The impact of Vasopressin on coronary microcirculation in ST-Elevation Myocardial Infarction

Ashfaq Mohammed

Thesis Submitted for the degree of Doctor of Medicine

2020

Newcastle University

Abstract

Background: When treating patients with an acute myocardial infarction, the coronary microcirculation is an area that still eludes understanding. One suspected mechanism for microvascular dysfunction in this patient group is ongoing coronary vasoconstriction during reperfusion. Animal models suggest that Arginine Vasopressin (AVP) has a vasoactive effect on the coronary microcirculation.

Aims:

1) To evaluate the blood levels of vasopressin in STEMI patients over the course of the myocardial infarction and during reperfusion.
2) To assess the impact of vasopressin on the coronary microcirculation in STEMI.

Methods: Arterial blood samples were taken from patients admitted to the Freeman Hospital, Newcastle with an acute STEMI, who subsequently underwent PPCI, over the time course of reperfusion. Copeptin, a precursor of vasopressin was measured. Cardiac MRI was performed to evaluate microvascular obstruction, infarct size and ejection fraction. Index of Microvascular resistance (IMR) was performed to measure microvascular dysfunction.

Results: In STEMI patients copeptin levels at baseline are markedly elevated (126.8 ± 13.94 pmol/l) with copeptin levels falling significantly by 90 minutes (86.15 ± 12.57 pmol/l) ($p < 0.0001$). Copeptin levels at 24 hours are significantly higher in patients where TIMI 3 flow was not achieved post PCI (<0.05). Copeptin levels were not related to the presence of microvascular obstruction or IMR, but higher copeptin levels resulted in significantly lower CFR ($p <0.01$). Higher copeptin levels at baseline and 30 mins post reperfusion were noted in patients with smaller infarctions ($p <0.01$).

Conclusion: In STEMI patients, circulating copeptin is elevated over the course of reperfusion, with increased levels at 24 hours signifying poor reperfusion. Copeptin does not impact on the coronary microcirculation but does significantly affect the coronary flow after reperfusion. Higher copeptin levels at baseline suggest a cardioprotective element with smaller infarctions.

Acknowledgements

This research project and thesis would not be possible without the support and guidance of Professor Ioakim Spyridoupoulos, who has been integral to my development and training as a cardiologist and been a superb mentor. Not only for his clinical and research support but also his pastoral support.

I would also like to take an opportunity to the following for their support during the recruitment and analysis of patients in the trials – Suzanne McCormack; Andre Nobian; Pedram Panahi; Adnan Ali and Luke Spray.

I would like to thank all the interventional cardiologists at the Freeman Hospital for helping to support the recruitment of their patients into the respective studies and being patient with me at all hours.

I would like to thank the team at the Centre for Life and Newcastle University for supporting and training me the software and equipment

I would also like to thank all the patient volunteers who have agreed to participate in the studies and made this piece of work possible

I also want to thank my family, my wife and my Amelia Noor for their support and understanding during this time.

Declaration

I declare that this thesis submitted for the fulfillment of the requirements of the award of a degree of Doctor of Medicine from Newcastle University is my own original work and has not been submitted elsewhere for a degree or diploma.

The research was performed by myself at Newcastle upon Tyne Hospitals NHS Foundation Hospitals and Newcastle University premises.

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Abbreviations

ACS	Acute Coronary Syndrome
ACTH	Adrenocorticotropic Hormone
ATP	Adenosine Triphosphate
AVP	Arginine Vasopressin
AVPR	Arginine Vasopressin Receptor
BMI	Body Mass Index
BP	Blood Pressure
CAD	Coronary Artery Disease
CFR	Coronary Flow Reserve
CMD	Coronary Microvascular Dysfunction
CMR	Cardiac Magnetic Resonance Imaging
CTFC	Corrected TIMI Frame Count
CVD	Cardiovascular disease
DAPT	Dual antiplatelet therapy
DCM	Dilated Cardiomyopathy
ECG	Electrocardiogram
HCM	Hypertrophic Cardiomyopathy
IMR	Index of Microvascular Resistance
LGE	Late Gadolinium Enhancement
LV	Left Ventricle
MBG	Myocardial Blush Grade
MI	Myocardial Infarction
MPTP	Mitochondrial Permeability Transition Pore
MACE	Major Adverse Cardiovascular Events
MCE	Myocardial Contrast Echocardiography
MCESI	Myocardial Contrast Echocardiography Score Index
MVO	Microvascular Obstruction
NSTEMI	Non- ST segment Elevation Myocardial Infarction
OTR	Oxytocin Subtype Receptor
PCI	Percutaneous Coronary Intervention
PPCI	Primary Percutaneous Coronary Intervention
ROS	Reactive Oxidative Species

SI	Salvage Index
SSFP	Steady-State Free Precession
STEMI	ST Segment Elevation Myocardial Infarction
TASH	Transcoronary Ablation of Septal Hypertrophy
TIMI	Thrombolysis in myocardial infarction
TTE	Transthoracic Echocardiogram

Chapter 1

1. Introduction

1.1 Myocardial Infarction

1.1.1 Prologue

Cardiovascular disease (CVD) continues to be the leading cause of death in the UK. In 2012, CVD was the most common cause of death in the UK for women, contributing to 28% of female deaths. For men, it was the second leading cause of death, behind cancer, causing 32% of deaths (1, 2). Cardiovascular disease includes coronary artery disease (CAD) as well as stroke. In 2016 it was estimated that cardiovascular disease contributed to 17.9 million deaths worldwide, the leading cause of mortality, with coronary artery disease being the largest component (3). Mortality and prevalence of cardiovascular disease are variable depending on the locality in the United Kingdom. In Scotland, death rates from CVD are one of the highest in the UK when standardised for age at 347 deaths per 100 000. The North of England follows shortly behind at 320 deaths per 100 000 (4). The prevalence of coronary artery disease is highest in the North East of England at 4.5%.

Myocardial Infarction (MI) is the most significant and acute form of coronary artery disease that results in irreversible damage to the myocardium as a consequence of prolonged ischaemia. An ST-elevation Myocardial Infarction is the most severe form of MI. This is the result of an acute total occlusion of one of the coronary arteries. Current treatments involve opening the occluded artery swiftly, but despite this myocardial damage continues, some of this can be a result of reperfusion of the myocardium.

The focus of this thesis will be investigating the role of intrinsic hormones, specifically vasopressin and its role in contributing to myocardial injury in the STEMI population.

1.1.2 Coronary Artery Disease

Coronary artery disease (CAD) is the result of atherosclerosis of the coronary arteries. Numerous plaques form within the intimal layer of the epicardial arteries as a result of chronic inflammation (5). The plaques are made up a lipid-rich core covered with a cap made of fibrous connective tissue. The core consists of foam cells, which are macrophages that are lipid-laden and extracellular lipids, together with smooth muscle cells (6, 7)**.** Smoking, elevated blood pressure and cholesterol, as well as diabetes and a family history of coronary disease, can accelerate the process of atherosclerosis.

The coronary artery plaques that develop initially are fibrous with a thick cap. These plaques are generally stable and grow into the lumen of the coronary artery. When the plaques are large enough, they limit the blood flow and cause an obstruction. This results in transient myocardial ischaemia during exertion, stable angina, one of the clinical manifestations of coronary artery disease (8).

1.1.3 Pathophysiology of Myocardial Infarction

When a plaque is fully developed, it has a lipid core that is separated from the artery lumen by the plaque cap. The core consists of cholesterol and its esters (9). In myocardial infarction, the fibrous cap can rupture exposing the lipid core to the arterial lumen. This results in platelet aggregation as well as fibrin formation. The flow of blood in the coronary artery is reduced, and coagulation pathways are activated. The resultant thrombus, consisting of erythrocytes and inflammatory cells trapped in the network of fibrin, can occlude the lumen of the coronary artery. (10-12). The thrombus occlusion of the vessel then results in ischaemia of the myocardium (Figure 1).

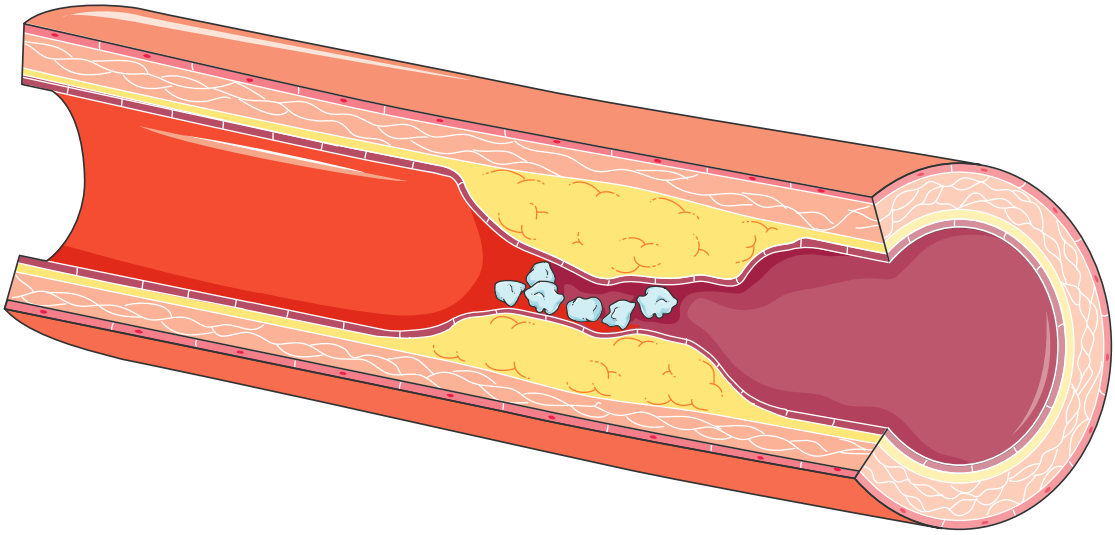

Figure 1: - Thrombotic occlusion of a coronary artery resulting in reduced distal flow

After 20 minutes of ischaemia, permanent damage occurs to the myocardium resulting in a myocardial infarction (5). This results in infarction in the subendocardial area of the myocardium initially, but as the ischaemia time lengthens then infarction spreads to involve the full thickness of the myocardial wall (13).

Most of the time, myocardial infarction is the result of unstable plaque disease. Clinically this can manifest as an acute coronary syndrome (ACS). This comprises of unstable angina, non-ST elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI). The duration and extent of obstruction of the coronary artery can delineate which of these pathologies develops (14, 15)

1.1.4 Presentation Acute Coronary Syndrome

Patients usually present with central chest tightness, sweats and nausea. The use of a 12-lead electrocardiogram (ECG) together with cardiac enzymes, such as troponin, can help to differentiate between the components of the spectrum.

Unstable angina occurs when there is an interruption of flow in the coronary artery resulting in cardiac chest pain and changes on the ECG. The duration of coronary occlusion and the degree of it is not enough to cause an increase in measurable biomarkers, including troponin T and troponin I (16).

ST-elevation myocardial infarction (STEMI) is usually the result of complete occlusion of the lumen of the coronary artery. This results in elevation on the ST segments of the ECG. A non-ST elevation myocardial infarction will not have these changes on the ECG, but the resultant myocardial damage will be detected in the cardiac enzymes, troponin I or T. This is usually due to partial occlusion of the coronary artery.

1.1.5 Management of Myocardial Infarction

Myocardial Infarction management involves limiting myocardial injury and damage by restoring and maintaining perfusion of the myocardium.

In STEMI, once the diagnosis has been established using an ECG, the patient will be given anti-platelets together with glyceryl-trinitrate, and oxygen if the saturations are low (17). Patients then undergo reperfusion therapy in the form of primary percutaneous coronary intervention (PPCI) or thrombolysis if they are in a region that does not provide this service (17).

Dual anti-platelet therapy (DAPT) is routine therapy in myocardial infarction (STEMI and NSTEMI). This typically consists of aspirin and a P2Y12 receptor inhibitor (Prasugrel/ Ticagrelor etc.) (14, 15). These drugs are vital as thrombus formation in the vessel contributes to the occlusion and subsequent myocardial infarction. Glycoprotein IIb/IIIa receptor inhibitors are potent anti-platelet agents that can be given intra-coronary and intravenously in some clinical cases (14).

Glyceryl-trinitrate works alongside other therapies to increase myocardial oxygen supply by dilatation of the coronary arteries. It also reduces myocardial oxygen demand by venous dilatation, resulting in a reduction in end-diastolic volume and preload. The combination of these effects is to reduce and limit ischaemia (15).

Revascularisation and restoring coronary blood flow urgently Is the most significant difference in the treatment of STEMI and NSTEMI. In STEMI total occlusion of the coronary artery is usually the case and re-establishing blood flow to the myocardium

is vital to salvaging it. In the 1980s reperfusion therapies in the form of thrombolytic agents, to break down the occlusive coronary thrombus, showed a reduction in mortality up to 25% in comparison to standard therapies (18-20). Unfortunately, there were significant complications with these therapies, including bleeding and in some instances intracranial bleeding resulting in a stroke (14).

1.1.6 Primary Percutaneous Coronary Intervention

Andreas Gruentzig developed percutaneous coronary intervention (PCI), also known as coronary angioplasty, in 1977, with the first procedure performed in Zurich in the same year (21). Coronary angioplasty involves passing a sheath into the femoral or radial artery through which a catheter is sited in the ostium of the occluded coronary artery. A wire is passed through the occlusion, over which a balloon is passed and inflated at the site of the occlusion. In the majority of cases, a stent is also deployed to scaffold open the occlusion and maintain coronary patency (8).

Primary PCI is the preferred method of reperfusion in patients who are having a STEMI, and this should be performed within 12 hours of the onset of symptoms (22). Reperfusion therapy is given swiftly to reduce the time that the myocardium is ischaemic. This is to reduce the size of the infarct and to salvage viable myocardium. Despite the time patients arrive in a hospital to being revascularised by PCI being reduced (door to balloon time) over the last few years, the in-hospital mortality in patients undergoing primary PCI has remained virtually the same (23).

It is felt that despite timely recanalisation of the infarct-related epicardial artery in PCI that coronary microvascular dysfunction continues to occur after reperfusion. Coronary microvascular dysfunction has been shown to increase the likelihood of cardiovascular events irrespective of the degree of epicardial disease. (24, 25) When there is epicardial coronary artery recanalisation, but there is not myocardial reperfusion, this is commonly known as ‘no-reflow’ phenomenon, and more recently this has been described as microvascular obstruction (MVO) (25).

In 30 - 40% of patients presenting with a STEMI, they do not get an adequate response of reperfusion of their myocardium despite recanalisation of the epicardial coronary artery (26). This is felt to be due to slow flow/no-reflow phenomenon that clinically can be assessed by ongoing pain, and failure of the ST segments on the electrocardiogram to normalise. It also can be assessed by the flow of contrast through a patent coronary artery post revascularisation, a thrombolysis in myocardial infarction grade (TIMI) less than 2 (27, 28).

Slow flow and microvascular obstruction is associated with a lower left ventricular ejection fraction and also larger infarct size (28). Microvascular dysfunction is a vital area to explore as part of the future treatments of patients with myocardial infarction.

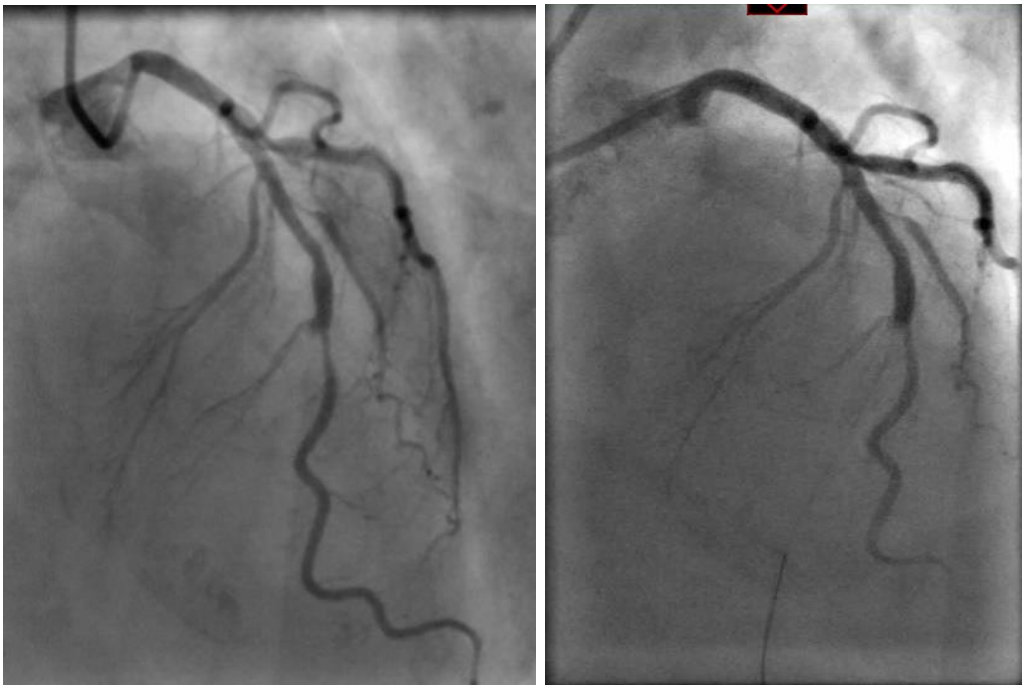

Figure 2: No Reflow Phenomenon: On the left shows an occluded mid LAD with TIMI 0 flow. Despite the successful deployment of a stent on the right side, we can see there is no contrast in the epicardial artery.

1.1.7 Clinical Consequences of Myocardial Infarction

With improving and more effective therapies, clinical outcomes from myocardial infarctions have improved over the years. Despite this mortality as a consequence of a STEMI remains approximately 13% at 6 months (29, 30)

Left ventricular failure is one of the most significant consequences of an acute myocardial infarction. Necrosis and loss of myocardial cells as a result of the infarction together with stunning of myocardial tissue can result in acute left ventricular failure (14). Left ventricular dysfunction is one of the strongest predictors of mortality in the setting of an ST-elevation myocardial infarction (14, 31). In 5 - 8% of STEMI cases, they present with cardiogenic shock as a consequence of the severity of the degree of LV dysfunction (32). This clinically manifests with persistent hypotension and end-organ hypo-perfusion. Mortality rates in cardiogenic shock as a consequence of a STEMI can be up to 50% (31).

Myocardial infarction is also a cause of chronic heart failure as well as acute left ventricular dysfunction. Studies have shown that after a myocardial infarction, 36% of patients experienced clinical symptoms of cardiac failure over a period of 8 years (33). After necrosis of myocardium and loss of myocytes cells after an acute myocardial infarction, there is a period of remodelling of the ventricle that involves scar formation and fibrous tissue developing at the infarction site. This remodelling process can result in a further decline in myocardial function and also impact on the function of viable myocardium as well (34).

Arrhythmias, including ventricular and supraventricular, are also significant sequelae as a consequence of a myocardial infarction. It has previously been noted that ventricular tachycardia and ventricular fibrillation has been detected in up to 20% of patients presenting with a STEMI (35). These rhythms are potentially life-threatening and frequently need cardioversion to restore sinus rhythm (14). Atrial fibrillation, which in the context of an acute myocardial infarction is associated with increased mortality, has been noted in up to 28% of patients with an acute MI (14, 36).

1.1.8 Left Ventricular Remodeling after Myocardial Infarction

When the size, function, shape and structure of the heart changes, it is termed ventricular remodelling (37). Remodelling can be physiological, such as in athletes, or pathological after the myocardium is injured, such as after a myocardial infarction (38). In a myocardial infarction, the degree of damage to the myocardium, and the location within the left ventricle can contribute towards the degree of LV remodelling (39). As a consequence of the infarction and resulting myocardial necrosis, the contractility in the affected myocardium is reduced. The increase in the haemodynamic burden on the affected myocardium results in ventricular dilatation and remodelling of myocardium that has not been infarcted (40). The dilatation is a consequence of a phenomenon called infarct expansion, where infarcted tissue stretches due to mechanical forces (40).

There are a number of stages when it comes to remodelling of the left ventricle in myocardial infarction. When the artery is occluded the myocytes in the infarct zone start to die through various processes including apoptosis, necrosis and autophagy. The aim of reperfusion is to halt this process (41). The next stage involves the healing of the myocardium. Inflammatory responses, where cells, including lymphocytes and macrophages infiltrate the tissue, remove the dead myocytes and initiate the healing phase (40). The next stage is the development of a fibrotic scar to replace the dead myocytes to strengthen that section of the myocardium to reduce the risk of rupture (42). Pressure on the myocardium results in hypertrophy where normal myocardium borders with the infarct zone and thinning of the fibrotic section and as a consequence dilatation of the ventricle (40). (Figure 2)

Left ventricular remodelling is associated with a poorer prognosis due to the correlation with heart failure (43). An increase in end-diastolic and end-systolic volumes is a measure of left ventricular remodelling (44).

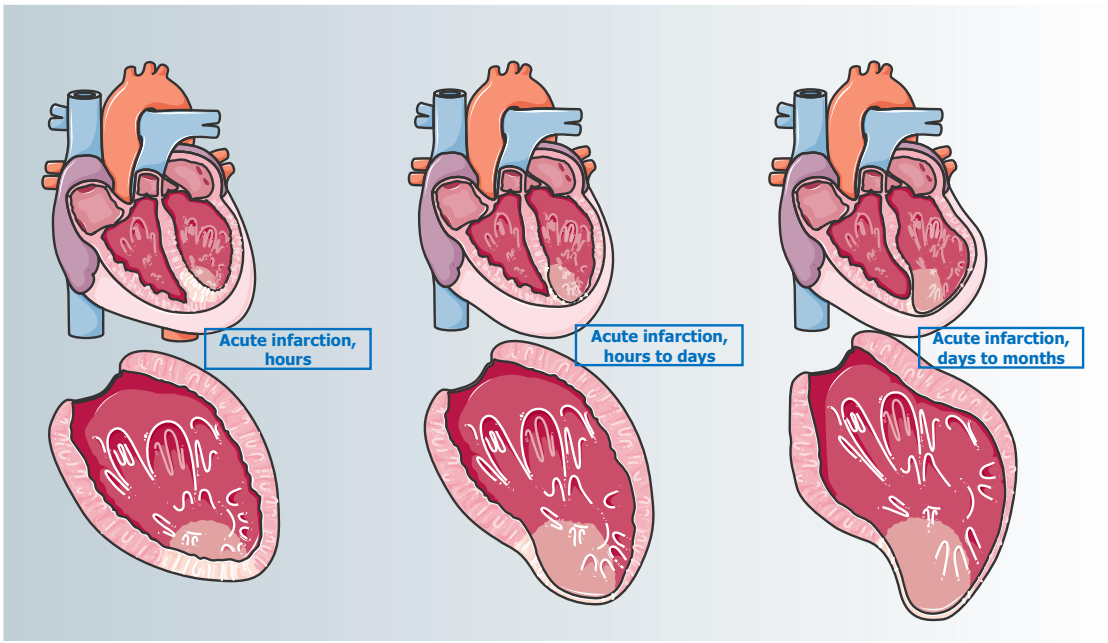

Figure 3:- LV remodelling post-infarction, showing LV dilatation and myocardial thinning

1.2 Coronary Microvasculature

1.2.1 Anatomy of the Coronary Vasculature

The coronary artery tree is made up of three compartments. The borders of the compartments are poorly defined from an anatomical aspect, and each compartment has a differing function (5). The compartments are described as the proximal, intermediate and distal compartments (45).

The proximal compartment consists of the large epicardial arteries that measure from 500 μm up to 5mm. These arteries do not provide much resistance to coronary blood flow, but the walls of the epicardial arteries have a degree of distensibility. This is often termed capacitance. The intermediate compartment consists of prearterioles measuring 100μm to 500μm. They are extramyocardial, and their main function is to sustain pressure at the origin of the distal compartment, arterioles (45, 46). The prearterioles are most responsive to intravascular pressure changes, to ensure the pressure into the arterioles is constant. The distal compartment, the arterioles are intramural and measure less than 100μm. They have an important role in the metabolic regulation of coronary blood flow (47). Blood flow in the vascular network is regulated by metabolic, myogenic and neural mechanisms. Metabolic mechanisms would include the impact on vascular flow by metabolites such as adenosine, which is a recognised vasodilator. Myogenic autoregulation of blood flow involves the impact of blood pressure on the stretch of vascular smooth muscle, and the subsequent constriction or relaxation of arterioles. The autonomic nervous system is also involved in blood flow regulation in vascular networks with alpha-1-adrenergic receptors responsible for vasoconstriction and beta-adrenoreceptors involved in coronary vasodilatation. An increase in blood flow would require an increase in vessel diameter of the microvasculature (46).

1.2.3 What is Coronary Microvascular Dysfunction?

The term microvascular angina was coined in 1985. It was felt that the coronary microcirculation was more sensitive to vasoconstrictor stimuli, and also the ability of the microvasculature to vasodilate is also limited (26, 48). It was felt that microvascular dysfunction was due to the small prearterioles and intramural arterioles (26). Coronary microvascular dysfunction has been described as a mismatch in the blood flow into the myocardium and the consumption of oxygen due to dysfunction in the coronary vessels that measure less than 500μm (45). The pathophysiology of coronary microvascular dysfunction is still elusive but felt to be due to a number of different mechanisms, including structural and functional. Structural contributors to microvascular dysfunction include vascular remodelling and perivascular fibrosis, whilst functional contributors include abnormalities in vascular smooth muscle function. The relevance of the individual mechanisms and their impact on the [patients depends on the cause of the microvascular dysfunction, although it is felt that multiple mechanisms are present in each patient (45).

1.2.4 Types of Coronary Microvascular dysfunction

Coronary Microvascular Dysfunction (CMD) can be classified into four types depending on the clinical scenario (26).

(1) CMD without myocardial disease and obstructive coronary artery disease.
(2) CMD in myocardial diseases.
(3) CMD in obstructive coronary artery disease.
4) Iatrogenic CMD

It is felt that multiple pathways contribute to coronary microvascular dysfunction, and there is an overlap between the mechanisms between the different subtypes (Table1).

