6.1. Change in NYHA and CCS

Change in NYHA and CCS classification in each group of the trial is shown in (Figure 3.18). Baseline NYHA and CCS classification did not differ between groups and there was no significant change in either groups NYHA or CCS classification over time.

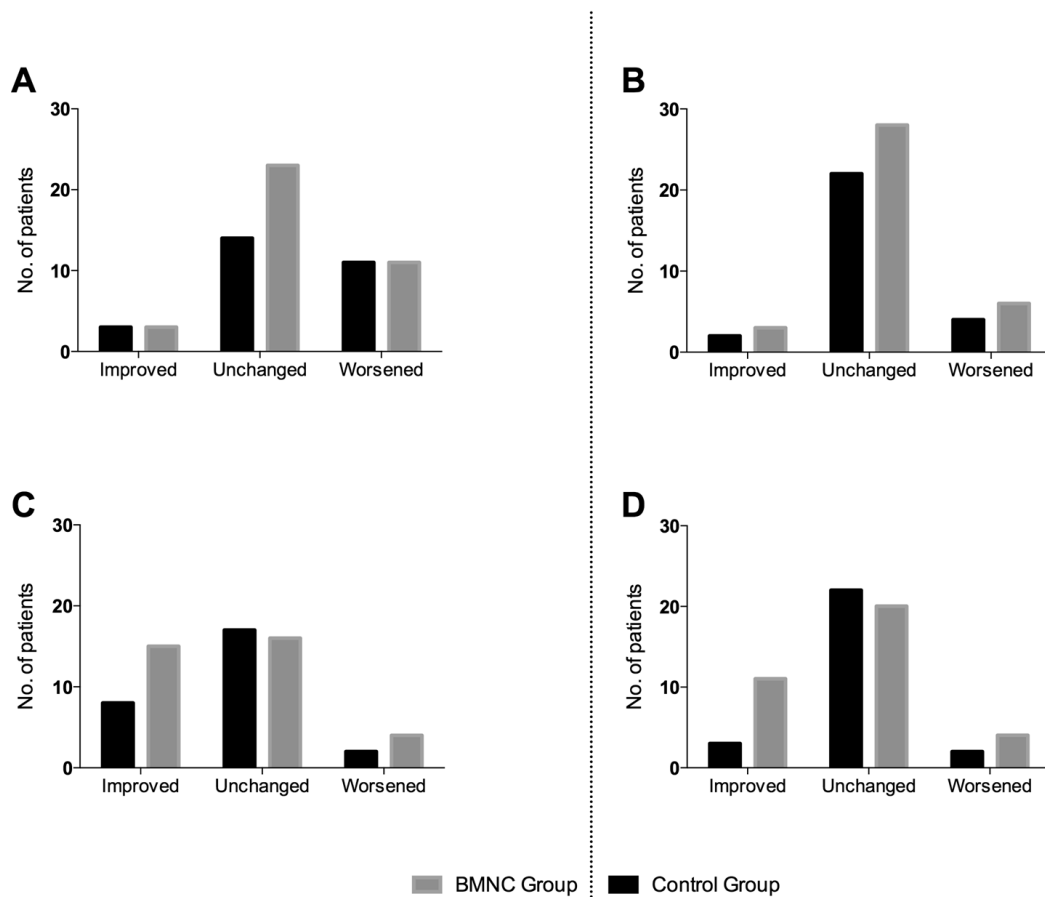


Figure 3.18. Changes in NYHA functional class and CCS Class

Change in CCS functional class in patients in both groups at 6 months (panel A) and 12 months (panel C), Change in NYHA functional class in patients in both groups at 6 months (panel B) and 12 months (panel D)

3.6.2. Change in Quality of Life

Patients undertook quality of life assessment using EQ5D, McNew and SF6 questionnaire at Baseline, 6 months and 12 months within UK based centres. At baseline there was no significant difference between both groups, and this pattern was mirrored at 6 and 12 month follow up in both groups and assessment tool used. Full assessment for quality of life assessment is shown in (Table 3.8 and 3.9).

Table 3.9. Mean scores and mean changes in EQ5D among the patient population

	Control (n = 25)	BMNC (n = 37)	Between Group p-value
6 months paired samples	(n = 25)	(n = 37)	
12 months paired samples	(n = 28)	(n = 37)	
EQ5D			
Index Score – Baseline	0.65 (0.52 – 0.79)	0.66 (0.53 – 0.79)	0.4611
Index Score – 6 months	0.78 (0.66 – 0.91)	0.73 (0.61 – 0.85)	
Index Score – 12 months	0.76 (0.63 – 0.89)	0.79 (0.71 – 0.87)	
In group p value (6 months)	0.0298	0.1149	
In group p value (12 months)	0.1735	0.0397	
Difference in Index Score			
6 months	0.11 (0.01 – 0.21)	0.10 (-0.03 – 0.23)	0.9167
12 months	0.10 (-0.05 – 0.25)	0.13 (0.01 – 0.26)	0.7484
VAS – Baseline			
VAS – Baseline	59.35 (48.11 – 70.59)	66.50 (58.95 – 74.05)	0.6793
VAS – 6 months	64.95 (53.95 – 76.42)	71.76 (62.68 – 80.83)	
VAS – 12 months	71.59 (63.51 – 80.40)	73.76 (66.06 – 81.47)	
In group p value (6 months)	0.1854	0.0070	
In group p value (12 months)	0.0325	0.0459	
Difference in VAS			
6 months	9.42 (-4.95 – 23.79)	9.00 (2.660 – 15.34)	0.9502
12 months	12.61 (1.15 – 24.07)	7.27 (0.14 – 14.39)	0.3941

Values are mean (95% CI). VAS – Visual analogue scale

Table 3.10. Mean scores and mean changes in SF36 and MacNew questionnaires among the patient population

	Control	BMNC	Between Group p-value
6 months paired samples	(n = 25)	(n = 37)	
12 months paired samples	(n = 28)	(n = 37)	
SF36®			
Physical – Baseline	38.13 (33.30 – 42.95)	38.68 (33.73 – 43.63)	0.6997
Physical – 6 months	44.84 (39.46 – 50.22)	40.29 (35.71 – 44.87)	
Physical – 12 months	44.17 (38.98 – 49.36)	41.69 (36.75 – 46.64)	
In group p value (6 months)	0.0144	0.2356	
In group p value (12 months)	0.0124	0.2535	
Difference in Physical			
6 months	6.34 (1.38 – 11.31)	2.88 (-1.96 – 7.72)	0.3279
12 months	6.03 (1.42 – 10.66)	3.02 (-2.25 – 8.29)	0.4039
Mental – Baseline	47.14 (41.85 – 52.43)	44.13 (44.36 – 53.90)	0.6221
Mental – 6 months	48.85 (42.53 – 54.16)	49.10 (44.64 – 53.56)	
Mental – 12 months	53.08 (49.10 – 57.07)	52.10 (47.86 – 56.33)	
In group p value (6 months)	0.6031	0.6021	
In group p value (12 months)	0.0060	0.2689	
Difference in Mental			
6 months	1.30 (-3.80 – 6.41)	-1.26 (-6.10 – 3.59)	0.4724
12 months	5.95 (1.85 – 10.04)	2.96 (-2.39 – 8.33)	0.4019
MacNew			
Mean Score – Baseline	4.74 (4.18 – 5.29)	5.36 (4.77 – 5.75)	0.3738
Mean Score – 6 months	5.51 (4.91 – 6.10)	5.31 (4.84 – 5.78)	
Mean Score – 12 months	5.69 (5.24 – 6.14)	5.53 (5.10 – 5.97)	
In group p value (6 months)	0.0155	0.7768	
In group p value (12 months)	0.0017	0.2838	
Difference in Mean Score			
6 months	0.59 (0.12 – 1.06)	0.06 (-0.38 – 0.50)	0.1081
12 months	0.96 (0.39 – 1.53)	-0.24 (-0.24 – 0.78)	0.0723

Values are mean (95% CI). VAS – Visual analogue scale

3.7. NT-proBNP

Blood analysis for NT-proBNP was performed on samples at baseline, 6 months and 12 month months for each patient. At baseline both the BMNC and Controls had similar concentrations. There was a significant decrease in the levels of NT-proBNP in the BMNC over time from 1304 ± 1069 pg/ml at baseline to 514.2 ± 1024 pg/ml at 6 months ($p=0.0028$) to 337.6 ± 615.2 pg/ml at 12 months ($p=0.0010$) and in the Control from 894.6 ± 994.7 pg/ml at baseline to 262.6 ± 225.6 pg/ml at 6 months ($p=0.0036$) to 214.3 ± 140.9 pg/ml at 12 months ($p=0.0005$) (Figure 3.19).

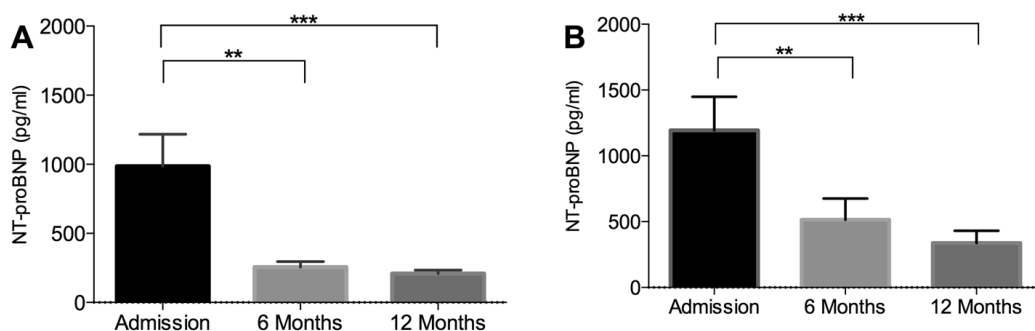


Figure 3.19. NT-proBNP and treatment group

Bar chart (Mean and SEM) Panel A: control, Panel B: BMNC. * = $P<0.05$, **= $P<0.01$, *** = $P<0.001$

At 12 months there was no significant difference between NT-proBNP concentrations (337.6 ± 615.2 pg/ml in the BMNC versus 209.1 ± 141.9 pg/ml in the control ($p=0.2439$).

3.8. Cell Type

The total number of mononuclear cell count in the pre processing bone marrow aspirate was 185×10^9 (IQR: 127-238.5) with a total of 40.6 (18.5-84.2) at the end of processing. The mean cell viability at the end of processing was 97.6%. There was no difference between groups of the baseline bone marrow aspirate and end product (Table 3.11).

Table 3.11. Characteristics of BMNC and cell treatment

	Control (n = 40)	BMNC (n = 51)	p-value (between group)
<u>CD34 Total</u>			
Pre-processing ($\times 10^6$)	3.7 (1.78-5.90)	3.46 (1.55-6.09)	0.8083
% Recovery post-processing	52.5 (36.5-61.1)	46.0 (33.9-60.5)	0.4557
Reinfused ($\times 10^6$)	-	1.32 (0.64-2.55)	-
<u>MNC Total $\times 10^9$</u>			
Pre-processing ($\times 10^9$)	207 (123.5-278.0)	184.8 (127.8-213.3)	0.1710
% Recovery post-processing	17.11 (7.72-32.96)	12.54 (6.97-32.99)	0.6497
Reinfused ($\times 10^9$)	-	31.5 (18.0-71.0)	-
<u>EPC Total $\times 10^2$</u>			
Pre-processing ($\times 10^2$)	60.0 (25.0-140)	57.43 (24.4-226.3)	0.6369
% Recovery post-processing	14.9 (8.4-23.7)	29.16 (6.1-54.6)	0.1638
Reinfused ($\times 10^2$)	-	25.4 (2.56-79.54)	-
Viability (%)	98 (97-99)	98 (97-98)	0.2495

Colony forming unit granulocyte-macrophage progenitor (CFU-GM) assessment was performed in a small number of patients (n=29). CFU-GM was performed prior and at the completion of cell preparation with no difference between either group (Table 3.12).

Table 3.12. CFU-GM assessment

	Control (n = 11)	BMNC (n = 18)	p-value (between group)
<u>Prior to Cell Processing</u> CFU-GM (x10 ⁵)	9.4±11.6	4.7±5.4	0.1418
<u>Completion of Cell Processing</u> CFU-GM (x10 ⁴)	20.5±25.3	24.3±31.5	0.7351

3.8.1. Catheter biocompatibility

Prior to the set up of the REGENERATE-AMI study infusions assessment were performed on a standard commercially available OTW balloon (Medtronic). No difference in the cell viability in any of the 10 samples assessed using a rapid infusion technique (within 10 seconds) or a prolonged infusion technique (over 3 minutes) were seen. The results for all 10 samples are shown below in table 3.13. This data set the standard infusion technique and catheter used for the REGENERATE-AMI study across all sites.

	Pre MNC (ul)		Post MNC (ul)		Pre viability (%)		Post viability (%)	
	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
Sample 1	477.76	477.6	306.78	152.8	66.3	88.3	80.7	82.4
Sample 2	450.72	450.72	176	186.66	70.5	70.5	91.3	88.9
Sample 3	278.85	278.85	176.24	129.62	69.1	69.1	87.5	84.6
Sample 4	442.19	442.19	205.08	161.22	68.9	68.9	89.5	84.2
Sample 5	256.07	256.07	458.85	384.78	73.9	73.9	93.3	91.9
Sample 6	984	984	769.64	757.4	75.3	75.3	72.9	73.3
Sample 7	935.31	935.31	1027.1	648.62	74.6	74.6	76.3	75.3
Sample 8	821.85	821.85	773.47	718.04	77.8	77.8	71	73.5
Sample 9	984.15	984.15	1198.56	755.53	81.4	81.4	71.4	71.8
Sample 10	938.98	938.98	1004.5	835.42	75	75	77	76.6

3.9. Summary

1. Intra-coronary BMNC infusion appears safe in the short term in patients who have undergone primary PCI for AMI with 24 hours
2. BMA can be performed safely in patients who have been anti-coagulated post primary PCI for AMI
3. In the trial population, BMNC infusion was associated with an improvement in LVEF at 12 months compared to placebo as assessed by advanced cardiac imaging however the improvement was smaller than expected
4. BMNC infusion was associated with a reduction in infarct size and improved myocardial salvage as assessed by cardiac enzymes (CK or Troponin T) or CMR imaging
5. There was an association between baseline LVEF level and effect of BMNC therapy however this did not reach statistical significance

Chapter 4

Changes in Cytokine and Growth Factors within REGENERATE-AMI

4.1. Background

Paracrine factors within the heart play an important role in the re-modelling that occurs after AMI. The cytokine hypothesis holds that the progression to HF is due to a cytokine cascade that exacerbates an indirect haemodynamic or a direct toxic effect on the heart. The role of cytokines in BMNC therapy has previously been seen in both animal and human studies (Deten, Volz et al. 2002, Nian, Lee et al. 2004). Cytokines, which are small molecules that are secreted by cells during times of stress play a role in apoptosis, differentiation and proliferation. Evidence has shown that the transplantation of progenitor cells decrease the release of inflammatory cytokines in response to stresses (Maltais, Tremblay et al. 2010). Additionally there is strong evidence to show that using BMNC can produce and secrete a wide range of cytokines and growth factors that can promote angiogenesis and reduce apoptosis in ischaemic conditions (Yoon, Wecker et al. 2005). This chapter investigates the changes in certain cytokine and growth factors after the early IC infusion of BMNC in patients who were part of REGENERATE-AMI, and to assess if these paracrine factors mediate the results seen.

For this sub-study the following cytokines were selected due to either their previous links as possible adjunctive targets or associations with BMNC therapy or the use of exogenous cytokine therapy in other REGENERATE trial (Hamshere, Arnous et al. 2015). IL-6, TNF- α and VEGF has been widely assessed in both AMI setting and the within cell therapy, therefore a comparison with these trials

would be possible. The exogenous use of G-CSF was once trialled for patients post AMI however as previous stated in this thesis no significant benefit was observed, the rationale behind selecting G-CSF was to assess if endogenous G-CSF played any significant role in conjunction with cell therapy. MMP plays an important spectrum in cardiovascular disease from AMI to heart failure and remodeling. With the progression of heart failure MMP levels increase, therefore we aim to assess if the use of cell therapy reduces the circulating levels of MMP-9.

4.2. Results

Of the 67 patients who were recruited at the London Chest Hospital to the REGENERATE-AMI trial, 64 patients (64% of total study) had bloods taken at baseline prior to BMA and re-infusion procedure. In addition to the baseline bloods, samples were taken at 3 months (n= 15, 23.4% of sub-study population), 6 months (n= 28, 43.8%) and 12 months (n= 36 (56.3%) (Figure 4.1). Baseline data is presented for the 64 patients in Table 4.1. Baseline blood samples were taken prior to BMA and any additional therapy other than standard management for an STEMI.

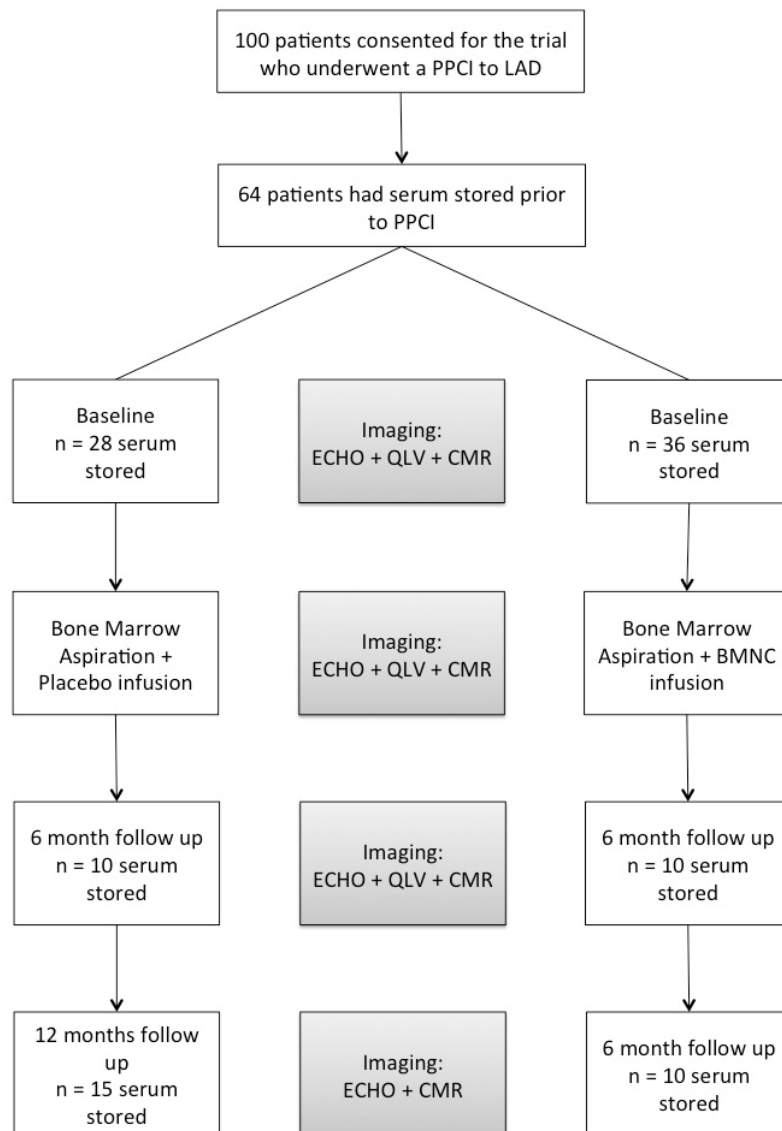


Figure 4.1. Consort diagram of cytokine study in stem cell therapy in acute myocardial infarction

Table 4.1. Baseline characteristics

	Control Group (n=28)	BMNC Group (n=36)
Age (yr), mean \pm SEM	56.54 \pm 2.21	54.64 \pm 1.66
Sex (M/F), (No.)	27/1	30/6
Ethnicity (Caucasian)	22 (78.6%)	28 (77.8%)
BMI, mean \pm SEM	26.19 \pm 0.76	26.58 \pm 0.54
Medical History prior to infarct:		
Hypertension	8 (28.6%)	17 (47.2%)
Hypercholesterolemia	5 (17.9%)	13 (36.1%)
Diabetes mellitus	3 (10.7%)	3 (8.3%)
Active smoker	13 (46.4%)	16 (44.4%)
Previous MI	0 (0%)	1 (2.7%)
Previous PCI	0 (0%)	1 (2.7%)
Family history	8 (28.6%)	17 (30.9%)
Timings:		
Chest Pain to PCI (min), median (IQR)	225.0 (144.8 – 324.5)	227.0 (151.3 – 379.5)
Door to PCI time (min), median (IQR)	34.5 (25.5 – 54.5)	33.25 (33.3 – 57.8)
PCI to reinfusion (min), median (IQR)	942.5 (468.5 – 1385)	589.0 (410 – 1370)
BM aspiration to infusion (min), median (IQR)	306.5 (285.5 – 357.0)	327.0 (280.8 – 373.5)
Baseline LVEF (%), mean \pm SEM	47.71 \pm 1.68	47.30 \pm 1.66
Baseline Infarct size (%), mean \pm SEM	20.79 \pm 2.02	14.91 \pm 8.43
Baseline AAR (%), mean \pm SEM	35.04 \pm 2.56	32.69 \pm 2.08
Sample number		
Baseline (No.)	28	36
Paired	15 (54.5%)	21 (58.3%)
Triplicate samples	9 (32.1%)	18 (50%)

Data are presented as No. (%) unless otherwise specified

4.2.1. Baseline cytokine

The BMNC and control group were similar with respect to baseline plasma concentrations of VEGF, IL-6, TNF- α and G-CSF. There was a significant difference in the baseline MMP-9 concentrations between the control group 155.2 ± 13.79 ng/ml and the BMNC group 68.71 ± 9.02 ng/ml (Table 4.2).

