# A Pilot Randomized Controlled Trial of Intravenous N-acetyl Cysteine in Patients Undergoing Pharmaco-invasive Reperfusion Early After an ST-segment Elevation Myocardial Infarction

by

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## ABSTRACT

**Background:** N-acetyl cysteine (NAC) effectively breaks down arterial thrombi in animal models and activates cardioprotective cellular pathways in STEMI models. Our study aimed to conduct a pilot trial to translate the beneficial effects of intravenous NAC observed in vascular occlusion models into clinical practice.

**Objective:** The objective was to evaluate the extent of myocardial salvage achieved through early administration of intravenous high-dose NAC in high-risk STEMI patients with a substantial area of myocardium at risk, in comparison to standard therapy.

**Methods:** This investigator-initiated, single-center, open-label trial involved the randomization of high-risk STEMI patients undergoing pharmaco-invasive reperfusion. Participants were assigned to receive either intravenous high-dose NAC or standard therapy. The primary clinical endpoint was the measurement of myocardial infarction size using late gadolinium-enhanced cardiac magnetic resonance imaging. The investigators responsible for assessing the primary study outcome remained blinded to the randomization.

**Results:** A total of 44 patients were randomized, with 22 patients assigned to the intravenous NAC group and 22 patients to the control group. All patients received thrombolytic therapy prior to reaching PCI-capable hospital, with a mean (SD) symptom-to-needle time of 1.5 (± 0.7) hours. There was no significant difference in myocardial infarct size between the two groups (mean [SD] infarct size as a percentage of LV myocardial mass: intravenous NAC: 19.7 [7.9] % versus control: 18.2 [10.1] %, p = 0.62). LV volumetric measurements, including LV end-diastolic volume index (intravenous NAC: 75.6 [17.5] ml/m2 versus control: 71.7[14.1] ml/m2, p = 0.78) and end-systolic volume index (intravenous NAC: 38.7 [11.7] ml/m2 versus control: 35.4 [9.5] ml/m2, p = 0.83), were similar between the two groups. Two patients in the intravenous NAC group developed

congestive heart failure. Myocardial microvascular obstruction was present in 6 (37.5%) patients in the intravenous NAC arm and 9 (42.9%) patients in the standard therapy arm (p = 0.74). There was no significant difference in myocardial salvage between the groups (intravenous NAC: 18.4 [7.2] % versus control: 18.4 [6.0] %, p = 0.99).

**Conclusions:** High-dose intravenous NAC administration did not lead to a reduction in myocardial infarct size in high-risk patients undergoing early reperfusion with a pharmaco-invasive strategy following a STEMI with a significant area of myocardium at risk. In conclusion, the translation of cardioprotective therapies from animal studies to clinical care for STEMI patients continues to pose significant challenges. However, this pilot randomized trial, which adheres to current translational research guidelines, stands as a valuable model for designing future trials aimed at evaluating cardioprotective therapies within the context of STEMI.

## PREFACE

This thesis represents the original research work conducted by MSc candidate Anoop Mathew. The study received ethical approval from the institutional research ethics board (REMO approval number Pro00087545). Additionally, it obtained administrative approval for clinical research in the Edmonton zone through the Northern Alberta Clinical Trials and Research Centre (approval #36349). Health Canada issued a no-objection letter for the protocol on 16 April 2019 (# HC6-24-c225757). The study was registered on clinicaltrials.gov with the identifier number NCT04023266. Dr. Michelle Graham served as the project guide. Co-investigators Dr. Haran Yogasundaram, Dr. Waleed Alhumaid, and Dr. Janek Senaratne were involved in patient recruitment, obtaining patient consent, and documentation. Dr. Richard Coulden and RN Emer Sonnex played a key role in performing and analyzing the cardiac magnetic resonance imaging (MRI) scans, as well as reporting the findings. Co-investigators Dr. Evangelos Mickelakis and Dr. Sean Van Diepen contributed to the development of the grant application. The supervisory committee included Dr. Michelle Graham, Dr. Janek Senaratne, Dr. Ian Patterson, and Dr. Sean Van Diepen.

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## List of Abbreviations.

STEMI- ST-segment elevation myocardial infarction

LV-Left ventricle

RV- Right ventricle

LV EF- Left ventricular ejection fraction

PPCI- Primary percutaneous coronary intervention

VWF-von Willebrand's factor

HM WM-high molecular weight von Willebrand's factor

IV NAC- Intravenous N-acetyl cysteine

CMR- Cardiac Magnetic Resonance Imaging

**Key Words –** ST Elevation Myocardial Infarction, Acetylcysteine, Myocardial Reperfusion

## WHAT'S NEW

- First randomized pilot trial to evaluate the cardioprotective and thrombolytic effects of NAC in patients with STEMI undergoing pharmaco-invasive reperfusion in the presence of a large area of myocardium at risk.
- This study attempts to establish a model for trial design for cardioprotective therapies in STEMI.
- Translating the preclinical benefit of cardioprotective therapies to clinically meaningful outcomes for STEMI patients remains challenging, with an unmet need for innovative trial designs.

# CHAPTER 1

# INTRODUCTION

## The unmet clinical need:

Acute myocardial infarction has a significant impact on the Canadian population each year. ST-segment elevation myocardial infarction (STEMI) can lead to irreversible transmural myocardial damage, resulting in substantial morbidity and mortality. Timely reperfusion, achieved through fibrinolysis, primary percutaneous coronary intervention (PPCI), or a combination of both, plays a crucial role in limiting infarct size and preventing irreversible deterioration of cardiac function[1]. While the implementation of effective reperfusion therapies, including PPCI and newer antithrombotic agents, has contributed to a decline in short- and long-term STEMI mortality rates, long-term mortality remains high[1,2]. Adverse outcomes, such as congestive heart failure, cardiogenic shock, recurrent myocardial infarction, and death, have been observed in up to 19.4% of reperfused STEMI patients in an Alberta-based cohort at one year[2]. Despite the increasing utilization of PPCI as the primary reperfusion modality, long-term STEMI mortality rates remain persistently high across different regions [3,4]. Moreover, STEMI can lead to significant long-term morbidity, with approximately 20-30% of patients developing heart failure within the first year following a myocardial infarction [5]. These factors highlight the unmet clinical need for the development of potential therapeutic agents to limit infarct size in STEMI patients.

Some of the adverse outcomes observed in STEMI patients may be attributed to advanced myocardial damage at presentation, as indicated by the presence of Q-waves on the surface electrocardiogram (ECG) [6]. Timely reperfusion, extending to the level of the coronary microvasculature, remains a crucial aspect of STEMI treatment. Paradoxically, reperfusion of the infarct-related artery can trigger cardiac myocyte cell death and irreversible tissue injury,

collectively known as myocardial reperfusion injury [7,8]. Myocardial reperfusion injury may contribute to ventricular dysfunction observed in some STEMI patients, despite prompt PPCI. Therefore, the prevention of myocardial reperfusion injury presents an appealing therapeutic target for limiting infarct size and improving clinical outcomes [9]. Although numerous therapeutic agents have been investigated in STEMI patients with the aim of reducing myocardial reperfusion injury, none of these agents have demonstrated improved clinical outcomes in significant clinical trials [10]. To effectively reduce infarct size and enhance post-myocardial infarction left ventricular function, it is essential to design trials that allow the therapeutic candidate to intervene before substantial myocardial injury has occurred.

**Standard of therapy for STEMI:**

The current standard of STEMI therapy emphasizes prompt reperfusion, ideally within three hours of the onset of chest pain, using primary percutaneous coronary intervention. Additionally, patients receive high-dose statin therapy along with dual antiplatelet therapy involving aspirin and either clopidogrel, ticagrelor, or prasugrel. Unfractionated heparin or low-molecular weight heparin is used as an adjunctive anti-thrombotic therapy. The utilization of beta-blockers, angiotensin receptor blockers, and mineralocorticoid receptor blockers depends on left ventricular function and presence of regional wall motion abnormalities among other factors. However, in countries such as Canada where cardiac catheterization lab services are regionally distributed across a large geographic area, patients residing in rural areas face challenges in accessing timely reperfusion services. For such patients, the most effective approach is a pharmaco-invasive reperfusion strategy involving the administration of thrombolytic agents by emergency medical services (EMS) within the initial hours of STEMI presentation, followed by

transfer to a hospital capable of performing PCI. This trial sought to improve the outcomes for such patients without timely access to catheterization lab-based reperfusion services.

**Von Willebrand factor as a therapeutic target in STEMI:**

One significant approach to limiting infarct size is by targeting more efficient thrombus resolution in both epicardial coronary arteries and the coronary microcirculation. Acute coronary syndromes typically involve a cascade of cellular reactions, with traditional thrombogenesis pathways including critical steps such as plaque rupture and exposure of the highly thrombogenic subendothelial matrix to platelets. A key mediator in thrombogenesis is von Willebrand factor (VWF), a multimeric glycoprotein produced by endothelial cells. VWF, a recognized marker of cardiovascular disease, plays a crucial role in clot formation, stabilization, and vascular permeability [11]. VWF can counteract the effects of both endogenous and exogenous thrombolytic agents and likely contributes to microvascular obstruction in patients with acute coronary syndromes [11]. Acute coronary syndrome patients exhibit elevated levels of VWF upon presentation, which correlate with the long-term risk of major adverse cardiac events[12]. Therefore, combining VWF blocking agents with antiplatelet and antithrombotic therapies represents a synergistic therapeutic strategy in STEMI.