	Clinical Setting	Main Pathogenetic mechanisms
Type 1: in the absence of myocardial diseases in obstructive CAD	Risk Factors Microvascular Angina	Endothelial dysfunction Smooth muscle cell dysfunction Vascular remodelling
Type 2: in myocardial diseases	Hypertrophic cardiomyopathy Dilated Cardiomyopathy Anderson-Fabry's disease Amyloidosis Myocarditis Aortic Stenosis	Vascular remodelling Smooth muscle cell dysfunction Extramural compression Luminal obstruction
Type : in obstructive CAD	Stable Angina Acute Coronary Syndrome	Endothelial dysfunction Smooth muscle cell dysfunction Luminal obstruction
Type 4: iatrogenic	PCI Coronary artery bypass Grafting	Luminal obstruction Autonomic dysfunction

Table 1: Classification of coronary microvascular dysfunction and proposed pathogentic mechanisms. Adapted from Crea et al, (26)

1.2.4.1 CMD without myocardial disease and obstructive coronary artery disease

This type of CMD is usually attributed to cardiovascular risk factors, including diabetes, hypertension and dyslipidemia, with the main pathophysiological pathways including endothelial dysfunction and vascular remodelling (26). This type of microvascular dysfunction is felt to be as a consequence of functional abnormalities and data suggest that it is reversible to some degree (45)

Diabetes has been found to be associated with microvascular dysfunction in multiple body organs, including the kidneys and the eyes, as well as the heart. Longstanding hyperglycemia has been found to be associated with reduced endothelial-dependent and independent function (45) .In patients with no obstructive coronary atherosclerosis, who had insulin resistance, when they were offered treatments to improve insulin sensitivity and function, studies showed an improvement in endothelial function and reduced myocardial ischaemia (49).

Smoking is known to be a risk factor for cardiovascular disease and affects many arterial beds, including coronary, cerebral and peripheral circulation. Endothelial dysfunction has been shown in the coronary arteries of long-term smokers (45). There is also evidence of microvascular disease in smokers with no coronary artery disease with a reduction in the coronary flow reserve by 21% when compared to non –smokers (50).

In asymptomatic patients with hyperlipidemia with normal coronary arteries, there have been studies that have shown they have a lower coronary flow reserve compared to those with normal cholesterol, that improves when they undergo cholesterol-lowering treatments (51, 52)

Patients with elevated C-Reactive Protein (CRP), which can be a biomarker for low-grade chronic inflammation, has also been felt to have a role in microvascular dysfunction (26). Patients with pathologies such as rheumatoid arthritis and other chronic inflammatory conditions, who have higher CRP levels, have been shown to

have lower coronary flow reserve levels, suggesting chronic inflammation contributes to microvascular dysfunction (53).

Patients who do not have obstructive coronary artery disease, or other myocardial diseases, can still present with typical anginal symptoms. Historically this was termed syndrome X in patients who also had evidence of ST changes on exercise or reversible ischaemia on a perfusion imaging (48). Studies have shown that there is a reduction in endothelial function and coronary vasodilatation (54). This form of coronary microvascular dysfunction is coined microvascular angina and studies have shown that there is a reduction in coronary flow reserve on invasive assessment and non-invasive assessment (26). When previously felt to be benign, it has been shown that women with normal coronary arteries and chest pain typical of angina have an increased risk of developing atherosclerosis and an adverse prognosis (26).

1.2.4.2 Coronary Microvascular dysfunction in myocardial disease

In patients with hypertrophic cardiomyopathy (HCM), coronary microvascular dysfunction has been shown to cause myocardial ischaemia in patients with normal coronary arteries (26). On cardiac MRI there is evidence of myocardial fibrosis, on late gadolinium enhancement images, in patients with hypertrophic cardiomyopathy that is felt to be due to chronic CMD and recurrent ischaemia resulting in necrosis and then fibrosis (26). The coronary flow reserve is shown to be significantly reduced not only in the hypertrophied septum but also in the left ventricular free wall, which has less left ventricular hypertrophy (45). On autopsy, there is remodelling of the intramural arterioles, with medial hypertrophy and decreased luminal size all contributing to CMD in HCM patients (45). The severity of the CMD in HCM patients is an independent predictor of death in this patient cohort (55).

Having normal epicardial coronary arteries is previously felt to be necessary to make a diagnosis of idiopathic dilated cardiomyopathy (56). There is increasing evidence that suggests that coronary microvascular dysfunction plays a role in this pathology, with a significantly reduced coronary flow reserve found in DCM patients (45). A reduction in myocardial blood flow has also been noted in early DCM patients (26).

As with HCM patients, the degree of CMD has been found to be an independent predictor of sudden death and the progression in heart failure in DCM patients (57).

In infiltrative cardiomyopathies such as Fabry's disease, an X-linked deficiency of alpha-galactosidase A, there is cardiac, renal and cerebrovascular deposition of glycosphingolipid (45). Patients with Fabry's commonly describe typical angina despite normal coronary arteries, and studies have shown that there is a blunted response in the coronary flow reserve (45). It is felt that glycosphingolipid deposition in endothelium may result in endothelial dysfunction and perivascular fibrosis. This then results in an increase in coronary microvascular resistance (45).

It has been noted that 50% of patients with severe symptomatic aortic stenosis will describe anginal symptoms and have normal coronary arteries on coronary angiography (58). CMD and reduction in the coronary flow reserve are felt to be multifactorial. There has been found to be a reduction in the density and number of capillaries at autopsy assessment (26). Studies have shown that the reduction in the coronary flow reserve is related to the aortic valve area (45).

1.2.4.3 Iatrogenic coronary microvascular dysfunction

Distal embolisation during coronary intervention can be marked. As the plaque is disrupted, it is washed into microcirculation and results in micro-infarcts and tissue necrosis (59). A meta-analysis showed that nearly one-third of patients undergoing PCI had a troponin elevation after PCI and that at follow up peri-procedural troponin rise was associated with a 50% increase in MACE and 2 fold increase in the risk of death (60). Also in patients undergoing percutaneous coronary intervention for stable coronary disease, there is evidence that the CFR does not recover straight away and that CMD persists despite success recanalisation of the epicardial stenosis, suggesting ongoing microvascular dysfunction (26).

1.2.5 Coronary Microvascular Dysfunction in Myocardial Infarction

Opening of the acutely occluded epicardial coronary artery in a STEMI swiftly is essential in salvaging the myocardium. Yet, the consequence of this intervention can result in injury to microvasculature and does not terminate the infarction immediately. In its most significant form, this is called no-reflow phenomenon.
It is felt that in ST-segment elevation myocardial infarction patients, that there are four interacting mechanisms that contribute to coronary microvascular dysfunction:

1) Ischaemic Injury
2) Reperfusion Injury
3) Distal atherothrombotic embolisation
4) Individual susceptibility of the microcirculation to injury (25)

1.2.5.1 Ischaemic Injury

In animal studies, the coronary microcirculation has been analysed with electron microscopes after the coronary arteries have been occluded for 90 minutes. This has shown damage to the capillaries as well as “blebs” blocking the capillary lumen. A further consequence of ischaemia is oedema of the myocardial interstitium, which can cause compression to the small arterioles and hence further disrupt blood flow (25, 61). The duration of ischaemia is felt to be the strongest predictor of the degree of ischaemia- induced injury (62).

1.2.5.2 Reperfusion Injury

Opening an occluded vessel in the context of an acute myocardial infarction swiftly is vital to save viable myocardial tissue that is at risk to infarction. It had previously been felt reperfusion contributed to the development of microvascular obstruction (MVO), as further myocardial injury occurs. It is recognised in patients who have had ischaemia lasting more than 3 hours (25). Vasoconstrictors are produced by

neutrophil-platelet aggregates. They also can cause the lumen of the vessel to be occluded further (63). Other mechanisms involved in reperfusion injury include the rapid restoration of pH, intracellular calcium overload, and oxidative stress (46, 64). Reperfusion Injury will be discussed in more detail in section 1.3.

1.2.5.3 Distal Atherothombotic Embolisation

Emboli of plaque and thrombus can migrate from the culprit epicardial artery into the microcirculation during primary PCI, causing mechanical obstruction of the vessel lumens. The micro emboli can contribute to a reaction locally within the microcirculation, with the release of inflammatory mediators as well as vasoactive metabolites (25, 65). Studies have shown that myocardial blood flow reduces irreversibly when microspheres block more than half of the coronary capillaries (58).

Studies have looked at the role of distal protection devices, with substantial debris being trapped in the device when visually inspected (66). Distal embolisation has been found to result in an 8 fold increase in mortality, but trials using protection devices have not shown any benefits in MACE or infarct size (67, 68).

1.2.5.4 Individual Susceptibility and Pre-existing dysfunction

There is progressive evidence to suggest that pre-existing CMD contributes to outcomes in patients presenting with a STEMI. With patients who had a normal coronary angiogram but with a low CFR, a marker of microvascular dysfunction, that they had a higher risk of developing a myocardial infarction (69). It is perceived that genetic factors can influence the structure, density and function of the microcirculation. For example, the VEGFA and CDKN2B-AS1 genes have shown an association with microvascular dysfunction (70). Predisposition can also be acquired as well as genetic. As previously discussed, diabetes and hypercholesterolemia have been shown to have impacts on CMD, and in animal models have shown to exacerbate microvascular injury (71).

Ongoing vasoconstrictor tone after revascularisation has also been felt to contribute to slow-flow and microvascular obstruction. In a study by Chilian et al, it was found that there was an inverse relationship between the initial diameter of a vessel with its ability to dilate. The larger pre-arterioles were able to dilate by a greater percentage compared to the smaller arterioles. It was also noted that coronary arterioles did not dilate to their maximal size when in a state of hypo-perfusion, suggesting ongoing vasomotor tone in the microcirculatory tree (72, 73).

As described above, microvascular dysfunction is exacerbated by vasoactive metabolites. Arginine vasopressin (AVP) is felt to have a vaso-constrictive effect of the coronary microvasculature (74, 75). AVP, when administered to patients, has shown cause a reduction in cardiac function and myocardial lactate production. It has also shown to cause ischaemic changes on the ECG as AVP contributes to coronary vasoconstriction and a similar state to a myocardial infarction (76).

1.2.6 Assessment of the Coronary Microcirculation

When treating patients for a STEMI, restoring flow in the epicardial coronary artery does not necessarily mean that coronary microvascular perfusion has been adequately restored. There are a number of different methods and techniques to assess this further.

1.2.6.1 Electrocardiographic ST – Segment resolution

The resolution in the ST-segment elevation on ha 12 lead ECG has been used as a marker of the success or the lack of success of reperfusion therapy. It has been shown that rapid resolution of the ST segment is a strong predictor of patency of the culprit coronary artery and preservation of the microvasculature. These patients have a lower mortality (77, 78).

Failure of the ST segment to resolve is associated with a 50% rate of culprit artery occlusion and a significant reduction in microvascular dysfunction. ST – Segment resolution of less than 50% has been shown to be an indicator of no-reflow phenomenon (79). Using the ECG provides limited information on the coronary microcirculation,

1.2.6.2 TIMI flow grade

The Thrombolysis in Myocardial Infarction (TIMI) flow grade can be assessed as at the time of coronary angiography. Once contrast is injected into the coronary artery, the flow can be assessed (80).

- Grade 0 – no perfusion or antegrade flow.
- Grade 1 – penetration but failure to opacify the entire distal bed.
- Grade 2 – partial perfusion with slow opacification of the distal bed and slow washout of contrast.
- Grade 3 – normal full perfusion.

The flow grade of < 3 is felt to be associated with the development of microvascular obstruction on CMR. Although there are a significant number of patients with TIMI 3 flow at the end of undergoing PPCI, who also have MVO, showing this technique has low sensitivity to identifying patients with coronary microvascular dysfunction (80).

1.2.6.3. TIMI Myocardial blush grade (MBG)

The myocardial blush grade is an assessment of the contrast opacification of the myocardium and the wash out after injecting down the epicardial artery. The MBG can be graded from 0 to 3, where zero represent no myocardial perfusion and a three represents an intense myocardial blush and rapid washout suggesting optimal microvascular perfusion (80). The myocardial blush grade is a strong predictor of mortality in patients undergoing PPCI who have TIMI 3 flow after revascularisation.

Patients with a MBG of 0 to 1 had larger troponin rise and lower ejection fraction (81, 82)

There have been some studies that have shown an association between MBG and other markers of microvascular dysfunction including ST-segment resolution and coronary pressure wire studies (82)

Unfortunately, MBG can be subjective and open to intra-operator variability. When MBG is compared with more sensitive and specific markers of myocardial damage, including echocardiography and CMR, then it has been shown to be inaccurate (83, 84).

1.2.6.4. Corrected TIMI Frame Count (cTFC)

cTFC was generated as an objective and reproducible method to assess coronary blood flow. The technique involves counting the number of cine images during angiography needed for the contrast to reach anatomical specific landmarks see on the screen (82, 85). It is more reproducible than TIMI flow grade alone, and CTFC has been found to be an independent predictor of survival over 5 years. The lower the cTFC. the greater the recovery from the myocardial infarction (86).

When used to assess microvascular dysfunction and compared with coronary blood flow assessment using a doppler wire, it was found that cTFC reflected epicardial blood flow and was not an accurate reflection of microvascular dysfunction (87).

1.2.6.5 Myocardial Contrast Echocardiography (MCE)

Myocardial contrast Echocardiography involves using micro bubbles together with a transthoracic echocardiogram (TTE). The bubbles, measure between 2 to 6 μm, and stay confined to the vasculature, hence can be used to assess perfusion in the microcirculation. The bubbles are biologically inert and can pass into the

microcirculation. The contrast intensity represents the capillary blood volume. The ultrasound waves from the echocardiographic assessment destroy the micro-bubbles, and the speed that they are replenished within the myocardium reflects myocardial blood flow. The slower the myocardial blood flow, the longer the replenishment time of the micro bubbles (88, 89).

No-reflow, when detected using intracoronary micro-bubbles and MCE in patients given thrombolysis therapy, had a significantly lower left ventricular ejection fraction at 30 day follow up (90). When myocardial contrast echocardiography is compared to cTFC, MBG and ST-segment resolution as a predictor of left ventricular dysfunction at 30 days, it was found to be the best predictor with a sensitivity of 88% and specificity of 74% (91).

MCE with intracoronary micro bubbles can be done at the time of the primary PCI for patients admitted with a STEMI, by injecting the bubbles down the guide catheter. The perfusion of the myocardium can be assessed using the myocardial contrast echocardiography score index (MCSEI). This involves using the 16-segment model for myocardial perfusion from the AHA (0 represents no perfusion visible; 1 represents some patchy uptake of contrast; 2 represents homogenous uptake of contrast) (92). The MCSEI is then calculated by taking an average of the single segment scores. An MCSEI of $\geq$ 1 is considered to be adequate reperfusion whilst an MCSEI of 0 represents microvascular obstruction and no-reflow. Those with an MCSEI of 0 had lower ejection fraction and more adverse remodelling at 6-month review (92).

Issues with this modality involve patients with poor echocardiographic windows due to patient body habitus or chronic lung disease giving poor visualisation. These patients were excluded from the trials (93). Also, due to the semi-quantitative nature of the assessment, there is a greater degree of operator variability in the interpretation, just as there is for routine TTE assessment.

1.2.6.5 Cardiac Magnetic Resonance (CMR)

Transthoracic Echocardiography is the routine imaging modality in clinical care to assess for left ventricular function post-myocardial infarction. Cardiac magnetic resonance imaging (CMR) allows for higher spatial resolution, producing high-quality images. This allows for accurate assessment of left ventricular dimensions and ejection fraction. The CMR images acquired also allow for assessing myocardial viability and perfusion. The images are highly reproducible and have become the gold standard in clinical research looking at left ventricular size, volumes and ejection fraction (94).

When assessing for infarction and microvascular dysfunction on cardiac MRI, late gadolinium enhancement images are taken. These are T1 –weighted images when during the scan; gadolinium contrast a paramagnetic metal ion is administered intravenously. There is gadolinium uptake in both normal and injured myocardium. In normal myocardium there is early wash out of the contrast, as opposed to myocardium that has sustained injury, where the wash out is slower, resulting in "delayed enhancement" of the myocardium. In myocardial infarction, the enhancement is seen subendocardial. Where there has been a full thickness infarct, the enhancement is transmural (95).

Where there has been microvascular dysfunction, and as a consequence microvascular obstruction (MVO), there are changes seen on MRI. Areas of MVO are seen as dark central zones within the hyper-enhanced LGE areas of a transmural infarct (96). The presence of no-reflow phenomenon and dark zones attributed to microvascular obstruction is a poor prognostic indicator (97).

The presence of MVO has been found in up to 50% of patients undergoing PPCI for STEMI. Studies have shown that the presence of MVO was a predictor of increase in MACE compared to those without MVO. Also, when MVO was adjusted for the size of the infarct as well has been shown to be prognostic for post MI complications (97-99). CMR is discussed in more detail in section 1.4

1.2.7 Invasive Coronary Pressure Wire Assessment of Microcirculation

1.2.7.1 Thermodilution

Invasive coronary assessment of microcirculation utilises the thermodilution technique with a wire in the coronary artery. Thermodilution is based on the indicator dilution principle (100). This principle states that injecting into the blood an indicator and then measuring its concentration at a site distal to the point of injection then volumetric flow can be calculated. According to thermodilution theory, then flow (F) equals vascular volume (V) divided by mean transit time (Tmn).

$$F = V / Tmn$$

Using this principle, coronary blood flow can also be calculated and measured. A coronary wire can be sited with a proximal thermistor and a distal sensor that measures pressure and temperature. V represents the volume from the tip of the guide catheter to the sensor on the distal end of the wire. Tmn represents the time taken for a bolus of room temperature saline, to get from the sensor in the proximal body of the wire, to the distal temperature sensor (101).

1.2.7.2 Thermodilution derived coronary flow reserve (CFR)

Flow within the coronary arteries can increase nearly four-fold when the myocardium is ischaemic (102). This is called the coronary flow reserve. It is defined as the ratio between hyperaemic coronary blood flow and baseline coronary blood flow (102).

Using thermodilution to assess for CFR was first validated in canine models (103). Absolute flow was compared to the inverse mean transit time (1/Tmn) using a 3ml bolus of normal saline that was a room temperature, and there was found to be a significant correlation between absolute flow and inverse mean transit time. There

was a strong correlation of CFR, calculated from the ratio of hyperaemic to resting flow velocities, to CFR from the ratio of resting to hyperaemic mean transit times (103).

CFR = Tmn at rest / Tmn at hyperaemia

This has been validated against Doppler wire assessment in humans and found a strong correlation between using Doppler wire assessment and thermodilution assessment in the calculation of CFR (104).
CFR has its limitations when it comes to the assessment of the coronary microcirculation. Although originally was formulated to assess epicardial coronary stenosis to assess the success of PCI, it was noted that was challenging due to the CFR assessing epicardial and microvascular compartments together (105). The inability to separate the epicardial and microvascular contributions to the CFR limits its use in microvascular assessment (106). Also, the CFR is dependent on haemodynamic factors including blood pressure and heart rate, and this affects the reproducibility of the data as well Ng (107).

1.2.7.2 Index of Microvascular Resistance (IMR)

Index of microvascular resistance uses thermodilution to measure microvascular resistance and function, in the same way CFR is measured. It uses a coronary wire to measure the mean transit time (Tmn) and the distal coronary pressure (Pd) during maximal hyperaemia.

IMR = Pd x Tmn (maximal hyperaemia)

Fearon et al, validated using IMR to assess microvascular resistance, in porcine models. They compared true microvascular resistance (TMR) with IMR. True microvascular resistance was defined as the pressure in the distal LAD divided by the absolute coronary flow. This was calculated by using an ultrasonic flow probe (108). Microspheres were then injected into the coronary arteries to cause microvascular dysfunction. The group found a significant correlation between IMR

and TMR. The study also showed that a higher IMR was related to more significant microvascular dysfunction irrespective of epicardial disease (108). Further studies went on to solidify the validation and data that IMR was a validated and independent marker of microvascular resistance, and not affected by epicardial stenosis (109).

As previously described a limitation of CFR is the impacts of the haemodynamics on the reproducibility. Studies looking at CFR and IMR on haemodynamic dependence have shown that IMR values do not change when haemodynamic conditions are varied, unlike with CFR (107). This means the IMR is not only more specific marker of microvascular resistance, with no influence from epicardial stenosis, but also a more reproducible assessment and thus a powerful tool in assessing coronary microvasculature (107).

Index of microcirculatory resistance (IMR), which looks at distal coronary pressure divided by inverse of the hyperaemic mean transit time, is a way of assessing microvascular resistance independent of any epicardial stenosis (108). Both CFR and IMR are both associated with microvascular pathology and microvascular obstruction in patients who have had a STEMI (110). IMR correlates more closely with LV remodelling and microvascular pathology than CFR (110).

A number of studies were collated to generate the normal values for IMR (111). An IMR > 25 is associated with microvascular dysfunction (112).

1.2.7.3 IMR in STEMI

There are a number of studies looking at measuring IMR in patients presenting with a STEMI undergoing PPCI. IMR has been shown to predict infarct size when measured by clinical biomarker (CK and Troponin), as well as 3 month wall motion score on echocardiography, where as TIMI flow, CFR and CTFC did not (113, 114).

When IMR was measured alongside CMR in STEMI patients, not only was it associated with MVO, it correlated with adverse LV remodelling as well as ejection fraction after 6 months (110). McGeoch et al, also showed that patients with MVO on MRI had a higher IMR compared to those that did not have MVO, solidifying its

role in assessing the coronary microcirculation (114). When IMR has been measured directly after PPCI, it has shown to be a predictor of infarct size when compared with CMR (115).

In patients presenting with a STEMI, an IMR > 40 is associated with poor long-term clinical outcomes including mortality and re-hospitalisation for heart failure (116).

Despite the invasive nature of calculating IMR, siting a coronary pressure wire and the administration of a medical therapy to induce hyperaemia, the procedure is safe in myocardial infarctions (117). IMR is readily available in the cardiac catheter labs and provides reproducible and specific data on the coronary microcirculation in STEMI patients.

1.2.8 Management of No-Reflow Phenomenon

No reflow phenomenon occurs when despite the epicardial artery being revascularised there is still insufficient reperfusion of the myocardium due to profound microvascular dysfunction. It can be easily diagnosed in the cardiac catheterisation lab during PPCI by a TIMI flow grade < 3 and a low MBG. It was found to occur in a third of patients undergoing PPCI, and these patients had poorer outcomes, including death and cardiogenic shock (118). There have been no specific therapies formulated for the microcirculation and for no reflow management, although operators may trial a number of strategies in the cardiac catheter lab.

1.2.8.1 Thrombus Aspiration

In STEMI patients there is often a thrombus occluding the epicardial artery, and the manipulation of the thrombus with balloons and stents is felt to contribute to embolisation of the thrombus down stream of the occlusion site, and contribute to no-reflow. Aspiration of the thrombus involves siting a thrombectomy catheter proximal to the occlusion and passing it through the occlusion multiple times.

The 2009 meta-analysis of studies showed that thrombectomy improved reperfusion of the myocardium, but this did not translate into improvements in clinical end-points including mortality (119). Most recently, the TASTE trial that randomised 5033 patients to thrombectomy versus PCI alone in STEMI patients, showed no improvement with thrombectomy in death or cardiogenic shock but did show thrombectomy caused more stroke (120).

Although thrombectomy does improve angiographic flow and distal embolisation, there is no data that shows that it improves clinical outcomes and its use is recommended when there is visible thrombus as opposed to routinely (118).

1.2.8.2 Pharmacotherapy treatments for No Reflow

Adenosine is commonly used in the catheter lab in patients with no-reflow. It mainly works as a smooth muscle relaxant in the coronary circulation, although it's mechanism on how it works on the coronary circulation is still not well understood (118). The AMISTAD and AMISTAD II trials showed a reduction in infarct size when intravenous adenosine was given to patients undergoing reperfusion with thrombolysis or PCI. This did not translate into improved clinical outcomes with respect to death, heart failure and re-hospitalisation (121, 122). Intracoronary Adenosine has also been trialled in the catheter lab for no-flow. The REOPEN-AMI study compared adenosine with intracoronary nitroprusside and placebo. The adenosine group had markedly improved outcome in the degree of ST segment resolution compared to nitroprusside, but there was no clinical benefit noted between the two groups at 30 days (123). The REFLO-STEMI trial, published in 2016, cast doubt on the benefits of adenosine, showing no reduction in infarct size or MVO and an increase in clinical outcomes compared to the control arm (124). Although still routinely used in catheter labs, there is still a lack of large study data on the role of adenosine in no reflow.

Sodium Nitroprusside (SNP) works on vascular smooth muscle via activation of guanylate cyclase, resulting in vasodilatation (118). When given intracoronary, via a distal catheter, it has been shown to improve no-reflow with also a marked improvement in MBG (118). A randomised trial by Zhao et al, showed an

improvement in ST segment resolution and also an improvement in LV function. TIMI flow was no different between the two groups, but we have appreciated this is not the most sensitive test for microcirculatory assessment (125). The pharmacological effects of nitroprusside last longer than that of adenosine, but the side effect profile is also more sustained as well (118). Although meta-analysis has suggested a noticeable benefit of SNP in microvascular dysfunction, this was not reflected in the REFLO-STEMI trial (124).

Calcium channel blockers, including verapamil and diltiazem, have been given intracoronary as part of the management for no-reflow by some cardiologists to STEMI patients (118). Meta-analysis of studies have shown a benefit in the no-reflow with calcium channel blockers (118). Verapamil is routinely given, as part of an intracoronary cocktail, when patients undergo rotational atherectomy to reduce no reflow in this patient group. There is still a lack of evidence from a large randomised control trial to explore the role of this class of drugs in more detail for microvascular dysfunction (118).

No –reflow a representation of severe microvascular dysfunction is not uncommon in STEMI patients and although there are some therapies that have shown improvements in the coronary flow, this has not reflected in large randomised studies and in improved clinical outcomes. This area of acute cardiology continues to be a target for new interventions and therapies.