Table 4.2. Baseline Cytokine concentrations

	Control (n = 28)	BMNC (n= 36)	p value
Baseline VEGF (pg/ml)	884.3 ± 109.3	746.2 ± 71.01	0.2756
Baseline IL-6 (pg/ml)	232.4 ± 50.33	264.9 ± 86.28	0.7635
Baseline TNF- α (pg/ml)	26.45 ± 8.05	25.37 ± 6.16	0.8527
Baseline G-CSF (pg/ml)	38.85 ± 16.51	35.27 ± 11.05	0.8527
Baseline MMP-9 (ng/ml)	155.2 ± 13.79	68.71 ± 9.02	<0.0001

Values expressed as Mean and SEM). Statistical comparisons performed using unpaired t-test.

4.2.1.1. VEGF concentrations over time points post primary PCI

In all patients the plasma level of VEGF was highest at baseline 806.6 ± 62.36 pg/ml with a significant change over time with the lowest levels seen at 3 months compared to baseline (259.4 ± 63.55 pg/ml versus 806.6 ± 62.36 pg/ml, $p=0.0004$). Levels at 12 months were similar to but not back to baseline levels (768.3 ± 75.81 pg/ml versus 806.6 ± 62.36 pg/ml) (Figure 4.2)

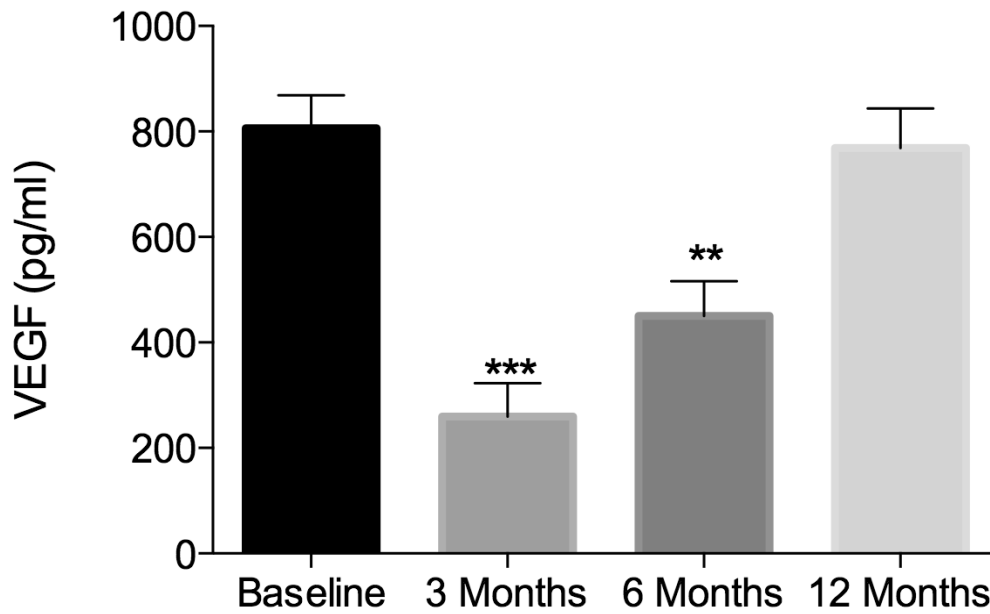


Figure 4.2 VEGF concentration change over time post primary PCI.

Bar charts of VEGF concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. VEGF concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. * denotes significance with $p < 0.05$, ** denotes significance with $p < 0.01$ and *** denotes significance $p < 0.001$ using 1-way ANOVA with Bonferroni's multiple comparison test.

No difference was seen in VEGF concentration when splitting patients in BMNC and control group after primary PCI ($p=0.3115$) (Figure 4.3).

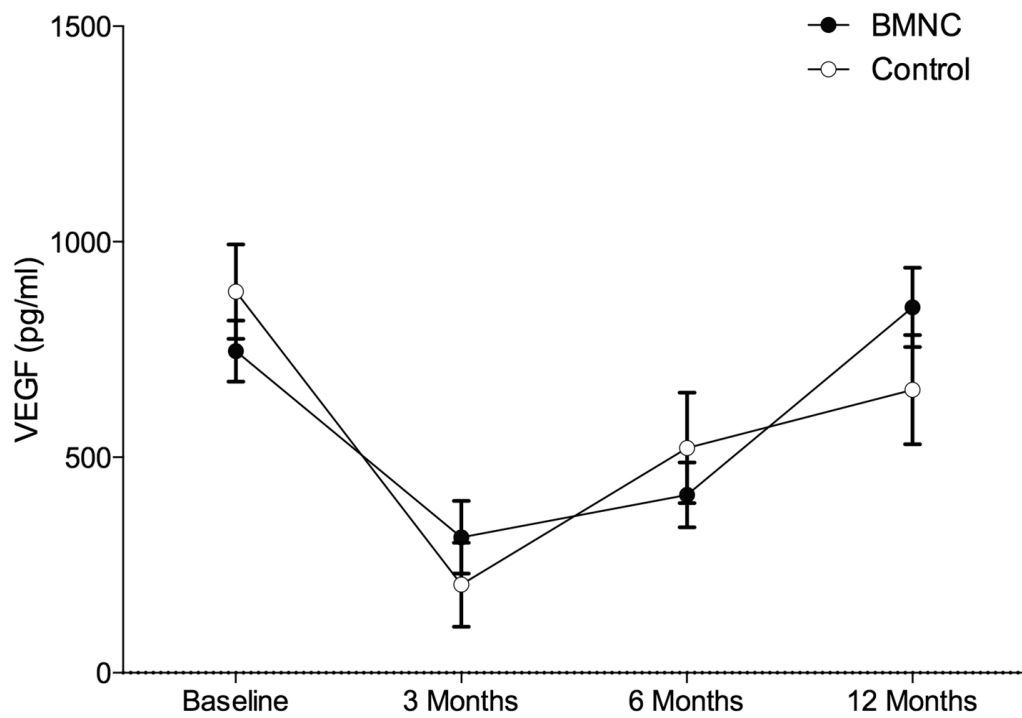


Figure 4.3. VEGF concentration over time by treatment group after primary PCI
VEGF concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. Figure displays VEGF concentration count over time showing BMNC treated vs control. VEGF concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. Statistical comparison performed using two-way repeated measures ANOVA.

4.2.1.2. IL-6 concentrations over time points post primary PCI

There is a decrease seen in IL-6 concentrations over time in all patients however this does not reach conventional statistical significance. Levels of IL-6 were highest at baseline (250.7 ± 52.97 pg/ml) with the lowest levels seen at 6 months although this did not reach significance (95.95 ± 13.17 pg/ml versus 250.7 ± 52.97 pg/ml, $p=0.0716$) (Figure 4.4).

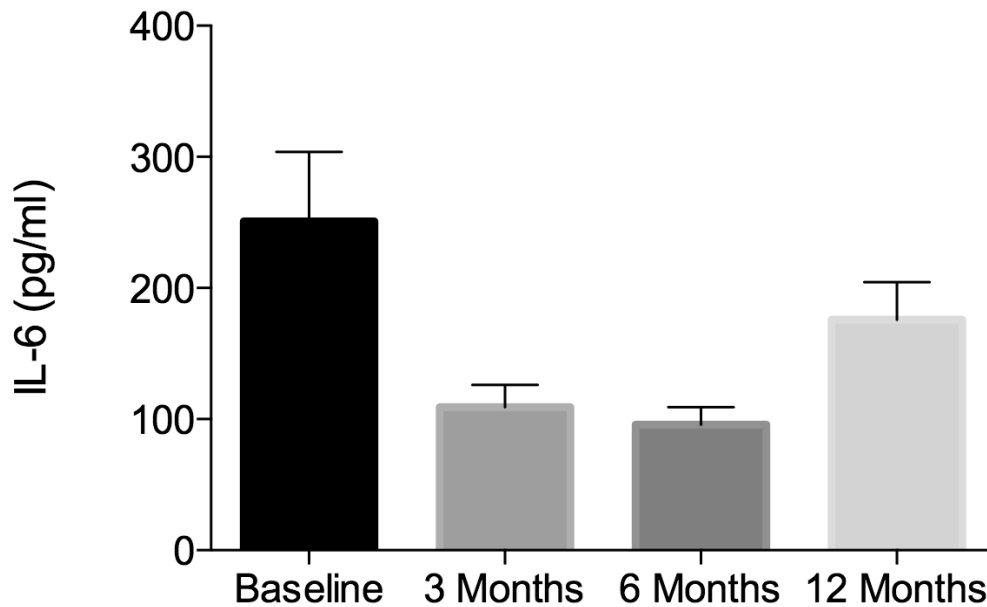


Figure 4.4. IL-6 concentration change over time post primary PCI

Bar charts of IL-6 concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. IL-6 concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. * denotes significance with $p<0.05$, ** denotes significance with $p<0.01$ and *** denotes significance $p<0.001$ using 1-way ANOVA with Bonferroni's multiple comparison test.

No difference was seen in IL-6 concentration when splitting patients in BMNC and control group after primary PCI ($p=0.1028$) (Figure 4.5).

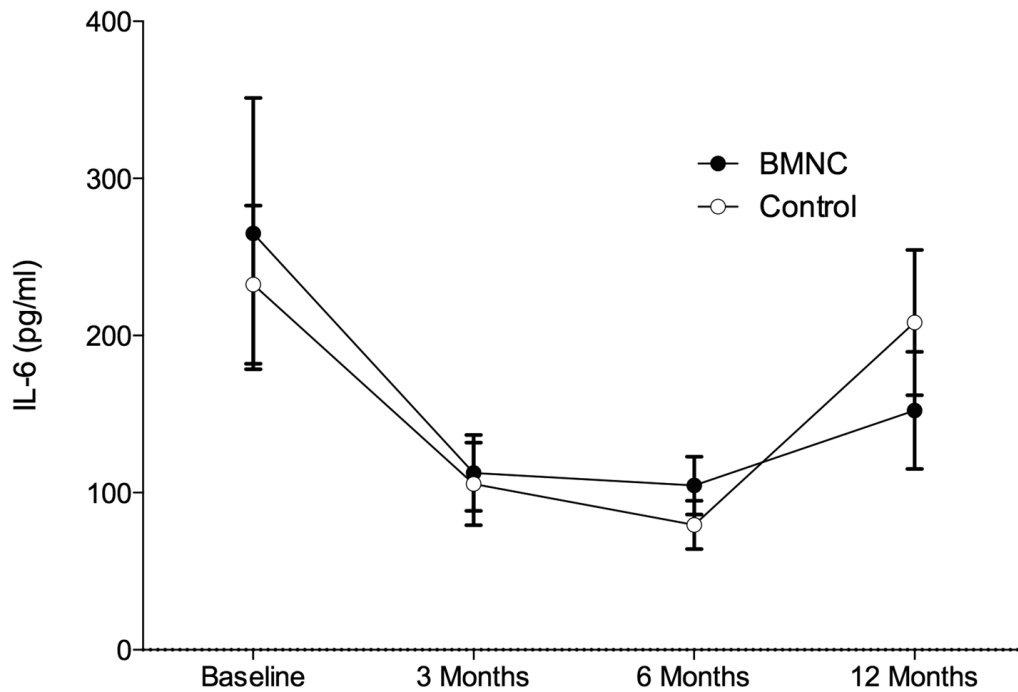


Figure 4.5. IL-6 concentration over time by treatment group after primary PCI

IL-6 concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. Figure displays IL-6 concentration count over time showing BMNC treated vs control. IL-6 concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. Statistical comparison performed using two-way repeated measures ANOVA.

4.2.1.3. TNF- α concentrations over time points post primary PCI

TNF- α appeared to increase at 3 months with a near significant trend for an increase in TNF- α from baseline over time however this did not reach conventional statistical significance ($p=0.0585$). Level of TNF- α was lowest at baseline (25.84 ± 4.90 pg/ml) with the peak level seen at 3 months (27.09 ± 11.39 pg/ml versus 25.84 ± 4.90 pg/ml, $p=0.0790$) (Figure 4.6).

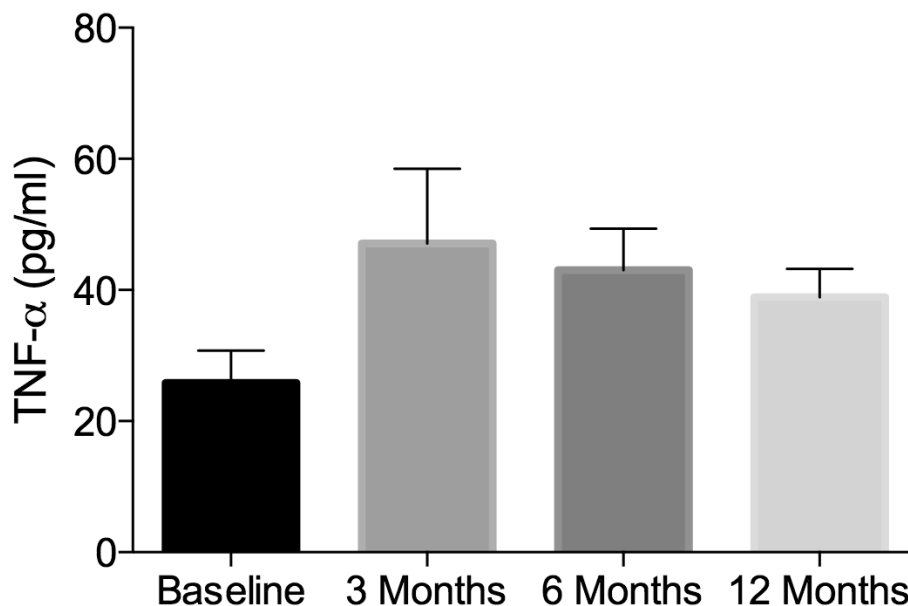


Figure 4.6. TNF- α concentration change over time post primary PCI

Bar charts of TNF- α concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. TNF- α concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM.

No difference was seen in TNF- α concentration when splitting patients in BMNC and control group after primary PCI (p=0.8822) (Figure 4.7).

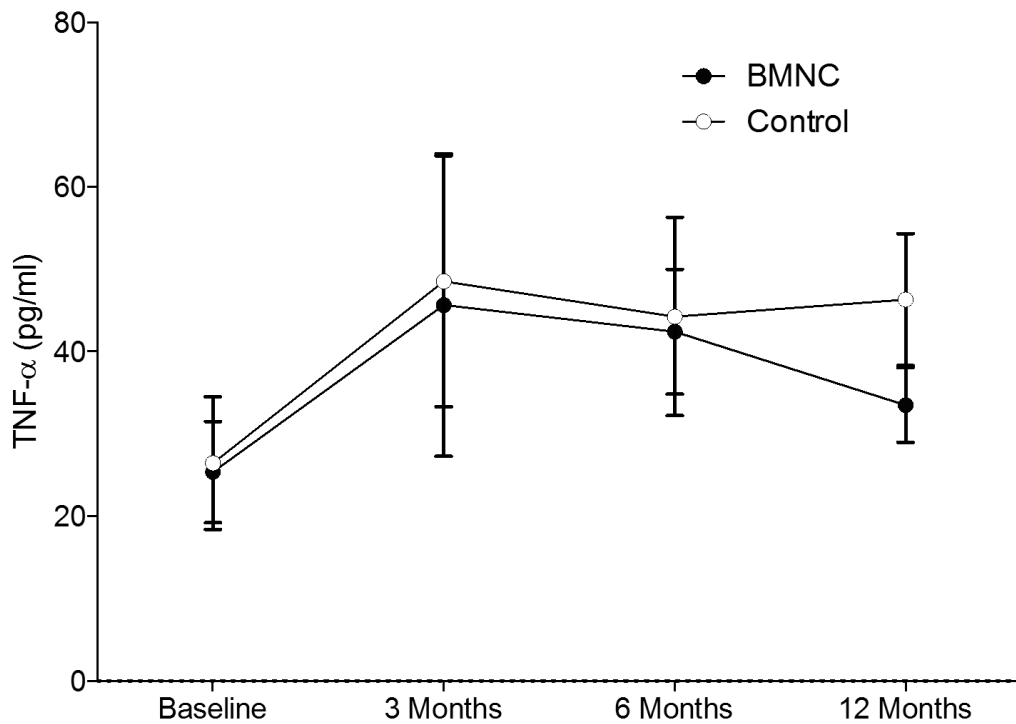


Figure 4.7. TNF- α concentration over time by treatment group after primary PCI

TNF- α concentration measured at baseline, 3 months 6 months and 12 months after primary PCI.

Figure displays TNF- α concentration count over time showing BMNC treated vs control. TNF- α concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM.

Statistical comparison performed using two-way repeated measures ANOVA.

4.2.1.4. Changes in G-CSF concentrations over time after primary PCI

No difference was seen in plasma G-CSF concentrations over time in all patients ($p=0.1273$). Level of G-CSF was lowest at baseline (36.84 ± 9.45 pg/ml) with the highest level seen at 12 months compared to baseline although this did not reach significance (91.25 ± 32.52 pg/ml versus 36.84 ± 9.45 pg/ml, $p=0.0666$) (Figure 4.8).

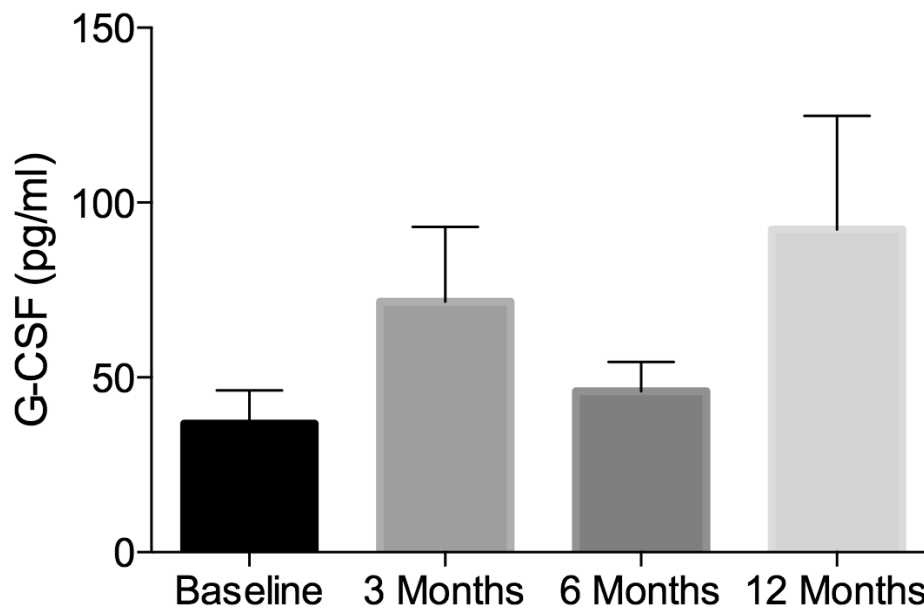


Figure 4.8. G-CSF concentration change over time post primary PCI

Bar charts of G-CSF concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. G-CSF concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM.

No difference was seen in G-CSF concentration when dividing patients in BMNC and control group after primary PCI ($p=0.8822$) (Figure 4.9).

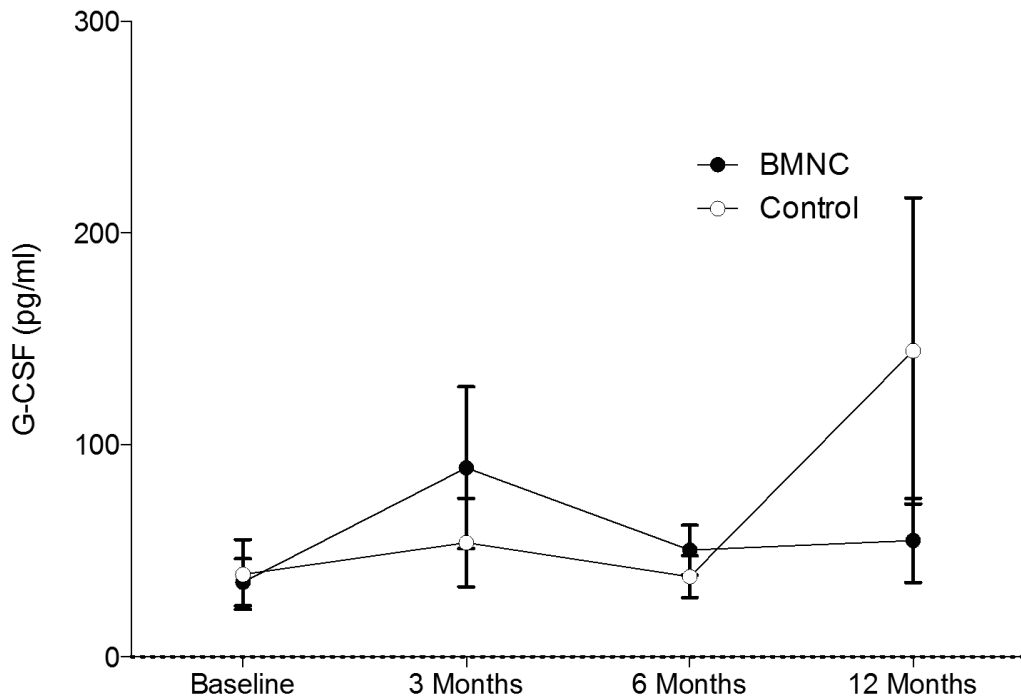


Figure 4.9. G-CSF concentration over time by treatment group after primary PCI

G-CSF concentration measured at baseline, 3 months 6 months and 12 months after primary PCI.

Figure displays G-CSF concentration count over time showing BMNC treated vs control. G-CSF concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. Statistical comparison performed using two-way repeated measures ANOVA.

