UNIVERSITÀ DEGLI STUDI DI MILANO

PhD COURSE IN TRANSLATIONAL MEDICINE
XXXVIII cohort

DEPARTMENT OF CLINICAL SCIENCES AND COMMUNITY HEALTH, CARDIOVASCULAR SECTION

PhD THESIS

Transcoronary cooling and dilution for cardioprotection during revascularisation for ST-segment elevation myocardial infarction: the STEMI-Cool pilot study.

Scientific-disciplinary sector: MED/11

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Academic Year
2024-2025

To my beloved Chiara,

forever grateful that I can share my successes with you.

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Introduction

ST-segment elevation myocardial infarction

Acute coronary syndromes (ACSs) are the consequence of a sudden reduction in myocardial blood flow or imbalance between oxygen supply and demand and are estimated to affect 7 million people worldwide each year[1,2]. Based on the European Society of Cardiology (ESC) guidelines, ACSs encompass a spectrum of conditions ranging from unstable angina, where there is myocardial ischaemia at rest or on minimal exertion in the absence of acute cardiomyocyte injury/necrosis, to an overt ST-segment elevation myocardial infarction (STEMI)[3]. This is the most dreadful of these syndromes and is characterised by the abrupt and sustained occlusion of a coronary artery, usually resulting from the rupture or erosion of an atherosclerotic plaque with subsequent thrombus formation. The complete interruption of blood flow leads to transmural myocardial ischaemia, which, if untreated, progresses to irreversible necrosis of the affected myocardial territory. Despite the therapeutic advancements introduced over the last decades and in particular the primary percutaneous coronary intervention (primary PCI or pPCI), STEMI is still associated with an excess mortality of 5.9% at 30 days and 2.1% at 10 years in 90-day survivors[4].

The hallmark of STEMI on the electrocardiogram (ECG) is persistent ST-segment elevation in two or more contiguous leads, reflecting ongoing transmural ischaemia. Biochemically, the diagnosis is confirmed by the rise and fall of cardiac-specific biomarkers, particularly high-sensitivity troponin,

which reflects myocardial necrosis. In practice, however, management decisions in the acute setting are primarily guided by symptoms and ECG findings, as biomarker confirmation normally requires time and should not delay treatment.

Epidemiology

Despite improvements in prevention, ACSs remain a major global health issue. Data from high-income countries suggest that the incidence of ACSs has decreased over recent decades due to declining smoking rates, better control of hypertension, and widespread use of statins[5]. However, the annual incidence of myocardial infarction is still as high as 550,000 new cases and 200,000 recurrent cases in the United States, approximately 38% of ACSs consisting of a STEMI[6,7]. In contrast, in many low- and middle-income countries, STEMI continues to represent the dominant form of ACS, reflecting differences in risk factor burden and healthcare infrastructure[8-10].

Men are affected more often than women, and STEMI typically presents 7–10 years earlier in men, although women who present with STEMI often have worse outcomes[11]. Traditional cardiovascular risk factors such as smoking, diabetes, hypertension, and hyperlipidaemia remain key contributors to the development of STEMI. Importantly, diabetes and smoking confer a particularly high risk of premature STEMI.

Clinical presentation

The classic presentation of STEMI is chest pain is typically described as crushing, substernal, or radiating to the arm, neck, or jaw, lasting more than 15-20 minutes and not relieved by rest or nitrates (Figure 1). However, presentations may be atypical, particularly in women, elderly patients, and those with diabetes, who may experience dyspnoea, fatigue, syncope, or even silent infarction. Physical findings are often non-specific but may include pallor, diaphoresis, hypotension, or signs of heart failure[3].