1.3 Myocardial Reperfusion Injury

1.3.1 What is Reperfusion Injury?

As previously described, recanalisation of an occluded coronary artery in the context of an acute STEMI is vital to salvage myocardium that is ischaemic and at risk of infarction. Studies have shown that reperfusion of the myocardium can cause further damage to the myocardium itself, described as ischaemia/reperfusion injury (126). Ischaemia/Reperfusion Injury has been shown to be responsible for up to half of the final infarct size. As a consequence, this can impact on the degree of left ventricular dysfunction and clinical outcomes for the patient (64).

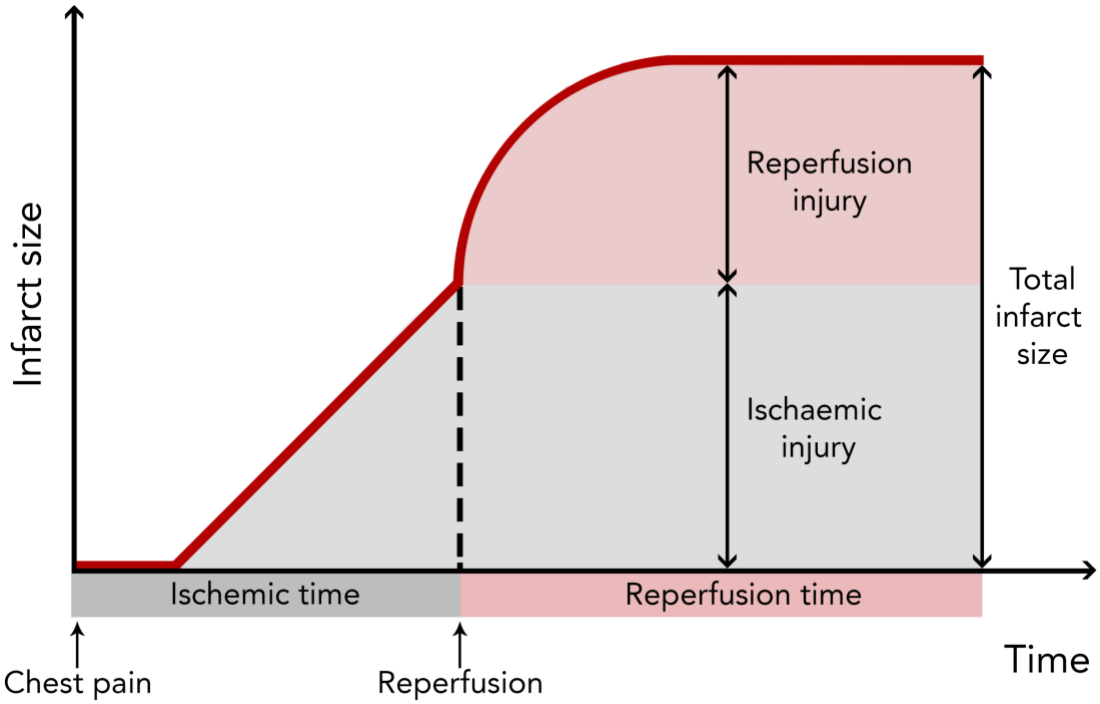

Figure 4: Ischaemia/Reperfusion Injury – When reperfusion is achieved, ischaemia/reperfusion continues, and the infarction propagates in size and can contribute up to 50% of the final infarct size. Adapted from Hausenloy et al, (64)

There has been a lot of discussion about whether myocardial reperfusion injury exists in humans. The theory was first coined in the 1960s when analysis of canines with perfused myocardial infarctions showed cellular swelling and myofibril

contracture together with calcium phosphate particles in the mitochondria (61) (127). Some studies looking at the development of MVO, a component of reperfusion injury, in patients who have had a STEMI with or without reperfusion therapies (medical, PPCI) showed that there were comparable amounts of MVO in all groups (128). This was regardless of whether recanalisation was achieved and that ischaemic time was a stronger contributor to the development of MVO as opposed to reperfusion injury. But other studies, including those by Staat et al, have suggested that ischaemia/reperfusion exists. They looked at ischaemic pre-conditioning in patients with myocardial by inflating a coronary balloon four times for one minute prior to stent deployment, resulting in a reduction in infarct size of 36% in STEMI patients (129).

Reperfusion Injury comprises of lethal myocardial ischaemia/reperfusion injury and microvascular obstruction (MVO), which cause irreversible damage that contributes to infarct size. Ischaemia/reperfusion injury also consists of myocardial stunning and arrhythmias that are short-lived and tend to be reversible (64).

1.3.2 Lethal Reperfusion Injury

When an ischaemic cardiomyocyte has reperfused, this then results in oxidative stress, rapid restoration of intracellular pH and causes cytosolic and mitochondrial calcium overload. This, together with depletion in adenosine triphosphate (ATP), causes the mitochondrial permeability transition pore (MPTP) to open, resulting in hyper contracture of the cardiomyocyte and lethal reperfusion injury (129).

When the myocardium is ischaemic lactic acid builds up as the cells are pushed into anaerobic glycolysis to maintain the availability of ATP. This causes a drop in intracellular pH, and to neutralise this, H+ is pushed out of the cells via Na^+/H^+ exchanger channels situated in the cell membrane, pulling Na^+ into the cells as part of the exchange (64, 130). The increase in intracellular Na^+ then acts on the $2Na^+/Ca^{2+}$ exchanger, exchanging Na^+ for Ca^{2+} and an accumulation of Ca^{2+} within the cells (130, 131). The damaging actions of intracellular calcium in reperfusion injury are initially inhibited during ischaemia due to the low intracellular pH levels. Once reperfusion occurs and pH is corrected then this protection ceases (130). The

build-up of calcium within the cells on reperfusion causes myocardial injury through hypercontracture and opening of the mitochondrial permeability transition pore. Elevated intracellular calcium also causes further cell damage through apoptosis (64).

Once reperfusion of the myocardium is restored, there is a flash of oxidative stress that causes cell death through a number of mechanisms (132). When the myocardium is ischaemic, the myocardial cells change their metabolism from aerobic to anaerobic. Once reperfusion is restored, there is a rapid switch back to aerobic metabolism. This is done through reactivating mitochondrial electron transport chains. When this occurs, there is an accelerated production of reactive oxygen species (ROS). The most plentiful reactive oxygen species is superoxide, and the volume produced surpasses the anti-oxidant system that converts superoxide to hydrogen peroxide. Hydrogen peroxide is then converted to water and then oxygen (130, 133). There is further production of reactive oxidative species through cellular xanthine oxidase. This works on the oxidation of hypoxanthine to xanthine and then to uric acid, and generates more reactive oxidative species in the process (133). When the myocardium is ischaemic hypoxanthine accumulates, resulting in a more reactive oxidative species forming through the above reaction (133). The reactive oxidative species propagate the reperfusion injury as they directly damage cells, DNA and enzymes. They also open mitochondrial permeability transition pores which also contribute to reperfusion injury, this will be discussed in more detail below (64, 130).

As previously stated, opening of the mitochondrial permeability transition pore (MPTP) is also a factor in contributing to reperfusion injury. This pore sits within the inner mitochondrial membrane and is usually closed but can be opened by excessive intracellular calcium (134, 135). Opening of the MPTP has a number of harmful effects, eventually resulting in cell death. After the channel opens the mitochondria membrane potential ceases, and this stops ATP synthesis from occurring via oxidative phosphorylation. This in turn, results in a decline in ATP (134-136) Also opening of the MPTP results in swelling of the mitochondria that lead to their rupture. These in turn, results in the release of pro-apoptotic substances such as cytochrome c, perpetuating further cell death (135, 137). The MPTP is opened by a number of factors including the production of reactive oxidative species,

increasing extracellular calcium and the recovery in cellular pH, which all occur in as a consequence of reperfusion (130). Minimising and preventing MPTP opening has been identified as a possible target for therapies to minimise reperfusion injury and total infarct size (64).

1.3.3 Microvascular Obstruction

As previously described and first noted in animal models, microvascular obstruction (MVO) is the result when there is good epicardial flow in the coronary arteries, but there is inadequate reperfusion of the myocardium supplied by it (61). In 1974 a study on canines it was found that after a period of prolonged ischaemia that there was uneven uptake of a marker stain, thioflavin S, on electron microscopy within the inside of the infarcted area (61). This is despite good flow in the epicardial coronary artery that the myocardium still did not enough perfusion to transmit the tracer.

Microvascular obstruction is a multifactorial process involving the blockage of the capillaries with platelets and leucocytes together with the swelling of the endothelial cells resulting in the obstruction of the capillary lumen as they protrude into it (61, 138). There is also compression of them capillaries externally as a result of swelling in the surrounding myocytes (61).

Although ischaemia can result in swelling in the myocytes and the bleb protruding into the capillaries and causing obstruction, it is felt that the platelet thrombi and leucocytes within the capillaries is a result of reperfusion, and washed down into the capillaries after the flow is restored (98, 139). This results in further obstruction and further injury to the myocardium (61, 140). The leucocytes occluding the lumen also fuel the increase in reactive oxygen species which themselves, as described above, cause further reperfusion injury (141). The ROS also can cause further reperfusion injury by causing further fibrin to accumulate within the lumen and cause more obstruction (141). Micro-embolisation of plaque debris has also been found within the microvasculature in patients undergoing primary PCI for STEMI, and as well as the luminal obstruction also fuels the inflammatory response, which is described below (142).

1.3.4 Inflammatory Response

Once reperfusion occurs in a myocardial infarction, there has been noted to be a profound inflammatory response. There has been shown to be a notable influx of neutrophils into the myocardium once it has been reperfused (143-146). The aggregation of neutrophils occurs almost straight away once reperfusion is performed and persists over six hours. There is a further peak in the inflammatory response after 24 hours (145, 146). It is not clear why the inflammatory response contributes to reperfusion injury, but data suggest that leucocytes are a significant contributory factor (147). When comparing myocardial infarction with and without reperfusion, in infarctions where reperfusion has not been performed then the concentration of neutrophils occurs more slowly with a peak noted after 2-4 days, and mainly located in the peripheries of the infarcted area (147). When reperfusion is performed, then the neutrophils concentrate in the centre of the infarcted area very rapidly and in a larger number (148).

As previously stated, there is a concentration and excess production of reactive oxidative species within reperfused myocardium, and they attract the neutrophil response (147). The neutrophils themselves then release further reactive oxidative species, and this perpetuates further damage to the cells and myocardium (147). It has also been noted that the flurry of leucocytes into myocardium also results in clogging up of the capillaries and contributes to the development of microvascular obstruction (147).

Animal studies, where anti-inflammatory agents are given when reperfusion is achieved, have shown a reduction in the overall infarct size, showing a direct role of the inflammatory response in reperfusion injury (147, 149). There is evidence in humans that the inflammatory response plays a role in reperfusion injury also. There is evidence that inflammatory markers such as C-Reactive Protein (CRP) correlates with the degree of MVO, and the also the degree of resolution of the ST segments, which both reflect reperfusion injury (138, 150, 151).

1.3.5 Reperfusion Arrhythmias and Myocardial Stunning

Unlike lethal reperfusion injury and microvascular obstruction reperfusion arrhythmias and myocardial stunning are forms of reperfusion injury that are reversible (61, 152). Once reperfusion occurs, arrhythmias can occur, the most dangerous of which are ventricular fibrillation and ventricular tachycardia, which can be swiftly treated with a cardioversion (14). The most frequently seen arrhythmia is that if an accelerated idioventricular rhythm, what is self-limiting and non-prognostic (14).

Stunning of the myocardium is a form of reversible reperfusion injury where there is a transient reduction in the myocardial function irrespective of sufficient perfusion (61, 152). This is believed to be through oxidative stress (61, 152). The myocardial function usually recovers over a number of weeks (153).

1.3.6 Pharmacotherapy treatments for Reperfusion Injury

Despite the increasing understanding of the pathophysiology of reperfusion injury and increasing potential targets for treatments to reduce infarct size, successful therapies have yet to be identified for use in regular clinical practice (64, 154). There have been a number of trials and explorative research in this area, but a successful treatment has yet to be discovered (64, 155).

There have been randomised controlled studies looking at reducing oxidative stress but using anti-oxidants reduce ROS, as well as using anti-inflammatory drugs, such as monoclonal antibodies, yet these trials have shown no benefit (64, 155-157). One of the hypotheses that the positive clinical outcomes seen in animal studies have not been replicated in human clinical trials, is felt to be due to the length of ischaemia time (147). In animal studies the ischaemia time is short, usually less than 2 hours, when compared to patients in clinical practice where the ischaemia time in patients is profoundly longer, and can be up to 12 hours. Thus it is felt that clinical patients may present too late for the therapeutic agents to have clinically recognised benefits (147).

As previously stated, elevated intracellular calcium contributes to reperfusion injury, and on the back of this studies have been done with calcium channel blocking therapies to reduce reperfusion injury. However, small randomized controlled trials showed some benefits, this as not replicated when performed in larger clinical trials (158-160).

There have been other pharmacotherapies that have been directed towards working on the MPTP. Adenosine has been trialled, which works on the reperfusion injury salvage kinase pathway. This protective pathway, which is activated during reperfusion, works on stopping the MPTP from opening (149). Large clinical trials with adenosine unfortunately did not show any benefit, when used in patients undergoing primary intervention form a STEMI (121). Insulin has also been used to try and target the reperfusion injury salvage risk pathway, and despite some positive results in smaller studies, larger trials did not show ant benefit when a glucose potassium infusion (GKI) was given in patients with a STEMI having PPCI (161). A small trial using glucagon-like-peptide, which also works on the same pathway have shown promising results in reducing infarct size, but larger clinical trials are needed to establish the clinical benefits (162).

There have been other studies that looked at drugs that act on the MPTP directly to stop it from opening, such as ciclosporin. A small randomised controlled trial showed a reduction in infarct size when a bolus of ciclosporin was given prior to reperfusion, and another showed a reduction in the adverse remodelling of the LV on CMR (163). Unfortunately, this was not reflected in the larger studies CIRCUS and CYCLE trials, where there was no clinical benefit of this therapy in STEMI seen (164). A small RCT of ciclosporin in STEMI by Spyridopolous et al, also showed no clinical benefit in the drug compared to placebo (165). All in all, the trial data of pharmacotherapies in STEMI working on reducing reperfusion injury has not been fruitful.

1.3.7 Mechanical Therapies for Reperfusion Injury

Animal models have shown that ischaemic pre-conditioning, prior to recannalising an occluded coronary artery, is protective of the myocardium. They have shown that transient ischaemia prior to reperfusion reduces the size of the infarct by reducing the reperfusion injury (166). Subsequent studies have also looked at the phenomenon of ischaemic post conditioning. They have shown a reduction in the infarct size when an occluded vessel is opened, and after reperfusion, there is a period of oscillating vessel occlusion and opening prior to the final vessel opening (125). It is felt that this protective mechanism and reduction in infarct size works by activating the reperfusion injury salvage kinase pathway (130, 149).

Ischaemic post conditioning in clinical practice is technically easy to perform in the context of primary PCI by re-inflating the coronary balloon used to restore flow intermittently. There has been mixed data when this has been researched in small clinical trials with some studies showing a benefit to treatments and others suggesting potential harm (129, 167, 168). Larger trials have not shown benefits of ischaemic post conditioning (169, 170).

There are other new and novel interventional therapeutic treatments that are being developed and researched to reduce reperfusion injury. Pressure-Controlled Intermittent Coronary Sinus Occlusion (PICSO) is a system that involves siting a balloon tipped catheter in the coronary sinus. The premise is that the balloon inflates and deflates, which causes the coronary sinus pressure to increase intermittently, and as a consequence, results in redistributing venous blood via collaterals (171). Animal studies have shown a significant reduction in the infarct size by nearly 30% (172). Early study data in patients presenting with a STEMI have shown a reduction in infarct size (173).

1.4 Cardiac Magnetic Resonance Imaging (CMR)

1.4.1 Introduction to CMR

For a number of years, cardiac MRI has become more accessible and used more prominently in cardiology research as well as clinical practice. Historically echocardiography was the imaging modality of choice, but cardiac MRI provides more value and benefits.

Cardiac MRI data is less subjective and provides more accurate and reliable measurements of cardiac function and the chamber measurements. CMR also allows for the analysis of tissue and hence the analysis and measurement of normal myocardium, infarcted myocardium as well as degree of microvascular obstruction. Cardiac MRI can also highlight myocardial oedema (174, 175).

This makes cardiac MRI a useful modality when assessing for ischaemia reperfusion injury.

1.4.2 Left Ventricular Size and Function

Left ventricular measurements and assessment of ejection fraction are reproducible and very reliable (174). When acquiring the cardiac MRI, a technique called steady-state free precession (SSFP) is used. These images have high temporal resolution and high spatial resolution that can be accurately analysed. Ventricular volumes are measured by the production of cine images, moving images of the heart, in a short-axis view of the heart. Accurate assessment can be made of the frames representing end-systole and end-diastole due to the high spatial resolution (174, 175).

The endocardial and epicardial borders of the myocardium can be traced and measured accurately giving the measurements of the cardiac volumes and allow the calculations of the ejection fraction and stroke volume.

1.4.3 Measurement of Infarct Size and Microvascular Obstruction

Cardiac MRI allows for the assessment of the different myocardial tissue characterisation, and this is possible as the different tissues respond to the T1 and T2 relaxation times differently.

Intravenous gadolinium, a paramagnetic contrast agent, is given to patients undergoing a cardiac MRI after a myocardial infarction. It is an extracellular agent that once given to the patient passes into the myocardium rapidly within a few seconds. When the myocardium is normal, the gadolinium passes through the tissue after 15 minutes, and hence is found in low concentrations in these cases. Gadolinium has a short T1 relaxation time which gives a high MR signal in myocardium when it accumulates in tissues (174, 175). When there has been a myocardial infarction and subsequent necrosis, the extracellular space is increased, and as a consequence, the gadolinium concentrations are higher in this tissue. This results in a greater T1 signal (174, 175). Late Gadolinium enhancement is a technique used to produce images of infarcted myocardium when T1 weighted images are taken 15 minutes after intravenous gadolinium is given. These produce images that highlight infarct as bright white against the nulled black normal myocardium (176). This allows for the infarcted territory to be accurately measured.

Using late gadolinium enhancement technique also can be used to identify MVO and measure the degree of MVO. On the LGE T1-weighted images microvascular obstruction appears as dark areas within the right hyper-enhanced infarcted segments (98, 175, 177). The hypo-enhancement of the MVO core occurs, as when the T1 weighted images are acquired 15 minutes after the administration of the gadolinium, the concentration of gadolinium is low within that territory of the myocardium (175, 177). Gadolinium cannot enter that area via perfusion due to the obstruction in the coronary microcirculation, therefore has to use passive diffusion, which is a slow process (98, 177).

1.4.4 Myocardial Salvage and Area At Risk (AAR) on CMR

Cardiac MRI can be used to measure the area at risk (AAR) in patients admitted with an acute STEMI who undergo PPCI (177, 178). The area at risk is a representation of the area of myocardium that is ischaemic prior to recanalisation of the coronary artery. This is a measure of the potential infarct size if revascularisation was not performed and the myocardial infarction completed (64). To assess the area at risk on cardiac MRI, then T2-weighted images are taken during the image acquisition. In this study, these were in the form short inversion time inversion recovery (STIR) images. Myocardial oedema accumulating within the area at risk can be identified on the images as the increased water content causes a highlighted appearance and higher signal compared to the normal, unaffected myocardium (175, 178). It has been shown that the highlighted myocardium, representing myocardial oedema, corresponds to the area at risk measured when histologically assessment was performed in animal models (154, 179).

In patients who have undergone reperfusion primary PCI in context of a STEMI and cardiac MRI has been used to measure the infarct size and area at risk then it is possible to quantify the degree of myocardium that has been salvaged by recannalising the occluded coronary artery. The area of salvaged myocardium can be calculated subtracting the infarct size from the area at risk (AAR) (167, 180, 181). When the degree of rescued myocardium is expressed as a ratio of the AAR, then this parameter is called the salvage index (SI). As the final infarct size is a combination of the damage to the myocardium before reperfusion as well as ischaemia reperfusion injury sustained after recanalisation, then reperfusion injury reduces the degree of myocardium that is salvaged (64). For this reason, studies have used salvage index as a research primary end-point when assessing different therapies and their potential to reduce reperfusion injury (167).

Cardiac MRI is a useful imaging modality that provides information on the infarct size and microvascular obstruction together with the salvage index. Although the degree of infarct due to reperfusion injury cannot be measured precisely the other measurements obtained can be used to give the impression of reperfusion injury and prognostic information (64).

1.4.5 Drawbacks of Cardiac MRI

There area number of strengths in the assessment of myocardial infarction and reperfusion injury using cardiac MRI, but there also a number of challenges that need to be considered as well. Performing a cardiac MRI can take one hour to acquire, and this is a time-consuming process that requires not only resources and access to a scanner but also needs cooperation from the patients and their time during the study. To get interpretable images the images need to be acquired during breath-holding for 10 seconds, and this is due to the effect that respiration has on the movement of the heart position in the thorax, and consequently the artefact that occurs if not performed adequately (176, 182). The patient undergoing the study needs to be able to perform multiple breath-holds during the acquisition of the images; otherwise the image quality may be too poor for analysis. This limits the patients who can have a cardiac MRI study performed, because if they struggle to breath-hold or have underlying respiratory diseases that restrict breath-holding, then an incomplete and uninterpretable scan may not be able to be analysed (183).

Having a permanent pacemaker as well as some other metal implants within the body are also contraindications to performing a cardiac MRI due to the interference of the magnet on these implants and potentially to pacemaker function. Another important contraindication to consider when performing a cardiac MRI is that of claustrophobia. The scan involves the patient being in a small-enclosed space for up to an hour, which some patients struggle to tolerate. Pennel et al, reported that 2% of patients suffered from significant claustrophobia during CMR image acquisition (176). This can potentially result in an incomplete scan and patients who not want a repeat scan if needed as part of the study protocol.

1.5 Arginine Vasopressin

Arginine Vasopressin (AVP) is a peptide hormone, consisting of nine amino acids, which is produced in the hypothalamus, in the paraventricular and supraoptic nucleus. AVP is then stored in the posterior pituitary gland or neurohypophysis (184). Vasopressin has two main functions, firstly to regulate water by retaining it to maintain serum osmolality within the normal range, and secondly acting as a vasoconstrictor (185). When the body is dehydrated, and there is a subsequent rise in plasma osmolality, receptors within the hypothalamus are activated, resulting in AVP being released from the posterior pituitary gland. AVP will then enter the systemic circulation after passing along the supraoptic - hypophyseal tract. AVP then acts on the kidney, resulting in increased water retention (185).

As part of a stress response, AVP is also released. Stress is defined as a nonspecific body response to any factor that disturbs homeostasis (186). A stress response results in neuro-hormonal activation, including sympathetic nervous activation, with a catecholamine release (185). AVP also contributes to the release of adrenocorticotropic (ACTH), which itself stimulates cortisol as part of the stress response (187). ACTH is a well-recognised stress hormone and has been measured in marked levels in a multitude of stressful situations, including pain, physical stress and neurogenic stress (188). As well as the catecholamine surge, AVP is also released to help sustain blood pressure during the periods when the body is under stress. Septicaemia, circulatory collapse and acute haemorrhage have shown to result in elevated AVP as part of a stress response (185, 189). There are also receptors in the left atrium and pulmonary arteries that can detect changes in intravascular volume. The receptors respond to pressure-induced stretch, and when activated results in an inhibitory effect on the release of AVP. Thus when the volume in the left atrium, and thus the pressure is low, the inhibitory response is suppressed, and AVP is released. The resulting retention of water increases intravascular volume to try to maintain the blood pressure (186, 188).

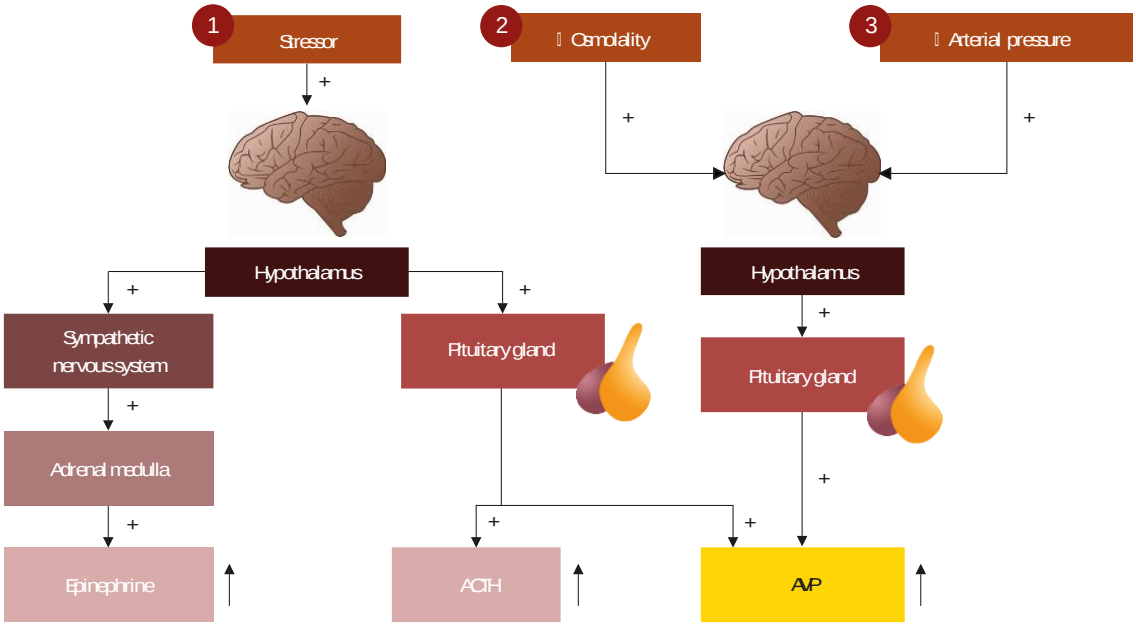

Figure 5: Illustration showing the three pathways to stimulate AVP from the posterior pituitary gland. (Arrow up represents an increase in the level; arrow down represents a decrease in the level; + represents positive stimulation)

1.5.1 Arginine vasopressin in the circulation

For body fluid homeostasis to be maintained, then AVP concentrations range between 1-5 pg/ml (190). At these levels, it is below the concentrations for AVP to have a vasoconstrictive effect. Studies in healthy subjects have shown that plasma levels of >50 pg/ml are needed to raise blood pressure with AVP's vasoconstrictive effect (190). AVP in normal circumstances has little impact on the maintenance of blood pressure (191). It can increase peripheral resistance, but the normal baro-receptor reflex would absorb the pressor effect of AVP (192, 193). In incidences where there is a failure of the autonomic nervous system or failure of the baroreceptor reflex (septic shock) then AVP has a more prominent role in BP regulation (193).