VWF regulates thrombosis through various mechanisms. Firstly, it increases the thrombogenicity of large and ultra-large VWF multimers. Secondly, VWF multimers stabilize the outer layers of the blood clot, providing a shield for fibrinogen within [13]. Plasma VWF multimers exist in different sizes, and their thrombogenicity varies based on molecular weight and size. The ultra-large and high-molecular-weight subtypes are particularly thrombogenic. Metalloprotease ADAMTS-13 plays a crucial role in regulating the size of plasma VWF by cleaving high-

molecular-weight VWF multimers [13]. Once secreted from Golgi bodies in response to shear stress, ultra-large multimers have a short lifespan as they are rapidly cleaved by ADAMTS-13. Hemostasis generally results from the balance between the biosynthesis of highly thrombogenic ultra-large VWF multimers and their regulation by the ADAMTS-13 metalloprotease [13]. Severe deficiency of ADAMTS-13 can lead to thrombotic thrombocytopenic purpura, characterized by widespread intravascular thrombus formation. Also, the levels of ADAMTS-13 drop after infusion of vasopressin analogue desmopressin, which is used in the treatment of hemophilia[14]. Conversely, a mutation in the A2 domain of VWF increases its susceptibility to cleavage by ADAMTS-13 and reduces the quantity of large VWF multimers, resulting in von Willebrand's disease type 2A, a bleeding disorder[13]. Additionally, the composition of thrombi varies within the blood clot, and VWF plays distinct roles in stabilizing the thrombi depending on its specific location within the clot. The more compact core of the clot is stabilized by fibrinogen interlinking platelets [15], while the outer layers exposed to shear stress are stabilized by high-molecular-weight VWF multimers, linking platelet clumps [15].

**Preclinical evidence on efficacy of N-acetyl cysteine in limiting infarct size:**

How do we target VWF to bring about efficient thrombolysis down to the microvasculature level? A therapeutic agent that can mimic the action of ADAMTS-13, thereby cleaving the high molecular weight VWF multimers, would be useful in managing patients with acute coronary syndrome and coronary thrombosis. N-acetylcysteine (NAC) may be one such agent that fits the bill. NAC has been proposed as a therapeutic option for patients with thrombotic thrombocytopenic purpura. The mechanism of action is by reducing disulfide bonds, thereby downsizing the high-molecular-weight multimers of VWF[15]. Recent studies have shown that

NAC is efficacious in mouse models of cerebral artery thrombosis. High-dose NAC infusion, 20 minutes after arterial occlusion, reduced cerebral infarct size by more than half in mouse models where thrombosis was initiated by injection of ferric chloride and by more than a third in a model created by direct intra-arterial thrombin injection[16]. Remarkably, the thrombolytic effect in the ferric chloride model was against a backdrop of lack of demonstrable thrombolytic effect with tissue plasminogen activator and unfractionated heparin. Also, in mice models, clot formation in the inferior vena cava induced by ferric chloride is dependent on platelet and glycoprotein Ib alpha-vWF interaction [17]. Additionally, blocking protein disulfide isomerase may be an additional mechanism that could yield significant anticoagulant action [18].

NAC has shown considerable promise in preclinical STEMI models. The beneficial effect of NAC on arterial thrombi may not just be limited to splitting the high-molecular-weight multimers of VWF (HMWvWF). In experimental rat myocardial infarction models exposed to tobacco smoke, created by ligation of the left anterior descending artery, NAC reduced infarct size and ameliorated the decline in fractional shortening[18]. NAC, in addition, resulted in a significant increase in the intra-cardiac mRNA expression of antioxidants [18]. Similar effects have been demonstrated in isoproterenol induced myocardial infarction in rat models [19].NAC also has significant antioxidant properties. As shown in a recent study, NAC may scavenge hypochlorous acid released secondary to activation of myeloperoxidase [20]. Myocardial salvage in patients treated with NAC was proportionate to myeloperoxidase levels in these patients[20]. High-dose NAC may effectively scavenge reactive oxygen species, thereby limiting oxidative stress and reperfusion injury [21]. In addition, NAC potentiates the vasodilator and anti-aggregatory effects of nitroglycerine. This potentiation accelerates the restoration of stable myocardial perfusion in the presence of subtotal epicardial coronary arterial obstruction[20].

**Hypothesis:**

We postulate that intravenous high-dose NAC would significantly reduce the myocardial infarct size, if administered very early, in STEMI patients allocated to a pharmaco-invasive reperfusion strategy in the presence of a large area of myocardium at risk.

**N-acetyl cysteine as a potential therapeutic agent to limit myocardial injury in STEMI:**

NAC has several potential therapeutic actions that may translate into clinical benefit in terms of limiting the infarct size in STEMI. The following are some of the proposed mechanisms of benefit:

1. **NAC is a potent thrombolytic agent:** In experimental mouse models, intravenous NAC administration promoted lysis of arterial thrombi that are resistant to conventional thrombolytics and anti-coagulants like recombinant tissue-type plasminogen activator, direct thrombin inhibitors, and anti-platelets [16,22]. This thrombolytic effect of NAC is mediated by reduction in the di-sulphide linkages that stabilize the HMWvWF found cross-linking platelets on the outer layers of the occlusive clot. NAC has been shown to be effective in treating microvascular thrombosis in preclinical and clinical models of acquired thrombotic thrombocytopenic purpura [22,23], a disease characterized by deficiency of ADAMTS-13 that cleave VWF multimers.

2. **NAC is shown to decrease myocardial infarct size in experimental animal models:** In experimental animal models of myocardial infarction, created by ligation of the left anterior descending artery, NAC reduces infarct size and ameliorates the decline in fractional shortening [18]. NAC, in addition, resulted in a significant increase in the intra-cardiac mRNA expression of antioxidants [18]. Similar effects have been demonstrated in isoproterenol induced myocardial infarction in animal models [19].

3. **NAC has significant antioxidant properties:** NAC is a scavenger of hypochlorous acid [24], via activation of myeloperoxidase. The extent of myocardial salvage in STEMI patients treated with high-dose IV NAC correlates with the myeloperoxidase levels present in plasma [20].

4. **NAC potentiates the effect of nitroglycerine:** NAC potentiates the vasodilator and anti-platelet effects of nitroglycerin [20,25,26].

**Clinical evidence for the utility of NAC in myocardial infarction:**

High dose intravenous NAC, in addition to nitrate therapy, reduced cardiac MRI assessed infarct size by 5.5% (absolute) in STEMI patients undergoing primary percutaneous intervention in a recent randomized placebo-controlled clinical trial [20]. Myocardial salvage was doubled in the NAC group compared to placebo [20]. However, there was no improvement in LV ejection fraction. Similarly, in STEMI patients undergoing thrombolysis with streptokinase, IV NAC in addition to nitrate therapy achieved a decrease in oxidative stress and a trend towards more rapid reperfusion and better preservation of LV function [27]. In patients with unstable angina, IV NAC in addition to nitrate therapy, lead to a lower rate of myocardial infarction [28]. Intracoronary administration of NAC may improve coronary perfusion and decrease peak high-sensitivity troponin levels [29].

Previous clinical studies attempting to replicate successful animal models of cardioprotective agents in STEMI failed due to several reasons. Firstly, timing of the experimental intervention matters. To achieve myocardial salvage, cyto-protective therapy must be administered prior to or at the time of onset of reperfusion[10]. The narrow window of 1 to 3 hours after symptom onset in patients presenting with STEMI is critical for achieving meaningful myocardial salvage [30].

Second, the absence of a large area of myocardium at risk may have contributed to negative outcomes of some studies. Cardio-protective therapies are likely to achieve better clinical outcomes in patients with a significant area of the LV at ischemic risk, such as in the case of an anterior myocardial infarction. Third, inadequate dosing of the therapeutic agent may be an issue. Only high dose NAC regimes have been shown to reduce myocardial infarct size. The NACIAM trial investigated the impact of NAC on the size of myocardial infarction in patients with STEMI who underwent percutaneous coronary intervention[20]. The study administered intravenous NAC in a total dose of 29 grams over a period of 48 hours[20]. The median total ischemic time for patients who received NAC in the NACIAM trial was 144 minutes with the highest quartile of patients receiving reperfusion therapy with primary PCI at 240 minutes or more [20]. Finally, STEMI patients presenting with markers of irreversible myocardial injury, including baseline Q-waves, have higher likelihood of in-hospital composite end point of death, congestive heart failure, cardiogenic shock, and re-infarction, irrespective of the time to reperfusion [6]. This group with baseline Q-waves may not respond favorably to cardio-protective therapies, since myocardium is already damaged. We considered the above-mentioned factors while designing our study.

We postulated that intravenous high-dose N-acetylcysteine could significantly reduce the myocardial infarct size, if administered very early prior to the development of Q-waves, in STEMI patients allocated to a pharmaco-invasive reperfusion strategy in the presence of a large area of myocardium at risk.

**Safety of intravenous N-acetylcysteine:**

Anaphylactoid reactions were reported in 8-17% of patients who received high-dose intravenous NAC for acetaminophen poisoing[31,32]. Majority of these reactions were cutaneous, occurring within the first few hours of initiation of NAC infusion [31]. Systemic reactions including respiratory distress and hypotension were rate (1 in 50 cases) [31]. Adverse reactions entail total discontinuation of NAC infusion in about 1 of 45 patients[32]. More commonly, the NAC infusion needs to be paused temporarily or slowed [33]. Another study looked at patients undergoing major vascular surgery and found that even though NAC decreased platelet aggregation and had anticoagulant effects, it did not result in increased intra-operative blood loss or transfusion requirement [34]. A randomized trial of intravenous NAC in patients undergoing PPCI did not show any evidence of increased major bleeding requiring blood transfusions in those receiving intravenous NAC[35]. Also, no safety concerns have been identified following the use of NAC in patients undergoing cardiac surgery[36,37].