4.2.1.5. Change in MMP-9 concentrations over time after primary PCI

In all patients the plasma level of MMP-9 was highest at baseline (106.5 ± 9.50 ng/ml) with a significant change over time with lowest level seen at 3 months compared to baseline (12.28 ± 7.20 ng/ml versus 106.5 ± 9.50 ng/ml, $p=0.0003$). Levels at 12 months were similar to baseline levels (101.6 ± 15.28 ng/ml versus 106.5 ± 9.50 ng/ml) (Figure 4.10).

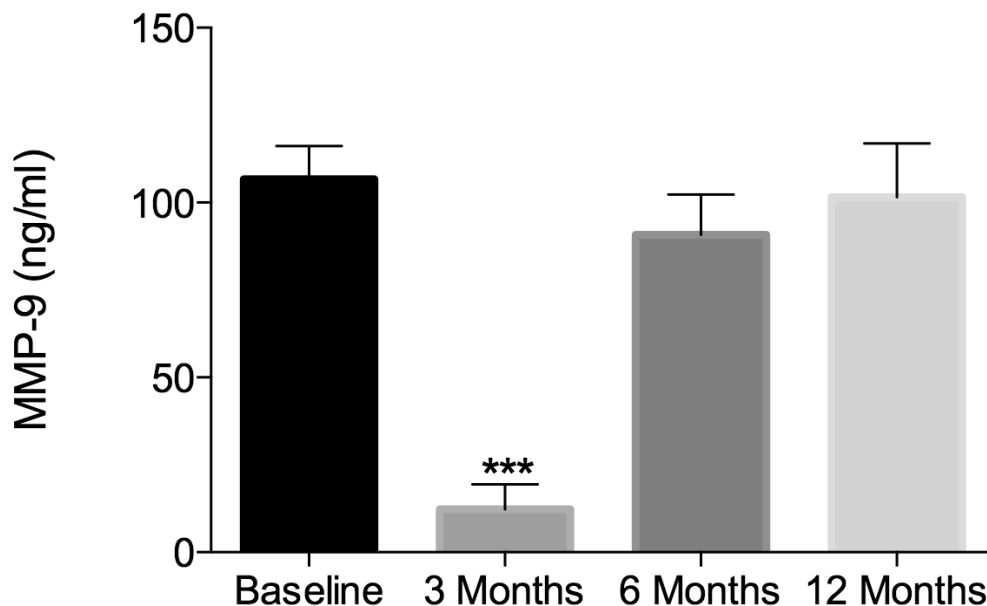


Figure 4.10. MMP-9 concentration change over time post primary PCI

Bar charts of MMP-9 concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. MMP-9 concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. *** denotes significance $p<0.001$ using 1-way ANOVA with Bonferroni's multiple comparison test.

There was a significance difference seen in MMP-9 concentration when splitting patients in BMNC and control group after primary PCI (p=0.0003) (Figure 4.11).

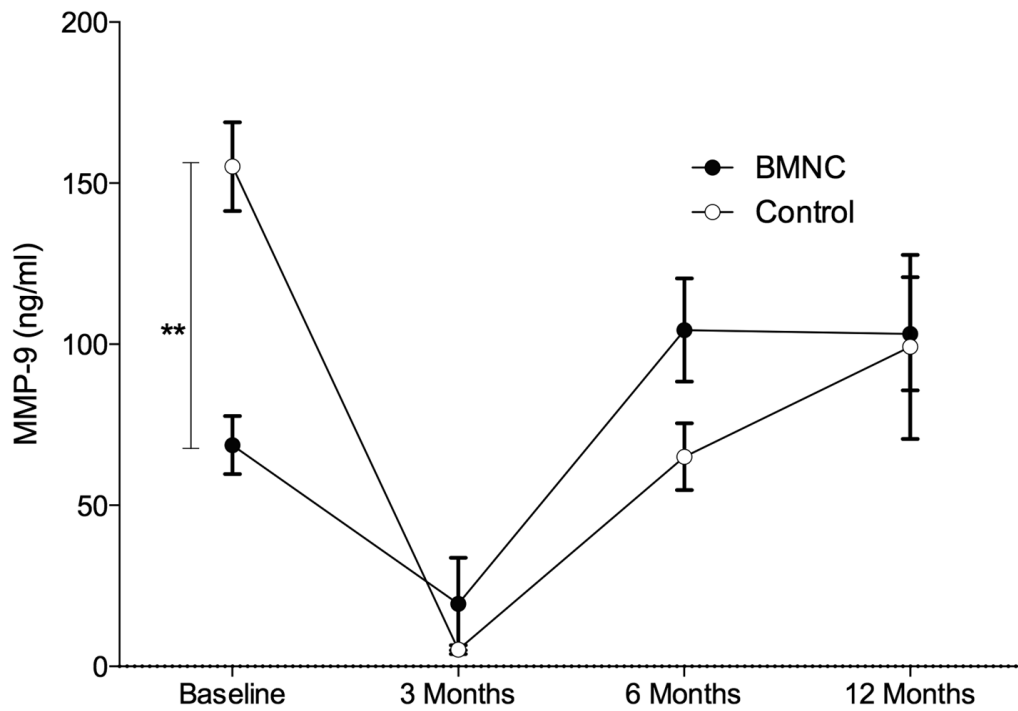


Figure 4.11. MMP-9 concentration over time by treatment group after primary PCI

MMP-9 concentration measured at baseline, 3 months 6 months and 12 months after primary PCI.

Figure displays MMP-9 concentration count over time showing BMNC treated vs control. MMP-9 concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. Statistical comparison performed using two-way repeated measures ANOVA. ** denotes significance $p < 0.01$

4.2.2. Relationship between cytokines and infarct size

Baseline imaging was performed on patients at day 3 after primary PCI. Analysis demonstrated that both baseline G-CSF and TNF- α concentrations showed a weak association with infarct size (Figure 4.12). No correlations were observed with MMP-9, IL-6 or VEGF (Table 4.3).

Table 4.3. Correlation matrix of cytokine concentrations to LVEF and change in cytokine concentration and change in LVEF over time

Cytokine	Infarct Size (correlation coefficient/ p value)
VEGF	0.2136 / 0.0927
IL-6	0.3650 / 0.0033 **
TNF- α	0.2628 / 0.0853
G-CSF	0.3162 / 0.0116 **
MMP-9	0.1195 / 0.3508

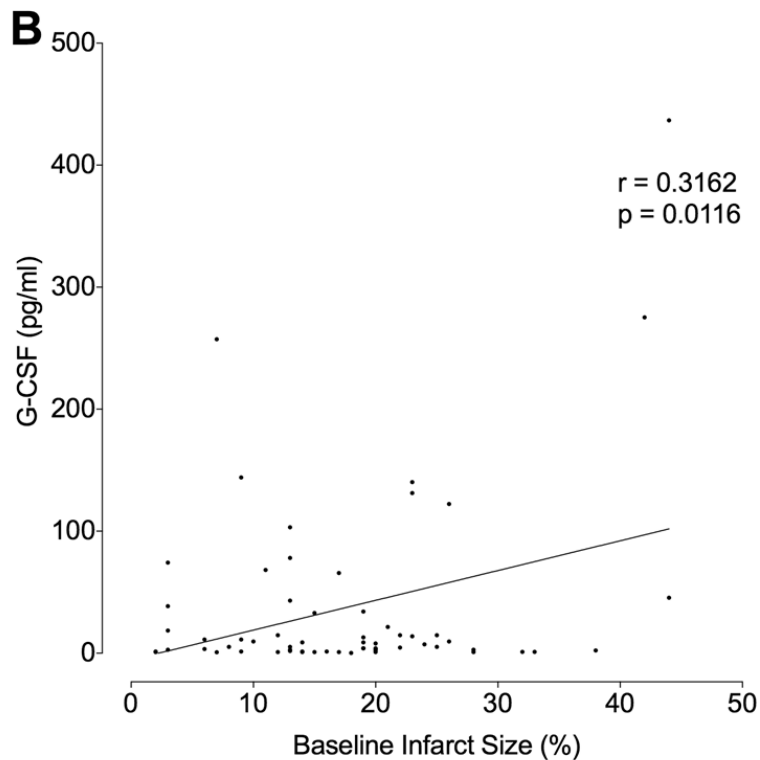
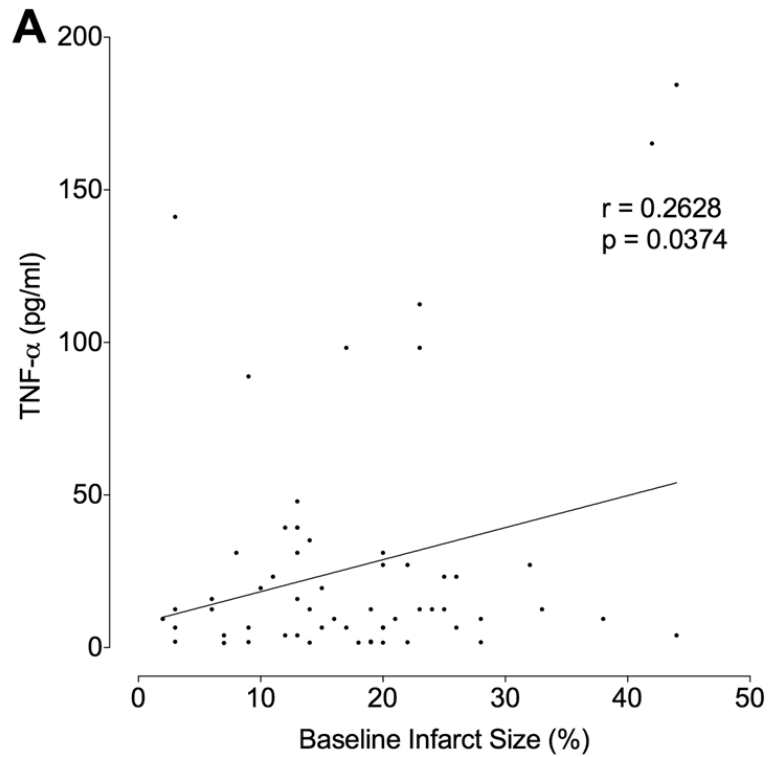


Figure 4.12. Associations between cytokine concentration and infarct size on CMR at baseline

There was a significant positive association between TNF- α concentration and LGE assessed infarct size on CMR at baseline, as shown in panel A. Panel B depicts a similar positive association between G-CSF concentration and LGE assessed infarct size on CMR at baseline

4.2.3 Relationship between cytokine concentration and Left Ventricular Ejection Fraction

There was a weak but significant relationship seen between combined IL-6 concentrations and all time point LVEF (Figure 4.13).

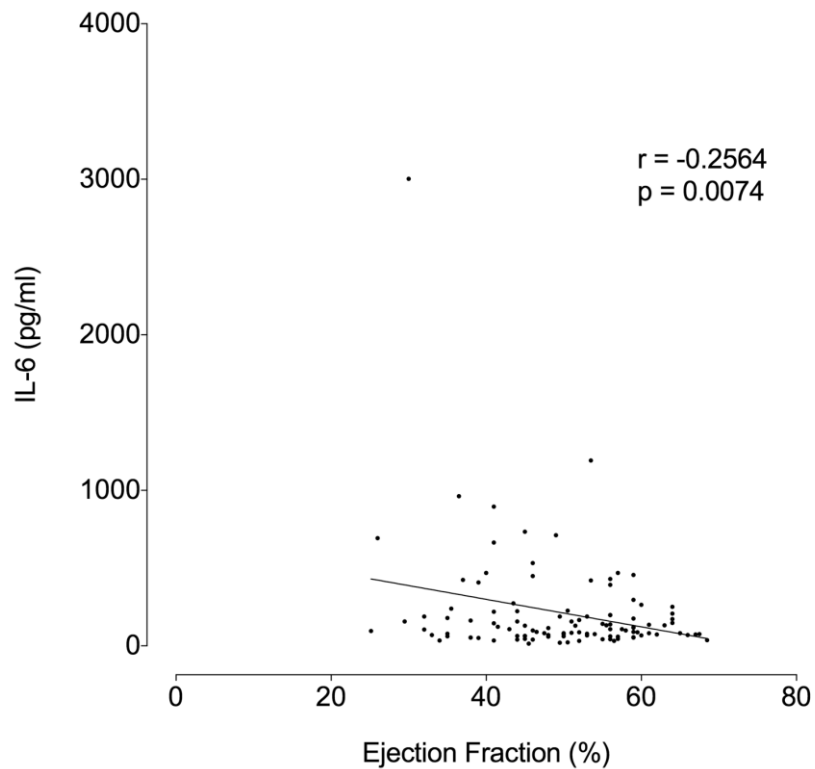


Figure 4.13. Associations between IL-6 concentration and LVEF on CMR at baseline

There was a significant weak negative association between VEGF concentration and LVEF assessed on CMR at baseline

No relationship was observed in the remainder of the cytokine concentration and LVEF or in all the changes in cytokine concentration or change in LVEF over time (Table 4.4).

Table 4.4. Correlation matrix of cytokine concentrations to LVEF and change in cytokine concentration and change in LVEF over time

Cytokine	LVEF (correlation coefficient/ p value)	Change in LVEF (correlation coefficient/ p value)
VEGF	0.0600 / 0.5358	-0.1627 / 0.3505
IL-6	-0.2564 / 0.0074**	-0.2294 / 0.1850
TNF- α	-0.0923 / 0.3419	0.0317 / 0.8565
G-CSF	-0.1076 / 0.2676	-0.0232 / 0.8949
MMP-9	-0.0727 / 0.4544	0.0655 / 0.7088

4.2.3.1. Correlation between change in cytokine concentration and area at risk

As previously described cytokine concentration changed over time in the total population. There was a strong negative correlation between change in VEGF concentration at 1 year and change in AAR at 1 year (Figure 4.14). No correlations were observed with IL-6, TNF- α , G-CSF and MMP-9 (Table 4.5).

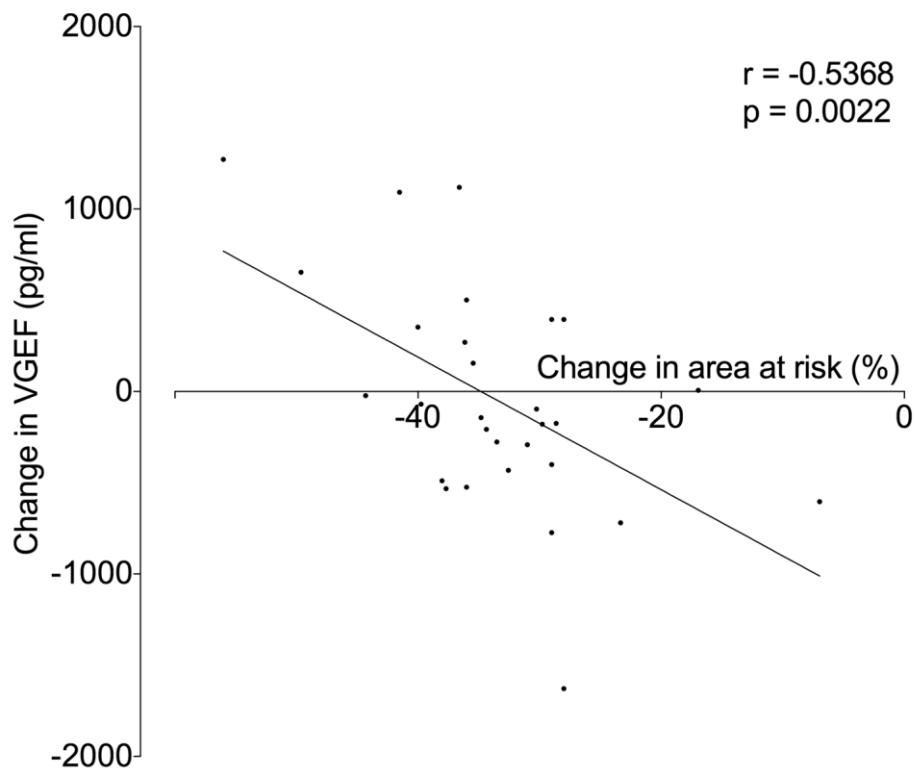


Figure 4.14. Associations between VEGF concentration and area at risk size on CMR at baseline

There was a significant positive association between VEGF concentration and myocardial oedema assessed AAR on CMR at baseline

Table 4.5. Correlation matrix of change in cytokine concentrations at 1 year and change in AAR at 1 year

Cytokine	Infarct Size (correlation coefficient/ p value)
VEGF	-0.3674 / 0.0420
IL-6	0.2304 / 0.2125
TNF- α	0.1909 / 0.3035
G-CSF	0.2008 / 0.2789
MMP-9	-0.1750 / 0.3463

4.2.4. Relationship between cytokine concentration and NT-ProBNP

Matched NT-proBNP samples were correlated with corresponding cytokine concentrations of the combination time point (baseline, 3 months, 6 months and 12 months). Analysis demonstrated that TNF- α was directly associated with NT-proBNP whilst MMP-9 demonstrated a negative association with NT-proBNP (Figure 4.15).

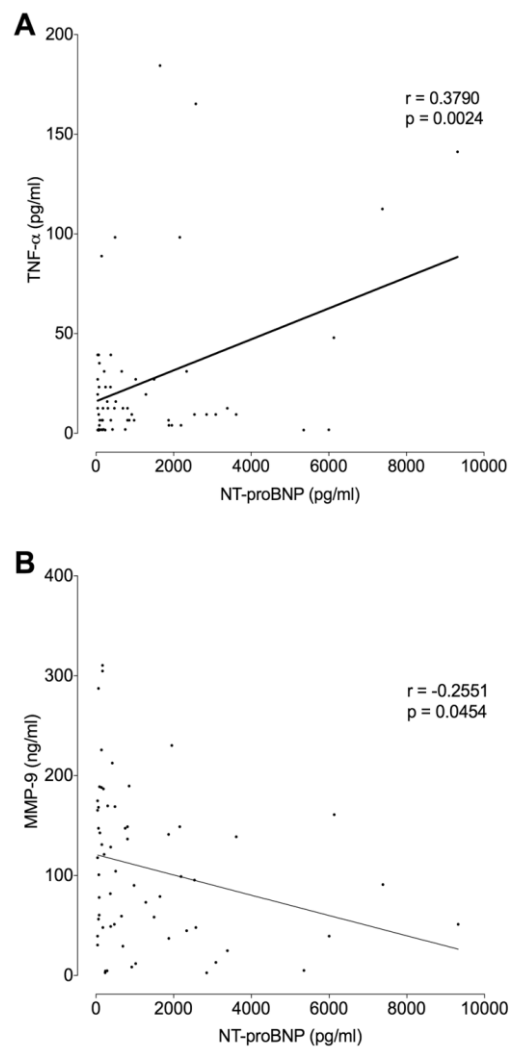


Figure 4.15. Associations between cytokine concentration and NT-proBNP

There was a significant positive association between TNF- α concentration and NT-proBNP, as shown in panel A. Panel B depicts a negative association between MMP-9 concentration and NT-proBNP concentrations

4.2.5. Cytokine concentration in BMNC responders

In order to test whether the cytokine concentration were related to changes in LVEF assessment of the BMNC group responders was performed. The median improvement of LVEF in all patients was 6% at 12 months. In those patient who received BMNC therapy 20 patients were deemed improvers with an LVEF improvement $\geq 6\%$ and 16 as non-improvers ($\leq 6\%$). There was no significant difference between baseline cytokines concentrations between both groups.

4.2.5.1. Change in VEGF concentrations over time in improvers vs non improvers

There was no significant difference seen in VEGF concentration when dividing patients into improvers and non-improvers ($p=0.5644$) (Figure 4.16).

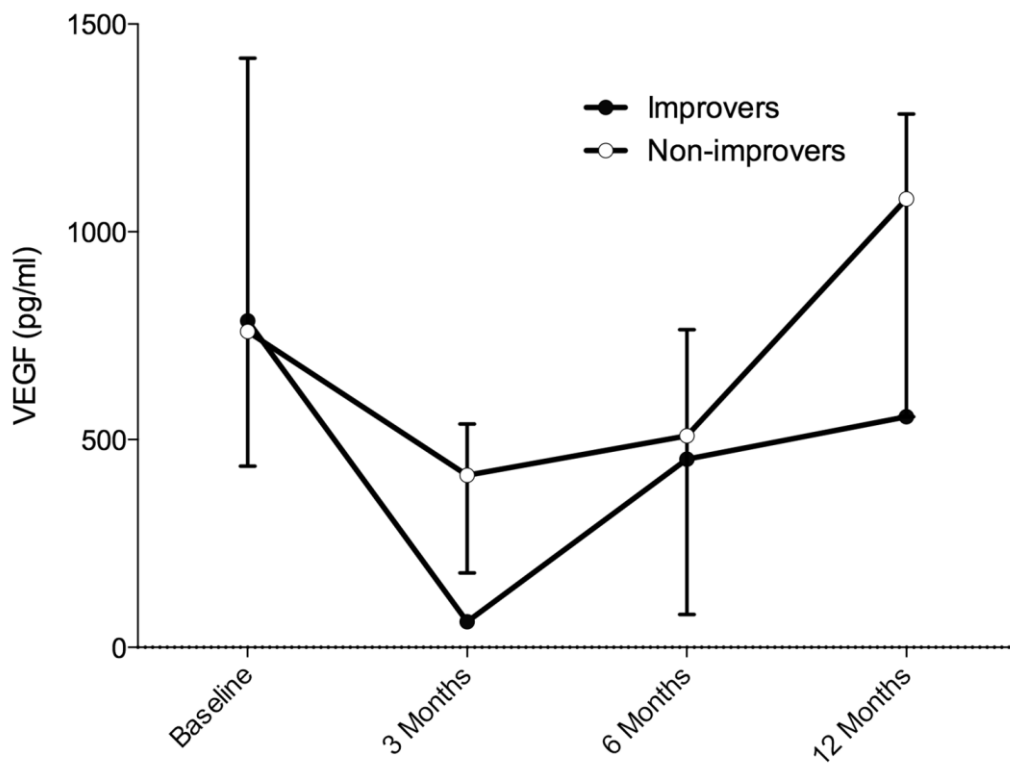


Figure 4.16. VEGF concentration over time by improvers and non-improvers

VEGF concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. Figure displays VEGF concentration count over time showing Improvers vs Non-improvers. VEGF concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. Statistical comparison performed using two-way repeated measures ANOVA.