Plasma levels of AVP rise further in acutely unwell patients with levels of >500pg/ml seen in patients with significant haemorrhage, and in patients with cardiac arrest levels have been measured > 450pg/ml (190). In vasodilatory shock such as septic shock, a combination of AVP infusion with norepinephrine has been shown to

restore tone within the vasculature (194).

During a myocardial infarction, it has been noted that there is a rise in AVP levels (186). This may be as a consequence of a stress response to the myocardial infarction (186). It has also been noted in animal models that during a myocardial infarction, there is an increase in activity in AVP secreting neurons (195). Coronary vasoconstriction has been shown to occur when serum AVP has been measured at levels between 10 and 1000pg/ml (76).

1.5.2 Arginine vasopressin and coronary vasculature in myocardial infarction

With elevated levels of AVP in patients with an acute myocardial infarction, the systemic benefits to maintain BP homeostasis may be negated by the subsequent impacts of AVP on vasoconstriction of the coronary circulation (76).

Elevated circulatory AVP has been shown in animal models to be associated with increased adverse effects in animal models. In one study where vasopressin boluses of 0.8nmol were given to canines, there was noted a fall in stroke volume and further impairment of left ventricular function by 18%. It was felt that coronary vasoconstriction in small non-diseased arteries was a contributing factor to the negative inotropic effect from the resulting AVP release (74). The vasoconstrictive effect has been noted to be more prominent on coronary microvasculature than the larger epicardial arteries. It is felt that this has the potential to exacerbate microvascular dysfunction and as a consequence, infarct size to be larger (74, 75). The vasoconstrictive effect of AVP is potentiated in subjects who have an acute stressor as opposed to subjects who are under normal conditions (189).

In mice studies where low dose vasopressin (the equivalent of 0.04 units/min in a 70kg man) was administered after a myocardial infarction and subsequent reperfusion, the mice who received the vasopressin displayed poorer cardiac function and an increase in mortality. Mortality nearly doubled at day 7 (196). This poses the concern that elevated levels of AVP in patients suffering from an acute myocardial infarction may result in an increase in microvascular dysfunction, and as

a consequence, more microvascular obstruction.

There have been other studies that have suggested that AVP may have a vasodilator effect on the coronary circulation. A study on pigs, which involved injecting AVP directly into the coronary arteries, showed a marked increase in vessel diameter (197). A study by Dusner et al, concluded that low dose AVP increased myocardial blood flow as a consequence of coronary vasodilation and increased perfusion pressure (194).

It has been recognised that the degree of coronary vasoconstriction increases with higher doses of AVP and at low doses in some studies has shown to have a positive inotropic effect (198). This combination of vasoconstriction and vasodilatation may be explained by the different receptors that AVP works on.

1.5.3 Arginine Vasopressin Receptors

There are a number of AVP receptors: receptor AVPR1a, receptor AVPR2, and AVPR3 (also known as AVPR1b). The AVPR1a receptor can be found in high abundance in the heart and is also located in vascular smooth muscle. Activation on AVPR1a is associated with vasoconstriction. Stimulation of AVPR2 in the tubules and collecting ducts of the kidney results in the retention of water. AVPR3 receptor activation, in the anterior pituitary, facilitates the release of ACTH as part of the stress response (199). AVP also actions on oxytocin subtype receptors (OTR) in the vascular endothelium and P2 Purinergic receptors in the cardiac endothelium (200).

When AVP actions on AVPR1a in arterial smooth muscle cells it results in an increase in ionised calcium in the cytoplasm as well as increased intracellular calcium levels in cardiac myocytes and subsequent vasoconstriction (201). Aoyagi et al, studied AVPR1a deficient mice and found that the vasoconstrictive effect of AVP was absent, showing the role of AVPR1a in vasoconstriction (202).

AVPR1a is also expressed on human platelets and its possible that stimulation of these receptors together with up-regulation of AVPR1a during

ischaemia/myocardial infarction potentiates the effect of AVP on the coronary microcirculation during an ischaemic insult and thus more coronary vasoconstriction (196, 198).

Oxytocin Subtype Receptors in the vascular endothelium respond to AVP and oxytocin equally, resulting in vasodilation due to nitric oxide release (198). This may explain the combined vasodilatory and vasoconstrictive properties of AVP. The degree of expression and activation of the different receptors (AVPR1a/OTR) could explain the variation of response in different clinical scenarios and at different doses of AVP (198).

1.5.4 Copeptin

AVP is unstable in the blood stream and more than 90% of AVP bound to platelets. It has a very short half-life of 10-15 minutes and as a consequence can make accurate measuring difficult with over/under estimation (203, 204). AVP is cleaved from a pre-provasopressin, a precursor, in the hypothalamus before it is transported to the posterior pituitary gland (184, 205). Copeptin is a fragment of pre-provasopressin that is in equimolar quantities to AVP in circulation once it has been cleaved. As a consequence of this, copeptin can be used, as an equivalent biomarker to AVP, and the levels in circulation are reflective of AVP levels (203, 205). Copeptin is a more stable biomarker, even days after being sampled, and hence more reliable and accurate measurement than AVP (206). Copeptin has a half-life of 82 minutes in comparison to AVP (197). Copeptin has been found to be stable at room temperature for 7 days and when stored at -4 degrees Celsius stable for 14 days (204).

Copeptin concentrations are not affected by exogenous AVP therapy, therefore are a true reflection of endogenous AVP production, and again making the measurement more accurate than assays measuring AVP (204).

A normal copeptin level is 1 – 4.5 pmol/l. In conditions that AVP would be elevated, including haemorrhage and sepsis, copeptin levels are also markedly elevated. In myocardial infarction, copeptin levels have also been increased and previously been

considered as potentially a diagnostic biomarker (2, 204, 207). Elevated copeptin levels, in the context of an acute myocardial infarction, are associated with higher mortality and correlate with infarct size as well as left ventricular function (208, 209). In the study by Ananth et al, where copeptin levels were measured in patients with STEMI, who had reperfusion by PCI and by pharmacotherapy, the copeptin level was also higher in patients with prolonged symptoms prior to reperfusion, as well as those with extensive coronary artery disease (208).

Little information is available on the release pattern of vasopressin levels in acute myocardial infarction. A study by Slagman et al, looked at the release of copeptin in STEMI and non-STEMI patients, taking blood samples in the ambulance, on admission, and at a number of time points after admission (2, 4, 6 and 12-36 hours). In the STEMI population the admission copeptin was the peak with a rapid decline after admission (median 70.0 pmol/l, IQR 22.0 – 144.8 pmol/l), and almost normalised by the 12 - 36 hour time point (210).

In the ZODIAC-31 study, an observational study, there was found to be an association with baseline copeptin and cardiovascular mortality and all-cause mortality in patients with type 2 diabetes mellitus (199). Other studies have looked at copeptin in patients with known coronary artery disease. In a study by Haehling et al, copeptin levels were measured in patients with symptomatic coronary artery disease, including stable angina and those with acute myocardial infarction. This study again showed that elevated copeptin levels are associated with a higher all-cause mortality, as well as an increased rate of recurrent myocardial infarction, and ischaemic stroke at three months (211).

Previous studies have shown an association with copeptin levels, taken 2 days after a STEMI, with larger infarct size determined by CMR as well as related to a higher risk of adverse remodelling (212). In the study by Reinstadler et al, the copeptin samples taken at 2 days after a STEMI showed a significant and inverse correlation with ejection fraction on CMR at admission and at 4 month follow up. The median copeptin level was 10.4 pmol/l, and it was noted that copeptin levels above the median were associated with larger infarcts, lower ejection fraction, and larger end systolic volumes (212). In the same study, 6 out of the 47 patients had features of adverse remodelling on CMR, with an increase in end diastolic volume of $\geq$ 20%

used as a surrogate for adverse remodelling. Higher copeptin levels were associated with adverse remodelling.

With copeptin being more stable, and measurements more accurate, it is a more favourable surrogate biomarker to AVP.

Chapter 2

2. Aims and Hypotheses

2.1 Overall Aim

The aim of this study is to investigate the role of vasopressin on microvascular dysfunction in patients admitted with acute ST elevation myocardial infarction. The second objective is to understand the trend of vasopressin release in myocardial infarction.

2.2 Hypotheses

1) Circulating Vasopressin is elevated in myocardial infarction and contributes to microvascular dysfunction
2) Elevated Vasopressin in patients with myocardial infarction results in higher MVO and infarct size

2.3 Objectives

1) Evaluate blood levels of AVP in STEMI patients over the course of the myocardial infarction and during reperfusion

2) Analyse infarct size, left ventricular dimensions, ventricular remodelling and MVO by MRI within 1 week of infarct and repeated at 12 weeks

3) Measure the IMR in a culprit coronary artery vessel in patients with STEMI as a maker of microvascular function, and correlate with vasopressin levels.

4) Evaluate blood levels of AVP in elective patients undergoing therapeutic septal artery ablation to ascertain how vasopressin is influenced by onset of ischaemia.

5) To measure ACTH and Epinepherine, as markers of a stress response, to correlate with vasopressin in patients who are having a STEMI.

Chapter 3

3. Methods

There are two studies to allow the objectives and aims to be addressed. For the first part of the study, the cohorts of patients were recruited into the CAPRI clinical trial. The second group of participants were recruited from the VASOPRESSIN study.

A formal induction was undertaken at both laboratory sites and all work was performed in accordance with Control of Substances to Health regulations.

3.1 CAPRI trial

The CAPRI ("Evaluating the effectiveness of intravenous ciclosporin on reducing reperfusion injury in patients undergoing primary percutaneous coronary intervention") trial was a single centre randomized, double-blinded controlled trial of ciclosporin vs placebo (saline) in patients presenting with a STEMI.

The trial had ethical approval passed through the National Research Ethics Committee (REC Reference 14/NE/1070). The trial was managed through the Newcastle Clinical trials Unit in correlation with the independent Trial Steering Committee.

Patients were recruited between March 2015 and December 2016 at the Freeman Hospital, Newcastle upon Tyne.

3.1.1 Study Population and recruitment

All the patients recruited presented to the Freeman Hospital with an acute STEMI and underwent primary PCI.

The inclusion criteria:

a) Patient above 18 years of age
b) Patient suitable for PPCI for treatment of STEMI
c) Onset of symptoms less than 6 hours

d) ST-segment elevation on ECG suggestive of STEMI (1mm ST elevation in concurrent limb leads, or 2mm in concurrent chest leads)
e) Culprit artery measuring at least 3mm in diameter with TIMI flow grade of 0 or 1 at the time of admission
f) Capacity to provide consent

The exclusion criteria:

a) Haemodynamic Compromise
b) Comorbidity (neoplastic pathology, inflammatory conditions, immunological dysfunction)
c) Contraindication to CMR
d) Previous Myocardial Infarction
e) Collateral coronary supply to the territory supplied by the culprit artery,
f) Cardiogenic shock
g) Unconscious patients
h) Uncontrolled Hypertension (>180/110mmHg)
i) Pregnant women, or those of childbearing age

Once a patient was identified according to the eligibility criteria, the consultant cardiologist performing the PCI obtained verbal consent in the presence of an independent witness in the cardiac catheterisation lab. The patient was then allocated a patient ID number as part of the CAPRI study, which was used to label the samples taken, and ensure anonymity.

3.1.2 Experimental protocol

Arterial blood samples were drawn from the guide catheter sited in the coronary artery at the following time points: Baseline (prior to PCI, and referred to as PRE from now on), then after reperfusion was sustained, at 5 minutes, 15 minutes, 30 minutes and 90 minutes. Venous blood samples were drawn at 24-48 hours post PCI and after 2 weeks. Reperfusion was determined when blood flow down the coronary artery was TIMI flow grade 2 or 3. Blood taken with a 20 ml syringe was decanted, using a BD Vacutainer Blood transfer device into 1 x 10ml BD Vacutainer

K2EDTA tube, 1 x 4ml BD Vacutainer K2EDTA tube and 2 x 5 ml BD Vacutainer SST II Advance Tube.

3.1.3 Blood samples

Blood samples were then transferred to the pathology lab at the Freeman Hospital. The 10ml BD Vacutainer K2EDTA samples were spun at 1000g for 15 minutes at 4 degrees Celsius. The plasma was then pipetted into a 15ml Falcon tube and stored in BD Biosciences transport bag along side ice packs.

One of the SST II Advance tube samples (serum) at each time point was left with the pathology department at the Freeman Hospital, where serum osmolality was measured as well as blood urea (mmol/l), serum creatinine (μmol/l) and troponin (ng/l).

Samples were then transferred to the Institute of Genetic Medicine (IGM) situated at the Centre for Life, Newcastle upon Tyne. On arrival the 4.0ml K2EDTA BD Vacutainer was placed onto a Stuart roller mixer SRT6. This was to ensure there was sufficient mixing of the samples.

The remaining serum sample (SST II Advance tube) is then centrifuged at the IGM at 1000g for 10mins at 15 degrees Celsius. The serum is then aliquoted into 15ml Falcon tubes.

220μl of plasma was then aliquoted, into individual 1000μl cryotubes. The serum samples were aliquoted as well. The cryotubes of plasma and serum were decanted for each individual time point. The samples are then stored in a freezer at the IGM at -80 degrees Celsius.

3.1.4 Hormone levels

Hormone concentrations were quantified by enzyme-linked immunosorbent assay (ELISA) on plasma samples: AVP (CUSABIO catalogue no. CSB-E09080h), ACTH (CALBIOTECH catalogue no. AC018T), and epinephrine (CUSABIO catalogue no. CSB-E08677h). ELISAs were done according to the manufacturers' instruction.

3.1.5 Copeptin

The copeptin levels were measured from the serum samples stored. The measurements were performed at the Blood Sciences Laboratory, at the Royal Victoria Infirmary, in Newcastle upon Tyne. 200μl of serum was used for the immunofluorescent assay. Brahms copeptin proAVP kit was used on a Kryptor Compact Plus automated system. The sandwich immunocomplex method was used on the on the assay, consisting of cryptate-conjugated anti-copeptin and XL-655-conjugated anti-copeptin. The signal, proportional to the level of copeptin, was determined with a nitrogen laser at 707nm.

3.1.6 Cardiac Magnetic Resonance Imaging (CMR)

The patients underwent 2 CMR scans, the first between 2-7 days post PCI, and the second 12 weeks post PCI (+/- 2 weeks). This was performed at the Freeman Hospital using a Siemens Avanto 1.5 Tesla MRI machine. All of the images were acquired during breath holding. Axial black blood HASTE images were obtained to define the anatomy as well as localizer images.

Cine images, moving images of the heart, are acquired using a steady-state free precession (SSFP) sequence. Cines of 2 chamber, 3 chamber and 4 chamber views are obtained. The repetition time was set in relation to the heart rate, the image matrix 144x192, and echo time at 1.19ms, with flip angle at 80^{o}.

The short inversion time inversion recovery (STIR) images are T2 weighted images acquired using a black blood segmented turbo spin echo technique. The images taken in the same views as the cine images. The repetition time was again set according to the heart rate with an echo time of 47ms and flip angle at 180 ^{o}
The Inversion was at 140ms and image matrix at 208 x 256.

More STIR images are then taken in end diastole in a short axis view, taking parallel slices (6mm slices with a 2mm gap between), which incorporate the entire left ventricle. Also cine views are also obtained using SSFP in the same short axis plane. This allows for the measurements of the ventricles and an assessment of the size, mass, and function.

The paramagnetic contrast agent used to assess for infarction size was Gadovist (gadolinium based contrast agent), which is administered intravenously at a dose of 0.1 mmol/kg. 10 minutes after the gadolinium Is given, short axis end diastolic late gadolinium enhancement images were obtained that reflect the same slices as the cine images and STIR images already obtained. The LGE images are acquired using an inversion recovery segmented echo sequence. The repetition time is set according to the heart rate with a flip angle of 25^{o}. The echo time was set at 3.41ms and the image matrix at 196 x 256. The inversion time was adjusted to null the normal myocardium and make it appear dark. The inversion time continues to be adjusted to ensure that the nulling is maintained.

The MRI scans were anonymised and analysed using CVi42 (MRI analysis software by Circle Cardiovascular Imaging). I performed the analysis of the MRI scans after undergoing a period of training in cardiac MRI analysis and using the CVI42 software package. The MRI scans were uploaded to CVi42 and the analysis performed in bundles. This was to reduce the potential for bias, and ensure anonymity was maintained.

The cine images were used to measure the left ventricular dimensions as well as the ejection fraction. The 2 chamber and 4 chamber views were used to ensure the longitudinal length of the left ventricle was accurately ascertained. The short axis stack view of the ventricle incorporating the calculated length of the ventricle is then

used to make the measurements. At the end of systole and diastole each left ventricle slice has the endocardial and epicardial borders mapped (213). The volumetric analysis was performed, measuring left ventricular size in systole and diastole, as well as left ventricular ejection fraction (LVEF) and stroke volume (SV).

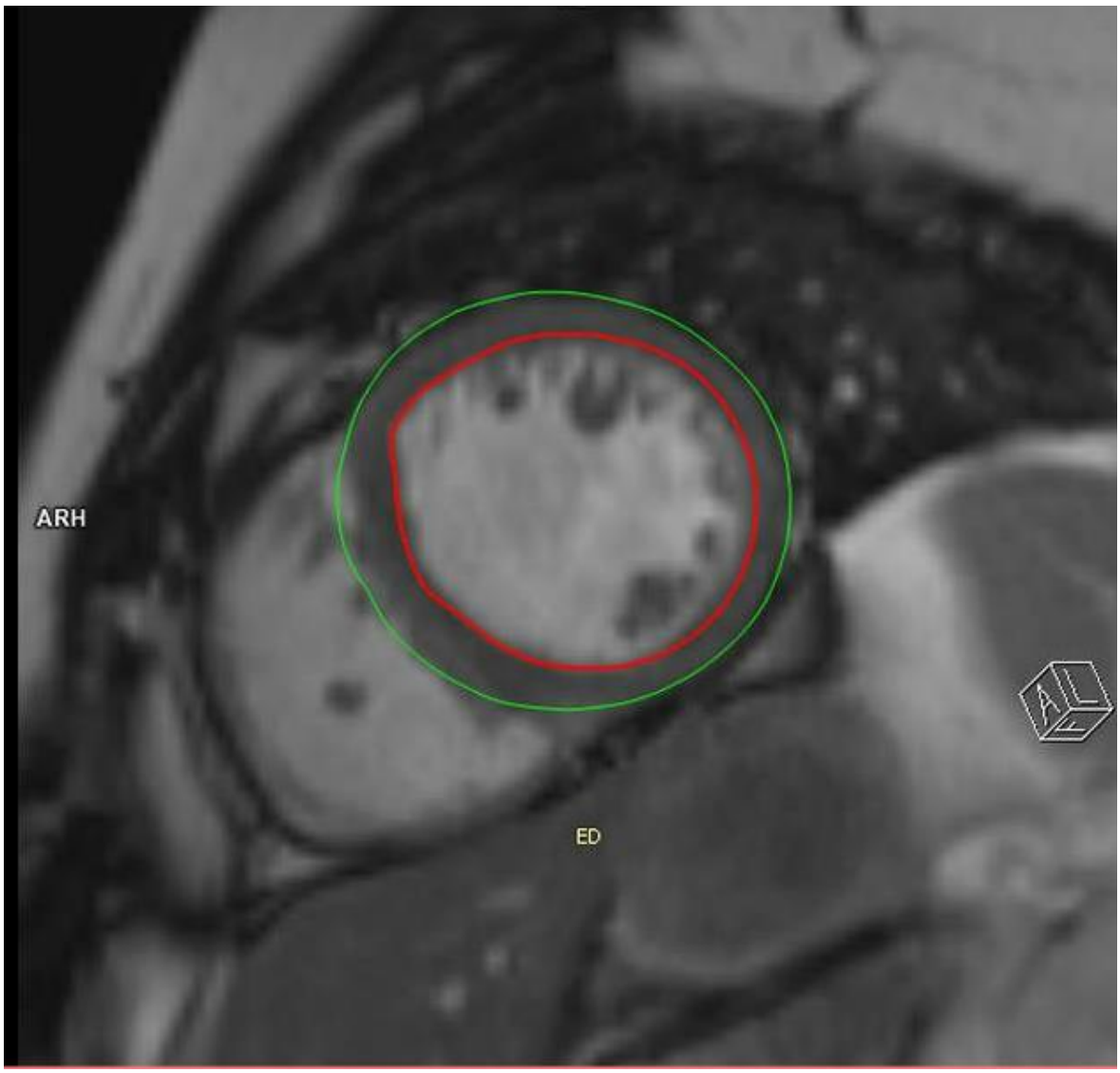

Figure 6: LV dimensions and function assessment with Cardiac MRI. Short axis images of the left ventricle are taken in end-systole and end diastole. The endocardial border is mapped (red border) and the epicardial border is mapped (green border).

In order to ascertain and measure the infarct size the short axis late gadolinium images were used. The borders of the epicardium and endocardium mapped out to isolate the myocardium and an area of nulled normal myocardium is identified. The

software package then automatically identifies areas of gadolinium enhancement that is more than 5 standard deviations in signal intensity of that of the nulled normal myocardium. This method of measuring the infarct size has been validated in other studies (214). The software quantifies the infarct size in mass and volume. Microvascular obstruction, identified as a central dark core within the infarct area, can also be measured by mapping out the borders of it. This measurement is then added to total infarct size.

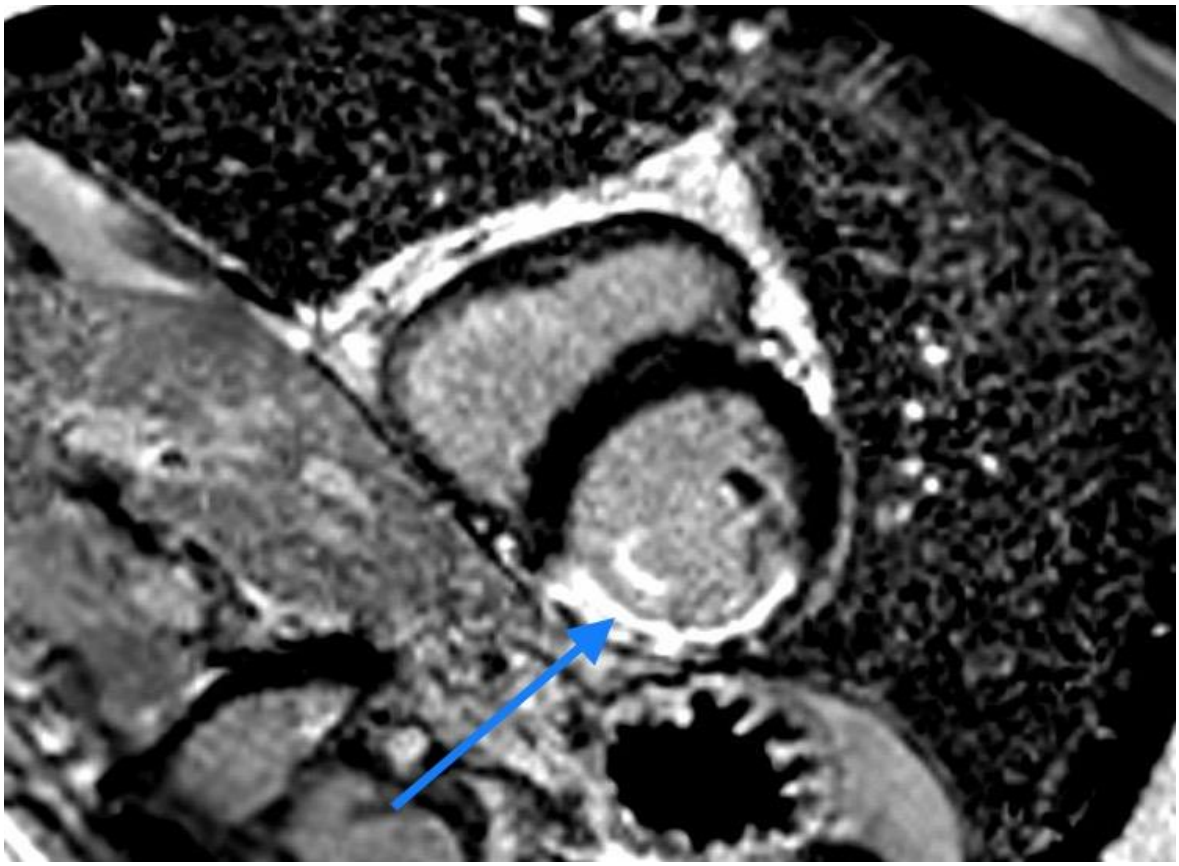

Figure 7: Late gadolinium enhanced images of a short axis view. The blue arrow showing the hyper-enhanced white infarct zone next to the dark normal myocardium.