**Why did we choose CMR-assessed infarct size as the primary endpoint?**

Cardiac magnetic resonance imaging (CMR) has become a valuable tool in quantifying infarct size and area at risk and myocardial salvage index during a single imaging session. Late gadolinium enhancement (LGE) imaging serves as a surrogate marker for infarct size, whereas T2-based imaging can be utilized for area at risk calculation. In addition, T1 imaging-based extracellular volume mapping contrast media administration also correlates well with LGE imaging assessed infarct size. Both these techniques are inherently limited by the rapid evolution of the infarct size over the initial week after the index infarct, making the time of measurement a key factor in standardizing infarct measurement. LGE imaging had better spatial resolution compared to extracellular volume mapping. Incorporating CMR-assessed myocardial infarct size

has several advantages. It not only provides a quantitative estimation of the cardioprotective effect of the drug therapy by considering both the area at risk and infarct size but also helps reduce the sample size in clinical trials evaluating cardioprotective therapies. Using a surrogate marker like CMR estimated myocardial infarct size enables design of more cost-effective trials with smaller sample sizes thereby accelerating the translation of experimental therapies to clinical practice.

Surrogate endpoints that demonstrate correlation with clinical outcomes have utility as endpoints in pilot clinical trials. Among CMR-derived endpoints, left ventricular ejection fraction stands out as the most significant, independently associated with major adverse events. Other CMR parameters, on the other hand, do not exhibit independent connections to "hard" clinical outcomes like death, myocardial re-infarction, or transplantation [38]. Nonetheless, microvascular obstruction, as identified by CMR following PCI in STEMI patients, shows a strong association with mortality and hospitalization for heart failure within one year [39]. Similarly, within the first month after primary PCI, infarct size as measured by CMR proves to be strongly linked to all-cause mortality and hospitalization for heart failure at one year [40]. Therefore, we adapted infarct size as an endpoint in this pilot clinical trial.

**Timing of cardiac MRI and its influence on myocardial infarct assessment:**

Following ischemia/reperfusion, dynamic changes occur in the tissue composition of the myocardium in the initial days after an infarct with temporal variations in size and structural composition of the myocardial infarct. Coronary artery occlusion and reperfusion, achieved using thrombolytic agents or primary PCI, triggers a rapid accumulation of myocardial edema,

leading to an increase in the area of irreversibly damaged myocardium. A distinctive feature of reperfusion injury is the pronounced extracellular edema that reaches its peak within the first 24 hours immediately after the infarct. This initial wave of edema, accompanying reperfusion, is also associated with swelling of cardiomyocytes. Subsequently, the intense extracellular edema undergoes rapid resolution, gradually diminishing over the first two days following the infarct accompanied by neutrophil infiltration. Around day three, another wave of edema occurs as part of the healing process and with lymphocyte and macrophage infiltration from days 3 to 4. By the end of the week, cardiomyocytes are replaced with collagen, resulting in significant tissue remodeling. Over time, there is progressive replacement of injured cardiomyocytes by collagen and extracellular matrix, leading to a notable reduction in myocardial thickness. This healing process gives rise to the late wave of edema, which can persist for days or even weeks depending on remodeling processes.

In the setting of CMR core-laboratories, two of the key factors contributing to variability in the quantification of infarct size is the subjectivity in delineation of endocardial and epicardial borders as well as the timing of the scan. The latter issue was examined in a study involving twenty-one patients who underwent primary percutaneous coronary intervention and received evidence-based therapy [41]. Each patient underwent three cardiac MRI scans at 48 hours, 3 weeks, and 6 months after the infarction. The study found that the presence of microvascular obstruction, a known unfavorable prognostic factor, affected the size of the peri-infarct zone. Both patients with and without MVO experienced a significant reduction in core infarct size over time. However, patients with MVO did not exhibit a significant change in PIZ size over time, while non-MVO patients showed a significant decrease in peri-infarct zone size as time progressed. These findings suggest that the size of the peri-infarct zone, like core infarct size,

varies depending on the timing of measurement [41]. Therefore, we elected to measure the cardiac infarct size at 3-5 days, a time period when the initial extensive periinfarct edema shows signs of resolution. The optimal timing for performing the acute CMR scan after the infarct remains a topic of debate, and there is currently no consensus in the field. Different proposed timeframes include 3 to 5 days and 4 to 7 days following PPCI [42,43].

# CHAPTER 2

# METHODS

## Study design:

A pilot open-label randomized controlled trial of high-dose intravenous NAC therapy as an adjunct to pharmaco-invasive strategy in patients presenting early after a large STEMI. Figure 1 depicts the study design.

## Study Population:

Per standard protocol, STEMI patients presenting within 3 hours of symptom onset, who are unlikely to receive primary percutaneous coronary intervention (PPCI) within 1 hour of first medical contact due to logistical reasons, were allocated to a pharmaco-invasive strategy utilizing the existing Vital Heart Response (VHR) system. The implementation of the VHR program in central and northern Alberta has established a crucial connection between Alberta Health Services Emergency Medical Services paramedics, emergency medicine physicians, and cardiologists within the Edmonton Zone. Operating around the clock, this program follows a hub and spoke model, with the University of Alberta Hospital serving as one of the key hub hospitals. Specifically, the VHR model adopted in the Edmonton region empowers paramedics with the necessary training to conduct symptom checklists and perform and interpret 12-lead ECGs in patients suspected of experiencing a myocardial infarction. These ECG readings are then transmitted over a dedicated software platform, where a physician is available on call to evaluate the ECG and checklist in collaboration with the paramedics via phone communication. Based on their assessment, the physician determines whether the patient is eligible for thrombolytic treatment, which is initiated by the paramedics. By implementing this regional Pre-Hospital

Myocardial Infarction program, significant improvements have been observed in terms of safety, reduced time to treatment, and enhanced patient outcomes.

These patients were initially treated medically with pre-hospital Tenecteplase, along with antiplatelet and systemic anticoagulant therapy, followed by coronary angiography and angioplasty as applicable within 2 to 24 hours. Such patients were screened for inclusion in the present trial on arrival at the PCI-capable hospital. If there was < 50% ST-segment resolution in the ECG lead with maximal ST-elevation (at 90 minutes post-thrombolysis) or clinical evidence of failed reperfusion within 90 minutes after fibrinolysis, rescue coronary intervention was performed.

**Inclusion criteria:**

Patients had to meet all the following criteria for inclusion in the trial.

1) Patients with anterior and/or inferior STEMI having >0.2mV ST elevation in two contiguous leads.
2) Onset of chest pain to administration of reperfusion therapy duration of <3 hours.
3) Absence of baseline Q-waves on the initial ECG: The presence of Q waves was using the Selvester QRS screening criteria at baseline [44] : a Q wave of ≥30 ms in lead aVF (inferior); ≥40 ms in leads I and aVL (lateral); or ≥40 ms in ≥2 leads V4 , V5 or V6 (apical); or any Q wave ≥20 ms or QS complex in leads V2 and V3 (anterior).
4) A pharmaco-invasive reperfusion strategy has been initiated.
5) Age more than or equal to18 years.

**Exclusion criteria:**

Patients meeting any of the following exclusion criteria were not randomized into the trial after the screening stage.

1) Previous myocardial infarction
2) Known to have moderate-to-severe LV systolic dysfunction (LV EF<45%)
3) Known allergy to thrombolytic therapy, NAC
4) Presence of left bundle branch block
5) Cardiogenic shock (Cardiogenic shock is defined as systolic blood pressure of <90mm Hg, for at least 30 minutes, not responsive to fluid resuscitation)
6) Permanent pacemaker or cardioverter defibrillator implanted previously.
7) Patients with contra-indications to thrombolytic therapy
8) Patients with loss of consciousness or confusion
9) Patients with known chronic kidney disease (GFR < 30ml/min/m2) or on dialysis
10) Current pregnancy

**Randomization:** Randomization was conducted using computer-based 1:1 randomization, employing a block randomization technique with randomly varied block sizes. The randomization procedure was facilitated through a validated instance of Redcap, a web-based secure electronic data capture platform that incorporates randomization capabilities.

**Treatment:** The ECG was transmitted by the emergency medical services (EMS) personnel to the VHR system physician and the coordinator. The VHR system is a centralized STEMI rapid response system for Northern Alberta which allows for triage, prompt diagnosis and rapid reperfusion – by allocating to either pre-hospital thrombolysis or primary percutaneous coronary intervention. If the VHR physician chose a pharmaco-invasive strategy and ordered pre-hospital

thrombolysis, the research coordinator would then assess the ECG criteria for inclusion in the trial. On arrival at the recruiting hospital, consenting STEMI patients allotted to a pharmaco-invasive strategy and fulfilling the eligibility criteria were randomized to either intravenous NAC bolus of 1200 mg followed by 1000mg/hour for the remaining 24 hours (in 5% dextrose) or standard therapy. Angiogram with or without coronary angioplasty within 2-24 hours of thrombolysis was recommended for all patients. Rescue primary coronary intervention was performed for patients who did not show ECG and clinical evidence reperfusion at 90 minutes following administration of pre-hospital thrombolytic.

**Outcomes:**

**Primary endpoint** was myocardial infarct size measured by late gadolinium enhancement CMR imaging at 3-5 days from first medical contact.

**Secondary efficacy end points**:

1) Myocardial salvage as measured by T2-weighted short tau inversion recovery on CMR assessed on day 3-5 days after first medical contact,
2) LV ejection fraction on CMR at 3-5 days

**Safety endpoints:**

1) Occurrence of anaphylactoid reactions
2) Bleeding (bleeding research consortium type II, III and V bleeding)[45]
3) Need for blood transfusions.

**Definitions:**

TIMI (Thrombolysis in Myocardial Infarction) risk index is a validated predictive clinical variable calculated from patient's age, blood pressure and heart rate at clinical presentation using the formula (heart rate × [age/10]$^2$/systolic blood pressure)[46]. Standardized definitions from the Bleeding Academic Research Consortium (BARC) were used to characterize bleeding[47].

The BARC bleeding criteria are categorized into five types[47]:

Type 0: no bleeding

Type 1: bleeding that does not prompt the patient to seek unscheduled medical interventions, including investigations, therapies and hospitalizations.