4.2.5.2. Change in IL-6 concentrations over time in improvers vs non improvers

There was no significant difference seen in IL-6 concentration when dividing patients into improvers and non-improvers ($p=0.7538$) (Figure 4.17).

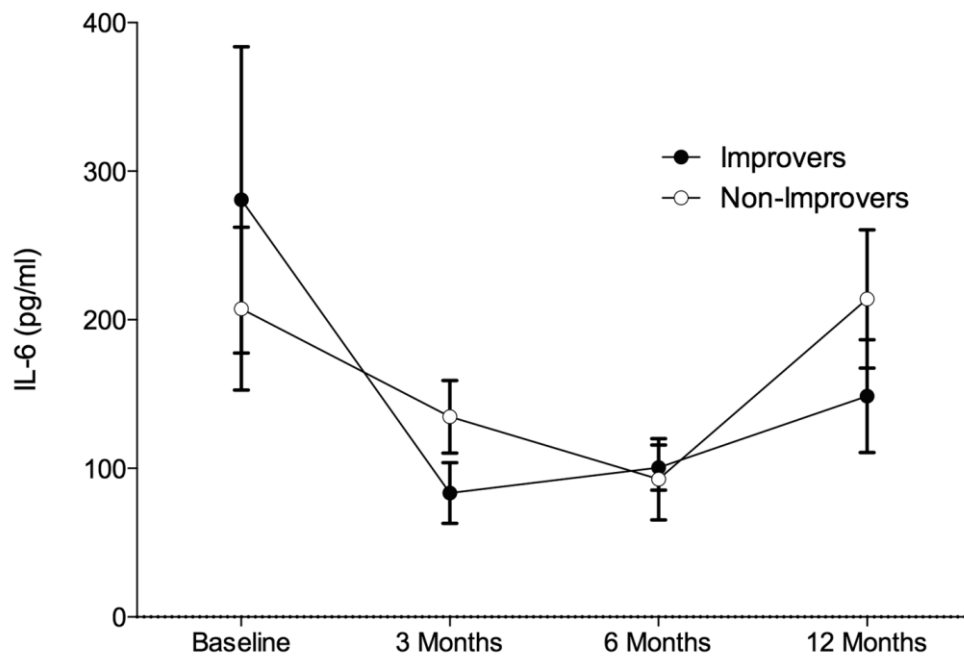


Figure 4.17. IL-6 concentration over time by improvers and non-improvers

IL-6 concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. Figure displays IL-6 concentration count over time showing Improvers vs Non-improvers. IL-6 concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. Statistical comparison performed using two-way repeated measures ANOVA.

4.2.5.3. Change in TNF- α concentrations over time in improvers vs non improvers

There was no significant difference seen in TNF- α concentration when dividing patients into improvers and non-improvers ($p=0.7538$) (Figure 4.18).

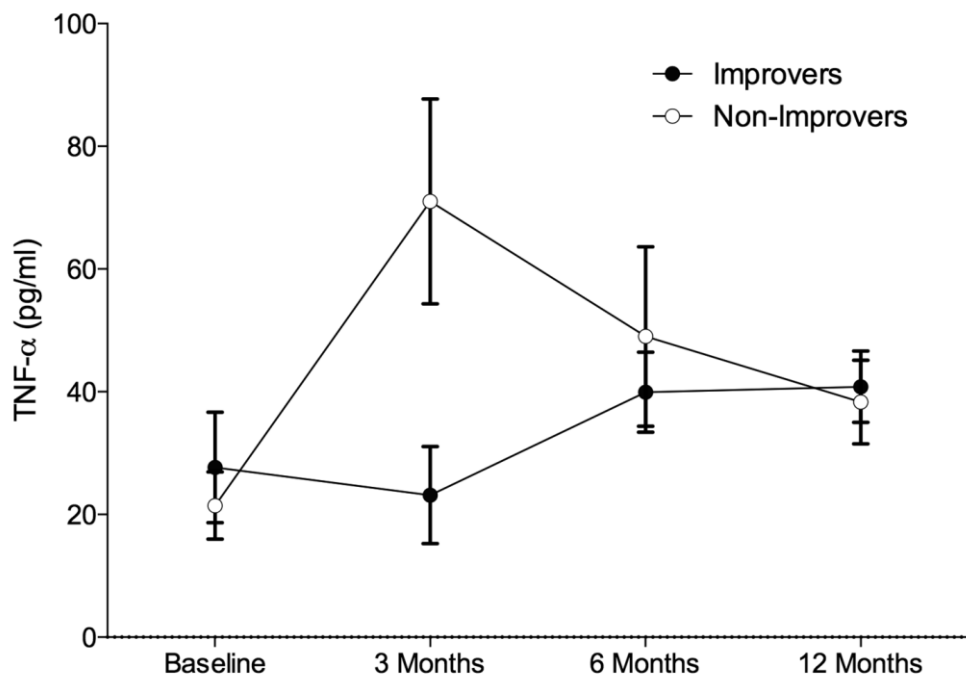


Figure 4.18. TNF- α concentration over time by improvers and non-improvers

TNF- α concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. Figure displays TNF- α concentration count over time showing Improvers vs Non-improvers. TNF- α concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. Statistical comparison performed using two-way repeated measures ANOVA.

4.2.5.4. Change in G-CSF concentrations over time in improvers vs non improvers

There was no significant difference seen in G-CSF concentration when splitting patients into improvers and non-improvers (Figure 4.19).

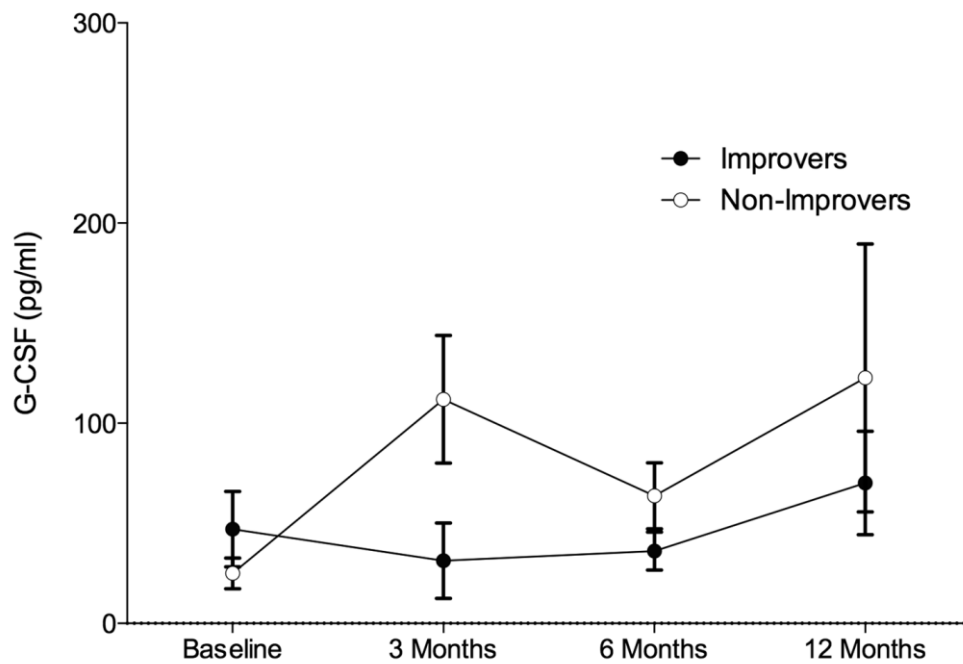


Figure 4.19. G-CSF concentration over time by improvers and non-improvers

G-CSF concentration measured at baseline, 3 months 6 months and 12 months after primary PCI.

Figure displays G-CSF concentration count over time showing Improvers vs Non-improvers. G-CSF concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. Statistical comparison performed using two-way repeated measures ANOVA.

4.2.5.5. Change in MMP-9 concentrations over time in improvers vs non improvers

There was no significant difference seen in MMP-9 concentration when splitting patients into improvers and non-improvers ($p=0.7538$) (Figure 4.20).

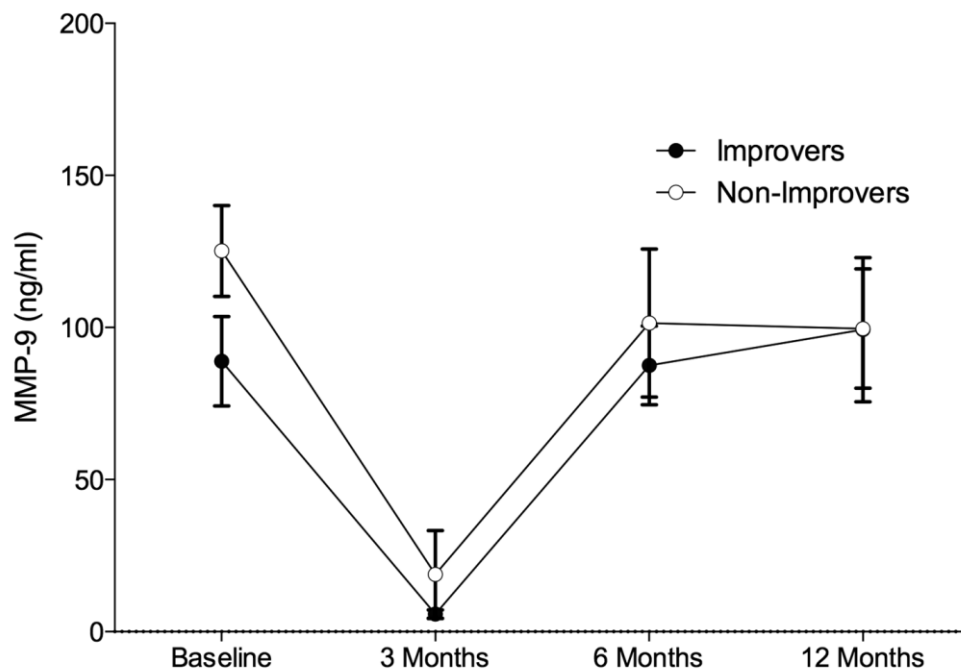


Figure 4.20. MMP-9 concentration over time by improvers and non-improvers
MMP-9 concentration measured at baseline, 3 months 6 months and 12 months after primary PCI. Figure displays MMP-9 concentration count over time showing Improvers vs Non-improvers. MMP-9 concentration assessed over time in all patients is shown. Data expressed as mean \pm SEM. Statistical comparison performed using two-way repeated measures ANOVA.

One year after AMI all acute changes seen on T2-STIR imaging had resolved. As stated in the previous chapter there was a significant reduction in the mean scar burden on follow up in the BMNC group with a significant mean over improvement in LVEF over time. Patients who were deemed non-improvers showed a negative correlation between changes in VEGF concentrations and changes in LVEF at 1 year ($r=-0.5656$, $p=0.0224$) that was not seen in improvers ($r=0.2583$, $p=0.2857$) (Figure 4.21). In addition in the non-improvers there was a significant positive correlation between changes in TNF- α concentrations and changes in infarct size over follow up ($r=0.6344$, $p=0.011$) which again was not seen in the LVEF improvers ($r=0.0989$, $p=0.7057$) (Figure 4.21).

Within the LVEF improvers sub-group there was a significant negative relationship between changes in IL-6 concentrations and changes in LVEF at 1 year ($r=-0.4727$, $p=0.0410$) that was not seen in the non-improvers group ($r=0.0796$, $p=0.7694$) (Figure 4.21).

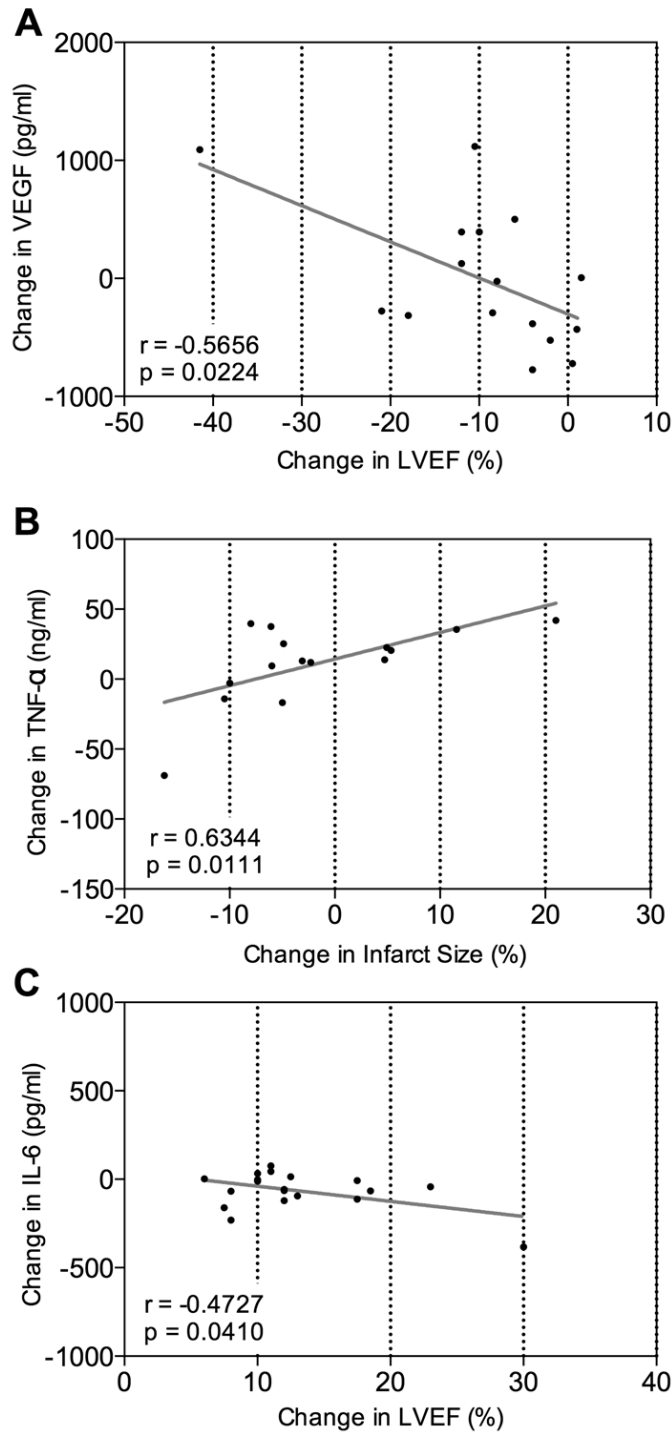


Figure 4.21. Association between change in cytokine and CMR assessed changes in infarct time and LVEF change

There was a significant negative correlation between change in LVEF assessed by CMR and change in VEGF concentration ($r=-0.5656$) in the non-improvers, as shown in panel A. Panel B depicts a positive association between change in TNF- α and change in infarct size as measured by CMR ($r=0.6344$). Panel C depicts a negative correlation in LVEF assessed by CMR and change in IL-6 concentration ($r=-0.4727$) in the improvers. Associations determined using Pearson's correlation coefficient assessment

4.3. Summary

1. In the whole cohort serum cytokine concentrations changed after primary PCI and by 12 months had returned to near baseline. Significant time related changes were seen in VEGF concentrations and near significant changes seen in TNF- α concentrations
2. MMP-9 concentrations were significantly higher at baseline and over time observed in the BMNC group compared to control
3. Significant relationships was seen in both baseline serum concentrations of TNF- α and G-CSF with CMR assessed infarct size
4. A significant positive correlation was observed between baseline IL-6 concentrations and LVEF measured by CMR
5. A significant relationship was observed between change in VEGF concentrations and change in AAR over follow up
6. Serum NT-proBNP showed a significant relationship with both serum TNF- α and MMP-9 concentrations
7. In a sub study of LVEF improvers vs. non-improvers there was a significant negative relationship observed between change in IL-6 concentrations and change in LVEF concentrations over time

8. In non-improvers there was a significant negative correlation observed between change in VEGF concentrations and LVEF and changes in TNF- α concentrations and infarct size

9. No significant changes were observed between BMNC group and control between VEGF, IL-6, TNF- α and G-CSF cytokine concentrations over all time points

10. No significant changes were observed over all assessed cytokines and the sub-study LVEF improves vs. non-improvers groups

Chapter 5

Use of Cardiac MRI in assessing myocardial pathology following Acute Myocardial Infarction

5.1. Background

CMR has become the gold standard in the quantification of ventricular volumes, function and tissue characterisation (Lorenz, Walker et al. 1999). It is now well established for the assessment of the myocardial AAR defined as 'the ischaemic myocardium during coronary artery occlusion i.e. the region that will be subject to infarction if the blood flow is not restored' after an AMI, using T2-weighted imaging to assess the myocardial oedema occurring in the ischaemic myocardium.

The quantification of the AAR is crucial in assessing the efficacy of reperfusion therapy and novel cardioprotective agents. Currently methods for assessing the AAR require coverage of the whole left ventricle with 10-12 myocardial slices acquired usually with a breath hold per acquisition. This can lengthen the CMR scan for patients early after a myocardial infarction and therefore techniques that shorten examination times are advantageous to improve patients' compliance and to reduce the risk for patients with AMI. Additionally despite advancements in semi-automated software, the analysis of the slices still requires contour detection and manual readjustments of multiple slices throughout the cardiac cavity remains a tedious process.

Thus the aim of this chapter is to assess the feasibility of 3-slice T2-STIR imaging for the assessment of the AAR following AMI and to compare 3-slice STIR imaging

to conventional 10-slice STIR imaging, the ESA method and angiographic AAR scores.

5.2. Study Design

Data was collected from patients who had undergone primary PCI for STEMI within 24 hours of symptoms onset. CMR scans were obtained from three clinical trials having the same time point of imaging performed at day 3. These trials were REGENERATE- AMI (NCT00765453), NITRITE-AMI (NCT01584453) and myocardial oedema in acute myocardial infarction (NCT00987259). Patient within the NITRITE-AMI study underwent IC infusion of nitire or placebo during primary PCI whilst patient within the myocardial oedema in acute myocardial infarction study only underwent primary PCI. CMR protocol for patients in the REGENERATE-AMI has previously been described in Chapter 2.7.3.1. Patients in the NITRITE-AMI and myocardial oedema in acute myocardial infarction underwent standard CMR imaging with conventional 10-slice STIR imaging. Angiographic risk assessment has previously been described in Chapter 2.7.3.4.2 and was calculated on all patients.

5.2.1. Statistical analysis

Baseline demographics and continuous variables are summarised for 3 slice and 10 slice groups. Continuous variables are presented as means \pm SD and categorical variables are presented as percentages. 95% confidence intervals (CI)

are given. Intra-observer variability and correlation between the methods were calculated using the coefficient of intra-class correlation. All p-values are 2-sided and a value <0.05 was considered to indicate statistical significance. Data plotting used in analyzing the agreement between the different methods was made with Bland-Altman analysis. All statistical analyses were performed using SPSS version 19 (IBM Corp. Armonk, NY, USA) and graphs produced using Graphpad Prism version 5.0 (GraphPad Software, San Diego, CA).

5.3. Results

5.3.1. General characteristics and Study population

167 patients presenting with AMI undergoing primary PCI at a single cardiac intervention centre were included in the analysis. The mean ages of patient was 56.2 ± 10.18 years and 88% were male. Clinical, CMR and angiographic characteristics of the population are shown in Table 5.1. Patients underwent either 3 slice (n=82) or 10 slice (n=85) T2-weighted STIR imaging for AAR assessment as previously described. These 2 groups (3 slice and 10 slice) were similar with regards to age, sex, LVEF and medical therapy, with the only difference between the 2 groups being a difference in culprit vessel treated with a greater number of LAD occlusions in the 3 slice group ($p < 0.0001$). CMR was performed at a median of 3 days (range: 2-3 days) after primary PCI. In all cases, increased signal intensity was detected in T2-STIR as well as in late enhancement sequences

Table 5.1. Baseline characteristics of the study population.

	3 slice (n=82)	10 slice (n=85)	P value
Age (yr)	56.95 ± 10.49	55.93 ± 11.35	0.6202
Sex (M/F)	71/11	75/12	0.3037
BMI (kg/m ²)	27.04 ± 3.79	27.44 ± 3.87	0.5277
Ethnicity (Caucasian)	66 (80%)	71(83.5%)	0.6113
Medical History:			
Hypertension	30 (36.5%)	28 (32.9 %)	0.6235
Hypercholesterolemia	24 (29.3%)	31 (26.4 %)	0.3251
Diabetes mellitus	10 (12.2%)	9 (11.6 %)	0.5766
Active smoker	42 (51.2%)	47 (55.3 %)	0.6004
Previous MI	1 (1.2%)	2 (2.4 %)	0.5841
Previous PCI	1 (1.2%)	3 (3.5 %)	0.3320
Family history	25 (30.5%)	19 (22.4 %)	0.2354
Culprit Vessel:			
LAD (No. (%))	82 (100%)	23 (27%)	<0.0001
LCx (No. (%))	0 (0%)	11 (13%)	
RCA (No. (%))	0 (0%)	51 (60%)	
Timings:			
Chest Pain to PCI (min)	194.5 ± 25.42	201.0 ± 32.16	0.8459

Data presented as No. (%) unless otherwise specified. BMI - body mass index, LAD - left anterior descending artery, LCx - left circumflex artery, RCA - right coronary artery, PCI - percutaneous coronary intervention.