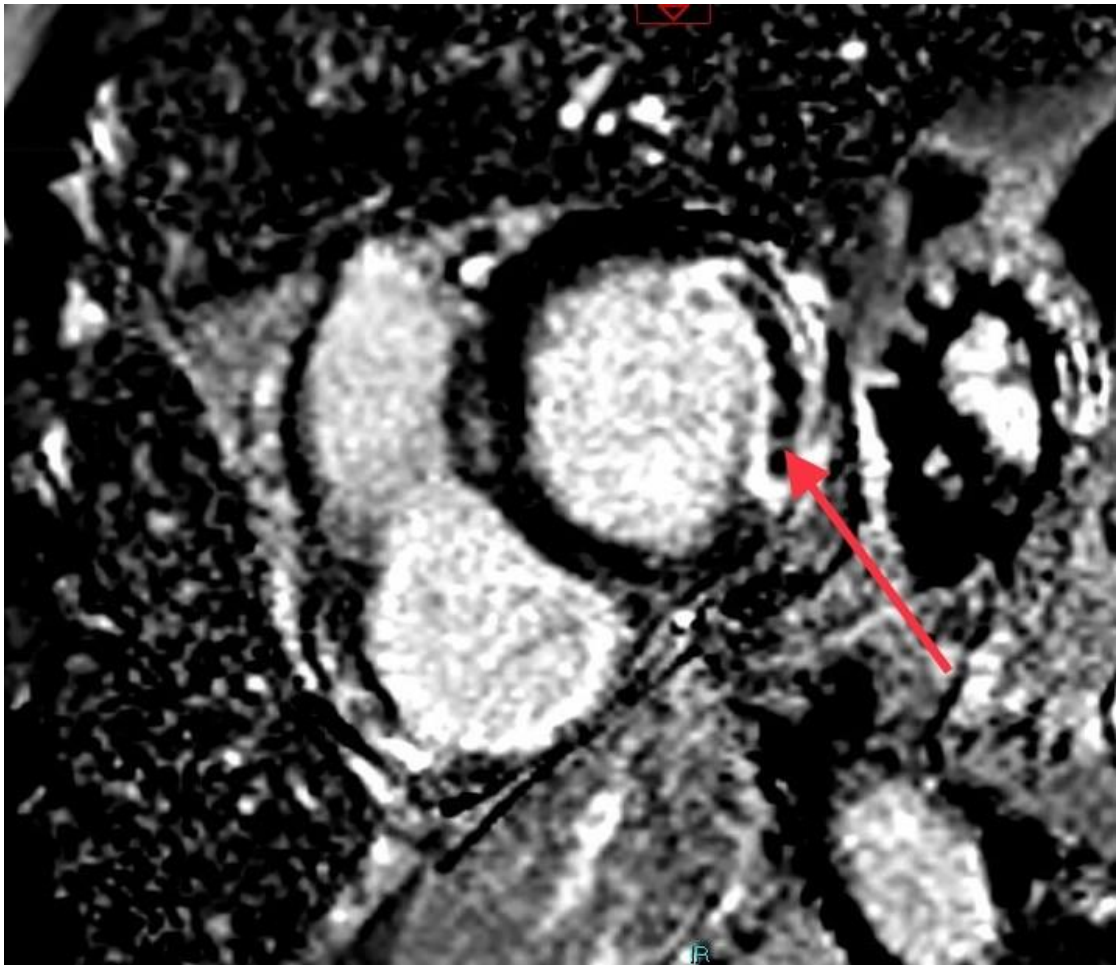

Figure 8: Late gadolinium enhanced imaging in a short axis view. The red arrow, highlighting the dark core of microvascular obstruction within the hyper-enhanced infarct zone.

To measure the area at risk and consequently calculate the salvage index the T2-weighted images were used. The borders of the myocardium again were traced out and as with the measurement of infarct size, an area of normal myocardium is identified. The area at risk is highlighted as an area that has signal intensity 2 standard deviations above the normal referenced myocardium (215). The myocardial salvage is quantified by subtracting the infarct size from the area at risk. The myocardial salvage index is calculated by diving the salvage index by the area at risk.

The qualities of the STIR images, used for quantifying the salvage index, were substandard and due to artefact, the AAR could not be identified in many of the infarcts, especially those that were non-anterior. In anterior infarcts the software package was a lot better in identifying the area at risk, visually a hyper-enhanced

area that surrounded the infarct zone on the equivalent late gadolinium enhancement short axis images. When the infarct was non-anterior the analysis would often pick up very remote areas of the myocardium and pick up the anterior wall as the area at risk. Due to the uninterpretable data, I chose not to pursue the ongoing measurements and assessment of myocardial salvage.

3.2. VASOPRESSIN Study

This was a single centre trial in patients presenting to the Freeman Hospital. The trial had ethical approval passed through the National Research Ethics Committee (REC 16/NE/0405) and underwent HRA approval.

I was involved in the completion of the IRAS forms and liaising with several different disciplines, including medical physics, to obtain the information requested by the ethics committee. This process took approximately 5 months.

3.2.1 Study Population and Recruitment

There are two patient cohorts in this element of the study; patients admitted for PPCI with a STEMI, and elective admissions for therapeutic alcohol septal ablation (TASH) in patients with hypertrophic obstructive cardiomyopathy.

The inclusion criteria for patients admitted for PPCI with STEMI to be enrolled in the study are:

a) Patients presenting with acute myocardial infarction (STEMI) and undergoing Primary PCI
b) ST-segment elevation on ECG suggestive of STEMI (1mm ST elevation in concurrent limb leads, or 2mm in concurrent chest leads)

c) Age above 18 years
d) Not involved in any other research projects
e) Able to understand and read English
f) Willing and able to provide written consent

The exclusion criteria:

a) Clinically unstable patients (Haemodynamically unstable, shock, unconscious patients)

b) Previous Myocardial Infarction
c) Patient lacks capacity
d) Collateral blood supply to the infarct territory
e) Sensitivity to Adenosine
f) Brittle Asthma

For the patients in the TASH arm of the study group the inclusion criteria are:

a) Patients presenting with hypertrophic obstructive cardiomyopathy, attending for elective alcohol septal ablation
b) Age above 18 years
c) Not involved in any other research projects
d) Able to understand and read English
e) Willing and able to provide informed consent

The exclusion criteria are:

a) Clinically unstable patients (Haemodynamically unstable, shock, unconscious patients)
b) Patient lacks capacity

For the patients presenting with a STEMI, the consultant cardiologist responsible for the procedure will obtain verbal consent from the patient, observed by an independent witness. The patient was then allocated a patient ID number as part of the VASOPRESSIN study, which was used to label the samples taken, and ensure anonymity. Once on the ward, and after the procedure I performed a formal written consent process. A copy was given to the patient along with the patient information sheet, and a copy also filed in the notes.

3.2.2 Experimental Protocol - STEMI Population

Arterial blood samples were drawn from the guide catheter sited in the coronary artery at the following time points: Baseline (prior to PCI, and referred to as PRE from now on), then after reperfusion was sustained, at 10 minutes, 20 minutes, 30

minutes, 40 minutes, 50 minutes, 60 minutes, 75 minutes, 90 minutes, 120 minutes and 180 minutes. Venous blood samples were drawn at 24-48 hours post PCI. Reperfusion was determined when blood flow down the coronary artery was TIMI flow grade 2 or 3. Blood taken with a 20 ml syringe was decanted, using a BD Vacutainer Blood transfer device into 1 x 10ml BD Vacutainer K2EDTA tube, 2 x 4ml BD Vacutainer K2EDTA tube and 2 x 5 ml BD Vacutainer SST II Advance Tube.

3.2.3 Blood samples

Blood samples were then transferred to the pathology lab at the Freeman Hospital. The 10ml BD Vacutainer K2EDTA samples were spun at 1000g for 15 minutes at 4 degrees Celsius. The plasma was then pipetted into a 15ml Falcon tube and stored in BD Biosciences transport bag alongside ice packs.

One of the SST II Advance tube samples (serum) at each time point was left with the pathology department at the Freeman Hospital, where serum osmolality was measured as well as blood urea (mmol/l), serum creatinine (μmol/l) and troponin.

Samples were then transferred to the Institute of Genetic Medicine (IGM) situated at the Centre for Life, Newcastle upon Tyne. On arrival the 4.0ml K2EDTA BD Vacutainer was placed onto a Stuart roller mixer SRT6. This was to ensure there was sufficient mixing of the samples.

The remaining serum sample (SST II Advance tube) is then centrifuged at the IGM at 1000g for 10mins at 15 degrees Celsius. The serum is then aliquoted into 15ml Falcon tubes.

220μl of plasma was then aliquoted, into individual 1000μl cryotubes. The serum samples were aliquoted as well. The cryotubes of plasma and serum were decanted for each individual time point. The samples are then stored in a freezer at the IGM at -80 degrees Celsius.

3.2.4 Copeptin

The copeptin levels were measured from the serum samples stored. The measurements were performed at the Blood Sciences Laboratory, at the Royal Victoria Infirmary, in Newcastle upon Tyne. 200μl of serum was used for the immunofluorescent assay. Brahms copeptin proAVP kit was used on a Kryptor Compact Plus automated system. The sandwich immunocomplex method was used on the on the assay, consisting of cryptate-conjugated anti-copeptin and XL-655-conjugated anti-copeptin. The signal, proportional to the level of copeptin, was determined with a nitrogen laser at 707nm.

3.2.5 Invasive coronary physiology Assessment

The lead operator, as per standard practice, performed primary PCI, which could involve the use of thrombectomy aspiration catheter to aspirate clot as well as intravenous glycoprotein IIa/IIIb inhibitors.

For the study a 0.014-inch pressure wire (St Jude Medical PressureWire Certus) was used together with the corresponding software and console (RadiAnalyzer Xpress by St Jude Medical). This pressure wire has a sensor 30mm from the tip of the wire, which allows for the measurement of pressure within the coronary artery. It also has a sensor located at the same site that measures temperature that is accurate up to 0.02^{o}C.

The pressure wire is calibrated outside of the patient's body by flushing the housing of the wire and leaving it flat for a minute prior to calibration. The wire is inserted into the guide catheter and the pressure/temperature sensor is placed at the ostium of the coronary artery and guide when it is then equalized. 200 micrograms of nitrate is injected intracoronary and flushed with normal saline, to minimize any arterial spasm. The wire was then carefully advanced into the distal third of the culprit artery.

The IMR is calculated by multiplying the thermo-dilution transit mean time (Tmn) at maximal hyperaemia by the distal coronary pressure (Pd). The operator would inject

3ml of room temperate saline, initially at baseline, ensuring the guide catheter is well engaged. Once this was done hyperaemia would be achieved by giving adenosine at 140micrograms/kg/min intravenously using an infusion pump through a venous cannula in the antecubital fossa. Adenosine activates receptors in the vascular smooth muscle and results in almost maximal coronary vasodilatation (216). The patient is observed for a physiological response to the adenosine as well as a symptomatic response. Once maximal hyperaemia was achieved then a further 3ml of saline would be injected a further three times and averaged to calculate the Tmn.

The serum and plasma samples were sent for assessment of copeptin levels as stated above.

Figure 9: Example of thermodilution curves at baseline and hyperaemia with Pa and Pd measurements and corresponding IMR and CFR

3.2.6 Experimental Protocol – TASH Population

Arterial blood samples were drawn from the guide catheter sited in the coronary artery at the following time points: Baseline (prior to wiring of the septal branch where alcohol planned to be administered), then after successful administration of

alcohol at 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, 75 minutes, 90 minutes, 120 minutes and 180 minutes. Venous blood samples were drawn at 24-48 hours post TASH. Blood taken with a 20 ml syringe was decanted, using a BD Vacutainer Blood transfer device into 1 x 10ml BD Vacutainer K2EDTA tube, 2 x 4ml BD Vacutainer K2EDTA tube and 2 x 5 ml BD Vacutainer SST II Advance Tube.

Blood samples were managed as stated above.

3.3 Statistical Analysis

SPSS (version 23; IBM) was used to perform the statistical analysis, and graphs were produced in GraphPad Prism (version 7). Shapiro-Wilk test was used to assess for normality and when it did not pass then non- parametric tests were used. Nonparametric Spearman correlation coefficient was used to assess for nonparametric correlations between parameters. Unmatched groups were compared using Mann-Whitney *U* test (two groups) or Kruskal-Wallis test with Dunn's multiple-comparisons test (three or more groups). Matched groups were compared using Friedman's test with Dunn's multiple-comparisons test. Data are expressed as mean ± SEM unless otherwise stated. A *P* value of less than 0.05 was considered significant.

Spearman test was used for multiple correlations due to the small sample size as well as this study was looking at factors known to impact on copeptin, microvascular dysfunction and infarct size. The hypothesis of this research was not to look at predictors of elevated or low copeptin.

3.4 My Involvement in the Study

I was heavily involved in the set up and ethical approval process for the Vasopressin arm of the trial. I screened all the patients for the study by liaising directly with the coronary care nurse triaging the STEMI patients. I was then involved in coordinating the timely blood sampling and supporting the clinician in performing the IMR

assessment. I then would go through the formal paperwork and consent with the patient, including arranging the MRI scans. I then would spin the blood at the Freeman site and transport the samples across to the laboratory at the Centre for Life where I would perform all the experiments required and freeze all the samples taken. For a substantial time during my research, I recruited out of hours and essentially was on call 24/7. This was due to competing STEMI trials that were being recruited for during normal working hours. I analysed all the MRI scans, both initial and at 3 months. There was no nurse support for this study.

I started the analysis of the MRI scans in March of 2016 whilst I was still working in programme as a cardiology SpR I then formally took time out of programme to start recruiting for the CAPRI arm of the trial in July 2016. I recruited for this study including 24/7 recruitment until December 2016 (then with 3 month follow up). Concurrently, the ethics process for VASOPRESSIN was undertaken and recruitment for this study started in May 2017 and continued until I returned into programme as an interventional trainee.

Chapter 4

4. Results

Statistical analysis was performed using IBM SPSS Statistics Version 24. Results were considered significant with p value of < 0.05. The following key is used in graphs to denote level of significance: ns – not significant, * (p-value <0.05), ** (p <0.01), *** (p<0.001)

4.1 Patient Population & Baseline Characteristics

A total of 72 patients were enrolled between the 2 patient cohorts, 52 from the first cohort and 20 from the second. All the patients recruited attended the Freeman Hospital, Newcastle with STEMI.

Of the 52 patients enrolled through the CAPRI study, 51 patients had an MRI within 7 days of admission with 49 patients having a follow up scan at 3 months. Copeptin levels were measured at all time planned time points in this cohort in 44 of the patients.

In the VASOPRESSIN cohort, out of the 20 patients recruited, 15 patients had successful calculation of the IMR. The unsuccessful assessments are described in the diagram below

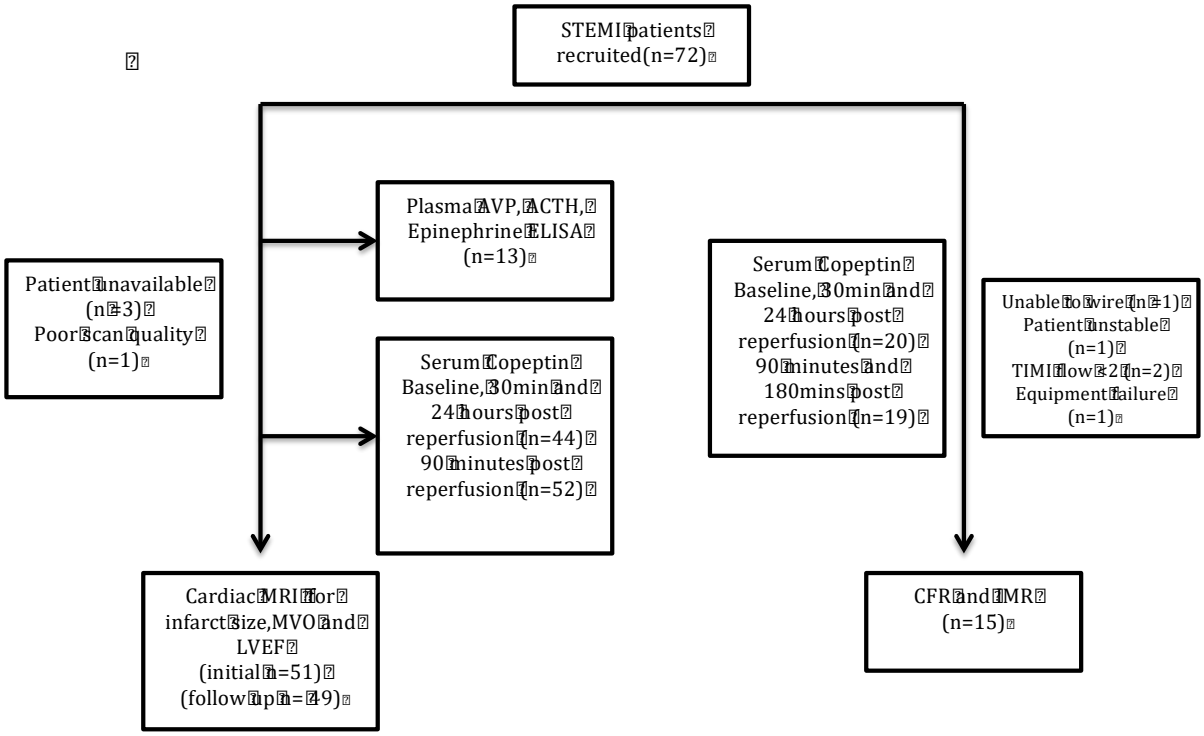

Figure 10: Consort diagram of patients enrolled into the two respective studies. CAPRI to left side of diagram and VASOPRESSIN to right side

The mean age of the participants was 63 years of age, of which 84.7% were male gender. With regards to conventional risk factors for coronary artery disease the mean BMI was 27.8 kg/m2, with the majority of patients being overweight. Although the North East of England has a higher density of the population of patients with an elevated BMI, this cohort's BMI is still lower than the national average, with a BMI of 29kg/m2 in men aged 55 to 64 across England (217). 25% of the patients enrolled had hypertension and 27.8% were active smokers. The Office for National statistics found that in 2018 14.7% of the UK population was active smokers (218).

Of the 72 participants in the study 29.2% of patients had an anterior STEMI. Previous studies have shown that the proportion of anterior STEMIs to be higher at 32.8% (219). The lower number of anterior STEMIs recruited can be explained by another study occurring at the centre that was specifically recruiting anterior STEMI patients.

	Data (n=72)
Age	63.1±10.6
Male Gender	61 (84.7)
BMI (kg/m2)	27.8±5.8
Diabetes Mellitus	6 (8.3)
Hypertension	18 (25)
Hypercholesterolaemia	6 (8.3)
Active Smoker	20 (27.8)
Anterior MI	21 (29.2)
Baseline Creatinine (μmol/l)	83.9±5.8
Peak Troponin (ng/l) **Normal reference 0 – 15 ng/l**	3776±2909
Pre PCI flow (TIMI 0/1/2/3)	60/10/1/1
Post PCI flow (TIMI 0/1/2/3)	1/3/1/67
Door to balloon time (minutes)	29.5±14.1
Onset to Reperfusion time (minutes)	190.6±92.8

Table 2 – Baseline characteristics of participants

4.2 AVP levels in circulation in Myocardial Infarction/Reperfusion

AVP levels at baseline are significantly elevated with a 14-fold increase when compared to normal circulating concentrations (70.3 ± 8.8pg/ml with normal levels at 1-5pg/ml). Plasma levels of AVP remained elevated after reperfusion but steadily declined (See figure 11) with levels significantly lower at 90mins post reperfusion (18.2 ± 4.0 pg/ml). At 24 hours post reperfusion the AVP were almost back to baseline but still elevated (7.5±1.2 pg/ml).

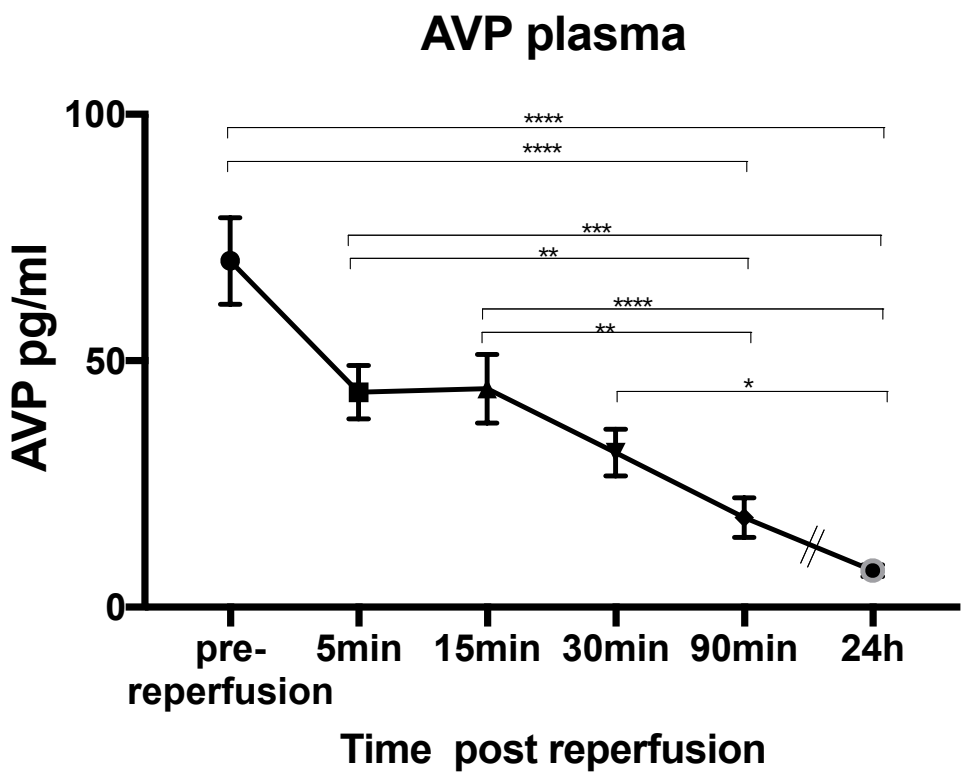

Figure 11: AVP concentrations in plasma prior to reperfusion and at various time points post reperfusion. Data is presented as mean ± SEM (n=13) p values were obtained from non-parametric ANOVA test (Friedman) with Dunn's multiple comparison test at α 0.05. * denotes $p < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.001$, and **** denotes $P < 0.0001$.

4.3 Copeptin levels during a Myocardial infarction and during Reperfusion

In STEMI patient's copeptin levels are markedly elevated prior to reperfusion at baseline (126.8 ± 13.94 pmol/l) with copeptin levels falling significantly by 90 minutes (86.15 ± 12.57 pmol/l) (p value for trend < 0.0001). Levels were at significantly lower levels at 24 hours (11.09 ± 1.63 pmol/l), and nearly back to baseline. A normal copeptin level is 1 – 4.5 pmol/l. In a subset of patients (n=18) a 180-minute post reperfusion time point was also taken. The drop in copeptin level from 90 minutes to 180 minutes was not significant in this cohort.

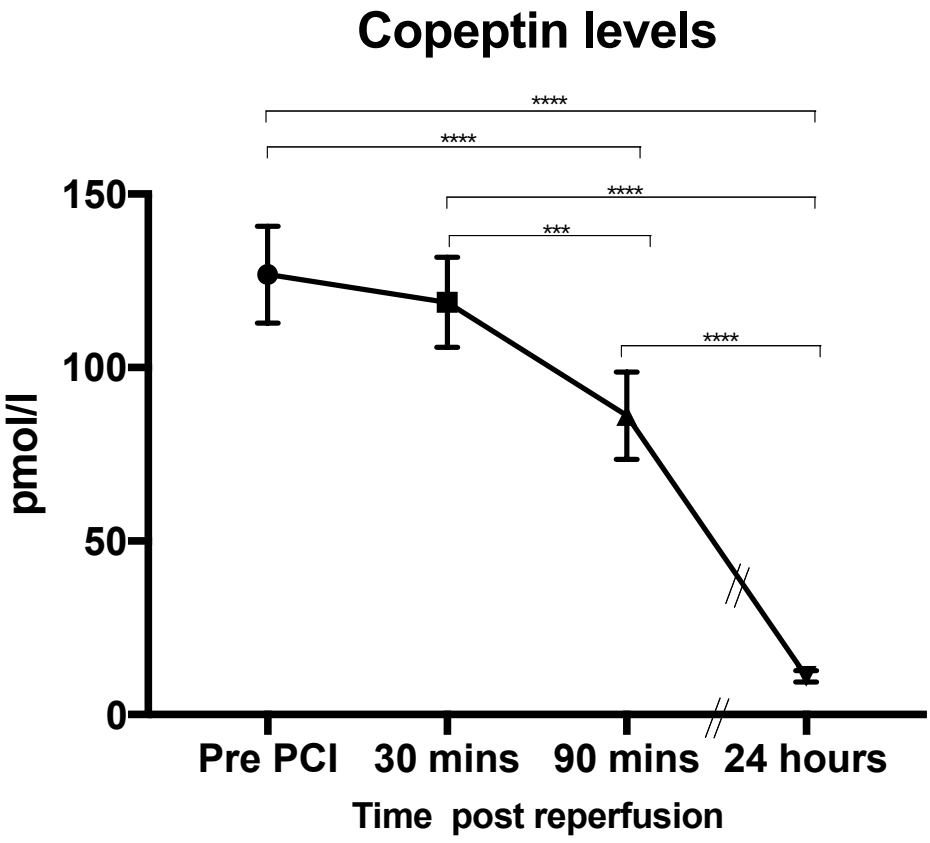

Figure 12. Copeptin levels in serum prior to and post-reperfusion in patients with STEMI. Data is presented as mean ± SEM (n=63). P values were obtained from non-parametric ANOVA test (Friedman) with Dunn's multiple comparison test at α 0.05. *** denotes P < 0.001, and **** denotes P < 0.0001.

4.4 Copeptin levels with baseline characteristics

Copeptin levels were higher in older patients at baseline, post reperfusion and remained higher after 24 hours ($p < 0.01$). There is also a strong correlation between peak troponin and 90minute copeptin level ($p < 0.001$), which is a surrogate for infarct size.

Body Mass Index did not have an impact on copeptin levels at any time point. The length of time between onset of symptoms and reperfusion, the ischaemia time, also did not correlate with copeptin levels and the trend post reperfusion. (Table 3)

	Copeptin Pre PCI		Copeptin at 90mins		Copeptin at 24 hours	
	r	P value	r	P value	r	P value
Age	0.33	0.007	0.348	0.003	0.319	0.01
BMI	-0.17	0.18	-0.52	0.667	0.097	0.446
Baseline Troponin	-0.145	0.254	0.13	0.282	0.267	0.033
Peak Troponin	0.307	0.014	0.405	<0.001	0.193	0.126
Ischaemic time	-0.271	0.03	0.028	0.186	0.039	0.759

Table 3: Correlation between copeptin and baseline characteristics. Correlation coefficient r and P values were obtained from nonparametric Spearman correlation analysis at α 0.05 (n = 72)

Patients who were active smokers showed significantly lower baseline copeptin level and a significantly lower copeptin level at 90- minute post reperfusion compared to those who had never smoked and ex-smokers.