Type 2: any overt, actionable sign of hemorrhage that does not fit the criteria for type 3, 4, or 5 but requires nonsurgical, medical intervention by a healthcare professional, leads to hospitalization or increased level of care, or prompts additional workup.

Type 3, which is further classified into subtypes 3a and 3b, indicating overt bleeding with a hemoglobin drop of 30 to <50 g/L or need for transfusion, and overt bleeding with hemoglobin drop ≥50 g/L, cardiac tamponade, need for surgical intervention for control bleeding, or need for intravenous vasoactive agents, respectively. Type 3c represents intracranial hemorrhage and intraocular bleeding that affects vision.

Type 4 indicates bleeding related to CABG, and

Type 5 represents fatal bleeding, which is further classified into probable or definite fatal bleeding based on clinical features, autopsy, or imaging evidence.

**Cardiac MRI protocol:**

CMR evaluation, per protocol, was mandated between day 3 to day 5 following first medical contact after STEMI. A 1.5T MRI system (Magnetom Aera, Siemens Medical Solutions, Erlangen, Germany) was used to perform the analysis. The MRI system was equipped with dedicated phased-array cardiac receiver coils. With the patient in the supine position, we obtained initial scout images to circumscribe the short-axis plane of interest. We utilized a pre-contrast T2-weighted triple-inversion fast-spin echo sequence to delineate myocardial edema. Native T1 mapping was performed at baseline. Study patients were administered a standard weight-based intravenous dose of gadopentetate dimeglumine (MAGNEVIST) as a contrast agent [48]. At 3 to 8 minutes following contrast administration, we acquired contiguous short-axis cine images scanning the entire left ventricle with an ECG-gated short-axis free precession sequence (post-contrast SSFP). Images were acquired at the end of the expiratory cycle and were electrocardiogram gated. Late gadolinium-enhanced phase-sensitive inversion recovery (PSIR) imaging sequence was used for myocardial infarct size estimation. Late gadolinium enhancement (LGE) was performed after 10 to 20 minutes of contrast administration. LGE was analyzed using a 2-dimensional phase-sensitive inversion recovery gradient-echo pulse sequence in short axis images corresponding to the short-axis cine SSFPs. All analyses were performed by a research radiology technologist and a radiologist. Imagers visually identified areas of left ventricular LGE. Imagers identified the mean grayscale signal intensity (SI) and pixel-by-pixel standard deviation (SD) of normal LV myocardium for each participant. Myocardial infarction size was estimated using Signal Threshold versus Reference Mean (STRM) techniques based on thresholds of >5 SD above mean signal intensity of reference myocardium. A sensitivity analysis was performed using corresponding thresholds of >6 SD above the mean signal intensity of the reference myocardium.

Acute myocardial infarct size estimated by cardiac MRI is a stronger predictor of clinical outcomes when compared to measures of LV systolic function[49]. Cardioprotective therapies when administered to STEMI patients can decrease both the myocardial infarct size as well as the extent of tissue edema[50]. A wide array of MRI parameters has been analyzed in patients with STEMI to determine their utility in quantifying the extent of myocardial injury, investigate the efficacy of cytoprotective therapies in achieving myocardial salvage, as well as predict adverse clinical outcomes. We utilized pre- and post-contrast T1 mapping to quantify the extracellular space. Also, native (pre-contrast) T1-mapping images were used as a sensitive marker of myocardial edema. All T1 maps were acquired in a single breath hold. Ventricular volumes and function were assessed using 3- to 8-minute steady-state free precession (SSFP) post-contrast cine images. Volume and mass parameters including LV end-diastolic volume, LV end-systolic volume, RV end-diastolic volume, RV end-diastolic volume, RV and LV ejection fraction and LV mass, were calculated from computerized measurements after delineating the endocardial and epicardial borders at end-diastole and end- systole. Late gadolinium-enhanced phase-sensitive inversion recovery (PSIR) imaging was used to determine infarct size. We used late gadolinium-enhanced frames, corresponding to the short-axis cine SSFPs, acquired 10-minutes post-contrast. Late gadolinium hyper-enhanced myocardium with a signal intensity of five SD above the mean intensity of normal myocardial tissue was considered as a myocardial infarct. We expressed infarct size both in absolute terms (in grams) and as a percentage of the left ventricular muscle mass. The extent of edema on T2 mapping is conventionally used as secondary endpoint to determine the efficacy of cardioprotective therapies in patients with STEMI [38,50]. Area at risk (AAR) is the myocardium subtended by an occluded coronary artery that becomes ischemic and remains at risk of turning

into an infarct [38,51]. Both T2-short tau inversion recovery images and contrast enhanced SSFP images provide identical estimates of area at risk and myocardial edema[52].

These estimates of area at risk remain constant over the first week following a myocardial infarction and are not typically affected by treatment[52]. Following delineation of the epicardial and endocardial borders for myocardial infarct size estimation we utilized a post-contrast mid-diastolic SSFP frame for AAR measurement. Frames corresponding to the matching PSIR infarct assessment images were used for AAR measurement. A signal intensity of 2 SD above the mean value of the normal myocardium was used as the cut off definition for AAR hyper-enhancement. AAR was also indexed to the total myocardial mass.

Microvascular obstruction indicates persistent lack of perfusion at the coronary microcirculation level despite re-establishing epicardial coronary flow [53]. The extent of area affected is dynamic and stabilizes over 48- to 72-hours. Microvascular obstruction can be identified as a dark hypointense core within areas of hyperenhancement on either early gadolinium enhancement (early microvascular obstruction) or conventional late gadolinium enhancement (late microvascular obstruction) sequences. Microvascular obstruction was delineated manually as an area of hypo-enhancement within the infarcted myocardium on PSIR images. Myocardial salvage was calculated as the difference between area at risk and infarct size. Myocardial salvage was measured using T2-weighted short tau inversion recovery images as well as postcontrast-SSFP images. Myocardial salvage index was expressed as ratio of myocardial salvage to the area at risk, expressed as a percentage. All CMR studies were analyzed offline using CVI42 software (V.5.6; Circle Cardiovascular Imaging, Calgary, Alberta, Canada).

## MRI Study endpoints

The primary study endpoint was late gadolinium enhancement extent on cardiac MRI expressed both in terms of absolute mass (in grams) and a percentage of left ventricular muscle mass, documented at 3-5 days after first medical contact.

Secondary MRI endpoints included myocardial salvage index, microvascular obstruction, and LV volumes and systolic function (LV ejection fraction), determined at 3-5 days following first medical contact.

**Statistical Analysis:**

In this study, we presented descriptive data in terms of frequency with percentages for categorical variables and mean values with standard deviation or median values with interquartile range for continuous variables, as appropriate. To assess the comparability of baseline characteristics between the treatment and control groups, we employed the Pearson $\chi 2$ test and Fisher's exact test for categorical variables, while continuous variables were compared using the Student's t-test or Mann-Whitney U test. A type I error rate of $< 0.05$ was considered statistically significant. Safety data were analyzed using summary statistics, including percentages and mean values with standard deviation. Efficacy analyses were performed using the intent-to-treat analysis, analyzing the data according to the randomized treatment allocation, regardless of the treatment received. The statistical significance for the efficacy analyses was determined using the Student's t-test, which assessed the differences in means between the treatment and control groups. Additionally, the Shapiro-Wilk test was employed to assess the normality assumption of the data. The study was underpowered for conducting inferential statistical analysis, which would have enabled a multivariate analysis using techniques such as

linear regression or Cox models. This limitation prevented the assessment of relationships between the exposure variable and the primary or secondary outcomes of interest. Statistical analysis was performed using Stata, specifically version 13.0 developed by StataCorp.

**Sample size calculation:** We assumed a mean infarct size of 16 % in the placebo group and a standard deviation of 3%. To detect a decrease in infarct size of 3%, with a type 1 error rate of 5% and power of 80%, we needed 16 patients in each group. Assuming 20% of patients in either group cannot undergo cardiac MRI, due to claustrophobia, we planned to enroll 20 patients in each group.