5.3.2. Comparison of 3-slice AAR size and 10-slice AAR size

The 10-Slice AAR group underwent both 10-slice and 3-slice analysis to directly compare the 2 techniques. When assessing the 3-slice acquisition in the 10-slice cohort, all scans had the same level basal, mid and apical slice assessed. Within the 10-slice AAR group the AAR ranged from 11.5 to 46.8% (mean 27.89 ± 7.33) and the 3-slice AAR assessment of the same patients showed an AAR range from 10.0 to 52.0% (mean 27.86 ± 8.26). There were strong correlations between 3-slice AAR and 10-slice AAR in this patient group ($r=0.9224$, $p<0.0001$) (Figure 5.1).

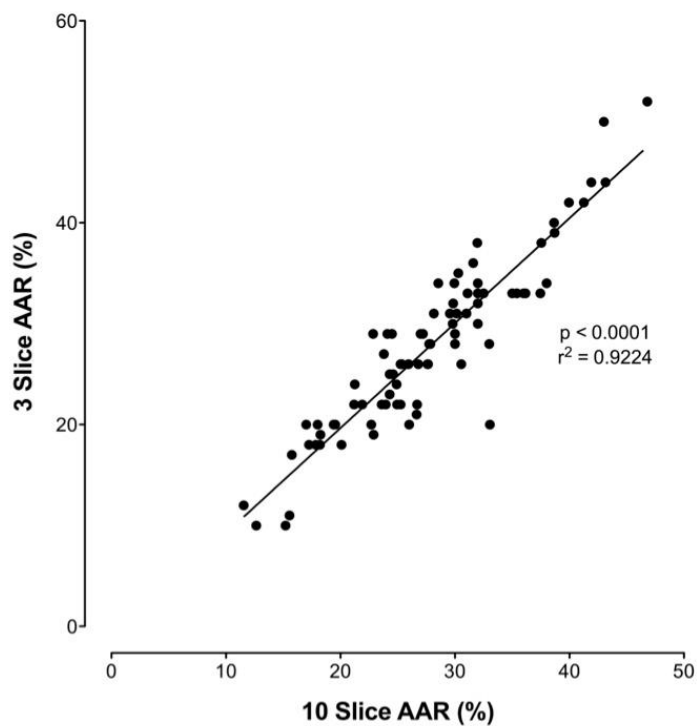


Figure 5.1. Association between 3 Slice AAR and 10 Slice AAR. Data presented shows the relationship between 3-slice STIR area at risk and 10-slice STIR area at risk assessed by CMR on sub group 10-slice STIR group

5.3.3. Comparison of CMR assessed AAR size and angiographic risk

The CMR assessed AAR in the 3-slice group ranged from 8 to 56% of the LV myocardium. Angiographic AAR was assessed for all patients, with the BARI risk score ranging from 21.0 to 47.4% (mean 35.32 ± 8.99) and the APPROACH angiographic risk score ranging from 27.5 to 47.8% (mean $37.6 \pm 7.6\%$) of the LV myocardium with good correlation between the 2 scores ($r=0.8639$ $p<0.0001$).

Oedema ESA was assessed for all patients, with the oedema ESA ranging from 2 to 59% (mean $31.47 \pm 11.60\%$). The intraclass correlation coefficients between the angiographic and CMR methods of 3-slice and 10-slice STIR imaging techniques for the assessment of myocardial oedema were performed (Table 5.2). There was significant correlation between 3-slice AAR and oedema EAS ($r=0.9126$, $p<0.0001$), in addition there was significant correlation between the 3-slice AAR and both the BARI angiographic risk score ($r=0.3049$, $p =0.01$) and the APPROACH angiographic risk score ($r=0.2530$, $p=0.0359$) (Figure 5.2).

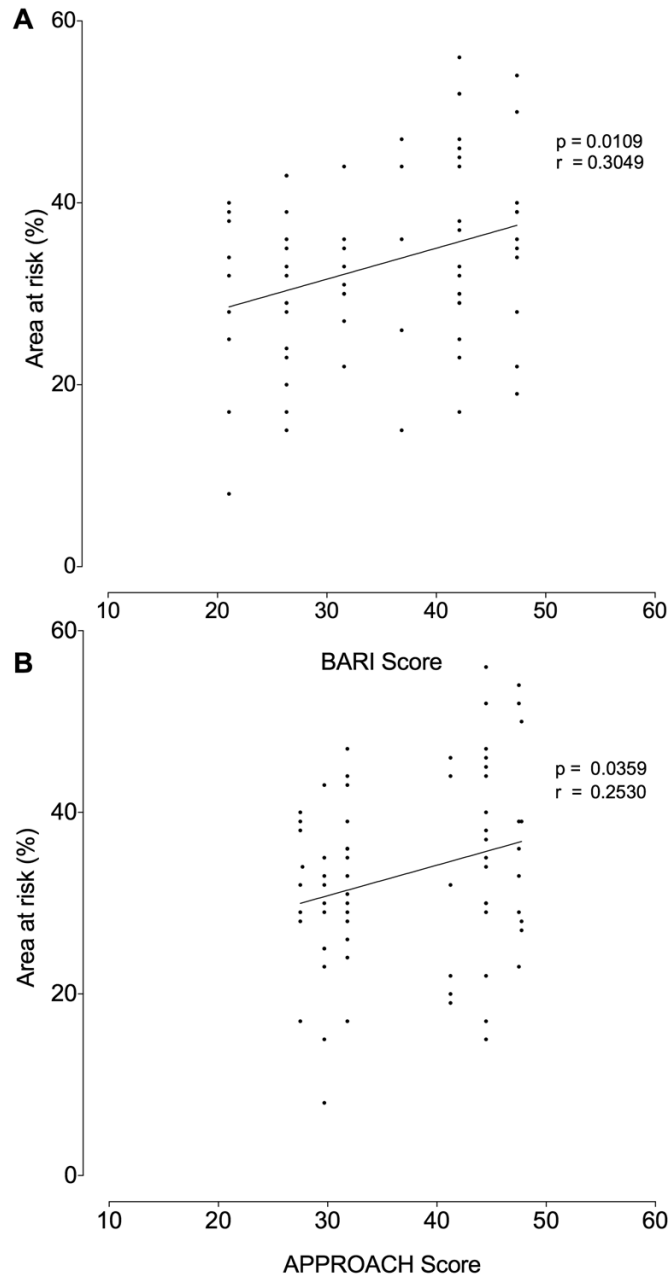


Figure 5.2. Association between angiographic risk score and 3-slice STIR AAR. Data presented shows a significant positive correlation between 3-slice STIR AAR assessed by CMR at 3 days and BARI angiographic risk at baseline ($r=0.3049$) as shown in panel A. Panel B depicts a similar association between the 3-slice STIR AAR assessed by CMR and APPROACH angiographic risk score ($r=0.2530$).

Table 5.2. Intraclass Correlation Coefficients Between Angiographic and Cardiovascular Magnetic Resonance Methods of 3-slice and 10-slice STIR imaging techniques.

3 Slice Assessment	T2 STIR	APPROACH	BARI
Infarct ESA	0.50 (0.30 – 0.66) ***	0.22 (-0.02 – 0.43)	0.16 (-0.07 – 0.37)
Oedema ESA	0.91 (0.86 – 0.95) ***	0.16 (-0.06 – 0.38)	0.26 (0.02 – 0.47) *
Infarct %	0.47 (0.26 – 0.64) ***	0.16 (-0.06 – 0.37)	0.16 (-0.07 – 0.37)
BARI	0.30 (0.07 – 0.51) *	0.86 (0.80 – 0.91) ***	
APPROACH	0.25 (0.01 – 0.46) *		

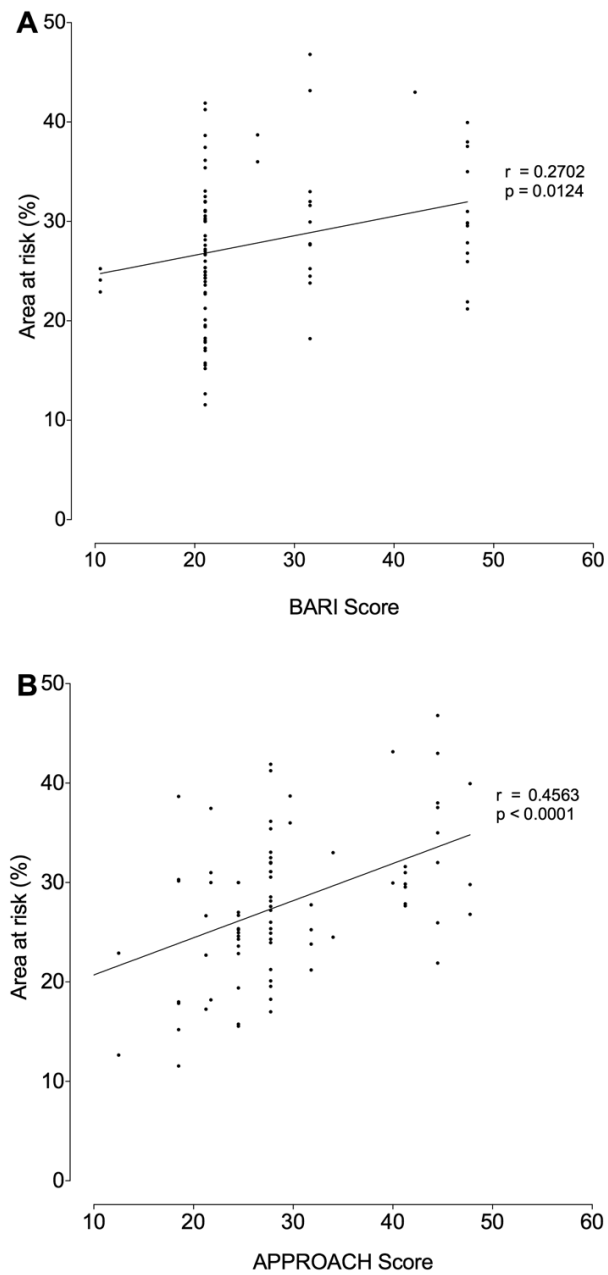
10 Slice Assessment	T2 STIR	APPROACH	BARI
Infarct ESA	0.64 (0.50 – 0.75) ***	0.44 (0.25 – 0.60) ***	0.28 (0.07 – 0.47) **
Oedema ESA	0.89 (0.84 – 0.93) ***	0.40 (0.20 – 0.56) ***	0.17 (-0.05 – 0.37)
Infarct %	0.73 (0.61 – 0.82) ***	0.50 (0.32 – 0.64) ***	0.33 (0.13 – 0.51) **
BARI	0.27 (0.06 – 0.46) ***	0.85 (0.78 – 0.90) ***	
APPROACH	0.46 (0.27 – 0.61) ***		

Data presented as intraclass correlation coefficients (confidence interval). p value <0.05 = *, <0.01 = **, <0.001 = ***. APPROACH, Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease; BARI, Bypass Angioplasty Revascularisation Investigation Myocardial Jeopardy Index; ESA, endocardial surface area; STIR, T2-short tau inversion recovery; LAD, left anterior descending artery

Within the 10-slice AAR group the CMR assessed AAR ranged from 11.5 to 46.80, the BARI angiographic risk score ranged from 10.5% to 47.4% (mean 26.56 ± 10.11), and the APPROACH angiographic risk score ranged from 6.5% to 47.8% (mean 29.24 ± 8.95%) of the LV myocardium. There was good correlation between the 2 angiographic risk scores ($r=0.8544$, $p<0.0001$). There was a significant correlation between the 10-slice AAR and both the BARI angiographic risk score ($r=0.2702$, $p=0.0124$) and the APPROACH angiographic risk score ($r=0.4568$, $p<0.0001$) (Figure 5.3).

To correct for the higher number of LAD infarcts in the 3-slice group a sub-group analysis of just patients within the 10-slice AAR group where the culprit vessel was the LAD showed only a significant correlation between 10-slice AAR and APPROACH angiographic risk score ($r=0.4247$, $p=0.0434$) (Figure 5.4).

Figure 5.3. Association between angiographic risk score and 10-slice STIR AAR.



Data presented as a significant positive correlation between 10-slice STIR AAR assessed by CMR and BARI angiographic risk at baseline ($r=0.2702$) as shown in panel A. Panel B depicts a similar association between the 3-slice STIR AAR assessed by CMR and APPROACH angiographic risk score ($r=0.4563$).

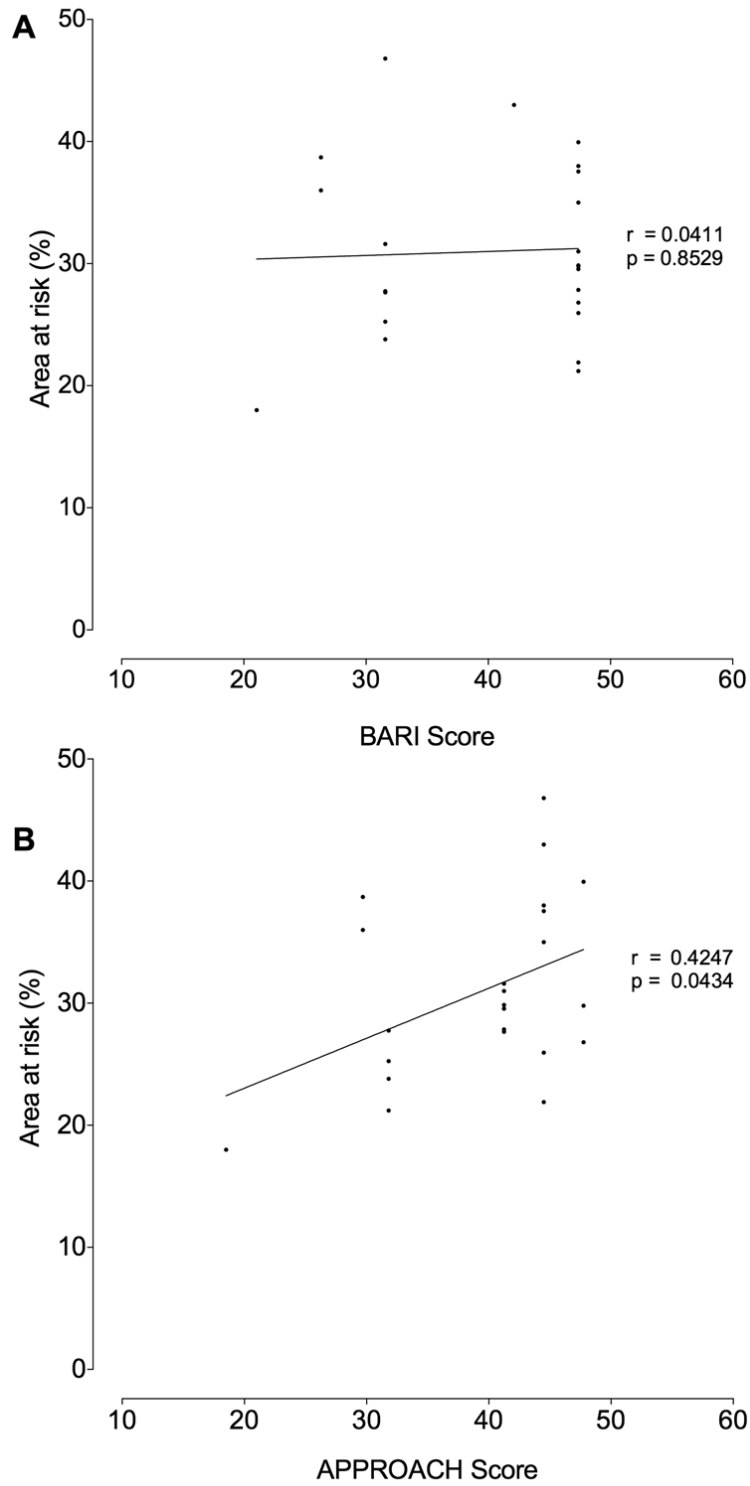


Figure 5.4. Association between angiographic risk score and sub-study LAD 10-slice STIR AAR.

Data presented shows a significant positive correlation between the sub-study LAD 10-slice STIR AAR assessed by CMR and APPROACH angiographic risk score ($r=0.4247$) as shown in panel B, that was not present between sub-study LAD STIR AAR and BARI score, panel A.

5.3.4. Comparison Between Infarct size and myocardial AAR Scores

Infarct size (assessed by LGE) ranged from 1% to 44% of the LV myocardium (mean $18.02 \pm 8.82\%$). There was a significant correlation between infarct size and 3-slice AAR ($r=0.4722$, $p<0.0001$) and infarct size and 10-slice AAR ($r=0.7324$, $p<0.0001$) (Figure 5.5).

Infarct size assessed by ESA ranged from 0 to 45% (mean $17.25 \pm 9.75\%$). There was significant correlation between infarct size ESA and 10-slice AAR ($r=0.4155$, $p<0.0001$) and 3 slice AAR ($r=0.4722$, $p<0.0001$). Infarct size assessed by ESA in the 3 slice AAR ranged from 1 to 57% (mean $17.28 \pm 10.01\%$). For 10 slice AAR group infarct ESA ranged from 0 to 45% (mean 17.25 ± 9.75). There was significant correlation between 10-slice AAR and infarct EAS ($r=0.4155$, $p<0.0001$).

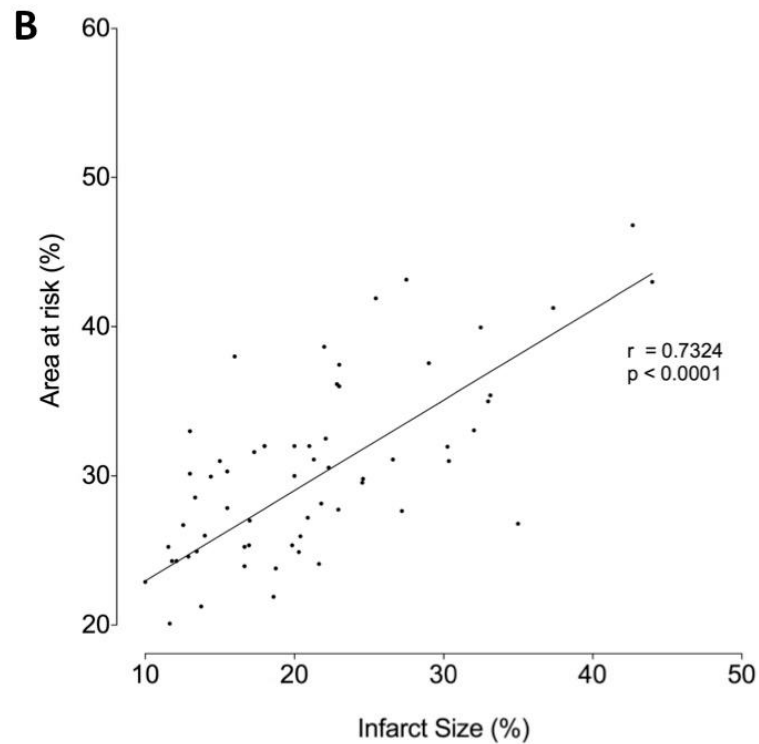
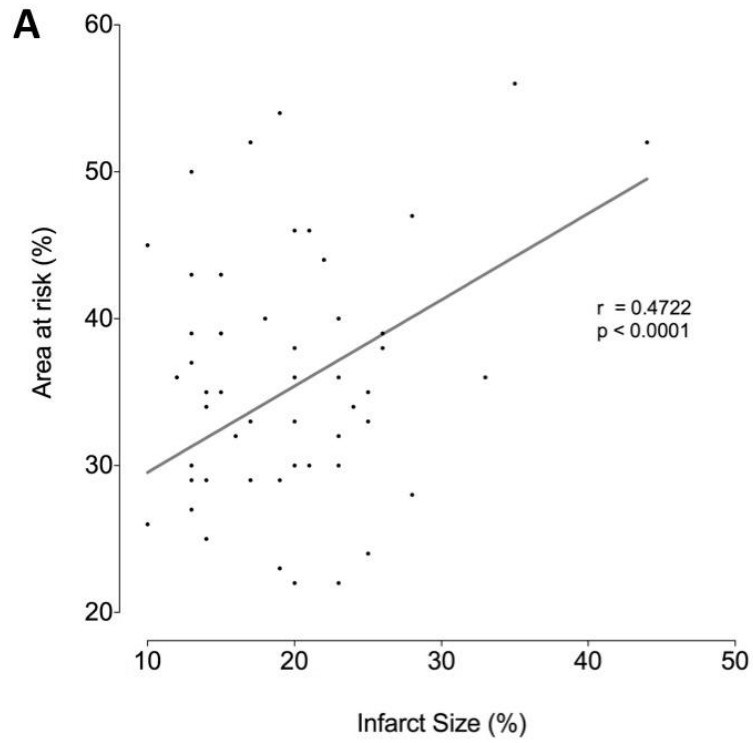


Figure 5.5. Association between infarct size and AAR.

Data presented as panel A shows a significant positive correlation between 3-slice STIR AAR assessed by CMR and infarct size assessed by CMR at baseline ($r=0.4722$) as shown in panel A. Panel B depicts a similar association between the 10-slice STIR AAR and infarct size assessed by CMR at baseline ($r=0.7324$).

5.3.5. Timing for acquisition and analysis of 3-slice versus 10-slice area at risk

The time taken for the acquisition of the T2-STIR sequences was assessed from the initiation of the scan to the start of the next imaging sequence. The 3-slice group had a median acquisition time of 100 seconds (range: 65-171 seconds) and the 10-slice group had a median acquisition time of 355 seconds (range: 275-603 seconds), with a significant difference seen between the two groups ($p < 0.0001$) (Figure 5.6)

For analysis the 3-slice group had a median time of 42.74 seconds (IQR: 36.04-55.17 seconds) and 10-slice group had a median time of 137.1 seconds (IQR: 133-143.5 seconds), with again a significant difference seen between the two groups ($p < 0.0001$) (Figure 5.6). The inter-observer timing variability correlated well between analysers; 3-slice $r = 0.9580$ $p < 0.0001$, 10-slice $r = 0.9288$ $p < 0.0001$).