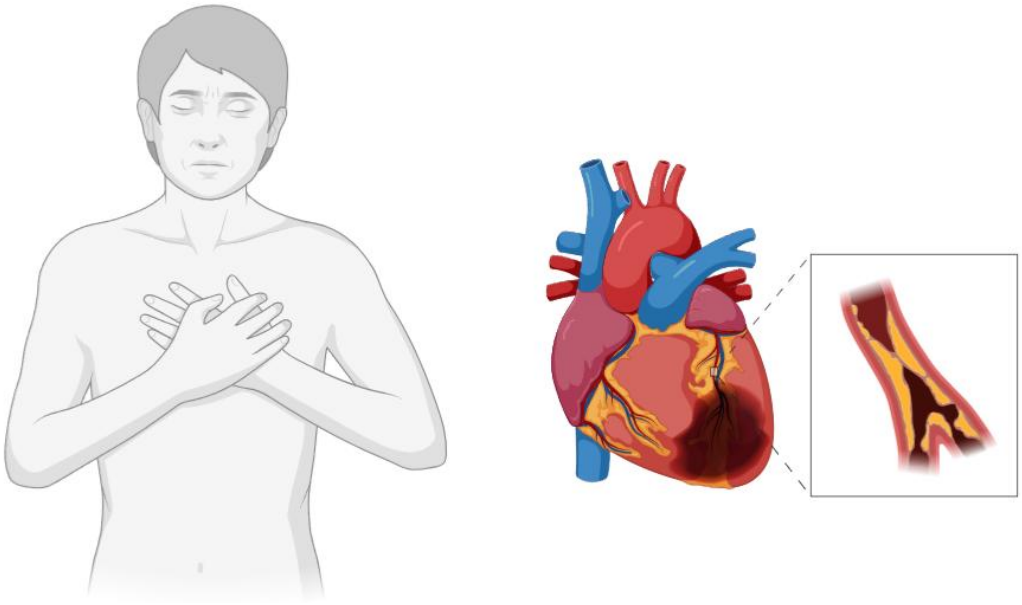


Figure 1 – STEMI pathophysiology and clinical presentation.

The most common pathophysiological mechanism of STEMI is the rupture or erosion of a coronary atherosclerotic plaque, although other mechanisms (e.g. coronary embolisation, or dissection) might be responsible. The typical clinical presentation of a STEMI is a sudden onset of central/left-sided pressure-like chest pain, often radiating to the left shoulder and jaw, not modifiable by posture, respiratory acts, or manipulation, and unresponsive to nitrates. Image created with Biorender.com.
STEMI = ST-segment Elevation Myocardial Infarction.

Diagnosis

A STEMI is generally diagnosed when a clinical presentation compatible with acute myocardial ischaemia meets the electrocardiographic hallmark of STEMI (Figure 2): new ST-segment elevation at the J-point in at least two contiguous leads (≥2.5 mm in men <40 years, ≥2 mm in men ≥40 years, or ≥1.5 mm in women regardless of age in leads V2–V3, and/or ≥1 mm in the other leads) in the absence of left ventricular hypertrophy or left bundle branch block. Equivalents such as new-onset left bundle branch block need to be treated as STEMI unless this can be clearly excluded. In practice, the ECG remains the cornerstone of rapid diagnosis, as it can be performed within minutes of presentation and guides immediate reperfusion decision. High-sensitivity

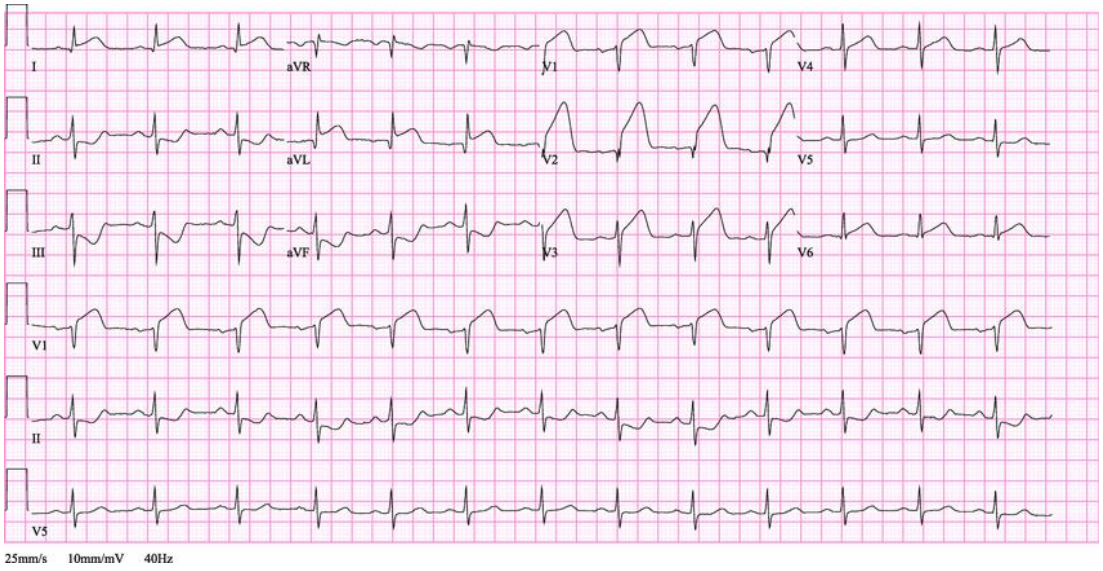


Figure 2 – Example of an ECG suggestive for STEMI.

This ECG, in the context of a clinical presentation suggestive for ACS, would be diagnostic for an anterolateral STEMI and would generally prompt immediate treatment without the need for further preliminary tests. Image source: de Bliek 2018[12].
ACS = Acute Coronary Syndrome; ECG = Electrocardiogram; STEMI = ST-segment Elevation Myocardial Infarction.

assays to measure cardiac troponins confirm the diagnosis when there is a clear uptrend or downtrend ("troponin curve") but, in the event of STEMI, reperfusion must be performed immediately without waiting for results. Likewise, supporting tests such as transthoracic echocardiography (usually documenting new regional wall motion abnormalities in the left ventricle) must not delay reperfusion[3].