**Figure 1. Study design**

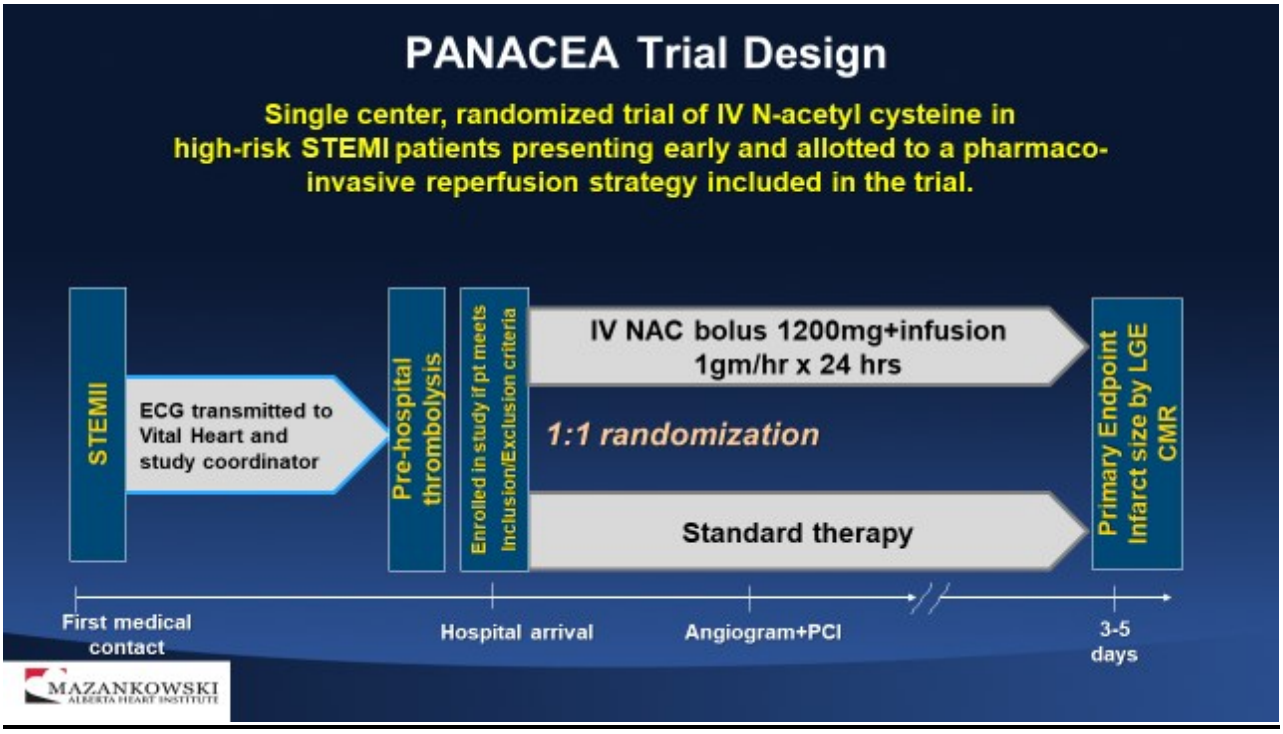
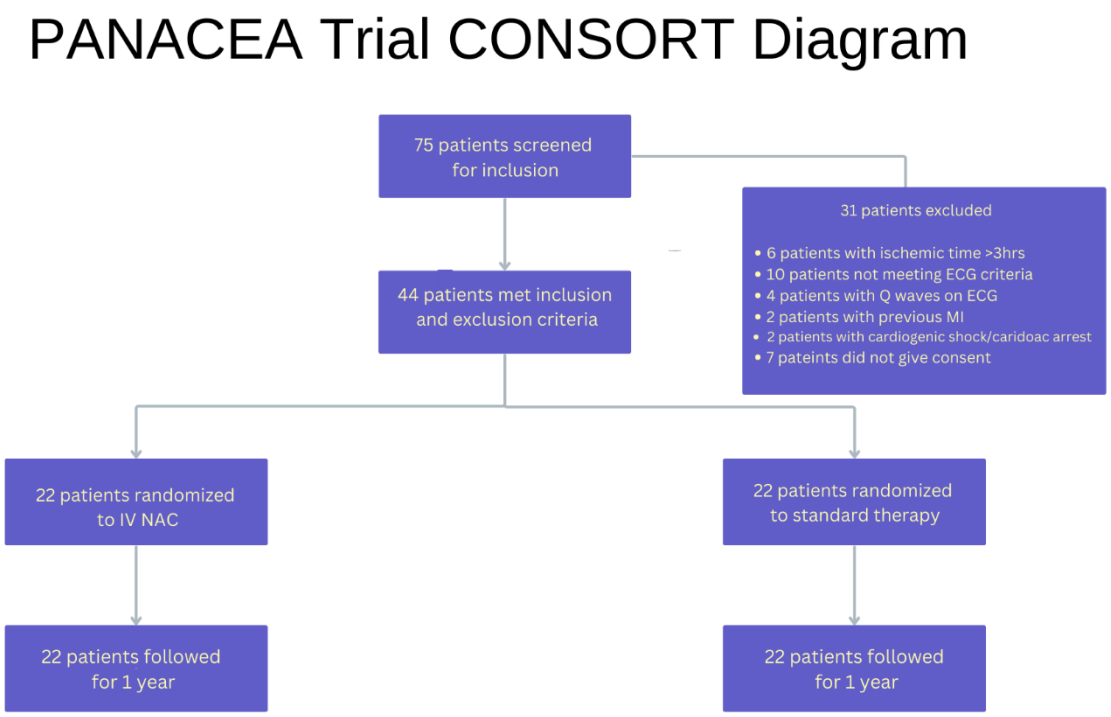


**Figure 2. CONSORT Diagram**



# CHAPTER 3

# RESULTS

## Baseline characteristics:

Table 1 depicts baseline characteristics of both group of patients. Figure 2 depicts the CONSORT diagram. We enrolled study patients from September 2019 to November 2020. All patients were clinically followed up for one year. Twenty-two patients were randomized to each of the two study groups: intravenous NAC group or the standard therapy (control) group. Baseline characteristics of both the treatment and control groups are listed in Table 1. Patients randomized to the intervention arm (intravenous NAC group) were similar to the control arm in terms of age (58.3[11.1] versus 57.1 [8.5] years, p=0.65), body mass index (29.9[5.8] versus 28.2 [3.5] kg/$m^2$, p=0.22), the proportion of women (13.6% versus 18.2%, p=0.68), and proportion with an anterior myocardial infarction (40.9% versus 45.5%, p=0.76). Emergency medical services (EMS) transferred 61.3% of patients from the community to PCI-capable hospital, with the rest presenting directly to the PCI-capable hospital. No difference in the proportion of EMS-transferred patients between the two groups. None of the patients had heart failure at admission. The heart rate (69±19 bpm versus 78±17 bpm, p=0.06), blood pressure (146±28 bpm versus 143±25 bpm, p=0.62) and TIMI risk index (16.8±7.6 bpm versus 18.9±7.1 bpm, p=0.17) documented at first medical contact were similar between the NAC and control groups, respectively. One patient in the NAC group was in complete heart block and another patient in the control arm had left bundle branch block at presentation. There was a trend towards lower number of patients in the NAC group being non-smokers compared to the control arm (18.2% versus 40.9%, p=0.05). The proportion of patients with comorbidities including diabetes (NAC:22.7% versus control:45.5%, p=0.11), hypertension (NAC:50.0% versus control:45.4%, p=0.76) and dyslipidemia (NAC:90.9% versus control:86.4%, p=0.51) were

similar across both groups. The proportion of patients with family history of premature coronary artery disease was similar across groups (NAC:18.2% versus control:22.7%, p=0.71). One patient in the control group had prior history of heart failure. More patients in the NAC group had a prior PCI compared to the control group (18.2% versus 0%, p=0.04). None of the patients in either group had prior coronary artery bypass graft surgery. No patient had a prior history of cerebrovascular accident, peripheral arterial disease, or paroxysmal atrial fibrillation. One patient in the control group was in atrial fibrillation at presentation. There was a non-significant trend for more patients in the NAC group to have underlying chronic obstructive airway disease (NAC: 22.7% versus control: 4.6%, p=0.08). Only one patient in the control group had cirrhosis or chronic liver disease at baseline. The glomerular filtration rate was similar across both the groups (NAC:77.6[18.4] versus control:83.5 [14.7] ml/min/1.73m$^2$, p=0.13), and none of the patients were on dialysis at baseline. No patient had a permanent pacemaker or other known contraindication to performing a cardiac MRI at baseline.

**Performance indicators:**

Table 2 depicts the performance indicators across randomized groups. All patients received thrombolytic therapy with a symptom-to-needle time of 1.5 (± 0.7) hours. Performance indicators including total ischemic time (NAC:1.5±0.7 hours versus control:1.6±0.7 hours, p=0.52), chest pain to first medical contact time (NAC: 0.8±0.6 hours versus control:0.9±0.6 hours, p=0.48) and first medical contact to electrocardiogram time (NAC:0.12±0.13 hours versus control:0.15±0.13 hours, p=0.48) were similar between both groups. The door-in door-out time for patients transferred from community emergency rooms (NAC:1.6±1.0 hours versus control:1.1±0.4 hours, p=0.17) were similar across groups. The time from arrival at catheterization lab to device time was similar across both groups (NAC: 0.24±0.13 hours versus

0.2±0.08 hours, p=0.18). A total of 42 (95.5%) patients underwent percutaneous coronary intervention after randomization. Of those undergoing PCI, 6 patients in the NAC group (27.3%) and 7 patients in the control group (35%) underwent staged PCI to a non-culprit artery. Percutaneous coronary intervention was performed after 16.7±16 hours following pre-hospital thrombolysis with not significant difference between groups (NAC: 17±18.5 hours versus 16.4±2.9 hours, p=0.27).

**Medications:**

Table 3 depicts adjunctive pharmacotherapy use across study groups. All recruited patients were administered reperfusion therapy with pre-PCI capable hospital administration of tenecteplase. Of these, weight-based dosing of Tenecteplase was used in all patients but for one (one patient in the standard arm administered half-dose Tenecteplase). Adjunctive low molecular weight heparin (LMWH) was administered in all patients at the time of thrombolysis, but for one patient in the standard arm. An intravenous dose of enoxaparin was administered in 21 (95.5%) patients in the NAC arm and 20 (90.91%) patients in the control arm, with similar dose used in both the groups (NAC: 30.9±4.4 IU versus 32.8±12.7 IU, p=0.7). A subcutaneous dose of enoxaparin was administered in 22 (100.0%) patients in the IV N-acetyl cysteine arm and 21 (95.5%) patients in the control arm, with similar dose used in both groups (NAC: 86.7±12.6 IU versus 79.3±18.4 IU, p=0.06). A similar proportion of patients were on aspirin at baseline in both groups (14.3% versus 4.6%, p=0.27). None of the subjects were on oral anticoagulation at baseline. All patients received a loading dose of aspirin in both the groups. The mean loading dose of aspirin administered was similar in both groups (181.5±48.6 mg versus 202.8±70.8 mg, p=0.87). A majority of patients were administered a loading dose of clopidogrel 300mg in both groups (100% versus 90.9%, p=0.49), at the time of thrombolysis. A

loading dose of 180mg ticagrelor was administered in slightly less than two-thirds of patients in both groups (57.1% versus 59.1%, p=1.0). All patients who did not receive an initial clopidogrel loading dose were loaded with ticagrelor. Glycoprotein 2b3a inhibitors were not used as part of PCI for any of the patients.