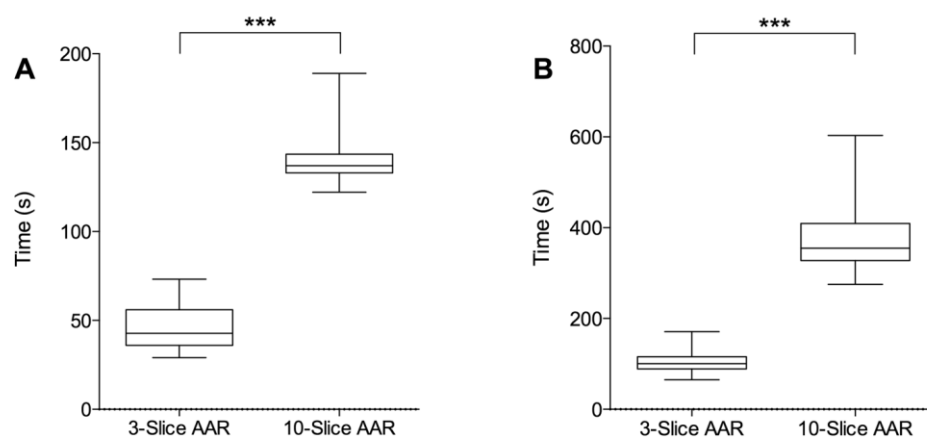


Figure 5.6. Acquisition and Analysis timings.

Data presented shows a significant decrease in acquisition time for the 3-slice AAR group compared to the 10-slice AAR group in Panel A. Panel B shows a significant decrease in analysis time for the 3-slice AAR group compared to the 10-slice AAR group. *** indicates p value < 0.001 .

5.3.6. Comparison of 3-slice and 10-slice AAR technique.

The comparison of the different correlation coefficients were compared using 2-tail Fisher's z-transformation statistical analysis. The analysis revealed that there was no significant difference in correlation between 3-slice STIR group and the 10-slice STIR group in regards to BARI angiographic risk score ($z=0.23, p=0.8181$), APPROACH angiographic risk score ($z=-1.42, p=0.1556$). Within the subgroup analysis patients there was no significant difference in correlation between 3-slice STIR group and the 10-slice STIR LAD culprit only group in regards to BARI angiographic risk score Within the subgroup analysis patients there was no significant difference in correlation between 3-slice STIR group and the 10-slice STIR LAD culprit only group in regards to BARI angiographic risk score ($z=1.07, p=0.2846$), APPROACH angiographic risk score ($z=-0.58, p=0.5619$). However there the 10-slice STIR assessment correlated better with infarct size percentage compared to the 3-slice STIR ($z=-2.53, p=0.0114$) (Table 5.3).

Table 5.3. Correlation of groups and Angiographic risk score.

3-Slice	10-Slice	10-Slice STIR (LAD culprit only)
BARI Angiographic Risk	0.8181	-
APPROACH Angiographic Score	0.9045	0.4533
Infarct Size	0.0114	0.1802

Data presented shows a Fisher's z-transformation of 3 slice STIR against 10-Slice STIR and LAD sub-study. APPROACH, Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease; BARI, Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy Index; ESA, endocardial surface area; STIR, T2-short tau inversion recovery; LAD, left anterior descending artery

5.3.7. Intra-observer and Inter-observer variability of 3-Slice STIR imaging

3-slice STIR imaging showed a low inter-observer variability. For the Bland-Altman plot, the 95% limits of agreement (-5.3%, 3.6%) contained 95.1% (78/82) of the difference scores. The mean bias of the measurements between observers was 0.08%, and the maximum and minimum difference was 8.0% and -7.0% respectively (Figure 5.7).

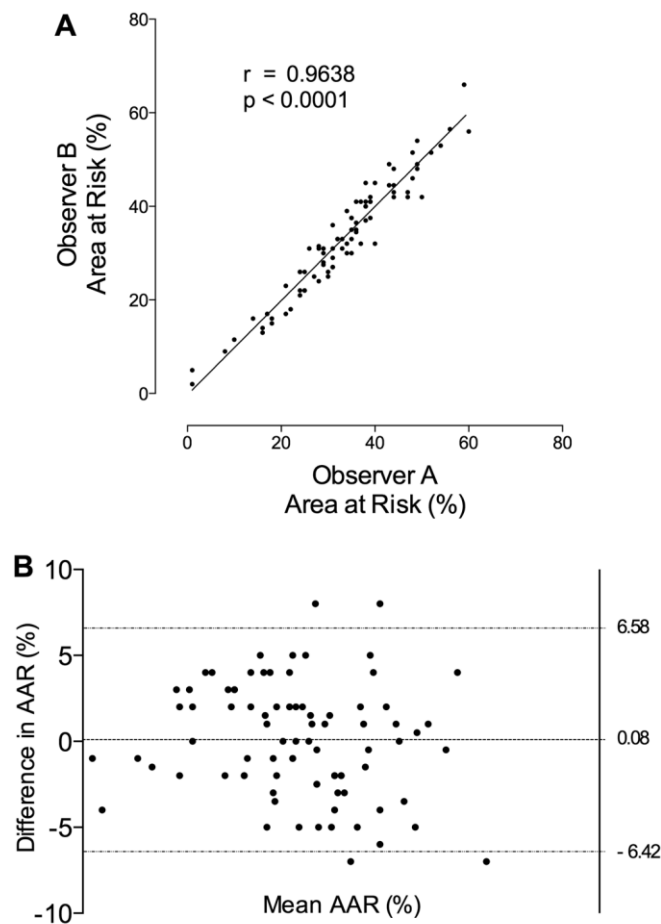


Figure 5.7. Linear regression and Bland-Altman plot for the interobserver variability for 3-slice group.

Data presented shows a significant positive correlation between the observer A and observer B when assessing 3-slice STIR imaging ($r=0.9638$) in Panel A. Panel B is Bland-Altman Plot for same data

The 10-slice STIR imaging showed low inter-observer variability. For the Bland-Altman plot, the 95% limits of agreement (-4.68%, 4.04%) contained 94.1% (80/85) of the difference scores. The mean bias of the measurements between observers was -0.32%, and the maximum and minimum difference was 5.0% and -5.6% respectively (Figure 5.8).

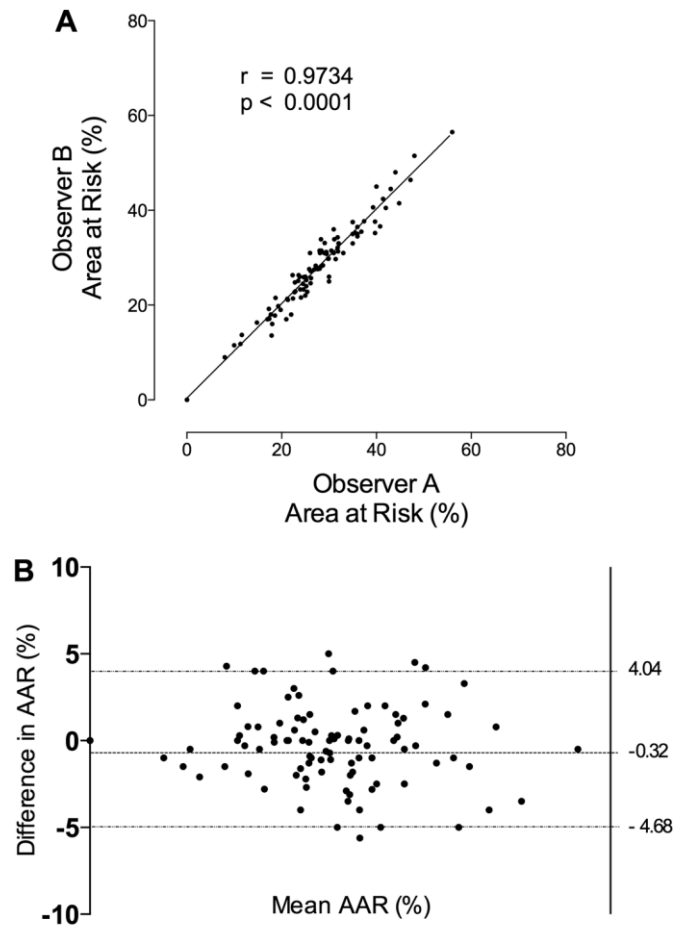


Figure 5.8. Linear regression and Bland-Altman plot for the interobserver variability for 10-slice group.

Data presented shows a significant positive correlation between the observer A and observer B when assessing 10-slice STIR imaging ($r=0.9638$) in Panel A. Panel B is Bland-Altman Plot for same data

5.4. Summary

1. The 3-slice AAR technique correlates well with the traditional 10-12 Slice AAR method
2. The 3-Slice AAR technique correlates well with angiographic AAR quantification methods (BARI and APPROACH)
3. The 3-slice AAR technique significantly reduces the acquisition time and hence leads to increased efficiency a CMR department and decreases patient exposure to the scan
4. The 3-slice AAR technique requires less time for analysis compared to the standard 10-slice method. In both the clinical and research setting this protocol could result in quicker analysis times without the loss of clinical information
5. The 3-slice AAR technique only images levels within the LV unlike the 10-12 slice AAR technique which can on occasion not image the LV. This reduced imaging technique stops any risk of a sequence not including the LV and wasting valuable time for both the patients and clinicians
6. Like 10-slice AAR technique the 3-Slice AAR technique provides good inter-observer variability

Chapter 6

Discussion

6.1. Introduction

This thesis examined the use of BMNC as a strategy to improve myocardial function after successful primary PCI applied within the standard length of hospital stay post AMI. Even with advances in the treatment of AMI a significant number of patients are left with substantial left ventricular impairment, HF and its associated morbidity and mortality. Few therapies have the ability to regenerate damaged myocardium or improve myocardial function after AMI and therefore BMNC are an appealing proposition.

The novel strategy of using the bodies own cells to regenerate the heart early post AMI is a key aim of regenerative medicine in CVD. Since early preclinical models demonstrated the potential benefit of early stem cell therapy after AMI the translation to trials in man have been rapid however all of these clinical trials have performed stem cell infusions over 24 hours post-coronary reperfusion with successful primary PCI (Table 1.3).

This thesis suggests that the intra-coronary infusion of BMNC is safe and tolerable in patients who have undergone infusion within 24 hours post successful primary PCI for AMI. Furthermore in patients who received BMNC within 24 hours of primary PCI it may improve myocardial salvage, and decrease infarct size.

This thesis also assesses the potential paracrine effect associated with BMNC infusion and the relationship to the improvements seen following AMI. The data presented proposes that BMNC infusion may have a direct effect on circulating cytokines that results in these beneficial effects. Additionally, this thesis assesses the potential role of a novel CMR imaging sequence (3-slice T2-STIR) for the assessment of the AAR following AMI compared to the standard 10-slice T2-STIR imaging approach. This sequence appears to correlate well with standard techniques with the added benefit of shorter acquisition and analysis times.

Three key aims were set out by this thesis, which will be discussed in detail during this discussion.

6.2. To assess whether the intracoronary infusion of autologous bone marrow derived progenitor cells within 24 hours of primary PCI following an anterior AMI is safe and feasible

A major concern regarding the early infusion of BMNC after AMI is the risk of bleeding complications and arrhythmias. Within the first 24 hours post AMI it is well established that patients are at a greater risk of developing cardiac arrhythmias, be it related to reperfusion or scar related mechanism(Manolis 2014). The early infusion of BMNC may potentiate these events with previous clinical trials in humans illustrating the safety and feasibility of IC stem cell infusion (Clifford, Fisher et al. 2012) only after 24 hours.

Performing BMA within 24 hours of primary PCI has the possibility of resulting in complications, such as bleeding secondary to anticoagulation and infection. Documented complications associated with BMA or bone marrow trephine have been well reported by the British Society for Haematology in the annual survey of biopsy-associated morbidity and mortality. It is usually a relatively safe procedure, with only 2 reported mortalities and serious adverse events in a study of 55,000 patients (<0.05% of procedures) (Bain 2003).

This thesis demonstrates that BMA performed within 24 hours of primary PCI was not associated with any significant serious events with only minor complications reported. There were a small number of patients who had complications during aspiration, with 5 non-critical events in total reported. This low number of events would reflect a similarity with patients who undergo BMA suffering from haematological disorders (Malempati, Joshi et al. 2009).

6.2.1. No adverse events were demonstrated with the re-infusion procedure

The IC infusion procedures appeared safe and were not associated with any adverse effects in patients within the whole trial population. The placebo or BMNC infusion procedure occurred after a median of 564.5 minutes following AMI and were in general well tolerated without major complications related to the reinfusion procedure. However one patient in both groups had intra-infusion PCI performed due to distal stent dissection prior to infusion, with a small

number of patients suffering chest pain with ECG changes. Importantly there was no evidence of further myocardial damage occurred during the IC infusion procedure as no significant changes in Troponin or CK were seen in either group.

The low number of adverse events reported in this thesis is reflected by previous studies in this field. The early studies such as BOOST and REPAIR AMI failed to fully describe events during BMA and stem cell infusion (Wollert, Meyer et al. 2004, Schachinger, Erbs et al. 2006). The trial with the most descriptive event profile was ASTAMI (Beitnes, Hopp et al. 2009), which reflects well with my results. In ASTAMI 1 patient had a significant complication with contaminated infusate requiring IV antibiotics. Of particular note is the number of patients who complained of chest pain or had ECG changes during coronary balloon occlusion. In ASTAMI 72% of patients had chest pain and 77% of patient had ST changes during the stem cell infusion procedure. Our data showed that only a small number of patients developed chest pain (4%) or ECG changes (6%) during the re-infusion procedure. The potential explanation for this substantial difference between the two trials is the time between MI and stem cell infusion. Within the ASTAMI trial the infusion time point was around 6 days in comparison to under 1 day in this thesis. This difference seen in ischaemic changes during coronary occlusion could possibly be due to the shorter interval after primary PCI and BMNC infusion due to post ischaemic conditioning (Vinten-Johansen, Yellon et al. 2005). This low rate of complications compares well to other published studies and shows that BMA and BMNC infusion in the early time-period post primary PCI is safe and better tolerated compared to later coronary re-infusion procedures.

6.2.2. The reinfusion of BMNC is logistically feasible following primary PCI

During the running of the trial, logistical issues with respect to the time from BMA to BMNC infusion not previously described in other trials were encountered. All recruiting centres were run within busy cardiovascular interventional units treating patients with AMI. Although in some cases the infusate was ready within the appropriate time-period there were delays due to the availability of angiography laboratories. Within this thesis the median time from aspiration to reinfusion was 372 minutes, however the longest time from aspiration to infusion was 713 minutes and this delay was due to unavailability of a free angiography laboratory. There were six patients who had a delayed BMNC infusion due to lab unavailability if these patients were removed from analysis the median time from aspiration to reinfusion was 314 minutes. Previously described trials such as BOOST and ASTAMI had a planned aspiration and infusion time making time delays less likely.

Although BMNC infusion is feasible in the acute phase there are relative drawbacks. The biggest issue with the early time point is the difficulty in planning for the procedures. Throughout medical practice planned elective therapies have a much lower event rate than those undergoing emergency therapies (Tottrup, Erichsen et al. 2012). The possible risk of complications due to unplanned or rapid turnaround after primary PCI could in theory result in

increased events, however this did not occur. The lack of difference between the event profile between the acute and the delayed time point trials, could suggest that the skillset within the recruiting centers and the safety profile of the infused agent counteracts the possibility for acute complications.

6.2.3. The lack of adverse events seen with BMNC therapy

This thesis was successful in demonstrating that the use of BMNC in the acute phase was both safe and feasible. When assessing the risk of MACE after BMNC therapy in patients who had undergone primary PCI for an AMI this thesis failed to demonstrate any difference. Within the 1-year follow up there were similar event profiles of 15.4% of patients in the control and 15.9% of patients in the BMNC arm suffering from a MACE. The possible reasons for the similar event profile are probably multifactorial. One of the main reasons is that this trial was only powered to demonstrate an imaging end-point, the numbers required to assess a difference in MACE are much larger than the numbers assessed here. In addition there was a wide range of baseline LVEF in this thesis from 25% to 68% meaning that patients with higher LVEF are at a lower risk of MACE (Wang, Xie et al. 2011).

To date no study has been able to demonstrate any difference in MACE due to the low numbers. Previous positive meta analysis in regards to LVEF changes have failed to demonstrate change in clinical outcomes such as MACE (Clifford, Fisher et al. 2012). To address this final potential benefit of BMNC therapy after an AMI

a large-scale trial is needed. The ongoing BAMI trial (BAMI, <http://www.bami-fp7.eu>) currently recruiting throughout Europe is the first Phase III trial designed to do assess BMNC therapy and all cause mortality by enrolling 3000 participants with all-cause mortality at 2 years as the primary endpoint.

6.3. The administration of autologous bone marrow derived progenitor cells does not lead to a significant improvement in LVEF compared to standard medical therapy alone.

LV function is the most important factor that affects mortality post AMI. The rapid restoration of epicardial flow following a period of coronary occlusion results in the preservation of cardiomyocyte integrity and function. In addition to cardiomyocyte damage caused by the period of hypoxia further damage caused by reperfusion injury leads to further LV dysfunction (Yellon and Hausenloy 2007). The proposed early infusion of BMNC could therefore possibly result in a decrease in the damage caused by both hypoxia and reperfusion injury. In Chapter 2, it was shown that the early infusion of BMNC resulted in a greater improvement in LVEF compared to patients who received placebo. After primary PCI both groups saw significant improvements in LVEF over time. There was however a signal that BMNC therapy resulted in a greater improvement, as at 1 year there was a 2.24% greater improvement in LVEF compared to the control group, although this did not reach statistical significance.

In addition to the improvement in LVEF there was a signal that the BMNC group had a reduction in MVO compared to the control group. Previous animal studies have demonstrated the reduction of MVO with the early allogeneic cardiosphere derived cells reducing the amount of MVO and infarct size (Kanazawa, Tseliou et al. 2015). This finding with the improvement in LVEF in REGENERATE-AMI may support the benefit of cell therapy however the with not all patients assessed this could result in bias.

Previous trials have demonstrated a greater improvement with BMNC post AMI in patients with greater LV impairment (Schachinger, Erbs et al. 2006). In a subgroup analysis of patients with baseline LVEF less than 45% there was a greater trend for improvement in LVEF at 1 year. These findings are not unsurprising, for example if you take a patient who has severely impaired LVEF with an ejection fraction of 30%, an improvement in LVEF of 6% represents a 20% improvement where as a 6% improvement in a patient with mild LVEF impairment (e.g. %) will only represent a 11% improvement in LVEF. It is highly unlikely that patients with mild to preserved LVEF will demonstrate any additional benefit with BMNC therapy in comparison to standard medical therapy due to the inability to measure these small improvements.

The findings of this thesis are consistent with previous meta-analyses and would suggest that the trend seen within the BMNC group is likely to be real but underpowered. This thesis was powered for the primary end point of change in LVEF determined by CMR at 1 year compared to baseline. The sample size was calculated using a two-sided unpaired t-test to detect an improvement in LVEF of

6%, powered at 90% with a significance of 5% with a standard deviation of approximately 8% based on previous studies (Wollert, Meyer et al. 2004, Janssens, Dubois et al. 2006). However as previously described myocardial stunning plays a significant role in LVEF impairment after AMI, and therefore in the control group you would expect to see an improvement of 1 to 3%(Surder, Schwitter et al. 2010). Therefore this would imply the difference in LVEF improvement from baseline to 1 year would have been $\delta=3\%$. Assuming that the SD would have been stable at 8% as previously described and using the same power calculation with the calculated difference the required study population would have been 300 patients. The original power calculations for this thesis would suggest a $\delta=6\%$, requiring a near 9% increase in the BMNC therapy arm, an improvement which has not been described before.

6.3.1. Highlighting the predictive value of animal models

The lack of benefit of IC BMNC therapy within the first 24 hours after AMI is not entirely unexpected. This thesis is based on previous pre-clinical models that assessed the early re-infusion showing beneficial effects of BMNC therapy. In these studies by Orlic et al the animal mice models had coronary ligation and then direct myocardial injections (Orlic, Kajstura et al. 2001). This study induced significant myocardial damage and impairment on LV function. However there have been significant short falls in the translation into human clinical trials. Although some of these trials are well designed it is difficult for true similarities between human and animals to be observed. Two major drawbacks with the

translation between the two is the technique of inducing MI and the methods assessing improvement. The majority of animal models have used the coronary ligation technique (Shao, Redfors et al. 2013) resulting in a mechanical occlusion of the artery requiring a thoracotomy potentially resulting in other myocardial damage that does not occur in AMI. The assessment of LVEF in animals is fraught with difficulties especially as the resting heart rate of rats are between 330 and 480 beats per minute resulting in possible difficulty in true LVEF assessment. This is especially true with echocardiography, which was used in early animal models. The use of CMR in human models and beta blockade results in a slower resting heart rate and better LVEF assessment that makes the methodology of the human trials superior to animal models.

All previous studies using stem cell infusions in AMI have performed these greater than 24 hours post PCI where LV assessment can be performed with greater accuracy, and removes the potential overestimation of LV dysfunction secondary to myocardial stunning. In this thesis the mean baseline LV function was $47.51 \pm 9.18\%$, which by definition is only mild LV impairment (Lang, Bierig et al. 2005). The baseline LV function in this thesis was similar in comparison to the majority of other studies, in BOOST and REPAIR-AMI where the baseline LVEF in the BMNC group were $50.0\% \pm 10.0\%$ and $46.9\% \pm 10.4\%$ respectively (Wollert, Meyer et al. 2004, Schächinger, Erbs et al. 2006). In a previous paper by Schächinger et al it was demonstrated that with greater LV dysfunction greater and more significant improvement is seen (Schächinger, Assmus et al. 2009) meaning that the greater the LV dysfunction the greater the need for

improvement and consequently with better LV function the less effect the therapy is going to be.