Location of the infarct did not have an impact on the copeptin levels pre- and post-reperfusion.

The participants who did not achieve TIMI 3 flow post reperfusion as a consequence of slow-flow/no reflow phenomenon showed a trend towards a higher copeptin level at 90 minutes post reperfusion, with a significantly higher copeptin levels at 24 hours post reperfusion.

	Subgroup	Copeptin Pre PCI		Copeptin at 90mins		Copeptin at 24 hours	
		Mean ± SEM	P value	Mean ± SEM	P value	Mean ± SEM	P value
Sex	Male	128.19 ± 14.60	0.29	88.19 ± 11.96	0.07	6.35 ± 1.81	0.04
	Female	110.25 ± 41.42		73.98 ± 34.8		11.84 ± 1.87	
Smoking history	Never	140.28 ± 17.46	0.048	83.83±10.82	0.001	12.47 ± 2.60	0.070
	Ex-Smoker	147.91 ± 33.56		127.85 ± 27.57		13.41 ± 3.96	
	Current Smoker	82.48 ± 19.73		36.47 ± 6.47		6.51 ± 0.81	
Infarct Location	Anterior	123.39±17.95	0.587	78.44 ± 19.79	0.850	10.31 ± 3.18	0.284
	Non-Anterior	126.29 ± 17.95		89.16 ±13.93		11.28±1.87	
TIMI flow Post	3	122.52 ±14.25	0.30	80.43 ± 11.36	0.088	9.97 ± 1.53	0.047
	<3	168.33 ± 57.50		159.42 ±75.76		26.20 ±10.07	

Table 4 – Comparison of copeptin levels between patients according to patient characteristics. P values were obtained from Mann-Whitney test and Kruskal-Wallis test at α 0.05. (n = 72)

The admission blood pressure recorded when the patient was admitted significantly correlates with the baseline copeptin levels, with a lower blood pressure resulting in a higher copeptin level.

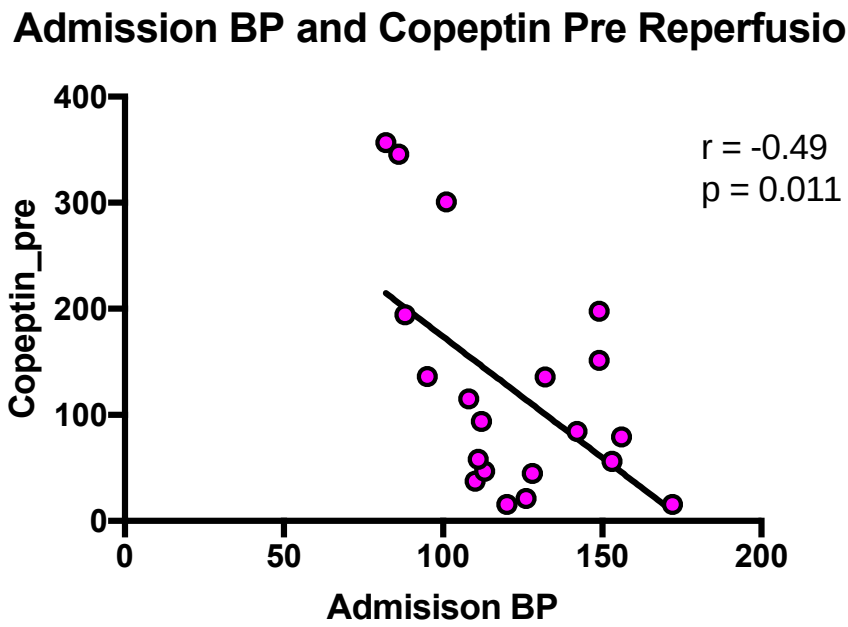

Figure 13: Correlation between copeptin and admission Blood Pressure (n = 20). Data is presented as a scatter plot with a line of best fit. Correlation coefficient r and p values were calculated from nonparametric Spearman correlation analysis at α 0.05. Simple linear regression was used for the line of best fit

When looking at the age of patients and the relationship with copeptin level. The cohort was split into three groups, less than 60 years of age, 60 – 70 years of age and more than 70 years of age. Between the age ranges there was no significant differences between the copeptin levels at any time point, with baseline copeptin measuring 101.8 ± 20.36 in < 60 group, 128.6 ± 25.88 ± 25.88 in 60 to 70 and 151.3 ± 23.45 in > 70 group showing the trend previously described, although no statistical difference between the two groups (Figure 14).

The trend is most marked between patients < 50 years old (n = 6) and those > 80 years old (n = 6). The copeptin levels were 58.37 ± 18.17 and 208.5 ± 36.46 respectively with a p value of 0.03.

There was no significant difference in the baseline characteristics between these two age brackets (Table 5).

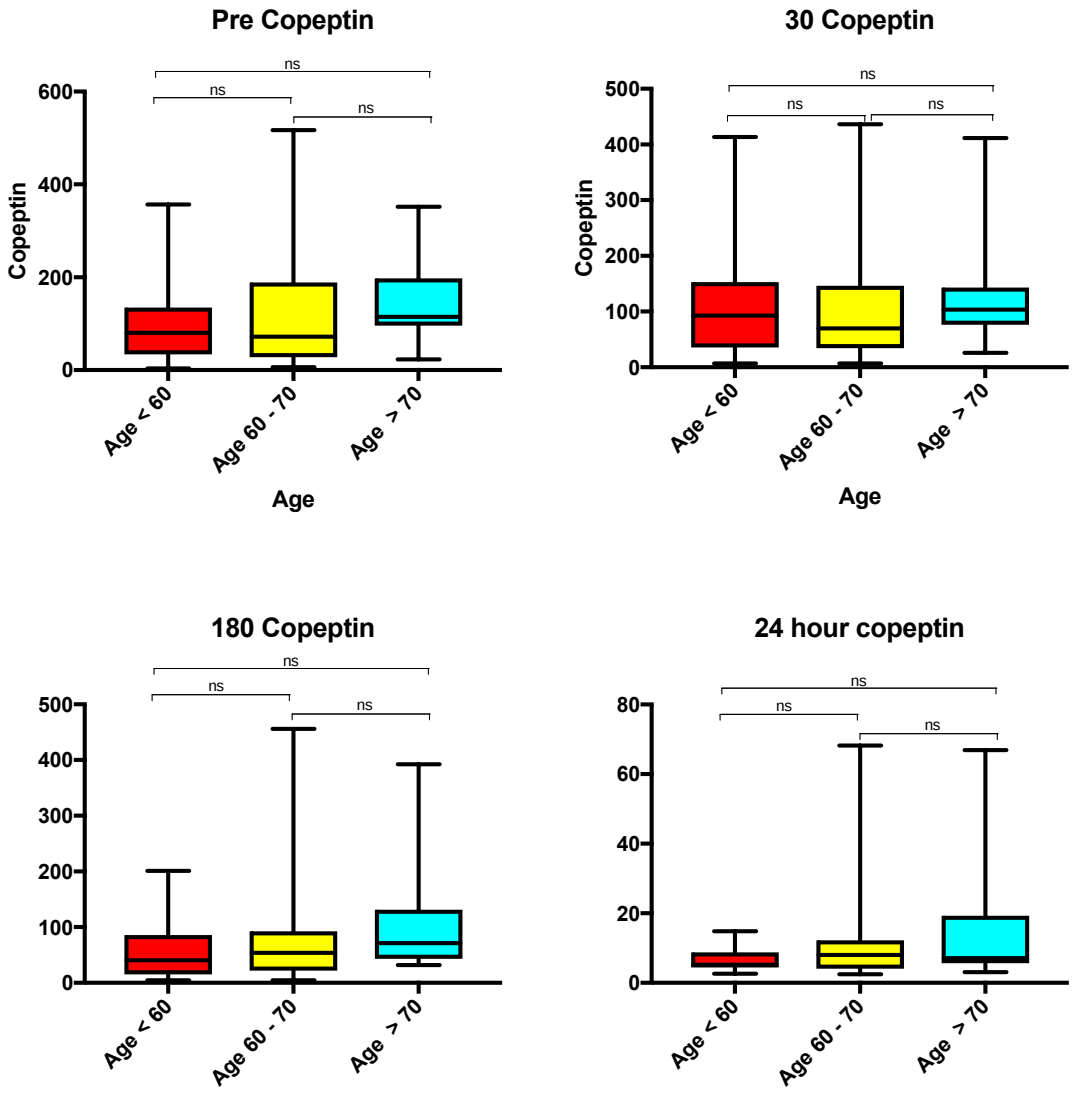

Figure.14: The relationship between copeptin levels and age in patients with STEMI. Copeptin concentrations in serum at multiple time points according to age (age < 60 n = 22, age 60 - 70 n = 25; age > 70 = 17). Data is presented as box (median, Kruskal-Wallis test at α 0.05. NS denotes not significant.

	Age < 50	Age > 80	p-value
n	6	6	
Age	45.5 ± 0.85	83.7 ± 1.67	**0.002**
Male sex	5 (83.5)	5 (83.5)	1.000
BMI	28.58 ± 2.44	25.10 ± 0.97	0.180
Diabetes Mellitus	0 (0)	1 (16.7)	1.000
Active Smoker	4 (66.7)	0 (0)	0.61
Hypertension	2 (33.3)	2 (33.3)	1.000
Hypercholesterolaemia	1 (16.7)	1 (16.7)	1.000
Anterior MI	1 (16.7)	1 (16.7)	1.000
Troponin T (ng/l)	2864 ± 882	4090 ± 1344	0.699
Treatment during PCI			
Door to balloon time (minutes)	30.83 ± 7.66	24.17 ± 3.75	0.699
Onset to reperfusion time (minutes)	146.7 ± 29.2	187.0 ± 30.45	0.310

Table 5: Baseline data for patients by age. Continuous variables expressed as mean ± SD and compared using Mann-Whitney U test. Categorical variables expressed as n (%) and compared using chi-square (χ^2) or Fisher's exact test as appropriate.

4.4 Stress Hormones during Myocardial Infarction and Reperfusion

Alongside Copeptin, Epinephrine and Adrenocorticotropic hormone (ACTH), two hormones associated with the stress response, were also measured (186). ACTH levels are normally 50pg/ml (220). ACTH levels were elevated on admission (191.4 ± 35pg/ml) and remained high after reperfusion initially. By 90 minutes post reperfusion the ACTH levels had dropped markedly and were back to normal levels by 24 hours. Epinephrine levels are normally less than 30pg/ml and again were markedly elevated on admission (80.4 ± 17.5pg/ml) (220). The decline in epinephrine levels was more gradual and at 24 hours still remained elevated compared to normal levels (38 ± 4.8pg/ml). (Figure 15).

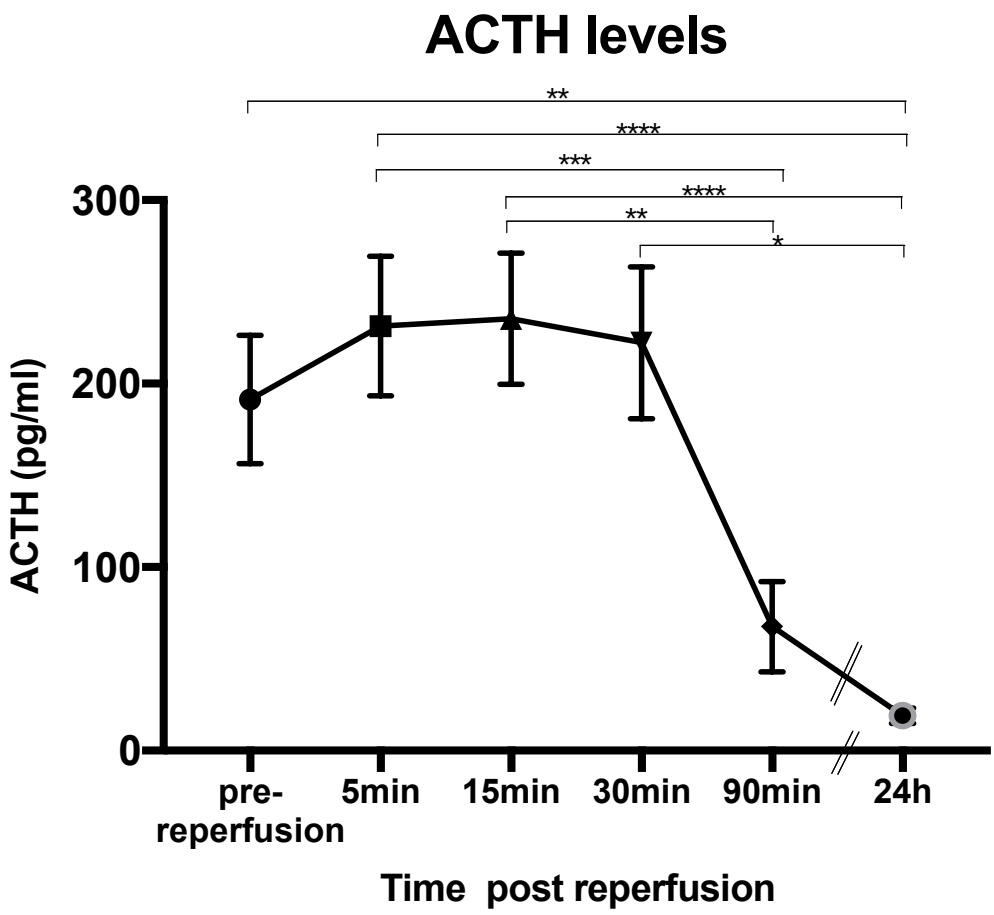

Figure 15. ACTH levels in plasma prior to and post-reperfusion in patients with STEMI. Data is presented as mean ± SEM (n = 13). P values were obtained from non-parametric ANOVA test (Friedman) with Dunn's multiple comparison test at α 0.05. * denotes P < 0.05, ** denotes P < 0.01, *** denotes P < 0.001, and ****

denotes P < 0.0001.

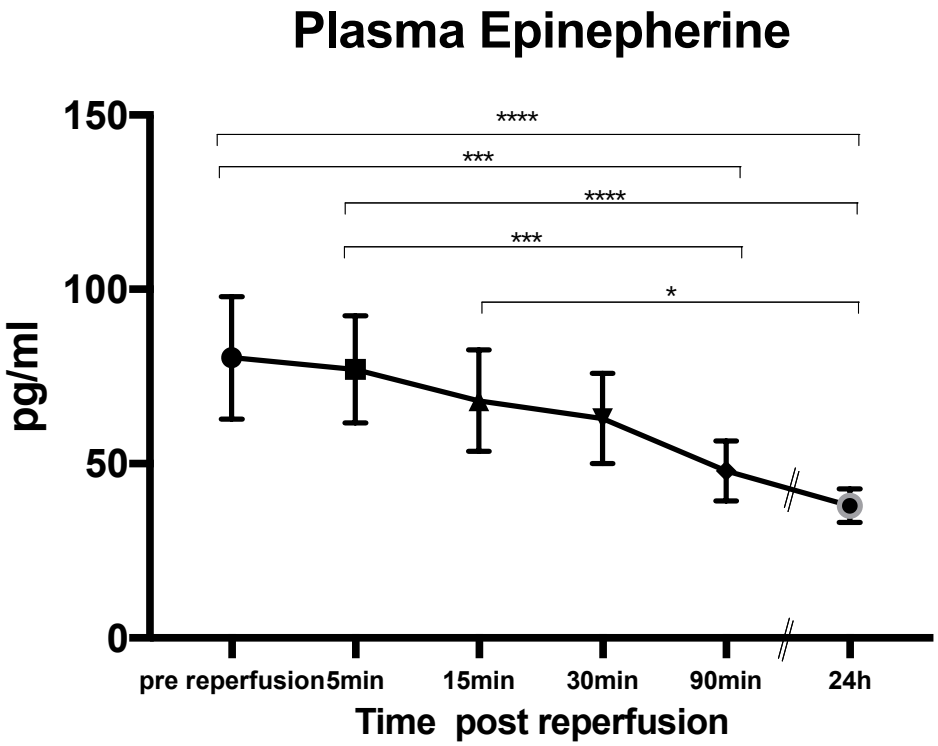

Figure 16. Epinephrine levels in plasma prior to and post-reperfusion in patients with STEMI. Data is presented as mean ± SEM (n = 13). P values were obtained from non-parametric ANOVA test (Friedman) with Dunn's multiple comparison test at α 0.05. * denotes P < 0.05, ** denotes P < 0.01, *** denotes P < 0.001, and **** denotes P < 0.0001.

4.5 Correlation between Copeptin and Stress Hormones

There was no significant correlation between copeptin and ACTH and copeptin and Epinephrine, apart from Copeptin and ACTH levels at 24 hours post reperfusion. The levels at baseline showed a tendency to positive correlation although not statistically significant.

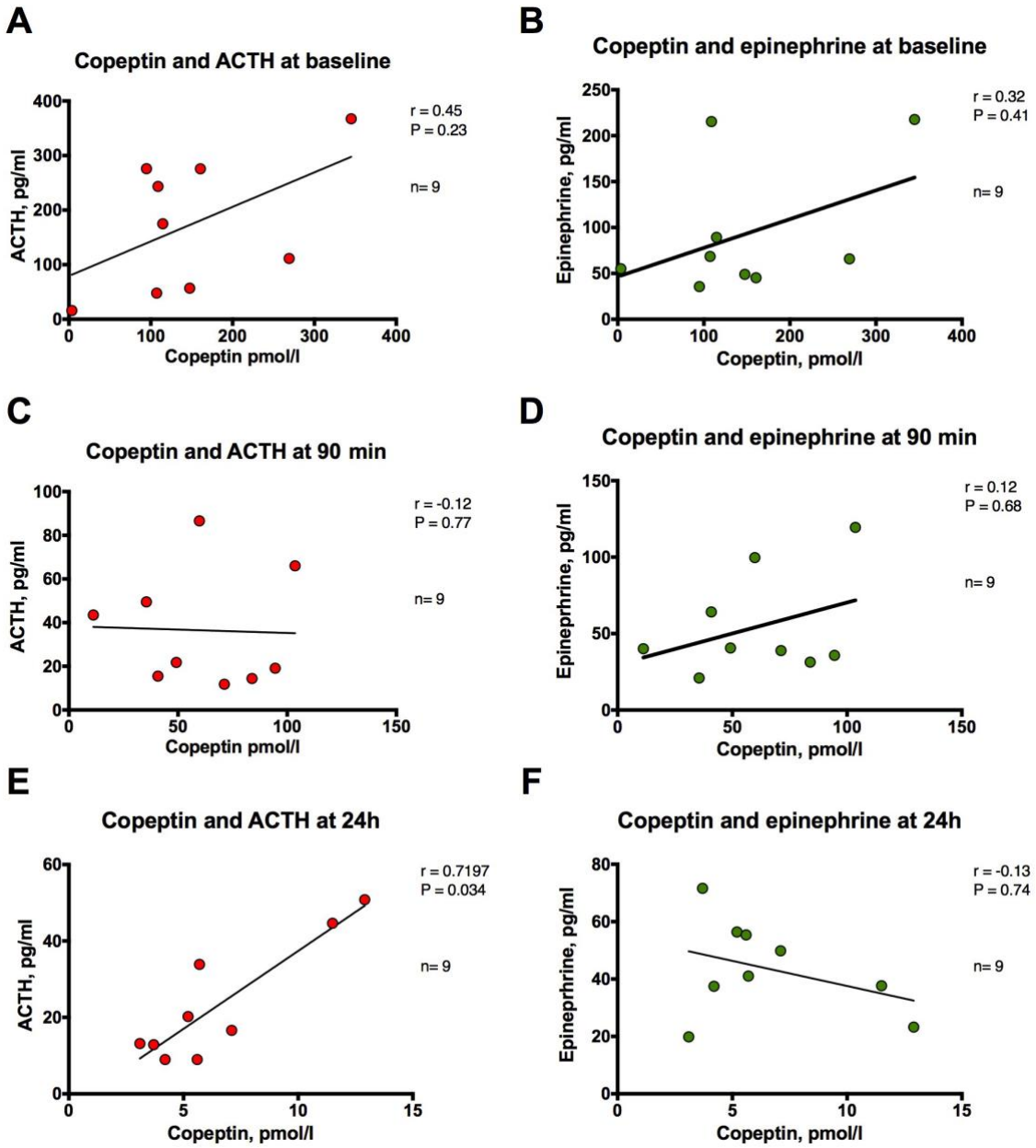

Figure 17: Correlation between copeptin and stress hormones. Data is presented as a scatter plot with a line of best fit. Correlation coefficient r and p values were calculated from nonparametric Spearman correlation analysis at α 0.05. Simple linear regression was used for the line of best fit (n = 9).

4.6 Copeptin levels and Index of Microvascular Resistance

Index of microvascular resistance was measured using a coronary pressure wire as described in the methodology. Out of the 20 patients enrolled into that arm of the trial IMR was not recorded in 5 patients. There was an equipment failure for one patient. There was one patient where the wire could not be sited successfully. Another patient became too unstable to tolerate the procedure. In 2 cases the TIMI flow was < 2 and the Tmn was too long for the software to calculate it.

An IMR > 25 is associated with microvascular dysfunction. IMR > 40 is associated with poor clinical outcomes after STEMI independently of infarction size. In the cohort the minimum IMR calculated was 10 with a maximum of 95. The mean IMR was 32.76 ± 5.617.

When IMR levels post PPCI was compared with copeptin levels there was no significant difference at any time point (Figure 18).

In patients who had a larger drop in copeptin from baseline to level at 90 minutes post reperfusion there appears to be a trend towards a lower IMR, but not statistically significant.

Peak troponin and IMR did not correlate and was not significant in this dataset as well (Figure 19)

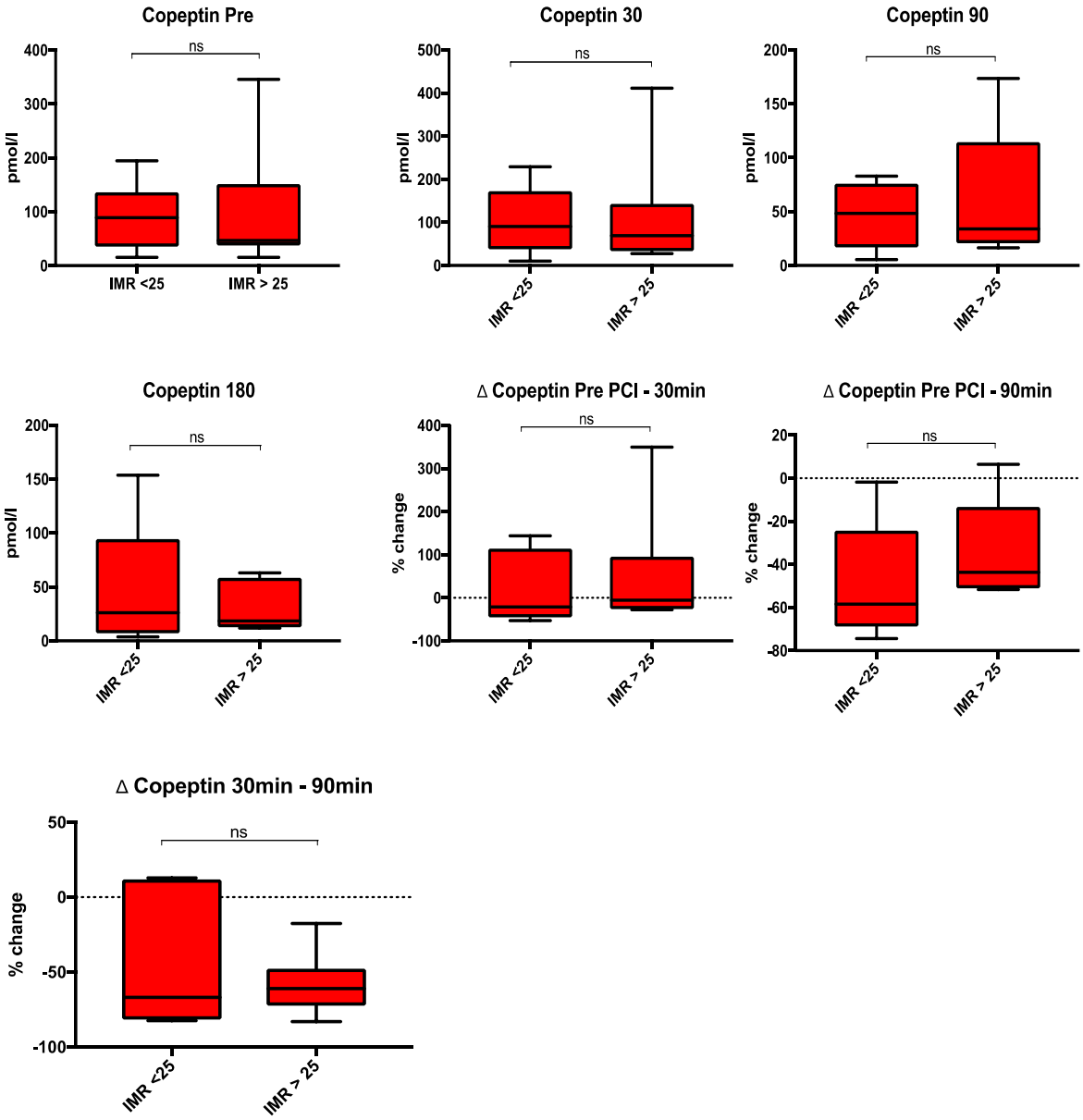

Figure 18. The relationship between copeptin levels and IMR in patients with STEMI. Copeptin concentrations in serum at multiple time points according to IMR level (<25 n=8, > 25: n=7). Data is presented as box (median, interquartile ranges) and whiskers (min and max values). P values were obtained from Mann-Whitney test at α 0.05. NS denotes not significant.