**Clinical outcomes:**

Table 2S in the supplementary section enumerates the clinical and safety outcomes across the two groups. No mortality was reported at 30 days or at 1-year of follow-up in either group. Only one patient, in the intravenous NAC arm, had contrast-induced nephropathy with increase in creatinine of 57 μmol/L. One patient in the control group had transient atrial fibrillation and another in the NAC group had resuscitated cardiac arrest/ ventricular fibrillation. No patient in either group had a recurrent myocardial infarction. Two patients in the NAC group developed congestive heart failure, one patient while in-hospital during the indexed STEMI episode and another patient requiring re-admission for heart failure on follow-up. No statistically significant difference in the incidence of congestive heart failure at one year between the two groups (NAC: 9.1% versus control: 0%, p=0.15). At 1 year, the clinical composite of death, congestive heart failure, and recurrent myocardial infarction were similar across NAC and standard therapy groups.

**Adverse and Safety Outcomes:**

The study drug bolus dose and infusion were well tolerated in all except one patient who had congestive heart failure necessitating discontinuation of NAC infusion. The study drug was initiated in all, but one patient, randomized to the NAC arm. Study drug (bolus and infusion) was not initiated in a patient randomized to intravenous NAC due to presence of a possible allergic

reaction to another drug causing oral and facial edema. None of the study patients experienced an anaphylactoid reaction or hypotension with study drug administration. No patient randomized to standard therapy in the control arm received N-acetyl cysteine. A total of 6 patients, three (13.6%) in each arm, experienced a bleeding event. In patients administered NAC, one patient had BRC type 2 (minor) bleed, a second patient had BRC type 3a bleed and yet another patient had type 4 (CABG-related) bleed. All 3 patients in the control group who experienced bleeds had BRC type 2 (minor) bleed. One patient in the NAC group had an ischemic stroke, whereas none of the patients in the standard therapy group had a stroke. No stent thrombosis was reported in the either group.

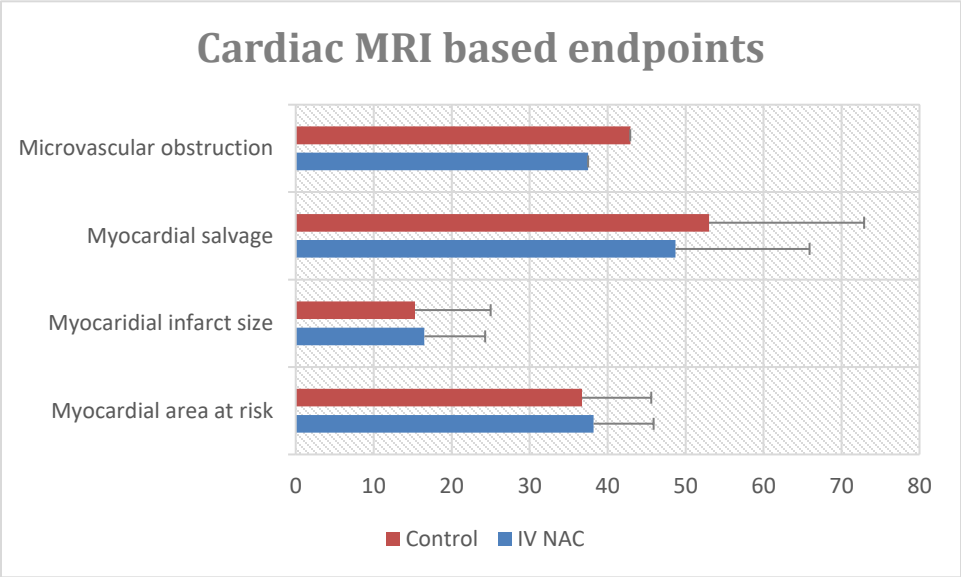
**Imaging outcomes:**

Table 4 depicts MRI variables across randomized groups. Figure 3 illustrates the cardiac MRI endpoints. The primary study endpoint of infarct size determined by late gadolinium enhancement extent on cardiac MRI did not differ significantly between the two groups (mean [standard deviation] infarct size as a percentage of LV myocardial mass, NAC: 16.5 [7.8]% versus control:15.3 [9.7] %, p=0.70). Median (inter-quartile range) time to cardiac MRI assessment after first medical contact was 3 (2, 5) days. LV volumetric measurements, including LV end-diastolic volume index (NAC: 75.6±17.5 ml/$m^2$ versus control:71.7±14.1 ml/$m^2$, p=0.78) and end-systolic volume index (NAC: 38.7±11.7 ml/$m^2$ versus control:35.4±9.5 ml/$m^2$, p=0.83) were similar between the two groups. Also, the LV stroke volume (NAC: 76.6±20.9 ml versus control:72.9±18.3 ml, p=0.54) and LV ejection fraction (NAC: 49.3±6.9% versus control:50.6±7.9%, p=0.58) were similar across both groups. Similarly, RV volumes including RV end-diastolic volume index (NAC: 64.6±16.1 ml/$m^2$ versus control:64.5±13.6 ml/$m^2$, p=0.98) and RV end-systolic volume index (NAC: 32.7±13.3 ml/$m^2$ versus control:30±9.2 ml/$m^2$, p=0.46)

were similar between the two groups. RV systolic functional indices including RV stroke volume (NAC: 68.2±19.1 ml versus control:71.1±17.1 ml, p=0.62 and RV ejection fraction (IV NAC: 52.9±7.6 % versus control:54.5±7.0%, p=0.49) were similar across both the groups. There was no difference in cardiac output across both groups (NAC: 4.8±1.1L/min versus control: 4.6±1.1L/min, p=0.53).

The myocardial area at risk was not significantly different across groups (NAC: 38.2±7.7 % versus control: 36.7±8.9%, p=0.59). Myocardial microvascular obstruction was present in 6 (37.5%) patients in the intravenous NAC arm and 9 (42.9%) patients in the standard therapy arm, p= 0.74. Myocardial salvage, estimated using a 5-SD cut-off for myocardial infarct sizing, was not significantly different across groups (NAC: 18.4±7.2%versus control: 18.4±6.0%, p=0.99). Similarly, the myocardial salvage index estimated using a 5-SD cut-off for myocardial infarct sizing was similar across both the groups (NAC: 48.7±17.2% versus control: 53.0±19.9%, p=0.49). Results were consistent irrespective of the 5-SD or 6-SD definitions being used to calculate the infarct size. Also, the myocardial salvage index as calculated by the postcontrast-SSFP was not significantly different between the two groups.

**Figure 3. Cardiac MRI outcomes:**




**Legend:** *IV NAC- intravenous N-acetyl cysteine. Microvascular obstruction expressed as percentage of patients showing evidence of microvascular obstruction on cardiac MRI. Myocardial area and risk and myocardial infarct size expressed as as a percentage of the left ventricular muscle mass. Myocardial salvage index was expressed as ratio of myocardial salvage to the area at risk, expressed as a percentage.*

**Table 1. Baseline characteristics of patients randomized to the N-acetyl cysteine treatment arm and the control standard treatment arm.**

| Characteristics | N-acetyl cysteine intravenous (n=22) | Control arm/standard therapy (n=22) | P value |
|---|---|---|---|
| | N (%) | N (%) | |
| **Age** (years) mean (SD) | 58.3 (11.1) | 57.1 (8.5) | 0.65 |
| **Male** | 19 (86.4) | 18 (81.8) | 0.68 |
| **Body mass index** ($Kg/m^2$) mean (SD) | 29.9 (5.8) | 28.2 (3.5) | 0.22 |
| **STEMI type** | | | |
| Anterior wall involved | 9 (40.9) | 10 (45.5) | 0.76 |
| Inferior wall involved | 13 (59.1) | 13 (59.1) | 1.00 |
| Posterior wall involved | 3 (13.6) | 4 (18.8) | 0.68 |
| Lateral wall involved | 2 (9.1) | 2 (9.1) | 1.00 |
| **Presentation** | | | 0.75 |
| EMS transferred from the community | 13(59.1) | 14(63.6) | |
| Directly presenting to ER | 9(40.9) | 8(36.4) | |
| Heart failure at admission, n (%) | 0(0) | 0 (0) | |
| Heart block at admission, n (%) | 1 (4.6) | 0 (0) | 0.31 |
| Heart rate at admission, mean (SD) | 69 (19) | 78 (17) | 0.06 |
| SBP at admission, mean (SD) | 146 (28) | 143 (25) | 0.62 |
| TIMI Index, mean (SD) | 16.8 (7.6) | 18.9 (7.1) | 0.17 |

| | | | |
|---|---|---|---|
| Left bundle branch block, mean (SD) | 0 (0) | 1 (4.6) | 0.31 |
| Pacemaker, n (%) | 0 (0) | 0 (0) | |
| Never smoker, n (%) | 4 (18.2) | 9 (40.9) | 0.05 |
| Current smoker, n (%) | 8 ((36.4) | 10 (45.4) | |
| Former smoker, n (%) | 10 (45.4) | 3 (13.6) | |
| **Comorbidities** | | | |
| Diabetes, n (%) | 5 (22.7) | 10(45.5) | 0.11 |
| Dyslipidemia, n (%) | 20 (90.9) | 19 (86.4) | 0.51 |
| Hypertension, n (%) | 11 (50.0) | 10 (45.4) | 0.76 |
| Prior PCI, n (%) | 4 (18.2) | 0 (0) | 0.04 |
| Prior CABG, n (%) | 0(0) | 0 (0) | |
| Family history of premature coronary artery disease, n (%) | 4 (18.2) | 5 (22.7) | 0.71 |
| GFR | 77.6 (18.4) | 83.5 (14.7) | 0.13 |
| Dialysis, n (%) | 0(0) | 0 (0) | |
| Peripheral arterial disease, n (%) | 0(0) | 0 (0) | |
| Prior CVA, n (%) | 0(0) | 0 (0) | |
| History of paroxysmal atrial fibrillation at baseline, n (%) | 0(0) | 0 (0) | |
| Atrial fibrillation at presentation | 0 (0) | 1 (4.6) | 0.31 |
| COPD | 5 (22.7) | 1 (4.6) | 0.08 |
| Cirrhosis | 0 (0) | 1 (4.6) | 0.31 |
| History of heart failure | 0 (0) | 1 (4.6) | 0.31 |

| | | | |
|---|---|---|---|
| **Baseline Medications** | | | |
| Aspirin | 3 (14.3) | 1 (4.6) | 0.27 |
| Oral anticoagulation | 0(0) | 0 (0) | |
| | | | |
| **Table 2. Reperfusion performance indicators across randomized groups** | | | |
| **Performance indicators** | | | |
| Total ischemic time | 1.5 (0.7) | 1.6 (0.7) | 0.52 |
| Chest pain to FMC | 0.8 (0.6) | 0.9 (0.6) | 0.48 |
| FMC to diagnosis | 0.12 (0.13) | 0.15 (0.13) | 0.48 |
| Door-in Door-out time for community emergency departments | 1.6 (1.0) | 1.1 (0.4) | 0.17 |
| Time from arrival at cath lab to device time | 0.24 (0.13) | 0.2 (0.08) | 0.18 |
| Time from fibrinolysis to device | 17.0 (18.5) | 16.4 (2.9) | 0.91 |