In this thesis the CMR imaging time point of 2 to 3 days after stem cell infusion, means that a 'true' baseline LVEF assessment was never performed using CMR. The use of regional wall motion abnormality on LV ventriculography after AMI for the inclusion criteria could have resulted in higher than expected LVEF after AMI particularly in a LAD cohort of patients. One of the issues with LV ventriculography is that the single view focuses on the anterior and inferior walls meaning that when ventricular measurements are performed overestimation of dysfunction could be a factor. In addition post AMI myocardial stunning generally shows regional wall motion abnormalities not necessarily be present at day 3 (Futterman and Lemberg 2000).

The infusion of progenitor cells into the recently infarcted myocardium also has its limitations. In the ischaemic myocardium there is an increase in free radicals, cellular osmolality and local circulating neutrophils factors which all may affect stem cell migration. Previous animal studies have demonstrated that stem cell transfer has poor survival and cardiac retention with the intra-arterial infusion of MSCs found to mainly arrest in the pre-capillary level with only 14% of progenitor cells surviving the micro-embolisation induced ischaemia by 72 hours (Toma, Wagner et al. 2009).

In a large anterior MI where there is significant LV dysfunction due to the loss of nearly 1 billion cardiomyocytes (Robey, Saiget et al. 2008), the role of early

BMNC therapy role to replace or repair these cells is the main aspiration. The previously described animal models by Olic et al used transmyocardial injection shortly after coronary ligation, this method may not translate into the clinical setting. In addition the majority of the animal models have used small models such as rats, rather than large animal models that could represent a better predictor in humans than rodents.

6.4. Reduction in infarct size with the administration of autologous bone marrow derived stem

The results of this thesis showed that the infusion of BMNC resulted in a reduction in myocardial infarct size (as assessed by CMR). The mechanism for this is unknown and it could be related to a reduction in I/R injury as previously postulated or related to the specific trial population. This section will consider the potential role BMNC therapy has within I/R as well as looking at other possible causes.

I/R results in an immune and metabolic cascade resulting in further myocardial damage. BMNC therapy has been shown to demonstrate both an anti-inflammatory, and immunomodulatory effect (Van Linthout, Stamm et al. 2011). Previous trials using earlier time points have demonstrated decreased infarct size compared to control without significant differences in improvement of LVEF (Janssens, Dubois et al. 2006). In addition to BMNC therapy there have been

multiple trials assessing novel therapies in the prevention of I/R. These trials have reflected similar results that were seen in this thesis with improvement in infarct size and improved myocardial salvage (Jones, Pellaton et al. 2015).

A challenge with the early infusion time point used in this thesis is that there is a potential for significant loss of infused progenitor cells due to free-radicle interaction. It is well recognized that oxidants and free radicals play a significant role in I/R (Zweier and Talukder 2006), and the use of progenitor cells in the early setting may result in an interaction with the free radicals decreasing the potential damage caused and promote repair, which may translate in this thesis as a decreased infarct size.

Patients within the BMNC groups had longer symptom to reperfusion times compared to the control group, suggesting larger potential infarct sizes with reduced potential for myocardial salvage. This however was not observed in the BMNC group compared to the control suggesting a potential effect of the BMNC therapy. A key factor that may have played a role in the reduction of infarct size is the relationship between reperfusion and reinfusion. The BMNC group had a shorter interval from reperfusion to the reinfusion procedure, suggesting possible beneficial effect of early infusion.

Within the BMNC group there was a nearly 50% reduction in the release of serum troponin compared to the control group however this did not reach statistical significance. The CMR data supported a potential reduction in infarct size however its important to highlight that the troponin values were taken at varied

time-points both before and after cell therapy making the data difficult to interpret. The relationship between troponin and infarct size is well documented and the signal seen within the BMNC group could therefore represent smaller infarct size due to other factors such as ischaemia time rather than any direct BMNC effect.

The design of the REGENERATE-AMI study however was not to ascertain the true mechanism of BMNC therapy rather than its impact on clinical outcomes therefore this stands as a hypothesis generating study.

6.4.1. Comparison with other studies using bone marrow derived progenitor cells in myocardial infarction

The results in this thesis are consistent with other published studies including those most similar to our trial design. Janssens and colleagues also demonstrated no benefit of IC infusion of BMNC on LVEF or infarct size and used the closest time point of 24 hours of all other studies assessing the IC infusion of BMNC post AMI in comparison to this thesis (Janssens, Dubois et al. 2006). There are similarities between this thesis and the study by Janssens and colleagues; both with respect to patient numbers (n vs n), patient baseline LVEF taken over 2 days post infusion and similar baseline LVEF ($47.51 \pm 9.18\%$ in this study vs $46.9\% \pm 8.2$ in Janssens et al). This study also demonstrated significantly smaller infarct size coupled with a greater MSI in the BMNC group at baseline compared to the

placebo group a finding which is consistent with other early phase I/II studies (Janssens, Dubois et al. 2006, Schachinger, Erbs et al. 2006).

There are differences between this study and those that found a significant improvement in LVEF (Assmus, Schachinger et al. 2002, Wollert, Meyer et al. 2004, Schachinger, Erbs et al. 2006). The largest scale studies assessing the effect of BMNC infusion by Schächinger and colleagues assessed a total of 204 patients presenting with AMI who received either IC infusion of BMNC (n = 101) or matching placebo (n = 103) with a primary endpoint of change in LVEF assessed by LV angiography. Although the improvement seen in LVEF was similar to that seen in our study ($5.07 \pm 7.27\%$ in this study vs $5.5 \pm 7.3\%$ in Schächinger et al), the use of LV angiography could possible result in over estimation of LV improvement compared to the advanced imaging techniques that have been used in negative trials (Traverse, Henry et al. 2011, Traverse, Henry et al. 2012).

The majority of trials showing favorable results have used similar BMNC preparation methods. The Ficoll–Hypaque centrifugation method for BMNC isolation that was designed by Schächinger et al were used in this study and REPAIR-AMI (Schachinger, Assmus et al. 2004). The use of closed automated Ficoll cell processing system (Aktas, Radke et al. 2008) has been used in multiple trials that have failed to show any benefit in BMNC therapy (Traverse, Henry et al. 2011, Traverse, Henry et al. 2012). The similarity in cell preparation between our study and positive studies suggests that cell processing is not the factor responsible for the negative results seen.

As previously stated this study is the first to assess the safety of IC BMNC infusion within 24 hours post AMI. It is important to note that the safety seen in this study is similar to those seen in other studies despite the early re-infusion. These results support the safety and feasibility of early reinfusion BMNC with a time point under 12 hours (median – within 10 hours). The concerns over early infusions have been a major reason why the majority of BMNC studies have used a protracted time point. In this study we were able to show that patients who underwent early BMA had few bleeding complications or arrhythmias during reinfusion.

It is possible that the lack of benefit seen in this study reflects the improvement in standard medical therapy for AMI. The majority of the positive trials occurred between 2002 to 2006 whereas the development of improved primary PCI services and the introduction of new antiplatelet medications/increased use of drug eluting stents (DES) could have lead to a potential differences in standard care and the reason for different outcomes compared to our study patients recruited during the later time period (2008 to 2012).

6.5. Mechanisms of changes in LV function, dimension and scar burden in patients treated with early intracoronary infusion of BMNC

The second exploratory aim of this thesis was to investigate if changes in LV function and infarct size after BMNC infusion were linked to the release of circulating cytokines. There has been evidence showing that paracrine factors released by ASCs particularly MSCs after BMNC infusion play a significant role in the regenerative process (Caplan and Dennis 2006). Pro-inflammatory cytokines such as TNF- α and IL-6 are not usually expressed in the normal myocardium and are up-regulated during myocardial ischaemia (Kapadia, Lee et al. 1995, Deten, Volz et al. 2002). The up-regulation of cytokines and subsequent return to baseline is dependent on the size of infarct. With larger infarcts a sustained up-regulation can be seen or a prolonged second wave of cytokine expression that can correspond to chronic remodeling (Ono, Matsumori et al. 1998). The cytokine up-regulation after MI precedes the increase in MMP and BNP (Deten, Volz et al. 2002). The mechanistic relationship has been demonstrated between the expression of myocardial MMP and LV remodeling (Spinale 2007).

In chapter 4, it was demonstrated that baseline cytokine levels were similar between treatment groups aside from a significantly lower serum circulating MMP-9 level seen in patients who received BMNC therapy. However this difference was seen at baseline prior to cell infusion and therefore should not be affected by this therapy. This difference at baseline although not connected to

BMNC therapy could be related to the difference in baseline LVEF seen between the groups.

Although categorical evidence that paracrine factors exert direct effects on LVEF and infarct size, there were trends seen that could suggest possible relationships between certain paracrine factors and the improvements seen. Elevated levels of TNF- α after MI have been associated with increased risk for recurrent coronary events (Ridker, Rifai et al. 2000). Smaller increases in TNF- α levels seen over time in the BMNC therapy group compared to control suggests that BMNC therapy reduced the up-regulation of TNF- α and subsequently reduced negative remodeling.

Increased serum circulating VEGF levels have been associated with improved angiogenesis (Deuse, Peter et al. 2009). We showed a trend to a difference in VEGF levels over 12 months between groups. The increase of 87.70 ± 569.4 pg/ml seen in the BMNC group compared to a large decrease seen in the control group of 303.6 ± 661.1 pg/ml. These results may reflect changes seen in the improved LVEF and decreased infarct size at baseline in the BMNC group. VEGF has been known to assert a cardioprotective influence by increasing the ischaemic tolerance of cardiomyocytes an effect which is reflected by the results seen in the BMNC group and would suggest a beneficial effect of BMNC therapy within the early infusion window.

G-CSF has been shown to induce myocardial regeneration by promotion of local stem cell activation (Orlic, Kajstura et al. 2001). In both groups there was an

increase in circulating G-CSF concentrations although this change was smaller in the BMNC group (17.59 ± 70.92 vs. 101.10 ± 288.40 in the control). A similar change was seen in IL-6 levels, a cytokine known to play an important role in the inflammatory process after an ischaemic event with elevated levels associated with increased risk of adverse events and LV impairment (Gabriel, Martinsson et al. 2004). The effects of these two paracrine factors on LV remodeling and patient outcome are not fully reflected in the results seen in this thesis. Patients with a greater level of G-CSF would be expected to have improved LVEF, which was not seen. This similar pattern was seen with IL-6 with a smaller decrease seen in the BMNC group (-54.73 ± 221.4 vs -12.94 ± 195.1 , $p=0.5531$).

The reason for the lack of association seen between cytokines and either LVEF improvement or stem cell therapy could be related to the use of peripheral blood samples for cytokine analysis. The proposed mechanism of IC BMNC therapy suggests that local increases in paracrine factors after infusion results in positive LV remodeling and cardioprotection. To detect significant difference in cytokine concentrations in the peripheral blood would require a monumental local increase in cytokine concentration. The optimum method for measuring the local cardiac concentrations of cytokines would have been ideally to use either coronary artery or coronary sinus blood sampling.

This sub-study failed to demonstrate any significant relationship between the outcomes within the REGENERATE-AMI study and measured cytokines. The minimal signal seen within the population could be related to sample selection

rather than a true mechanistic effect. In future studies the assessment of cytokines therapy should be a significant focus to ascertain the mechanistic effect of BMNC therapy.

6.6. Optimising Imaging End Points in Clinical Trials

In Chapter 1, it was discussed that the primary endpoint in the majority of BMNC trials is the assessment of LVEF and that there are several ways to do this. Early clinical trials used a mixture of non-invasive and invasive imaging modalities. For the majority of trials the use of simple 2D echocardiography has been an essential tool for the assessment of LV function in the acute setting. One of the greatest issues with this imaging modality is the low reproducibility and inaccuracy that can result in foreshortened views of the LV. The development of nuclear imaging, CT and CMR has given greater information regarding the effect of BMNC therapy after AMI, although these modalities still have drawbacks.

In the majority of early trials, LVEF assessment was performed using either echocardiography or LV angiography. The use of invasive LV angiography has been used regularly in AMI patients, as LV assessment can be performed within minutes after primary PCI. The use of LV angiography has been used in TOPCARE-AMI (Assmus, Schachinger et al. 2002) and REPAIR-AMI (Schachinger, Erbs et al. 2006) and both have demonstrated positive effects of BMNC therapy on changes in LVEF over time. As previously discussed LV angiography uses a single 2D image to assess LV function and in patients who have infarction

effecting the lateral or septal walls the LVEF maybe underestimated. In addition the requirement for a stable cardiac rate is essential for LV assessment with LV angiography, after a premature ventricular contraction the LVEF can be over estimated.

In chapter 1.7.2, three different imaging modalities were used to assess LVEF at different time points. Echocardiography and LV angiography were used in the first 24 hours and CMR performed 3 days post primary PCI. The use of these different modalities was included to historically compare with previous positive trials. The use of LV angiography is now thought to be outdated and in theory has a small but significant risk to the patient. Although LV angiography was not performed at 6 months in all patients, the safety data seen is comparable to that seen in REPAIR-AMI (Schachinger, Erbs et al. 2006). Echocardiography was performed at multiple time points and was the most non-invasive imaging modality used. As previously stated, the issue with echocardiography is the skillset needed to acquire a clear image that can be analysed for LVEF assessment. Since CMR has become more readily available, its use has been common within BMNC studies with the added benefit that it can assess more than just LVEF.

In chapter 3, it was shown that the three imaging modalities were safe to use and well tolerated in most patients. These imaging modalities demonstrated a very strong correlation between the assessment of LVEF with CMR and echocardiography, although significantly only a moderate correlation was observed between the LVEF assessed with LV angiography and by

echocardiography. Due to the improvement in image quality of CMR and the ability to assess more than LVEF it has become the gold standard imaging modality in stem cell trials.

Since the development of infarct analysis by positron emission tomography CT or late gadolinium enhancement CMR the possibility for a new cardiovascular trial endpoint has been assessed. In addition newer imaging modalities provide the ability to give mechanistic insights into the processes occurring with BMNC therapy. Questions have now been raised about the best imaging end point for phase II stem cell trials.

The assessment of infarct size is now widely used when CMR is performed, and as with this thesis and previous studies (Janssens, Dubois et al. 2006). The potential benefit of using infarct size or myocardial salvage in an investigational medicinal product (IMP) AMI study is the reduced requirement for multiple imaging assessments. This potential for decreased follow up times rather than the protracted study designs that requires an assessment in changes in LVEF may benefit these trials so that they are both cheaper and more product in reaching a satisfactory imaging end point.

6.7. To assess the feasibility of 3-slice T2-STIR short axis stack imaging compared to conventional 10-slice T2-STIR short axis stack imaging techniques

The assessment of myocardial oedema by CMR using T2-STIR has become a popular technique for the quantification of AAR. This study demonstrated that 3-slice oedema CMR AAR quantification remains accurate for the detection of myocardial oedema after AMI when compared to conventional 10-slice oedema CMR with similar inter-observer variability, however with quicker acquisition and analysis time compared to the conventional 10-slice approach without loss of clinical information.

The use of T2-STIR imaging remains popular for assessing myocardial oedema (h-Ici, Ridgway et al. 2012) and the AAR after AMI, with studies showing the superiority of the clinical use of oedema CMR imaging in both AMI and chronic heart disease (Friedrich, Abdel-Aty et al. 2008, Abdel-Aty, Cocker et al. 2009). The benefit of oedema CMR assessment over alternative AAR assessment such as single-photon emission computed tomography (SPECT) is the lack of ionising radiation or the need for tracer administration. The majority of these previous studies have used 10-12 slice short axis stacks covering the whole left ventricle, however due to the need for adequate breath holding and the possibility of artifact the overall image acquisition time is long for the 10-slice STIR imaging technique. The benefit of the 3-slice T2-STIR sequences in comparison to 10-slice T2-STIR sequencing is that it has a quicker acquisition time. The shorter acquisition time of under 2 minutes 30 seconds may have some clinical benefit with the use for only 3 breath holds in comparison to the 10 without loss of required clinical information is of benefit especially in patients who have issues including claustrophobia or who are unable to lie flat for extended periods.

Previous studies have demonstrated the suitability of 3-slice imaging in the assessment of myocardial scar as well as LV volumes (Kuoy, Nguyen et al. 2014). In Chapter 5, it was tested whether or not AAR can also be assessed using a 3-slice technique, where it was demonstrated that the technique is comparable to the conventional 10-slice approach. This 3-slice approach is not only beneficial to the patient but also to the operator with shorter image interpretation times. Additionally shorter scan times and image analysis times can have important cost implications for scans performed for research with AAR an increasingly important assessment made when testing potential cardioprotective therapies.

6.7.1. To assess the feasibility of 3 slice T2-STIR short axis stack imaging for assessing myocardial oedema and the correlation to area at risk compared to angiographic risk scores (BARI and APPROACH) in patients following AMI.

The assessment of AAR after AMI has become important as it has allowed for accurate assessment of myocardial salvage that occurs after reperfusion. In this thesis BARI and APPROACH scores correlated well with each other in their assessment of AAR. Previous animal models have shown that T2-STIR weighted imaging is able to delineate the AAR (Aletras, Tilak et al. 2006). Since its early development within cardiovascular imaging T2-STIR sequencing has become firmly established for the visualisation and quantification of myocardial oedema in patients post AMI (Simonetti, Finn et al. 1996, Abdel-Aty, Zagrosek et al. 2004).

Since its introduction T2-STIR sequencing quality has improved resulting in better image quality and decreased acquisition time.

Angiographic risk scores such as BARI and APPROACH scores were developed for prognostic purposes (Graham, Faris et al. 2001). In this study we over estimated the AAR in our patient with the angiographic risk scoring in comparison to T2-STIR AAR quantifications. Although there was a significant but low correlation between T2-stir imaging and angiographic risk scores. This is probably in part to the short falls in angiographic risk scoring. The angiographic scoring system especially with LAD infarcts rely on TIMI 0 flow, however there was a high percentage of patients who had TIMI >0 flow.

6.7.2. The reproducibility of 3-slice T2 STIR imaging is similar to 10-slice imaging

Over the past decade the use of oedema quantification has increased and is now an important part of outcome assessment after AMI (Friedrich 2010). As previously stated the CMR imaging provides both a functional and structural assessment of the heart. With oedema quantification the issues about quality of the images secondary to blood pooling has been raised. The use of semi-automated quantification process has led to quicker analysis of these images without loss of clinical information (Sjogren, Ubachs et al. 2012). In this thesis, there was a low degree of variability in the different image techniques for quantitative assessment of oedema. The oedema quantification from either the 3-

slice or 10-slice sequences of T2-STIR correlated well with each other and different infarct zones. Of note, the region of infarct correlated better with LAD artery territory infarctions.

Inspection of intra-observer performance revealed an overall high degree of correlation for both 3-slice technique ($r=0.9638$, $p<0.0001$) and 10-slice technique ($r=0.9734$, $p<0.0001$), with a slightly stronger association seen in the 10-slice technique. The exceptionally low intra-observer and inter-observer variability for both imaging protocols and the reproducibility was similar to previous studies assessing 3-slice techniques for LVEF assessment (Nguyen, Kuoy et al. 2015).

6.7.3. 3-slice T2 STIR imaging technique reduces acquisition time and analysis time without loss of clinical information

The introduction of automated and semi-automated techniques for CMR analyses has improved the reproducibility and the speed of analysis. As previously stated majority of SAX have used 10-12 slices to cut at 1 cm intervals along the LV. When analyzing these sequences the length of analysis time is dependent on the number of images that are assessed. With the 10-12 slice technique some of the slices do not fall within the LV or have a mixture of LV and non -LV mass, which require close manual exclusions.

It is easy to see how the reduction of SAX slices to 3-slice reduces both acquisition and analysis time. In general it requires approximately 10 seconds per acquisition slice, and 14 seconds per analysis slice, with similar timings per slice between the two groups. This study has shown that with this reduction in time there is no loss of clinical information. An issue that is present with 10-12 slice techniques is that some segments are cut through the mitral and LV outflow tract (LVOT). These areas can be issues when undergoing analysis; the need for close analysis and manual exclusion could result abnormal estimations of oedema mass.

A particular issue with T2 STIR imaging is the false signal that occurs in area of blood pooling especially in patients with poor LV function (Higgins, Lanzer et al. 1984). In the apical segments of the LV there is increased blood pooling causing

greater sub-endocardial bright artefact during myocardial quantification. The imaging technique for 10-slice acquisition takes single contiguous 1 cm slices from mid atria to true apex, whereas the 3-slice acquisition takes a slice in the basal, mid and apical segment of the LV therefore reducing true apical blood pooling. The observer variability was similar in the 3-slice and 10-slice acquisition groups. This implies that the use of 3-slice acquisition imaging may not result in the loss of important clinical information and may reduce the issue of increased signal from pooled blood in comparison to 10-slice acquisition. The use of 3-slice technique would in theory reduce all these possible artifacts. The 3-slice technique removes the LVOT and apical slices due to the SAX acquisition process and with this technique as we have shown in this study does not result in loss of clinical information.

6.7.4. Translation of 3-slice T2 STIR to clinical practice

The benefit in the clinical setting of the 3-slice T2 STIR technique in comparison to the standard 10-slice oedema CMR is a quicker acquisition time. The average shorter acquisition time of just less than 2 minutes 30 seconds may have some clinical benefit. The use of only 3 breath holds in comparison to 10 or 12 without the loss of clinical information is of benefit especially in patients who have issues including claustrophobia or who are unable to lie flat for extended periods. These factors are common in patients who have recently suffered from AMI especially when patients are suffering from HF.