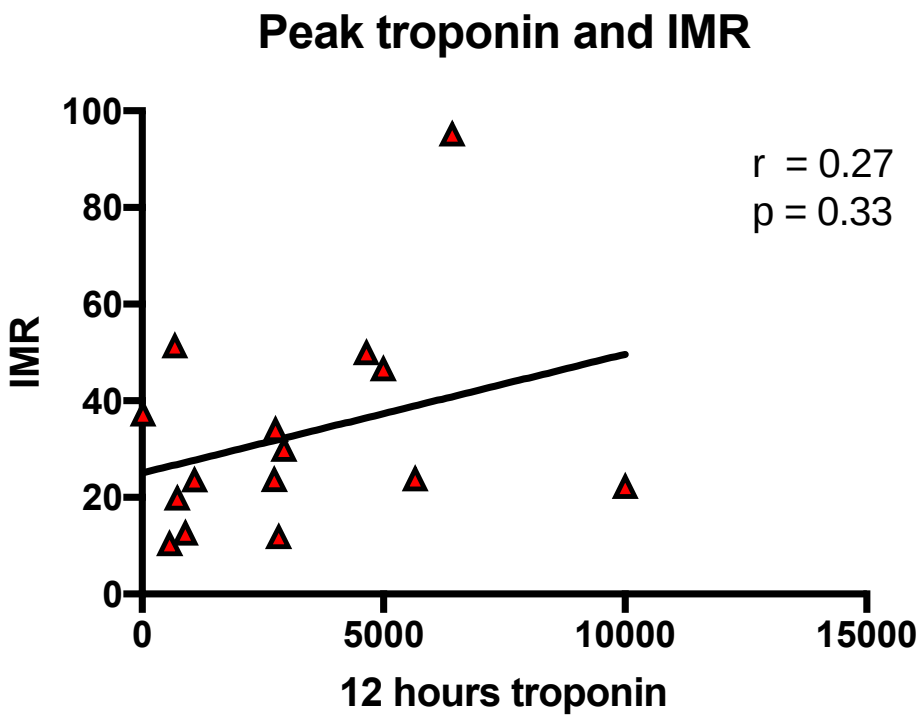

Figure 19 - Correlation between troponin and IMR. Data is presented as a scatter plot with a line of best fit. Correlation coefficient r and p values were calculated from nonparametric Spearman correlation analysis at α 0.05. Simple linear regression was used for the line of best fit (n = 15).

The dataset was analysed based on a low IMR < 25 and high IMR of more than 25. There was no significant difference between the baseline characteristics between the two groups, including risk factors previously known to contribute to CMD including diabetes and hypercholesterolemia. Although I appreciate the sample size is small.

	Low IMR (< 25)	High IMR (> 25)	p-value
n	8	7	
Age	54.38 ± 6.05	60.43 ± 10.86	0.197
Male sex	7 (87.5)	6 (85.7)	1.000
BMI	28.43 ± 8.31	27.95 ± 2.73	0.487
Diabetes Mellitus	1 (12.5)	1 (14.3)	1.000
Active Smoker	4 (50)	2 (28.6)	0.608
Hypertension	3 (37.5)	3 (42.9)	1.000
Hypercholesterolaemia	1 (12.5)	2 (28.6)	0.569
Anterior MI	3 (37.5)	2 (28.6)	1.000
Troponin T (ng/l)	3062 ± 3280	3210 ± 2323	0.728
Treatment during PCI			
Door to balloon time (minutes)	24.13 ± 9.00	35.43 ± 26.17	0.684
Onset to reperfusion time (minutes)	169.88 ± 94.67	208.14 ± 137.74	0.563
Admission Bloods			
White Cell count	11.32 ± 2.71	12.55 ± 3.56	0.563
Haemoglobin	146.50 ± 10.84	146.86 ± 14.55	0.862
Platelets	309.63 ± 114.46	274.14 ± 71.12	0.487
Lymphocyte Count	2.34 ± 0.86	2.34 ± 0.95	0.954
Serum Glucose	7.93 ± 1.85	8.11 ± 2.65	0.867

Table 6: Baseline data for cohort patients undergoing IMR, divided by IMR level. Continuous variables expressed as mean ± SD and compared using Mann-Whitney U test. Categorical variables expressed as n (%) and compared using chi-square (χ^2) or Fisher's exact test as appropriate.

4.7 Copeptin and Coronary Flow Reserve

The coronary flow reserve takes into account both epicardial and microvascular vasodilatory properties. A normal CFR is deemed to be > 2.0 (116, 221). Having split the cohort into those with a CFR < 2 and those > 2 after the culprit vessel has been revascularised. There is a significant difference in the copeptin levels at every time point after reperfusion, most noticeably at 24 hours (p value <0.001). A higher copeptin level is associated with a lower CFR. At 30 minutes post reperfusion those with a CFR < 2 had a copeptin of 148.99 ± 34.54 compared to a CFR > 2 with copeptin measuring 34.6 ± 9.33.

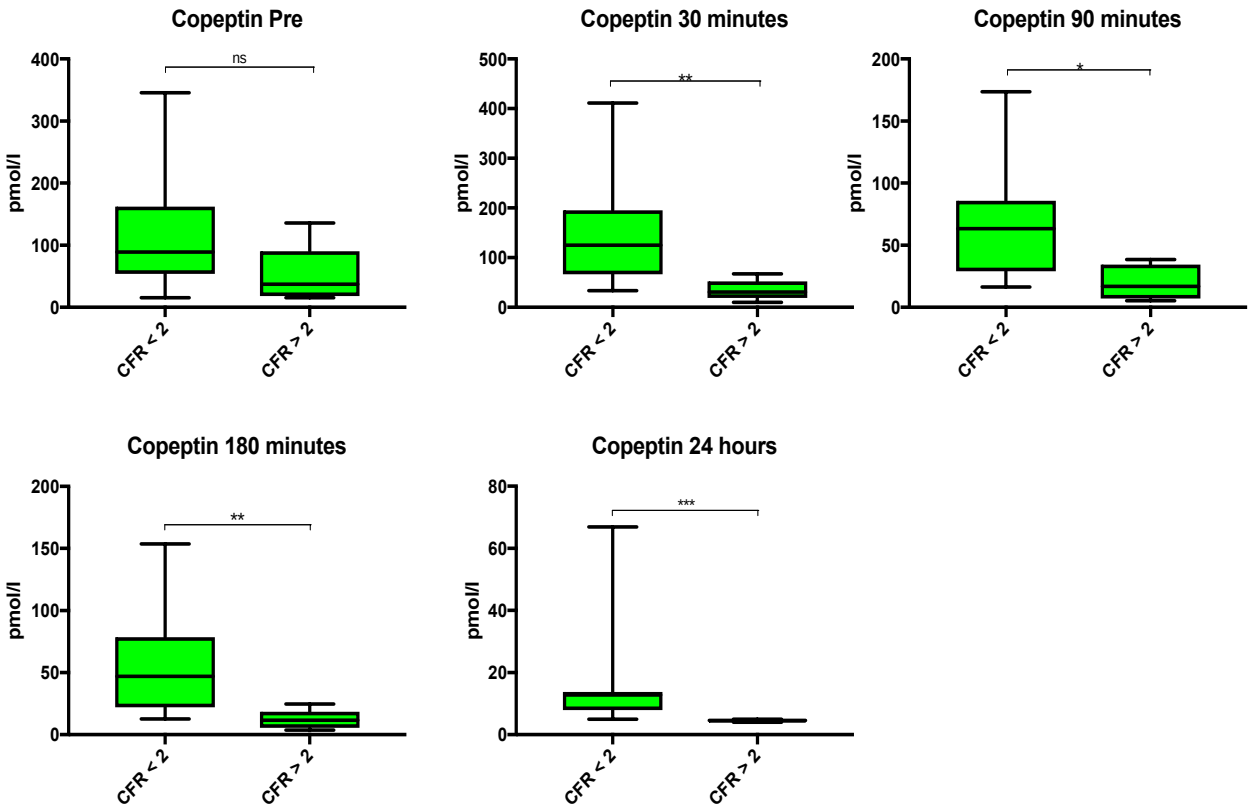

Figure 20. The relationship between copeptin levels and CFR in patients with STEMI. Copeptin concentrations in serum at multiple time points according to CFR level (< 2: n=10, > 2: n = 5). Data is presented as box (median, interquartile ranges) and whiskers (min and max values). P values were obtained from Mann-Whitney test at α 0.05. NS denotes not significant, * denotes P < 0.05, ** denotes P < 0.01, *** denotes P < 0.001

There was no significant difference in age between patients with a CFR < 2 and CFR > 2 although the trend appeared to show older patients tended to have a lower coronary flow reserve (Figure 21).

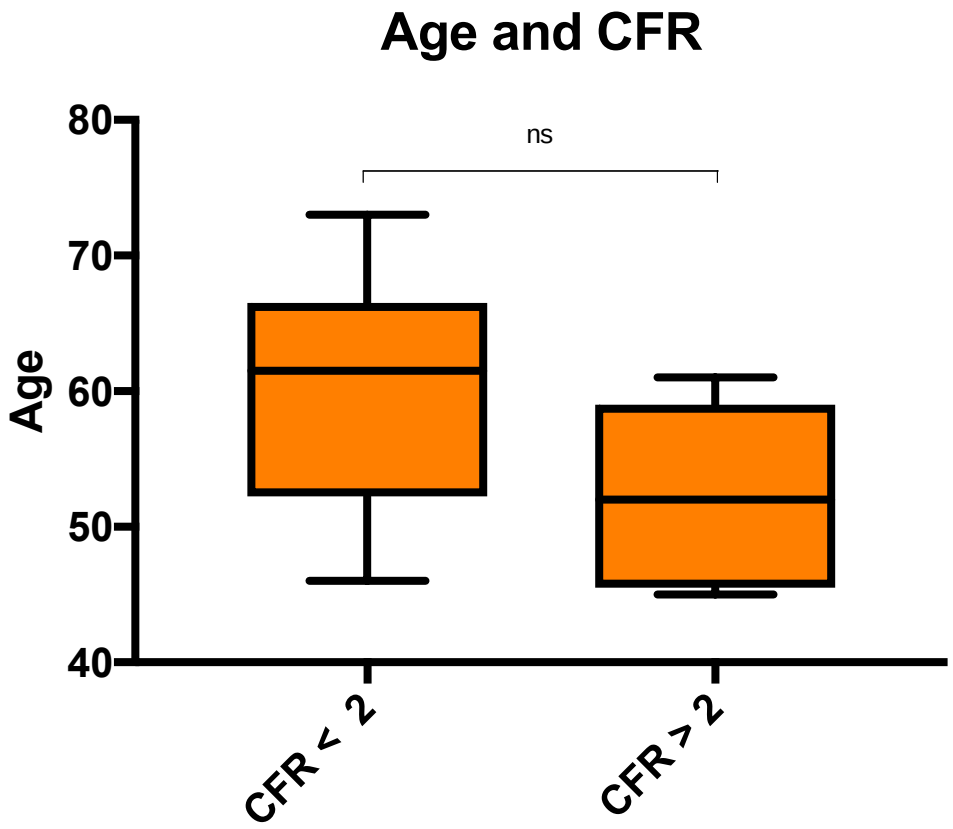

Figure 21: The relationship between age and CFR in patients with STEMI. (CFR < 2: n = 10, CFR > 2: n=5). Data is presented as box (median, interquartile ranges) and whiskers (min and max values). P values were obtained from Mann-Whitney test at α 0.05. NS denotes not significant

When looking at the baseline characteristics and separating the cohort based on CFR being < 2 or > 2 there were no statistically significant differences between the groups (Table 7).

	Low CFR (< 2)	High CFR (> 2)	p-value
n	10	5	
Age	59.7 ± 8.96	52.2 ± 6.90	0.126
Male sex	9 (90)	4 (80)	0.796
BMI	27.48 ± 3.18	29.86 ± 10.27	0.806
Diabetes Mellitus	1 (10)	1 (20)	0.796
Active Smoker	2 (20)	4 (80)	0.056
Hypertension	3 (30)	3 (60)	0.274
Hypercholesterolaemia	1 (10)	2 (40)	0.330
Anterior MI	3 (30)	2 (40)	0.726
Troponin T (ng/l)	3736 ± 3060	1920 ± 1765	0.254
Treatment during PCI			
Door to balloon time (minutes)	34.2 ± 21.79	19.8 ± 6.90	0.310
Onset to reperfusion time (minutes)	168.80 ± 66.80	225.6 ± 181.06	0.859
Admission Bloods			
White Cell count	12.44 ± 3.18	10.79 ± 2.86	0.594
Haemoglobin	146.90 ± 14.2	156 ± 146.2	0.594
Platelets	273.50 ± 59.7	332.2 ± 144.2	0.859
Lymphocyte Count	2.17 ± 0.84	2.68 ± 0.93	0.254
Serum Glucose	8.15 ± 2.36	7.74 ± 2.04	0.679

Table 7: Baseline data for cohort patients undergoing CFR, divided by CFR level. Continuous variables expressed as mean ± SD and compared using Mann-Whitney U test. Categorical variables expressed as n (%) and compared using chi-square (χ^2) or Fisher's exact test as appropriate.

4.8 Copeptin and Microvascular obstruction

Cardiac MRI was performed initially within the first week of their STEMI with a follow up scan at 12 weeks ± 2 weeks. 51 patients had their initial scan and 49 patients a follow up scan. 4 scans were not available; on 3 occasions patients were not available for their scan and on 1 occasion issues with the scan resulted in uninterpretable results.

Microvascular obstruction was measured and quantified on CMR imaging within 1 week of their infarct. And the cohort divided into two groups depending on the presence of MVO or not.

There was no relationship between copeptin levels and the presence of MVO, as there was no significant difference at any time point, including at baseline (see figure 22).

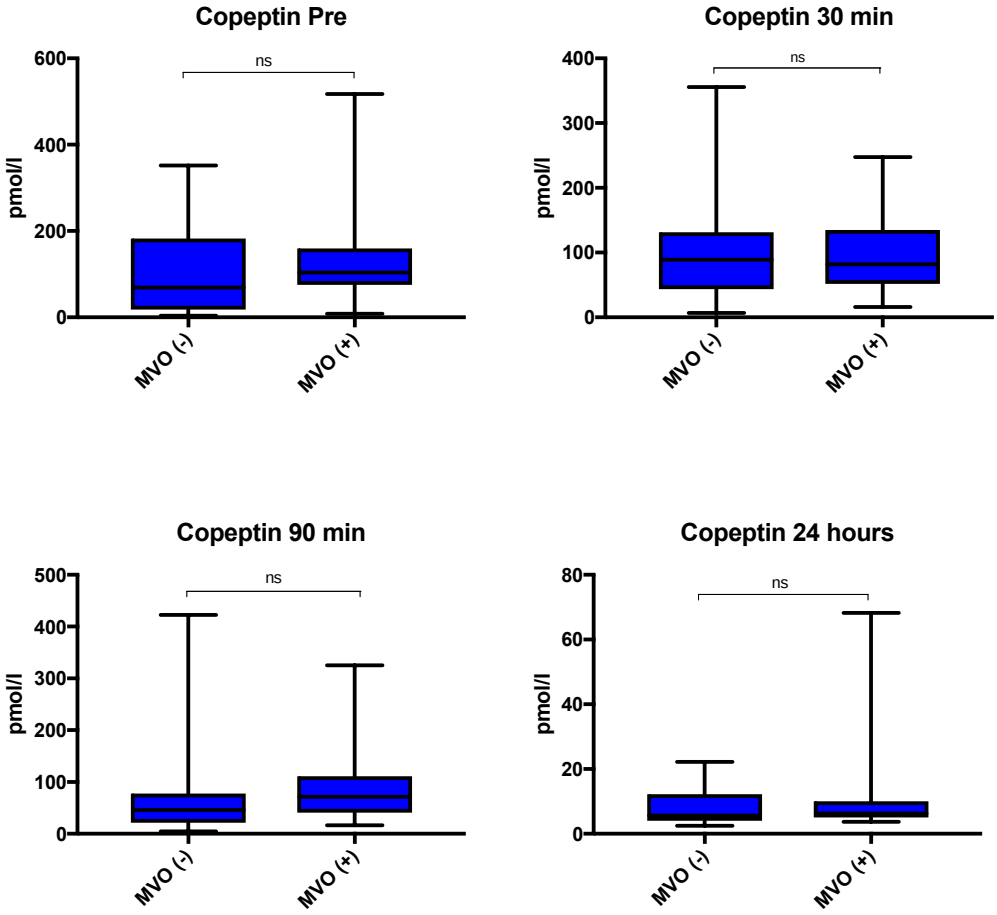

Figure 22. The relationship between copeptin levels and MVO in patients with STEMI. Copeptin concentrations in serum at multiple time points according to whether MVO was absent (-) or present (+) (Pre, 30min, 24 hour - MVO absent: n = 23, MVO present: n=20; For 90 min time point: MVO absent n = 29, MVO present: n = 22). Data is presented as box (median, interquartile ranges) and whiskers (min and max values). P values were obtained from Mann-Whitney test at α 0.05. NS denotes not significant

	No MVO	MVO Present	p-value
n	29	20	
Age	63.34 ± 1.99	65.41 ± 2.10	0.407
Male sex	22 (75.9)	21 (95.5)	0.117
BMI	29.4 ± 4.14	25.7 ± 7.27	0.36
Diabetes Mellitus	4 (13.8)	0 (0)	0.124
Family History of CAD	11 (37.9)	8 (36.4)	1.000
Active Smoker	6 (20.7)	6 (27.3)	0.741
Hypertension	4 (13.8)	4 (18.2)	0.713
Hypercholesterolaemia	3 (10.3)	1 (4.5)	0.625
COPD	0 (0)	2 (9.2)	0.181
Anterior MI	6 (20.7)	9 (40.9)	0.135
Troponin T (ng/l)	2843 ± 2123	5900 ± 3132	**<0.001**
Door to balloon time (minutes)	28.66 ± 8.23	32.77 ± 17.33	0.932
Onset to reperfusion time (minutes)	195.10 ± 85.96	197.82 ± 95.47	0.962
Treatment during PCI			
Thrombus Aspiration	6 (20.7)	7 (31.8)	0.518
Glycoprotein IIa/IIIb	19 (65.5)	14 (63.6)	1.000
Pre-admission Treatment			
Statin therapy	5 (17.2)	6 (27.3)	0.498
Beta Blockers	0 (0)	2 (9.1)	1.000
Aspirin	2 (6.9)	2 (9.1)	1.000
ACE-Inhibitor	3 (10.3)	3 (13.6)	1.000
MRI Parameters			
End-Diastolic volume (ml)	152.08 ± 21.55	169.15 ± 30.39	**0.024**
End-Systolic volume (ml)	69.92 ± 15.82	96.47 ± 24.94	**<0.001**
LV Ejection Fraction (%)	58.26 ± 7.46	47.85 ± 7.43	**<0.001**

Infarct size (% of LV)	7.01 ± 5.59	12.55 ± 7.31	**0.003**
MVO mass (g)	0	4.34 ± 7.86	**0.020**

Table 8: Baseline data for cohort patients undergoing cardiac MRI, divided by MVO. Continuous variables expressed as mean ± SD and compared using Mann-Whitney U test. Categorical variables expressed as n (%) and compared using chi-square (χ^2) or Fisher's exact test as appropriate.

MVO was present in 41 % of the initial scans performed. When MVO was present, that cohort had a higher troponin blood sample at 12 hours, 5900 ± 3132 (ng/l) in MVO group compared to 2843 ± 2123 (ng/l) in the no MVO group ($p < 0.0001$). There were no other differences noted in the baseline characteristics.

There were significant differences between the two groups in the MRI parameters measured. MVO was associated with larger infarcts, worsening in the ejection fraction and increased dilatation in the left ventricle (Table8).

4.9 Copeptin and Infarct Size on CMR

When assessing the relationship if copeptin with infarct size, the patient cohorts were divided into 2 cohorts based on the median value (measured at 8%) and found that higher copeptin levels at baseline and at 30minutes post reperfusion were associated with a lower infarct size (p value 0.04 and 0.008 respectively – see Figure 15). At 30 minutes reperfusion the patients with a smaller infarct size had a copeptin level of 131.3 pmol/l ± 15.9 as opposed to the large infarcts with copeptin measuring 89.7 pmol/l ± 18.7.

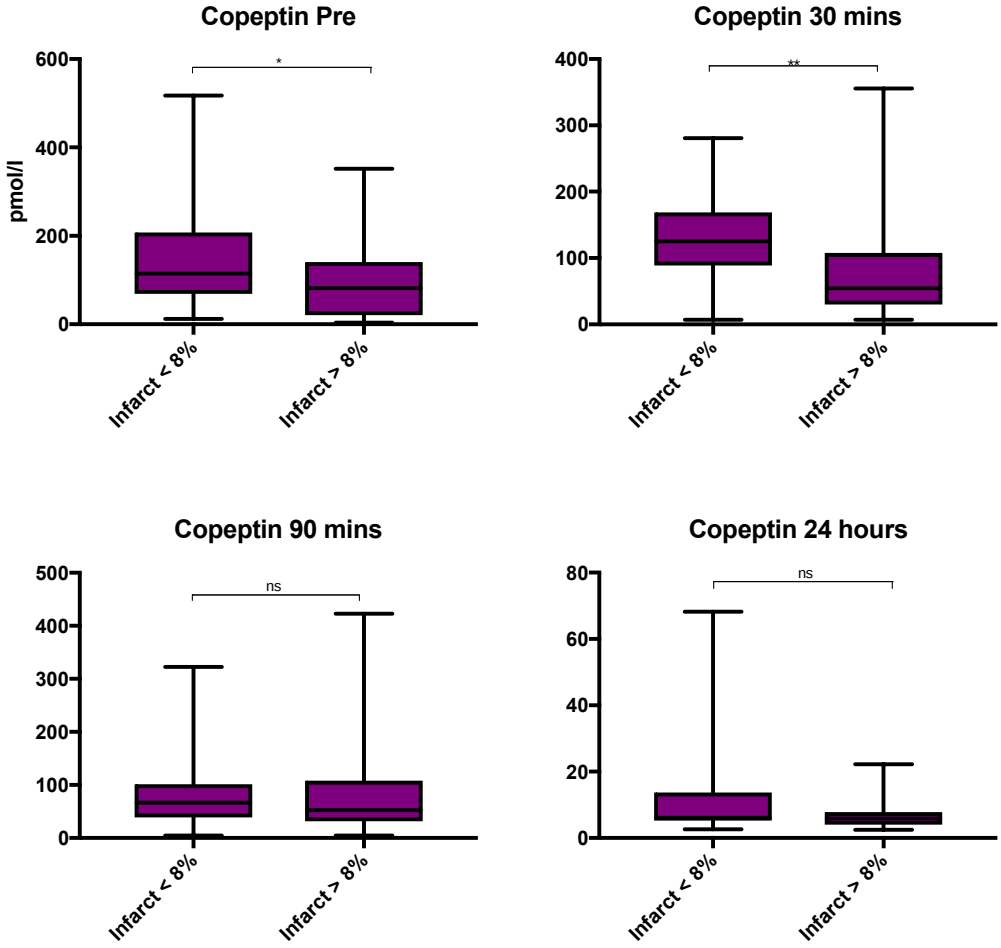

Figure 23. The relationship between copeptin levels and final infarct size in patients with STEMI. Copeptin concentrations in serum at multiple time points according to infarct size (small < 8% or large > 8%) (Pre, 30min, 24 hour – small infarct <8% :n =19, large infarct > 8% : n=25; For 90 min time point: small infarct n=22, large infarct: n=30). Data is presented as box (median, interquartile ranges) and whiskers (min

and max values). P values were obtained from Mann-Whitney test at α 0.05. NS denotes not significant * denotes P < 0.05, ** denotes P < 0.01.

	Small Infarct Size (< 8%)	Large Infarct Size (> 8%)	p-value
n	22	27	
Age	64.14 ± 10.27	64.37 ± 11.11	0.365
Male sex	19 (86.4)	22 (81.5)	0.715
BMI	27.73 ± 3.96	27.96 ± 7.26	0.393
Diabetes Mellitus	0 (0)	4 (14.8)	0.117
Family History of CAD	7 (31.8)	11 (40.7)	0.565
Active Smoker	5 (22.7)	6 (22.2)	1.000
Hypertension	3 (13.6)	5 (18.5)	0.715
Hypercholesterolaemia	1 (4.5)	2 (7.4)	1.000
COPD	0 (0)	1 (3.7)	1.000
Anterior MI	5 (22.7)	8 (29.6)	0.748
Troponin T (ng/l)	3075 ± 2588	4916 ± 3153	**0.029**
Door to balloon time (minutes)	25.32 ± 6.40	34.26 ± 16.23	**0.039**
Onset to reperfusion time (minutes)	171.91 ± 86.35	218.30 ± 89.39	0.056
Treatment during PCI			
Thrombus Aspiration	15 (68.2)	17 (63.0)	0.769
Glycoprotein IIa/IIIb	5 (22.7)	6 (22.2)	1.000
Pre-admission Treatment			
Statin therapy	5 (22.7)	6 (22.2)	1.000
Beta Blockers	0 (0)	2 (7.4)	0.495
Aspirin	1 (4.5)	4 (14.8)	0.362
ACE-Inhibitor	4 (18.2)	3 (11.1)	0.685

MRI Parameters

End-Diastolic (ml)	157.63 ± 28.83	163.00 ± 25.41	0.127
End-Systolic (ml)	78.97 ± 25.49	84.16 ± 24.03	0.106
LV Ejection Fraction (%)	56.02 ± 8.44	52.87 ± 9.80	0.34
Infarct size (% of LV)	3.49 ± 2.58	13.73 ± 5.84	**<0.001**
MVO mass (g)	0.29 ± 0.84	2.8 ± 7.20	**0.016**

Table 9: Baseline data for cohort patients undergoing cardiac MRI, divided by infarct size groups. Continuous variables expressed as mean ± SD and compared using Mann-Whitney U test. Categorical variables expressed as n (%) and compared using chi-square (χ^2) or Fisher's exact test as appropriate.