**Table 3. Adjunctive pharmacotherapy across randomized groups**

| **Treatment** | **IV NAC** | **Control arm** | **p-value** |
|---|---|---|---|
| Thrombolysis | 22 (100.0) | 22 (100.0) | 1.0 |
| LMWH, n (%) | 22 (100.0) | 21 (95.5) | 1.0 |
| IV LMWH, n (%) | 21 (95.5) | 20 (90.9) | 1.0 |
| SC LMWH, n (%) | 22 (100.0) | 21 (95.5) | 1.0 |
| IV LMWH dose | 30.9 (4.4) | 32.8 (12.7) | 0.7 |
| SC LMWH dose | 86.7(12.6) | 79.3 (18.4) | 0.06 |
| Aspirin loading | 22 (100.0) | 22 (100.0) | 1.00 |
| Aspirin loading dose | 181.5 (48.6) | 202.8 (70.8) | 0.87 |
| Clopidogrel loading | 22 (100.0) | 20 (90.9) | 0.49 |
| Clopidogrel loading dose | 300 (0) | 300 (0) | |
| Ticagrelor loading | 12 (57.1) | 13 (59.1) | 1.0 |

**Table 4. Magnetic resonance imaging variables across the two randomized groups**

| MRI outcome variable | IV NAC | Control arm | p-value |
|---|---|---|---|
| Area at risk, %LV, mean (SD) | 38.2 (7.7) | 36.7 (8.9) | 0.59 |
| Infarct size, %LV | 19.7 (7.9) | 18.2 (10.1) | 0.62 |
| Myocardial salvage, %LV | 18.4 (7.2) | 18.4 (6.0) | 0.99 |
| Myocardial salvage index | 48.7 (17.2) | 53.0 (19.9) | 0.49 |
| Early microvascular obstruction present, n (%) | 6 (37.5) | 9 (42.9) | 0.74 |
| LV ejection fraction, % | 49.3 (6.9) | 50.6 (7.9) | 0.58 |
| LV end-diastolic volume index, ml/m2 | 75.6 (17.5) | 71.7 (14.1) | 0.78 |
| RV ejection fraction, % | 52.9 (7.6) | 54.5 (7.0) | 0.49 |
| RV end-diastolic volume index, ml/m2 | 64.6 (16.1) | 64.5 (13.6) | 0.98 |

LV = left ventricular, RV = right ventricular, SD = standard deviation

# CHAPTER 4

# DISCUSSION

In this investigator-initiated, single-center, double-arm pilot randomized controlled trial of high-dose intravenous NAC in high-risk STEMI patients undergoing early reperfusion using a pharmaco-invasive strategy, the study drug did not significantly reduce early myocardial infarct size when compared to standard therapy. Moreover, neither myocardial salvage, microvascular obstruction, nor indices of cardiac function differed considerably between the two study groups. Safety outcomes, including BRC type 2, 3, and 5 bleeding as well as allergic reactions, did not differ significantly across groups.

The translation of cardio-protection observed in animal STEMI models into the clinical setting has proven to be exceptionally challenging. There are notable differences between experimental animal models and actual STEMI patients. These differences encompass a wide range of clinical and pathological factors, such as age, comorbidities, the presence of an inflammatory environment within the coronary arteries, the mechanism of coronary occlusion (plaque rupture versus vessel ligation), duration of ischemia, method of reperfusion, use of anesthesia, adjunct pharmacotherapy, pre-existing coronary artery disease, and outcome measures [9,54–56]. Small animal models used in experimental trials often lack genetic predisposition and conventional risk factors associated with coronary artery disease in humans [57]. Also, advanced age and multiple comorbidities in STEMI patients can potentially diminish or eliminate the cardioprotective effects provided by adjunct therapies [58,59].

Rossello and Yellon have shown that clinical proof-of-concept studies have a crucial role to play in connecting the evidence of cardio-protection observed in clinically relevant animal studies to the large-scale clinical outcome trials [60]. These studies serve as a bridge, facilitating the transition from preclinical research to the evaluation of cardioprotective strategies in real-world

multi-center trial settings. In our study, we adhered to several guiding principles to increase the likelihood of demonstrating significant myocardial salvage with the study drug in STEMI patients. Firstly, we recognized that prolonged total ischemic time could hinder the cardioprotective effects of adjunctive therapies [61]. There is significantly less myocardial salvage in STEMI patients who undergo reperfusion after 120 minutes from symptom onset[62]. To address this, we enrolled patients who received reperfusion therapy within 3 hours of symptom onset, aiming to minimize the impact of prolonged ischemic time. Secondly, we aimed to maintain a homogeneous study population by limiting the inclusion criteria to patients undergoing early pre-hospital thrombolysis followed by a pharmaco-invasive management strategy. Both pre-hospital fibrinolysis and timely primary PCI have demonstrated similar rates of reperfusion and survival in patients presenting early after a STEMI [63,64]. Thirdly, we excluded patients who presented with baseline Q-waves following a STEMI. This was based on the understanding that established Q-waves may serve as a surrogate marker for completed myocardial infarction and are associated with poor outcomes [6,65]. By excluding such patients, we aimed to ensure a study population with a higher likelihood of responding predictably to cardioprotective therapies. Fourth, we recognized the importance of establishing the efficacy of the investigational cardioprotective drug through multiple proof-of-concept clinical trials in various settings before proceeding to large-scale clinical outcomes trials [66]. This approach ensures that the drug's effectiveness is validated across diverse patient populations and clinical scenarios, bolstering the evidence base and justifying the progression to larger trials assessing clinical endpoints. Since intravenous NAC was previously evaluated in the primary PCI setting[20], we decided to study pharmaco-invasively managed STEMI.

We felt that it would be beneficial to have an investigative agent that can potentiate both endogenous as well as pharmacologically administered thrombolytic activity and prevent reperfusion injury. Intravenous NAC mediates both actions. Reperfusion injury is maximal within

the initial 3 hours following reperfusion[67]. The timing of drug administration to prevent reperfusion therapy is paramount in reducing infarct size. We, therefore, mandated that the study drug be administered as soon as the patient presents to the recruiting hospital after pre-hospital thrombolysis. Thus, with this study design, given the timing of study drug administration we achieved, may have relied more on the thrombolytic potentiation effect of high-dose intravenous NAC than reperfusion prevention.

Eight trials have evaluated the clinical efficacy of NAC in patients with STEMI. Of these eight trials, six used either intravenous (3 trials) or oral (3 trials) administered NAC; another trial used both intravenous and oral administered NAC, and yet another used intravenous as well as intracoronary NAC. A total of 1740 patients were enrolled across these trials. Studies evaluating oral NAC, without intravenous bolus, in STEMI have been primarily neutral. In a trial by Thayssen et al., the composite of cardiac death, myocardial infarction, and target vessel revascularization at 30-month follow-up did not differ significantly between the groups, irrespective of the use of oral NAC[68]. A Japanese trial evaluating oral NAC in STEMI did not report any in-hospital difference in the proportion of patients developing clinical congestive heart failure. Notwithstanding the trial results of Talasaz et al.[69], oral NAC is unlikely to be efficacious in patients with STEMI. This is not surprising since the bbioavailability of oral NAC is low, with significant first-pass metabolism in the gut wall and liver. Bioavailability varies between 6-10%[70,71]. Also, patients with STEMI may have nausea and vomiting, making oral NAC poorly tolerated. Therefore, oral NAC, at conventionally administered doses, is unlikely to achieve a high enough concentration in the coronary circulation at the time of or immediately after reperfusion.