In addition the timing for analysis was significantly shorter within the 3-slice approach, which may be of benefit when multiple analysis are required reducing analysis times in large quantity analysis.

6.8. The Potential Pitfalls of Cell Therapy

Section 1.6.2. highlights that when considering the most appropriate stem cell therapy multiple factors can effect the beneficial effect of this therapy. Within this quandary are three major deciding factors, timing of infusion, route of infusion and cell type used.

Despite many positive results in both small non randomised and large randomised trials many in vivo animal studies have demonstrated that 90% of cells are not viable within hours of infusion (Hill, Dick et al. 2003). In addition Hou et al. were able to demonstrate that within a couple of hours only 2.6%, 3.2%, and 11% of the intracoronary, interstitial retrograde transvenously, and direct intramyocardial injected cells respectively were retained within the myocardium (Hou, Youssef et al. 2005). This poor retention of cells is probably a mixture of cell phagocytosis and apoptosis within the myocardium or migration of the cells to the peripheral circulation, especially when used with the intracoronary route (Freyman, Polin et al. 2006).

As mentioned timing of cell delivery is a significant challenge of stem cell therapy and is a fine balance between the safety of cell delivery to occur and the feasibly

of the processes. The optimal time window for stem cell infusion post AMI remains unclear, with multiple trials using different time points. Depending on the timing of infusion, stem cells are subjected to a varied environment. Within the first few hours infused stem cells are subjected to an ischaemic and inflammatory environment, which would potentially result in cell death but has the benefit that may reduce local cardiomyocyte death. The later time points are likely to have less toxic local inflammatory processes occurring but have less potential for salvaging viable cardiomyocyte but potentially have a role in reduction of late remodeling.

To date the majority of cell types have been bone marrow derived mainly due to the ease of harvesting and the relative abundance. However as previously described the environment in which they are infused can potentially be harsh and results in rapid cell death. Therefore a stem cell that can thrive in a diseased environment should be considered or introducing an method to prime them to withstand the rigors of the post ischaemic environment is required.

Attempts were made to address these 3 variables within the REGENERATE-AMI study. As this study aimed to answer the early infusion time point the methods were steered toward a safer application method. Bone marrow aspiration in patients with significant clotting issues has been performed for a long time in haematological patients therefore due to its ease and relative safety this cell was used, in addition it did not require complicated cell preparation. The use of intracoronary cell infusion was routinely used in the early cell therapy trials

within the AMI setting therefore the REGENERATE-AMI study aimed to be similar to these trials.

Until a specific delivery route is designed to increase local retention and a method to reduce the effects of the local environment on the stem cells it will be difficult to truly conclude the beneficial effect of the true optimal time point for cell therapy in AMI

6.9. The Future of Progenitor cell therapy and Acute Myocardial infarction

The original goal of stem cell therapy in heart disease was to discover a way to regenerate lost cardiomyocytes and improve LV function. Many issues have been raised regarding the results of previous trials especially with regard to the outcomes associated with the improvement in LVEF (Francis, Mielewczik et al. 2013). There is now a need to move away from surrogate endpoints to more substantial hard clinical outcomes such as mortality, as the relationship between markers such as LVEF and mortality is not well defined.

With the results of REGENERATE-AMI trial the final question regarding the optimal timing of infusion seems to have been answered. Previous trials that have infused BMNC between time of infarct and day three have failed to

demonstrate any significant improvement in LVEF (Janssens, Dubois et al. 2006). In addition timing points beyond 1 week post AMI have failed to show any significant benefit however trials using a time point between 3-7 days have shown significant effects (Wollert, Meyer et al. 2004, Surder, Schwitter et al. 2010). These results taken with the results of preclinical trials ultimately show that the administration of BMNC result in some beneficial effect after an AMI assuming the correct time-point is used. However there is now a need for larger Phase 3 trials to assess clinical outcomes such as the on-going BAMI trial (BAMI - <http://www.bami-fp7.eu>), which aims to enroll 3000 patients assessing all-cause mortality at 2 years as the primary endpoint.

Although not specifically addressed in this thesis, the route of stem cell delivery in most trials of BMNC delivery for AMI is the IC infusion. The use of G-CSF or peripheral injections of progenitor cells have failed to result in any significant value towards the patient (Ince, Petzsch et al. 2005). With the perceived risk of rupture with IM injection post infarct and the benefit of a stented scaffold protecting the coronary artery during IC infusion the future method of delivery seems to lie with the method used in REGENERATE-AMI.

With the development of other fields within regenerative medicine the combination of stem cell therapy with additional therapies remains a potential option. Gene therapy has been suggested but both ethical issues and public concerns have been raised with regard to this over the last decade. One of the major concerns is the use of viral vectors for delivery with the potential for toxicity or the virus infective property remaining (Sesti and Kloner 2004). A

possible solution for this therapy is the use of progenitor cells as the new vehicle for gene transfer into the target area. In this scenario a group of progenitor cells that has been manipulated to express VEGF gene may result in improved local angiogenesis, which has been seen similarly in murine models post myocardial infarction (Madonna, Petrov et al. 2015).

6.10. Limitations

Important limitations may have affected the results in this study. The first of which is the long recruitment period meaning that the advancement of medical therapy for AMI including the introduction of new antiplatelet drugs and the greater access to primary PCI centers could have resulted in different outcomes in patients in the latter stages of the trials. In addition to this the sample size was based on BOOST trials 6% improvement (Wollert, Meyer et al. 2004), which is representative of the time when the REGENERATE-AMI study was designed. More recent meta analysis have demonstrated a smaller improvement of 3.96% (Jeevanantham, Butler et al. 2012), therefore suggesting this thesis was underpowered to detect this smaller improvement and potential benefit of BMNC therapy.

6.11 Conclusion

In summary the early infusion of BMNC is safe when delivered with 24 hours after AMI with no adverse events seen during BMA and BMNC infusion. Although

there was a greater improvement seen in LVEF in the BMNC group this was not significant compared to those in the control group. Infarct size was lower in the BMNC group compared to control even with a prolonged ischaemia time and a similar AAR, which could suggest a potential beneficial effect of cell therapy however as no true baseline CMR was performed this cannot be conclusively answered.

The hypothesis generating assessment of the effect of BMNC on certain paracrine factors and their functions failed to demonstrate any significant association. The observation that levels of VEGF and TNF- α increased may potentially reflect the reason behind the positive outcome in patients who received BMNC therapy as these factors are associated with the systemic inflammatory response and angiogenesis that are reflected with the trend to improvement in LVEF and reductions in infarct size.

A new suitable imaging modality in the form of 3-slice STIR imaging that could improve scan times and possible cost benefit without the loss of clinical information was shown and can be used as a surrogate end point in clinical studies.

This thesis aimed to answer the final question of the suitable time point for BMNC therapy for AMI. With no significant difference seen with the early infusion of BMNC, it could be argued that there is little rationale for recruiting patients within the first 2 days post AMI and definitely not within the first 24 hours. This early time point, although safe is unlikely to be most beneficial to patients and

future studies should aim to infuse between 3-7 days consistent with positive study data. A standard hospital admission post AMI ranges from 2-4 days, the time point assessed in this thesis implies that although having some beneficial outcome in patients who received BMNC therapy there is currently not enough evidence to change clinical practice for its application.

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Appendix

APPENDIX I: Summary Patient Information Sheet

REGENERATE AMI Trial

Randomised Controlled Clinical Trial of the use of Autologous Bone marrow Derived Progenitor Cells to Salvage Myocardium in Patients with Acute Anterior Myocardial Infarction

Protocol number 2.4

Summary Information Sheet

This is a summary of the research study. Please read the attached information which explains the study in more detail before making the decision whether or not to take part.

- You are invited to join a research study recruiting patients having a heart attack and being admitted to the hospital via the Heart Attack Centre, to see if their own stem cells help the damaged heart muscle to recover.
- If you agree to take part in the study, following the angioplasty procedure to treat your heart attack, we would perform a 'bone marrow aspiration'. A needle is inserted into your hip bone under strong local anaesthetic and bone marrow is withdrawn. This should be an uncomfortable but not painful procedure.
- On return to the ward, you will be given a full explanation of the study and an information sheet to read. You will have some time to decide whether or not you want to take part in the study, and will be encouraged to speak to your relative(s) about it. The research team will be available to answer any questions.
- If you agree to take part, the Stem Cell Lab will have processed your bone marrow and will either send back your stem cells or placebo (saline). Neither you nor anyone involved in your treatment will know which it is. If you do not wish to take part in the study we will ask you whether or not we can use your cells for research purposes. If not they will be destroyed.
- If you have agreed to the study, as soon as the stem cells or saline is ready you will return to the angio lab for infusion into the coronary artery which was previously treated to see if this will lead to an improvement in heart function.
- Following this, you will return to the ward and receive standard care for all patients having had treatment for a heart attack, aiming for discharge as usual between 3-5 days.
- Follow up for the study is a visit at 3 months, another angiogram at 6 months, a visit at 1 year and yearly telephone calls for the next 4 years.

APPENDIX II: Patient Information Sheet

REGENERATE AMI Trial
**Randomised Controlled Clinical Trial of the use of Autologous Bone marrow
Derived Progenitor Cells to Salvage Myocardium in Patients with Acute
Anterior Myocardial Infarction**

Protocol number 2.4
NORTH EAST LONDON HEALTH AUTHORITY
Information to Participate in a Research Project

We invite you to take part in a research study, which we think may be important. The information, which follows, tells you about it. It is important that you understand what is in this leaflet. It says what will happen if you take part and what the risks might be. Try to make sure you know what will happen to you if you decide to take part. Whether or not you do take part is entirely your choice. Please ask any questions you want to about the research and we will try our best to answer them.

What is the purpose of this study?

This research study involves a new treatment which we hope will help the heart muscle to recover following a heart attack.

Why have been invited?

This study involves 100 patients in centres in the UK and Europe who are admitted as an emergency with a heart attack ('myocardial infarction'). This occurs when a blood vessel of the heart ('coronary artery') suddenly becomes blocked by a clot (thrombus), which usually forms on top of existing area of narrowing inside the artery. The resulting impaired blood flow to the heart muscle leads to chest pain, indicating that part of the heart muscle is not getting enough oxygen. This can lead to damage and loss of function to this part of the heart. The treatment is to reopen the artery as soon as possible, to restore blood flow and reduce heart muscle damage. This is called Percutaneous Coronary Intervention (PCI – the balloon treatment) and is part of the standard care we offer to patients like yourself who are having a heart attack.

You are about to have or have recently undergone this PCI procedure which will stop the chest pain and prevent further damage to your heart muscle. However, not all of your damaged heart muscle may recover.

In this study we aim to inject special cells from your own bone marrow into the damaged area of your heart muscle. Bone marrow is a soft material in the middle of certain bones in the body. The bone marrow makes and stores red and white blood cells and cells called platelets. The cells in the bone marrow which are able to make these blood cells are called 'stem cells'. These stem cells are unique as they can become many types of cells and help to maintain and repair tissue in the body. It is hoped that these stem cells will have the ability to repair or replace the damaged heart muscle.

This study will be 'randomised'. This means that half of the patients will receive stem cells and the other half will receive a 'placebo' or 'dummy'. The placebo used for this study will be saline. The decision is made in a way similar to 'flipping a coin' and neither you nor anyone involved in your care will know which treatment you have received. All patients are then followed up in the same way. This enables us to compare both groups to assess the effectiveness of this new treatment.

Do I have to take part?

It is up to you to decide. We will describe the study and go through the information sheet, which we will then give to you. You will then be asked to sign a consent form to show that you have agreed to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What happens if I take part in this study?

Following a successful PCI procedure we will have decided that you are suitable for the study according to the condition and treatment to your coronary arteries. We will take regular blood tests which will help monitor the condition of your heart (an extra teaspoon of blood will be taken in addition to the routine blood samples taken before your treatment and at discharge). We also ask that we can test your blood for HIV and hepatitis viruses as part of the protocol (a further 3 teaspoons of blood). You will receive counselling for these tests.

Following the PCI procedure you will have an echocardiogram. This involves putting some jelly onto the chest and placing a small plastic probe over the heart that bounces ultrasound waves into the heart following the injection of some dye (similar to that used during your PCI). This is a very safe test.

At the end of your PCI you will have 100mls of bone marrow taken from the hip bone (bone marrow aspiration). These bone marrow cells are then processed in a specialised laboratory. If you agree to take part in the study the bone marrow is processed and either the stem cell infusion or placebo (saline) is returned. This means that half the patients will receive stem cells and the other half will receive placebo. We will not be told which it is.

For patients in UK: If you have been chosen to receive a placebo infusion, your stem cells will be stored in ice at the laboratory for 10 years after which they will be destroyed unless you state differently. If the blood tests which were taken for HIV, hepatitis, syphilis and other venereal disease are positive, the Stem Cell Lab will be unable to store your stem cells.

If in the future the study shows that the treatment works and you develop a condition in which it will be beneficial to you, e.g. another heart attack, an infusion of your stem cells can be given to you.

In the future we may want to use stem cells which have been stored, for further research. We will ask you to sign a separate consent form to allow us to store your stem cells if you receive the placebo, but we will contact you again for your permission before any future research is performed.

For patients outside UK:

If you are randomised to receive placebo, your stem cells will be discarded.

For all patients:

Following your PCI, as soon as the stem cell or placebo suspension is received from the Stem Cell Lab (ideally between 6-8 hours), you will undergo another angiogram. At this time the stem cells or placebo will be injected down the treated coronary artery.

If you do not wish to participate in the study after you have had bone marrow aspirated, you will be asked if the cells can be used for research purposes. If not, they will be destroyed.

Some of your blood samples and bone marrow may be used for research purposes but will not be tracked back to you. If these samples are to be used for future research but will be tracked to you, you will be contacted to obtain your consent.

Before discharge from hospital (at least 3 days following your procedure), you will undergo an echocardiogram and an MRI scan. An MRI scan uses magnetic radio waves to scan the body. This will further assess the pumping ability of your heart. If you are not suitable for an MRI scan (e.g. if you have had a pacemaker put in), a Cardiac CT scan will be performed. A CT scan uses X-rays and takes lots of pictures of your heart the same as an MRI scanner.

Follow-up:

During your hospital stay and throughout the study, you will be followed up closely. This will include ECG's and blood tests which will help to assess the health of your heart and regular assessments by your doctor.

You will be required to attend the hospital for a visit at 3, 6 and 12 months following your procedure.

At 3 months you will need to attend the hospital for a day to have a blood test followed by an MRI scan.

At 6 months you will be asked to return to the hospital for the day to have a left ventriculogram to assess the condition of your heart muscle. This is similar to an angiogram but looks only at the pumping ability of the heart and not at the coronary arteries. At this time you will also have a physical examination, blood tests (4 teaspoons of blood), an ECG, and an echocardiogram.

At 12 months you will attend the hospital to have a physical examination, blood tests (4 teaspoons of blood), an ECG, an echocardiogram, and an MRI scan.

In centres in the UK only, you will be asked to complete questionnaires which will help us to assess your state of health at discharge, 6months and 12 months.

Following this, you will be contacted by telephone yearly for the next 4 years to ask about your health.

Risks and side effects

As always, your health is our priority. If at any time you experience a recurrence of symptoms, or your condition requires a different treatment strategy, you will receive appropriate treatment regardless of the study. This research is at the forefront of our understanding and technology and as such, potential risks apart from those mentioned below, are unknown. However, given our current understanding of this technology we do not foresee any major problems.

The risks mentioned below are minimised as procedures are performed by experienced staff.

- **Left Ventriculogram**

A routine angiogram carries the risk of death 1 patient in 1000. However, we will not be looking at the coronary arteries so this risk will be considerably less. The catheter is inserted through an artery in the groin, which in a very small number of patients may cause a blood blister (aneurysm) or injury to the artery.

- **Bone marrow aspiration**

The procedure carries a risk of bleeding, infection and damage to the area where the needle is inserted. The skin around the puncture site is cleaned and numbed prior to the procedure. The aspiration is performed by inserting a needle into the hip bone to obtain the bone marrow. The needle will need to be inserted into the hip bone more than once through the same puncture site in the skin. There may be some pain during the aspiration of the bone marrow but this only lasts a few seconds and is lessened by aspirating more slowly. Any residual pain rarely lasts more than a few days and can be relieved by taking pain killers. If it becomes difficult to aspirate any bone marrow we will stop the procedure.

There is a risk of infection during the transfusion of the stem cells or placebo. However, the infusion is prepared and given in very strict sterile conditions. Because the bone marrow is your own there is no risk of rejection as may be seen with the injection of cells or organs from a different donor.

- **Echocardiogram**

This test carries an extremely small risk of a heart attack or death, (1 in 10,000).

- **MRI Scan**

The MRI scan does not use radiation. There are no known side effects or risks attached to MRI

- **Cardiac CT Scan**

The CT scan uses radiation. Some patients may experience side effects due to allergic reactions to the liquid dye injected into the veins during this scan. This can be treated easily with medicines.

In very rare cases, this dye has been known to damage already weakened kidneys. The doctors performing this scan will be told if you have weak kidneys and will be able to prevent any problems.

- **Radiation**

During this study you will receive more exposure to radiation from X-rays than during standard treatment for a heart attack. You will receive a small amount of exposure when the stem cells/placebo are injected, at 6 months for the repeat angiogram and also if you have been selected to have a CT scan. It is estimated that there is a small risk of patients developing cancer in the future but having coronary artery disease carries a greater risk.

Benefits

Although the pilot study performed using this treatment is very promising, there is a need for this further research. There may or may not be any benefit to taking part in this study. You will however have received the standard optimal treatment for your condition. The fact that you are having your blocked artery treated by balloon angioplasty leads to a 50% reduction in your chance of a future event (e.g. heart attack).

Confidentiality.

All aspects of this trial are strictly confidential. Your records will be kept at the hospital but you will be identified by your initials, a number allocated to you and your date of birth. Your identity will be unknown to anybody outside the hospital helping to process the information obtained. If you agree to participate, the results of your treatment may be published for scientific purposes, however your identity will not be revealed. Copies of your tests, including echo, MRI, CT scans and angiogram will be sent to a corelab in the UK to be analysed. Every attempt will be made to make these copies anonymous but it may not be possible in some centres. However all information will be treated in the strictest of confidence under the guidance of the 1998 Data Protection Act.

What happens if something goes wrong?

We would not expect you to suffer any harm or injury because of your participation in this study. If you are harmed by taking part in this study, there is no special compensation arrangement. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay your legal costs. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

Please contact Patient Advisory Liaison service (PALS) if you have concerns regarding the care you have received, or as an initial point of contact if you have a complaint. Please telephone 020 7377 6335, minicom 020 7943 1350, or email pals@bartsandthelondon.nhs.uk. You can also visit PALS by asking at any hospital reception.

You don't have to join the study. You are free to decide not to be in this trial or to drop out at any time. If you decide not to be in the study, or drop out, this will not put at risk your ordinary medical care.

What happens if you get worried or if there is an emergency?

You will always be able to contact a member of the research team to discuss your concerns and/or get help:

Dr Anthony Mathur, Consultant Cardiologist, via Hospital Switchboard

Dr Steve Hamshere, 0208 983 2475

Katrine Bavnbek, 0208 983 2475

APPENDIX III: REGENERATE-AMI Consent Form

CONSENT FORM (Version 1.6 Dated 06.10.09)

Title of project: REGENERATE - AMI

Investigator: Dr Anthony Mathur

Centre Number: Study Number: Patient Identification Number for this trial:

Please initial box to indicate agreement

1.	I confirm that I have read and understand the information sheet dated 06.10.09 (version 1.6) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3.	I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from the Barts and the London, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
4.	I understand that some of my bone marrow , stem cells and blood samples will be used for research purposes as long as they remain anonymous and cannot be linked to me. If there is any research on any of these samples in the future which can be linked to me I understand that I will be contacted and asked to sign a separate consent form	
5.	I consent to the storage of my stem cells for 10 years after which they will be destroyed (UK sites only)	
6.	I agree to my GP being informed of my participation in the study.	
7.	I agree to take part in the above study.	

Name of Patient

Date

Signature

Name of Person taking consent
(if different from Investigator)

Date

Signature

Investigator

Date

Signature

APPENDIX IV: REGENERATE-AMI HIV Consent Form

REGENERATE – AMI
**Randomised Controlled Clinical Trial of the use of Autologous Bone Marrow
Derived Progenitor Cells to Salvage Myocardium in Patients with Acute
Anterior Myocardial Infarction**

Name_____ DOB_____ Study no_____

INDICATIONS FOR FURTHER COUNSELLING

Gay or bisexual men	
Current or ex IDU	
Unprotected sex in countries where there is a high rate of heterosexual transmission	
Partner of the above	
Positive partner	
Other possible risk	
Other indications for further counselling (please specify)	

POINTS TO COVER IN PRE-TEST DISCUSSION

Previous HIV, hep B&C, syphilis tests (when/result)	
How HIV, hep B&C & syphilis is transmitted	
Difference between HIV and AIDS	
Window period (3 months – need for retest or wait)	
Confidentiality of records	
Insurance	
How would patient respond to +ve or -ve result?	
Who will they tell about the test and result?	
Does patient need more time to think?	
Risk reduction: Safer sex/drug use	

REGENERATE - AMI

**Randomised Controlled Clinical Trial of the use of Autologous Bone Marrow
Derived Progenitor Cells to Salvage Myocardium in Patients with Acute
Anterior Myocardial Infarction**

CONSENT FORM FOR HIV ANTIBODY, HEPATITIS B&C AND SYPHILLIS TESTS

Patient sticker

I agree that I have received adequate information and hereby consent to blood tests for HIV antibody, Hepatitis B&C and syphilis

Patient signature: _____ Date_____

Advisors signature: _____ Date_____

Advisor name (block capitals) _____