When looking at the baseline characteristics of the two groups, smaller and larger infarcts. The patients with larger infarcts had a significantly higher troponin level, and larger mass of MVO, as expected. Larger infarcts were also found in patients with a longer door to balloon time. Although the difference in ischaemia time was not statistically significant there was a trend towards larger infarcts in patients who underwent revascularisation after a longer duration; 218.30 ± 89.39 minutes in larger infarcts, and 171.91 ± 86.35 minutes in smaller infarcts (Table 9).

4.10 Copeptin and Left Ventricular Function

Left ventricular function was quantified on cardiac MRI. When looking at the relationship between copeptin and left ventricular function, there was no significant relationship at any time point, and the degree of change in copeptin did not contribute to the presence of left ventricular dysfunction.

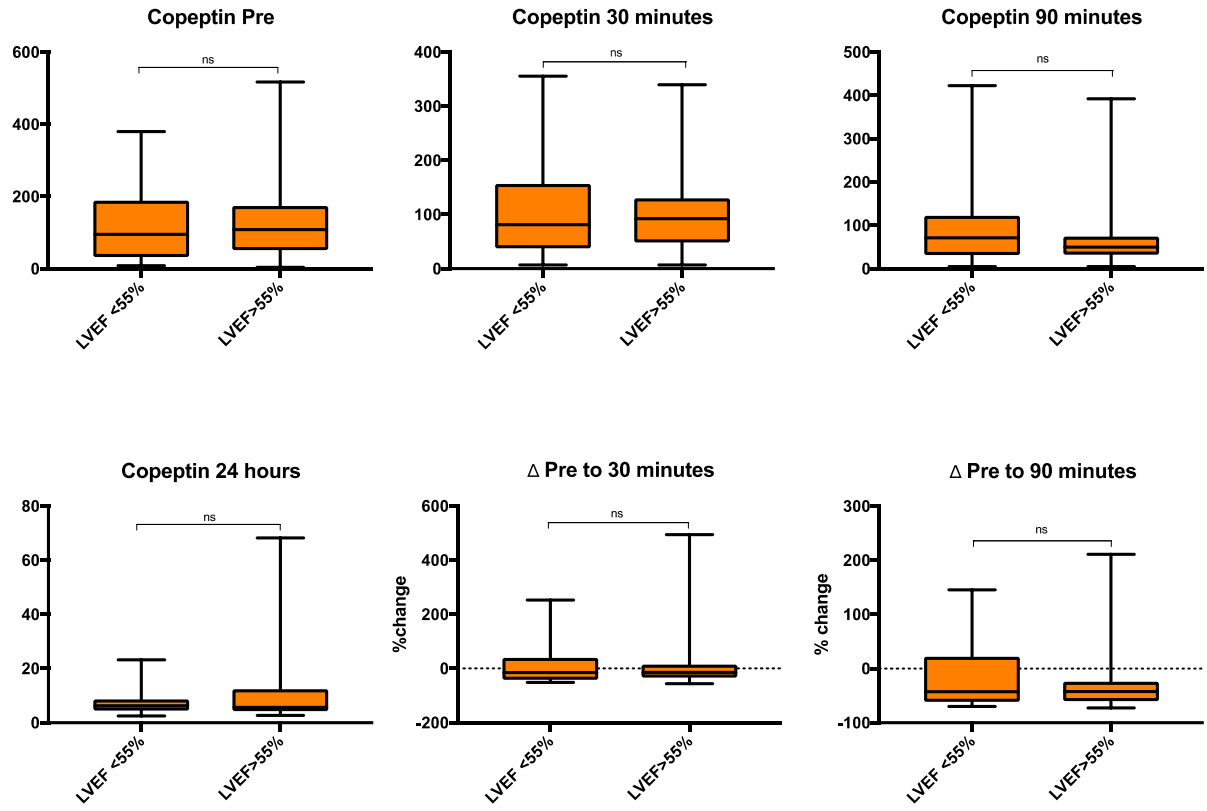

Figure 24: The relationship between copeptin levels LVEF in patients with STEMI. Copeptin concentrations in serum at multiple time points according to LVEF (< 55% or > 55%) (Pre, 30min, 24 hour; < 55% n=22, > 55% n=22; For 90 min time point: < 55% n=25, >55%: n=27). Data is presented as box (median, interquartile ranges) and whiskers (min and max values). P values were obtained from Mann-Whitney test at α 0.05. NS denotes not significant

4.11 Publications associated with the Project

Further work since completion of my research has resulted in more data and subsequent publication in the Journal of the American Heart Association

Al-Atta A, Spray L, Mohammed A, Shmeleva E, Spyridopoulos I. Arginine Vasopressin Plays a Role in Microvascular Dysfunction After ST-Elevation Myocardial Infarction. Journal of the American Heart Association. 2023 Sep 19;12(18):e030473.

Chapter 5

5. Discussion

5.1 Regulation of AVP during Myocardial Infarction in STEMI patients

We measured and investigated AVP in the setting of an acute STEMI and reperfusion. There are markedly elevated levels of circulating AVP on admission and during the course of the patient's admission after reperfusion. The elevated levels of AVP are in keeping with findings from previous studies (222, 223).

Copeptin levels, a precursor of AVP, and more stable to measure ex-vivo, mirrored those of AVP. The patterns of copeptin levels after reperfusion are similar to those of AVP.

This study had found that during a STEMI, endogenous stress occurs. There are elevated levels of ACTH and Epinephrine in our patient cohort. The body is in a state of stress during a myocardial infarction as a consequence of acute pain and myocardial injury and inflammation. This has also been shown in other studies (223, 224). Pain occurring during myocardial infarction is transmitted, via cardiac sympathetic and vagal afferents, to the nucleus tractus solitarius (224). The nucleus tractus solitarius transmits this input to the hypothalamus where the integrated stress response, including the release of AVP, ACTH and Epinephrine occurs (225).

Primary PCI has shown to lower the stress state; it continues to persist despite reperfusion and does not abruptly stop after reperfusion is achieved. It can be seen in Figure 17, that higher levels of stress, reflected by higher epinephrine and ACTH levels, show a trend towards higher levels of AVP. As the hypothalamus regulates each hormone independently, correlation is not intended to show a regulatory relationship. There is evidence that myocardial infarction promotes AVP release indirectly through an integrated stress response.

There was a significant relationship between ACTH and Copeptin at 24 hours, with a higher copeptin in patients with a higher ACTH ($p = 0.034$). A higher copeptin was found in patients with poor reperfusion when assessed by TIMI flow grade. Previous studies have shown an association with copeptin levels, taken 2 days after a STEMI, with larger infarct size determined by CMR as well as related to a higher risk of

adverse remodelling (212). Although the data from our studies did not show this at 24 hours, the trend is in that direction. Poorer reperfusion results in ongoing stress, and hence a higher ACTH and thus a higher copeptin. A study by Nito et al, showed that there were elevated levels of ACTH in patients in the days after an infarct in those that had larger infarcts. Those that underwent reperfusion therapy had a quicker recovery in their ACTH levels to normal (226).

The hypothalamus regulates ACTH and Copeptin independently, and the correlation at 24 hours is not intended to show a regulatory relationship. Together with epinephrine, they make up part of the stress response, which is triggered by a myocardial infarction.

5.2 Copeptin Trend in Myocardial Infarction

Copeptin levels are markedly raised in patients with a myocardial infarction. The highest levels were prior to reperfusion of the coronary artery with a reduction in the copeptin level by 32% 90 minutes after reperfusion ($p < 0.0001$). This would suggest that reperfusion results in a marked reduction in the copeptin level. As previously discussed copeptin levels at 24 hours were significantly lower (11.09 ± 1.63 pmol/l), but still not back to baseline (1 – 4.5 pmol/l). Myocardial reperfusion injury continues to keep copeptin levels elevated although they start to plateau at this stage with those with a poor epicardial flow having a higher copeptin level.

The proposed plan to recruit patients who were undergoing an alcohol septal ablation, to understand the trend or copeptin in a patient undergoing a “myocardial infarction” without reperfusion, did not occur due to logistical issues within the department. Liebetrau et al, looked into the release kinetics of copeptin in 21 TASH patients, with 12 samples measured in a 24-hour period prior and after the therapy. They found a peak copeptin level at 90 minutes after ablation and returning to baseline at 24 hours (227).

This data suggests that copeptin will fall irrespective of whether reperfusion occurs, and this can be seen in our patient cohort in patients who had severe no re-flow where an IMR was no able to be performed, that despite poor epicardial

flow copeptin falls. The speed that copeptin falls and return to baseline appears to reflect the adequacy of reperfusion.

Although TASH doesn't represent myocardial ischaemia, more so instant myocardial necrosis, animal models would also suggest that copeptin levels drop quickly even whilst coronary occlusion continues (206). This early drop has been interpreted to be caused by copeptin being secreted centrally and early in the myocardial infarction not being released from the myocardium (206).

The rapid and early release of copeptin has also been explored in the role as a diagnostic biomarker. It has been shown to be superior to troponin in early acute myocardial infarction, especially in the first three hours of infarction (205, 228)

Admission copeptin significantly correlated with peak troponin level, and previous studies have looked at copeptin use as a biomarker for rule out of myocardial infarction in combination with troponin (203). Reichlin et al, showed marked levels of copeptin having an acute myocardial infarction In patients admitted with an ACS those with a STEMI on admission had the highest levels of copeptin (45.5 pmol/l) (203).

Copeptin level at 90 minutes post-reperfusion also significantly correlates with peak troponin in this patient cohort. There are mixed studies on the prognostic value of peak troponin in STEMI. Some studies have shown peak troponin has not been associated with major adverse cardiovascular events (MACE) (229). Other studies have shown that peak troponin correlates with troponin size and infarct size and LV function (230). Copeptin levels can be used as a surrogate marker for peak troponin and used to risk stratify patients alongside with troponin release.

5.3 Age and Copeptin in Myocardial Infarction

In this study, older patients had a significantly higher copeptin level at baseline (p 0.007) and all the other time points. This was driven by patients over the age of 80, who had a significantly higher copeptin level compared to patients < 50 years of age. The copeptin levels were 58.37 ± 18.17 in < 50 age group, and 208.5 ± 36.46 in the > 80 age group, with a p-value of 0.03.

There have been a number of clinical studies looking at copeptin levels in a number of pathologies. Some have shown that copeptin levels have not been shown to correlate with age in healthy individuals (231). With regards to those with myocardial infarction again there has been a mixed picture, with studies showing no correlation between age and copeptin, whilst others have shown that older patients have higher copeptin level (207, 231).

There are several complex care issues which could contribute to the variable results in studies. Older patients presenting with a STEMI are more likely to have acute heart failure, as evidenced by an admission Killip class of > 2 at presentation in 11.7% of under 65 years old, whilst nearly half (44.6%) of those over 85 years old (232). Older patients are more likely to have a delayed presentation. In the Global Registry of Acute Coronary Events (GRACE registry) median time from symptom onset to presentation was 2.3 hours in those under 45 and 3.0 hours for those over 85 (233). The older patients in this study had longer ischaemia times at 187.0 ± 30.45 minutes compared to 146.7 ± 29.2 minutes, although not statistically significant (p = 0.310). With the copeptin trend data seen above you, would expect to find a longer ischaemia time to be associated with a lower copeptin level as the baseline copeptin is taken later after the onset of the infarct and further down the descending arm of the curve. This is not the case suggesting an increase in copeptin release in older patients. Older patients also had higher troponin levels, a reflection of the longer ischaemia time, but again this was not statistically significant (p = 0.699).

The mortality in STEMI increases with age as well. The 30-day mortality was 10-fold greater in GUSTO-1 in older patients (3.0% in under 65 compared to 30.3% in over 85 year old patients (234). Elderly patients presenting with a STEMI are also

more likely to have cardiogenic shock on admission with 10% of patients over 75 found to be in shock on admission (31, 235). The ageing heart also increased diastolic dysfunction, increased arterial stiffness and endothelial dysfunction resulting in altered vascular tone (236).

All of the above features may explain why higher copeptin is associated with higher mortality, as it is higher in older patients who are more likely to have complications associated with myocardial infarction and adverse features.

Studies have shown that older members of the population have higher AVP levels (228). Mechanistically this is felt to be due to changes in water regulatory capacity and the impairment in the kidney to concentrate urine with increased age (237). Animal studies have also found that with age the ability to release vasopressin increases with age as well (237). This may explain the higher copeptin levels with age, as this cohort not only have a higher baseline level, but also the propensity to release vasopressin is also greater.

5.4 Copeptin and Smoking

In this study, active smokers had lower levels of copeptin. It is likely active smokers being younger in age drive this. The participants who were active smokers had an age of 57.0 ± 2.1 (Mean ± SEM), whilst non-smokers were older 66.5 ± 2.4 and ex-smoker also older at 64.3 ± 1.5. Above we have explained why older participants may have a higher copeptin level. Studies have shown that smoking does not impact on infarct size (238).

Active smokers are more likely to have inferior STEMI as opposed to anterior STEMIs (239). Anterior STEMIs due to an occlusion of the left anterior descending artery are more likely to result in larger infarcts (240). This also may explain why the copeptin levels were lower in smokers.

Smoking and nicotine consumption causes activation of the hypothalamic-pituitary axis, and consequently, an increase in vasopressin acutely after a cigarette is

smoked (241). Although in this study, we did not document when the last cigarette was smoked by the participants.

5.5 Hypotension and Copeptin

Patients presenting with a lower systolic blood pressure had significantly higher copeptin levels. AVP is released to help maintain blood pressure during stress and hypotension. As previously stated, there are receptors in the left atrium and pulmonary arteries that can detect changes in intravascular volume and systemic hypotension. The receptors respond to pressure induced stretch, and when activated results in an inhibitory effect on the release of AVP. Thus when the volume in the left atrium, and thus the pressure is low, the inhibitory response is suppressed, and AVP is released (186, 188). Shock states have shown to have marked levels of copeptin, and this has been shown in cardiogenic shock as well (189).

There has been some question into whether hypotension is the trigger for copeptin release as opposed to myocardial ischaemia and stress associated. Copeptin is elevated in a number of shock states and studies on monkeys have shown that when a haemorrhage was induced, that the level of vasopressin was related to mean arterial pressure in the iliac artery, and not to the volume of blood lost (206). Also, Slagman et al, who measured mean arterial pressure (MAP) and copeptin in pigs undergoing a myocardial infarction, found that the drop in MAP was the trigger for copeptin release. In this study, copeptin did not correlate with the infarct size (206). This has also been reflected in other animal studies involving rats where the trigger for copeptin release was the drop in MAP (206). This is contrary to the results by Reinstadler et al, who found a correlation between copeptin and infarct size, measured by cardiac MRI (212).

5.6 Copeptin and Infarct Size

Patients with a higher copeptin level at baseline and at 30 minutes post reperfusion had significantly smaller infarcts. This significance was not present at the other time points. Reinstadler et al, showed that elevated copeptin levels at 2 days after STEMI corresponded with larger infarcts (212). Reperfusion in our cohort showed a rapid decline in copeptin levels, and those where TIMI flow was less than 3 showed significantly higher levels in copeptin levels. Higher levels of copeptin at day 2 are likely a reflection of inadequate reperfusion and hence larger infarcts (242). Doganay et al, found copeptin levels were associated with patency of infarct related artery in ST elevation myocardial infarction patients (243). Although in our patient cohort copeptin levels at 24 hours did not correlate to infarct size, this is a reflection of adequate perfusion in the majority of cases. 93% of patients in the cohort achieved TIMI 3 flow after reperfusion therapy.

A study by Nazari et al, where ischaemia-reperfusion injury was replicated in rat heart, and various doses of AVP were administered showed that AVP administration was cardio-protective and resulted in smaller infarcts (244). This can explain why higher copeptin prior to reperfusion and early after the infarct related artery is recanalised is associated with smaller infarcts.

There was no difference in copeptin levels in patients with or without left ventricular dysfunction. This is a surrogate for the degree of infarction and infarct size. It is noted that the ischaemia time was low, with a mean of 190.6 ± 92.8 minutes. This will result in smaller infarcts overall and again reflect on smaller changes in left ventricular function (245).

Increased copeptin, prior to reperfusion as a response to infarction, and a drop in MAP, appears to be cardioprotective prior to reperfusion and contributes to smaller infarcts. Once reperfusion is achieved though, sustained higher AVP levels at 24 hours is noted in larger infarcts, whether this is a contributor to reperfusion injury, or a reflection of poor reperfusion +/- reperfusion injury is yet to be ascertained.

5.7 Copeptin and Microvascular Dysfunction

Microvascular obstruction was present in 41% of our patients who underwent a CMR scan. This prevalence of MVO on LGE is in keeping with the range seen in previous studies where STEMI patients underwent PPCI (between 25 and 69%) (25, 97, 98). In a meta-analysis by Van Kranenberg et al, 56% of patients who had a CMR in context of PPCI for STEMI had MVO (246).

The presence of MVO was unsurprisingly associated with a larger infarct size, poorer LV function, and with larger LV end-diastolic volumes. This is in keeping with previously published studies (247).

Index of microvascular obstruction was measured in 15 patients. The mean IMR was 32.76 ± 5.617. 7 subjects had an IMR of > 25, and 4 subjects had an IMR > 40. 27% of patients had an IMR > 40, which is lower than seen in other studies (248). In the OxAMI study, 40% of the patients recruited had an IMR > 40.

In our cohort of patients, we did not find any difference between copeptin levels in patients with or without microvascular obstruction, at any time point. When splitting the cohorts by into two groups by IMR level, to IMR < 25, which is deemed to be normal, and IMR > 25 there was no difference in the copeptin levels at any time point. The change in the copeptin level also had no relationship to the IMR level. When splitting the cohort into IMR < 40 and IMR > 40, there was no significant difference in baseline characteristics or copeptin levels at any time point as well.

The MVO outcomes, together with the IMR suggests that copeptin does not impact on the degree of microvascular dysfunction. This insignificance could be related to a smaller volume of anterior STEMI as compared to non-anterior as well as a small case number. The proportion of patients with an IMR > 40 is low compared to other studies. This may reflect the speed the institute reperfuses the coronary arteries, with one of the shortest door to balloon times in the country.

5.8 Copeptin and Coronary Physiology

Prior to reperfusion, there is no change in copeptin levels associated with a high CFR (> 2) and a low CFR (< 2). Once reperfusion is achieved a higher copeptin level results in a lower CFR at every time point.

The coronary flow reserve is the ratio of maximal/hyperemic flow down the coronary artery to the resting flow (249). The CFR takes into account the epicardial artery as well as the coronary microcirculation. If there is an epicardial stenosis upstream or downstream of the stented culprit plaque, then this was result in a lower CFR (108). A higher copeptin level may be the result of an additional stenosis in the culprit artery; this exacerbates the ischaemia and reperfusion injury in that vessel. This then results in a higher copeptin level. Bax et al, found CFR strongly correlated with LV function recovery in anterior STEMI (250).

It has been established that vasopressin does not have an impact on the coronary microcirculation, but the low CFR level suggests that it may act on the epicardial aspect of the artery and contribute to vasoconstriction of the epicardial artery. The epicardial artery can be assessed using fractional flow reserve (FFR) and instantaneous wave free ratio (IFR).

Fractional Flow Reserve (FFR), an invasive pressure wire assessment of the coronary artery, can be used to assess epicardial stenosis and the significance of them. FFR looks at the pressure in the distal coronary artery comparative to the aortic pressure, during maximal hyperaemia. A study by Hoole et al, looked at FFR assessment of the culprit artery in STEMI, found that only 10% of patients in their study had an FFR of > 0.80 (251). An FFR of < 0.80 suggests flow limiting disease. It is not recommended to perform FFR assessment in STEMI due to the inaccuracies associated with it due to microvascular dysfunction, and recommendations to wait at least 6 days before FFR measurements are taken in a STEMI culprit artery (252).

There is limited data on the accuracy of IFR as well in the infarct area in STEMI. This makes epicardial assessment and interpretation of the low CFR challenging as it is the collective combination of the epicardial and microvascular circulation.

The other relationship between copeptin and CFR is that the low CFR drives the higher copeptin levels. Coronary flow reserve represents the amount of additional blood flow that can be supplied to the heart above baseline blood flow. If this is reduced, and we have established that copeptin falls more rapidly with more adequate reperfusion, then then the higher copeptin levels could represent poorer flow to that coronary bed rather than contribute to it. Copeptin may be a marker or poor reperfusion, represented by the low CFR, as opposed to contributing to it via epicardial vasoconstriction. This area needs further exploration.

The reliability of CFR and the interpretation of it can be challenging. Coronary flow reserve is reliant on calculating resting blood flow. Therefore changes in haemodynamic status, such as heart rate, blood pressure and left ventricular contractility can all impact on the CFR level as a result in the change in the resting flow (108). De Waard et al, found that CFR falls in patients after an acute MI due to an increase in baseline blood flow and a decrease in hyperemic blood flow. In the same study, a lower CFR was significantly associated with a larger infarct size, as a percentage of the left ventricle (253).

The MRI data did not suggest that copeptin had an impact on infarct size, and as previously stated, this can be explained by the smaller territory infarcts with branch vessel STEMIs recruited into the trial (obtuse marginal/ diagonal vessel occlusions), and the low ischaemia time. The relationship between copeptin and CFR would suggest that copeptin has an impact on the epicardial artery and could contribute to larger infarcts.

Chapter 6

6. Limitations

The studies that comprise this body of research were performed in a single centre, and the sample size for this study was small. The patients enrolled therefore are not necessarily representative of a “real world” population.

The Freeman hospital has one of the shortest door to balloon times nationally. This coupled with smaller territory occlusions, and short ischaemia time results in expected smaller infarcts overall in the cohort recruited. More anterior STEMI and higher area of myocardium at risk may have shown more measurable MVO.

The first CMR was performed within 2-7 days of the PCI being performed. Some studies have shown that the proportion of MVO can deteriorate over this time period (254). The range of time for CMR was required due to the availability of the MRI scanner for patients enrolled into the study. This could result in MVO appearing smaller in patients who had an MRI at day 7 as opposed to those where the MRI was done sooner.

Only 15 patients were able to have an IMR detected, and hence the data set for these patients is small.

In 2019 De Maria et al, found evidence of MVO in patients with an IMR < 40 in nearly a third of cases (248). Both IMR and CMR are useful surrogates to assess microvascular dysfunction but look at different aspects. CMR and IMR data is not available for all the patients.

Patients underwent PCI as per the operator regular practice, with some patients receiving Ticagrelor and others Prasugrel as a second antiplatelet. Not all the patients underwent thrombectomy, and some were given glycoprotein IIb/IIIa inhibitors. Small studies have suggested that these factors do not impact on the IMR, but that was in a different STEMI population (255).

I was unable to enrol patients undergoing therapeutic septal ablation for hypertrophic cardiomyopathy due to issues with cath lab capacity and staffing, resulting in patients being deferred for this treatment during my research.

The COVID 19 pandemic had a measurable impact on the write up of my thesis and work. I unfortunately caught covid in the early in the pandemic in 2020 which had significant impact of my breathing and concentration for months. Also during this time I was supporting the PPCI rota as a consultant cardiologist which was time consuming and fatiguing as well due to the frequency of clinical cover and lethargy of wearing PPE for long durations.

7. Future Work

The relationship between copeptin and microvascular disease should be explored further. A larger cohort of patients in a multicenter study would allow for further understanding and clarification of the work we have already done. For future studies, I would suggest focusing on Anterior infarct STEMI cases. The larger infarcts and myocardium at risk would result in a less heterogenous population or infarct size. The limitation of competing trials in a single centre would be diluted by a multi-centre approach.

My work suggests that elevated copeptin pre revascularisation may be cardio-protective and result in smaller infarct size. A randomised trial where low dose vasopressin is given to patients, or a placebo, would test this hypothesis. Cardiac MRI would be used to assess the infarct size.

After reperfusion, higher copeptin levels are seen in patients with poor reperfusion and a lower TIMI grade. An AVP antagonist could be given post reperfusion in a randomised trial to assess the impacts of blocking AVP on outcomes post STEMI including measuring troponin, infarct size and MACE (major adverse cardiovascular events). There are currently two AVP antagonists available, conivaptan (a combined AVPR1a and AVP2 antagonist), and tolvaptan (an AVPR2 antagonist). AVP receptor antagonists have been recommended in the treatment of heart failure, to reduce cardiac afterload. Studies have shown a short course of an AVPR1a antagonist, in heart failure patients after a myocardial infarction, have shown to reduce systemic vascular resistance and improve cardiac output (201). A study by Boyle et al, involved giving an AVP antagonist to hypoxic rat hearts after an AVP infusion. This showed a marked increase in coronary flow, negating the AVP-mediated effects on cardiac contractile function (76). This is an interesting and exciting area that warrants further exploration in patients' post-acute myocardial infarction.

8. Conclusion

The research I carried out had a number of important findings. AVP and copeptin levels are markedly elevated in patients with an acute myocardial infarction. It also showed that reperfusion of the infarcted territory and recanalisation of the occluded artery resulted in a significant fall in copeptin levels. Copeptin levels remain significantly elevated after reperfusion where TIMI flow was less than 3. Previous studies have shown that elevated Copeptin levels are related to larger infarcts and poor LV function, and this likely represents that the culprit artery remains occluded or significant slow flow phenomenon. Although the patient cohort in my study did not have a relationship with copeptin and infarct size, this likely reflects the smaller territory infarcts and low ischaemia time in this patient group. Previous trials have found a relationship with elevated copeptin and larger infarcts when measured 2 days after a myocardial infarction.

My research shows that Copeptin and Vasopressin do not interact solely on the microcirculation of the coronary artery with no difference in the relationship detected with MVO and IMR. Copeptin did have an effect on the whole coronary artery, including the epicardial component, as shown by a higher copeptin level resulting in a lower CFR. This study appears to be the first looking at the time course of copeptin and the relationship with coronary physiology.

The study is also the first to plot copeptin levels post reperfusion closely. It suggests that copeptin may initially be cardio-protective in the acutely occluded coronary artery, but sustained vasopressin after recanalisation may result in larger infarcts.

This opens the door for exploring potential new therapies such as low dose vasopressin in the prehospital setting on the lead up to revascularisation, and AVPR1a antagonists post reperfusion, as well as considering the role of copeptin as a biomarker to assess recanalisation and reperfusion of the infarct territory.

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