Other trials have evaluated intravenous NAC, administered intravenously only or along with oral or intracoronary doses (table 5). In the study by Marenzi et al., 11% of the control group

died compared to 4% of the standard-dose NAC arm and 3% of the high-dose NAC arm (p=0.02)[35]. This study used intravenous bolus followed by oral NAC[35] The NACIAM trial demonstrated the efficacy of intravenous NAC and intravenous nitroglycerine in achieving myocardial salvage in STEMI patients undergoing primary PCI[20]. It is unclear if intravenous NAC would achieve myocardial salvage without the concomitant use of intravenous nitroglycerin as a potentiating agent. In contrast, the LIPSIA-N-ACC trial did not report a significant difference in myocardial salvage index, mortality, or major adverse cardiac events with intravenous NAC compared to placebo[72]. There are substantial differences in the duration of follow-up and mortality rates reported across these small trials. Overall mortality rates range from 0-9.6%[72,73].

| Author | Year of publication | Geographic location | Number of randomized patients | | Type of reperfusion | Mortality in intervention and control group | Mode of administration of NAC |
|---|---|---|---|---|---|---|---|
| | | | NAC | Control | | | |
| Marenzi[35] | 2006 | Italy | 235 | 119 | PPCI | 3.4% (NAC) vs. 11% (control), in-hospital | Standard NAC arm (600-mg IV bolus prior to PPCI and 600 mg orally twice daily x 48 hours after PPCI), double dose arm: 1200mg IV bolus NAC and 1200mg orally twice daily x 48 hours after PPCI |
| Thiele[72] | 2010 | Germany | 126 | 125 | PPCI | 9.6% (NAC) vs. 9.6% (control), at 6 months | NAC arm: IV bolus of 1200mg prior to PPCI and 1200 mg IV twice daily for the 48 hours after PPCI (cumulative dose 6,000 mg) |
| Tanaka[73] | 2011 | Japan | 38 | 38 | PPCI | No deaths in either group, in hospital | NAC arm: 705 mg administered orally prior to and at 12, 24, 36 hours after PPCI |
| Sochman[74] | 1996 | Czech | 18 | 16 | Thrombolysis/ streptokinase | Not reported | 50 mg/Kg intravenous NAC bolus; 50 mg/Kg intravenous NAC infusion over 30 minutes |
| Pasupathy[20] | 2017 | Australia | 53 | 59 | PPCI | 0% (NAC) vs. 3.4% (control), in-hospital | Intravenous NAC (29 g over 48 hours) and low-dose nitroglycerin (7.2 mg over 48 hours) |
| Thayssen[68] | 2014 | Denmark | 353 | 362 | PPCI | Individual group mortality not reported | Two of the four arms used NAC 1200 mg orally before PPCI followed by 1200 mg daily for 48 hours |
| Eshraghi[75] | 2016 | Iran | 50 | 50 | PPCI | 0% (NAC) vs. 9.6% | Intravenous NAC 100mg/kg IV bolus followed by intracoronary NAC 480mg |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | (control), at 1-month | during PPCI and IV NAC infusion of 10mg/Kg for 12 hrs. |
| Talasaz [69] | 2014 | Iran | 50 | 48 | Thrombolysis or PPCI | 4% (NAC) vs. 10.4% (control), at 1-year | NAC 600mg oral twice daily for 3 days |

Table 5. **Previous trials using NAC in STEMI**

The inconsistency in the reduction of clinical events seen across trials may be related to differences in ischemic time, the timing of NAC initiation, the route of drug administration, initial NAC intravenous bolus dosage, the proportion of patients with a large myocardial infarction, sample size, and the clinical profile of patients recruited into the trial. In addition, differences in the extent of myocardial salvage may be due to variance in MRI protocols used for measuring the area at risk[50]. It is appealing to consider proceeding with an adequately powered large study to evaluate the effect of high-dose intravenous NAC on clinical outcomes in STEMI. Before proceeding with a large clinical trial, we should be able to achieve consistent results in meaningful surrogate endpoints across smaller trials. Given the divergence in results across previous small trials, the likelihood of a neutral trial result is high.

Unfortunately, we could not replicate the results of the NACIAM trial in this STEMI population undergoing pharmaco-invasive reperfusion. There are several reasons for this apparent lack of benefit. Total ischemic time with reperfusion by pharmaco- invasive strategy is shorter than with primary PCI[76]. Ischemic time may account for differences in the extent of reperfusion injury and thrombus composition. As a result, differences in ischemic time across studies may account for differences in infarct size reduction with adjunctive therapies. The total ischemic time in our study of 1.4 [IQR:1.0-2.8] hours was shorter when compared to that of the patients receiving IV NAC in the NACIAM trial (2.4 [IQR:1.67-4] hours). Furthermore, intravenous NAC may have reduced not

only the myocardial infarct size but also the extent of myocardial edema or the area at risk. This impact on the degree of myocardial edema can translate to there being no significant improvement in myocardial salvage. Also, intravenous NAC was combined with nitrates for a synergistic effect in the NACIAM trial[20]. Since we mainly aimed to achieve myocardial salvage through the thrombolytic potentiating effect of NAC, we did not combine intravenous NAC with nitrate infusion. Additionally, we may have measured myocardial salvage and infarct size using cardiac MRI too early. Measurements too early or late during the myocardial infarct can affect measurements. Clinical stability of the patient and logistic considerations, including preference for a shorter hospital stay and cardiac MRI slot availability, may impact the timing of cardiac MRI in larger trials. Also, patients who are clinically unstable from a massive myocardial infarction may be deemed too sick to undergo cardiac MRI. Such patients may not receive a cardiac MRI, or receive a delayed scan, thereby impacting cardiac MRI reported outcomes. Also, the timing of PCI was not standardized in our trial. PCI may result in a second wave of micro-thrombi release, from the plaque, resulting in microvascular obstruction. Finally, the extent of microvascular obstruction may vary depending on the antecedent use and timing of anti-thrombotic and anti-platelet therapy. In this trial, NAC was administered only after thrombolysis prior to the patient reaching the PCI-capable hospital. Delayed administration of IV NAC may have resulted in the drug no longer mitigating the arterial thrombosis in a meaningful way. The results obtained in this study should be regarded as exploratory in nature, as we aimed to generate a hypothesis to identify a suitable STEMI population that will benefit from this molecule and explore potential relationships between variables. However, it is important to note that these exploratory findings are susceptible to the risk of false-negative results or type II errors.

## 5. Conclusions:

High-dose IV NAC did not reduce myocardial infarct size in high-risk patients reperfused early with a pharmaco-invasive strategy after a large STEMI with significant amount of myocardium at risk. The translation of cardioprotective therapies from animal studies to the clinical care of STEMI patients continues to pose significant challenges. This pilot randomized trial adheres to current guidelines for translational research and provides a valuable framework for trial design when evaluating cardioprotective therapies in STEMI cases.

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# Appendices

**Table 1S. Additional MRI Data**

| MRI variables | IV NAC | Control arm | p-value |
|---|---|---|---|
| Left atrial volume index, ml/m$^2$ | 39.3 (14.2) | 40.1 (11.4) | 0.42 |
| LV end diastolic volume, ml | 157 (43.1) | 145.2 (36.4) | 0.82 |
| LV end systolic volume, ml | 80.4 (27.6) | 72.4 (23.4) | 0.83 |
| LV end systolic volume index, ml/m$^2$ | 38.7 (11.7) | 35.4 (9.5) | 0.83 |
| LV myocardial mass, mean (SD), g | 137.3 (30.8) | 130.6 (32.6) | 0.51 |
| LV mass index, mean (SD), g/m$^2$ | 65.8 (9.6) | 64.2 (11.5) | 0.64 |
| Cardiac output, L/min | 4.8 (1.1) | 4.6 (1.1) | 0.53 |
| RV end diastolic volume index, ml/m$^2$ | 64.6 (16.1) | 64.5 (13.6) | 0.98 |
| RV end systolic volume, ml | 62 (19.7) | 60.7 (20.7) | 0.84 |
| RV end systolic volume index, ml/m$^2$ | 32.7 (13.3) | 30 (9.2) | 0.46 |
| LGE myocardial volume, mL | 96.8 (18.2) | 101 (26.5) | 0.58 |
| Infarct size based on LGE > 6SD, %LV | 16.5 (7.8) | 15.3 (9.7) | 0.70 |
| Infarct size based on LGE > 5SD, %LV | 19.7 (7.9) | 18.2 (10.1) | 0.62 |
| No reflow volume, mL | 0.9 (3.1) | 0.5 (1.1) | 0.65 |
| Microvascular obstruction present, n (%) | 6 (37.5) | 9 (42.9) | 0.74 |
| Myocardial area at risk (AAR), mean (SD) % | 38.2 (7.7) | 36.7 (8.9) | 0.59 |
| Myocardial salvage (AAR%-5SD%) mean (SD) % | 18.4 (7.2) | 18.4 (6.0) | 0.99 |
| Myocardial salvage index (5SD), mean (SD) % | 48.7 (17.2) | 53.0 (19.9) | 0.49 |
| Myocardial salvage (AAR%-6SD%) mean (SD) % | 21.7 (8.0) | 21.4 (6.6) | 0.88 |
| Myocardial salvage index (6SD), mean (SD) % | 57.2 (18.2) | 60.9 (20.3) | 0.56 |

| | | | |
|---|---|---|---|
| SSFP Myocardial volume, mL | 99.6 (17.7) | 102.5 (25.9) | 0.70 |
| SSFP no reflow | 1.0 (2.9) | 0.9 (2.4) | 0.95 |
| Myocardial salvage, SSFP-6SD | 17.8 (11.5) | 15.0 (11.7) | 0.46 |
| Myocardial salvage index, SSFP-6SD | 49.3 (24.5) | 45.2 (37.7) | 0.70 |

Table 2S. **Clinical Outcomes and 30-day Safety outcomes across randomized groups**

| **Outcomes** | Overall (n=44) | NAC (n=22) | Standard therapy (n=22) | p-value |
|---|---|---|---|---|
| | N (%) | N (%) | N (%) | |
| Mortality at 1 year | 0 | 0 | 0 | --- |
| Congestive heart failure, in hospital | 1 | 1 | 0 | 0.31 |
| Heart failure re-admission | 1 | 1 | 0 | 0.31 |
| Heart failure, at 1 year | 2 | 2 | 0 | 0.15 |
| Recurrent myocardial infarction | 0 | 0 | 0 | --- |
| Resuscitated cardiac arrest after reperfusion | 0 | 0 | 0 | --- |
| **Adverse events** | | | | |
| Bleeding | 6 (13.6) | 3 (13.6) | 3 (13.6) | 1.0 |
| Bleeding type (BRC) | | | | |
| 2 | 4 | 1 | 3 | |
| 3A | 1 | 1 | 0 | |
| 3B | 0 | 0 | 0 | |
| 3C | 0 | 0 | 0 | |
| 4 | 1 | 1 | 0 | |
| Allergic reactions | 0 | 0 | 0 | --- |