Complications and prognosis

Without treatment, the prognosis of STEMI is poor: 30-day mortality of non-reperfused STEMI is estimated at 25%[13]. The introduction of reperfusion therapy — initially with fibrinolysis and subsequently with pPCI — has dramatically improved survival[14]. However, STEMI survivors remain at risk of significant morbidity. Left ventricular dysfunction is the main determinant of long-term prognosis, predisposing to chronic heart failure (HF), arrhythmias, and sudden cardiac death. Other complications include mechanical sequelae such as ventricular septal rupture, papillary muscle rupture leading to acute mitral regurgitation, and free wall rupture causing cardiac tamponade, although these have become less common with modern reperfusion therapy[15,16].

Global burden and health system implications

The global burden of STEMI extends beyond acute mortality. Many survivors live with reduced quality of life, recurrent hospitalisations, and disability. In Europe, cardiovascular disease — including myocardial infarction — accounts for an estimated €210 billion annually in healthcare costs, lost productivity, and informal care[17]. The challenge is particularly acute in regions where timely

access to reperfusion therapy remains limited. Delays in presentation, geographic barriers to PCI-capable centres, and limited healthcare infrastructure contribute to higher mortality rates in low- and middle-income countries[10].

STEMI treatment – primary PCI

From fibrinolysis to primary PCI

Before the widespread adoption of reperfusion therapy, in-hospital mortality following STEMI exceeded 20–30%. The introduction of fibrinolysis in the 1980s represented a major step forward, reducing early mortality compared to conservative management. However, fibrinolysis has important limitations: incomplete reperfusion in a significant proportion of patients, higher risk of intracranial haemorrhage, and recurrent ischaemia. Therefore, pPCI later emerged as a superior alternative. In a landmark meta-analysis of 23 randomised trials, pPCI was shown to significantly reduce mortality, reinfarction, and stroke compared with fibrinolysis, establishing it as the gold standard reperfusion method[18].

International guidelines stress the importance of rapid diagnosis and reperfusion in STEMI. The European and American societies both recommend immediate reperfusion therapy — preferably with pPCI within 120 minutes of first medical contact. If this is not possible, a fibrinolytic agent (e.g. alteplase) can be administered as the initial strategy, followed by early PCI[3,19].

Over the last 3 decades, pPCI has become the cornerstone of STEMI management, supported by robust evidence from randomised clinical trials and large registries, and reaffirmed by the 2023 ESC Guidelines for the management of ACSs[3].

Procedure description

The principle of pPCI is to mechanically reopen the occluded coronary artery, restore epicardial and microvascular flow, and thereby limit myocardial necrosis and adverse remodelling. This involves several critical steps, delivered by a skilled interventional team in a catheterisation laboratory equipped for emergency procedures.

Vascular access: Either radial or femoral access can be used, though radial access is generally preferred due to reduced bleeding complications and improved outcomes[20]. This procedure is normally performed with Seldinger technique under local anaesthesia and, in case of radial access, is generally complemented intra-arterial administration of verapamil to relax the artery and prevent spasm.

Coronary angiography: a coronary diagnostic or guiding catheter is advanced over a J-tip wire to the aortic root, under fluoroscopic guidance. The wire is then removed, and the catheter is maneuvered to engage the left/right coronary ostium. A bolus of iodinated contrast medium is injected to visualise and record the coronary anatomy. In case of a STEMI, the culprit coronary artery is normally occluded by a thrombus at the level of the plaque

rupture/erosion, which angiographically appears as an abrupt cut-off of the contrast column within the vessel lumen.

Lesion wiring and preparation: the occluded segment is crossed with a specialised guidewire, restoring some flow and enabling delivery of interventional devices. Balloon angioplasty is usually performed by advancing a semi-compliant balloon on the guidewire to facilitate stent delivery (pre-dilation). Thrombus aspiration is occasionally performed in case of high thrombotic burden.

Stent implantation: a drug-eluting stent is a small metal mesh tube, coated with anti-proliferative drugs (e.g. tacrolimus, everolimus) to prevent myointimal hyperplasia and early stent restenosis. This comes mounted on a dedicated balloon which is inflated at the level the lesion to expand the stent to the desired size. The stent can be further expanded with a non-compliant balloon (post-dilation) if needed.

Adjunctive pharmacology: acetylsalicylic acid, a P2Y12 inhibitor (ticagrelor or prasugrel as first choice), and unfractionated heparin are administered periprocedurally to prevent stent thrombosis. Glycoprotein IIb/IIIa inhibitors (e.g. tirofiban) are an additional anti-platelet therapy reserved for high thrombotic burden and/or bail-out scenarios[21].

Reperfusion injury

The role of reperfusion injury

Despite the unquestionable benefits of the pPCI in terms of short- and long-term survival and reduction of the final infarct size, this technique does not always suffice to prevent the insurgence of HF. Depending on several factors including time from STEMI onset, culprit vessel and segment affected, and

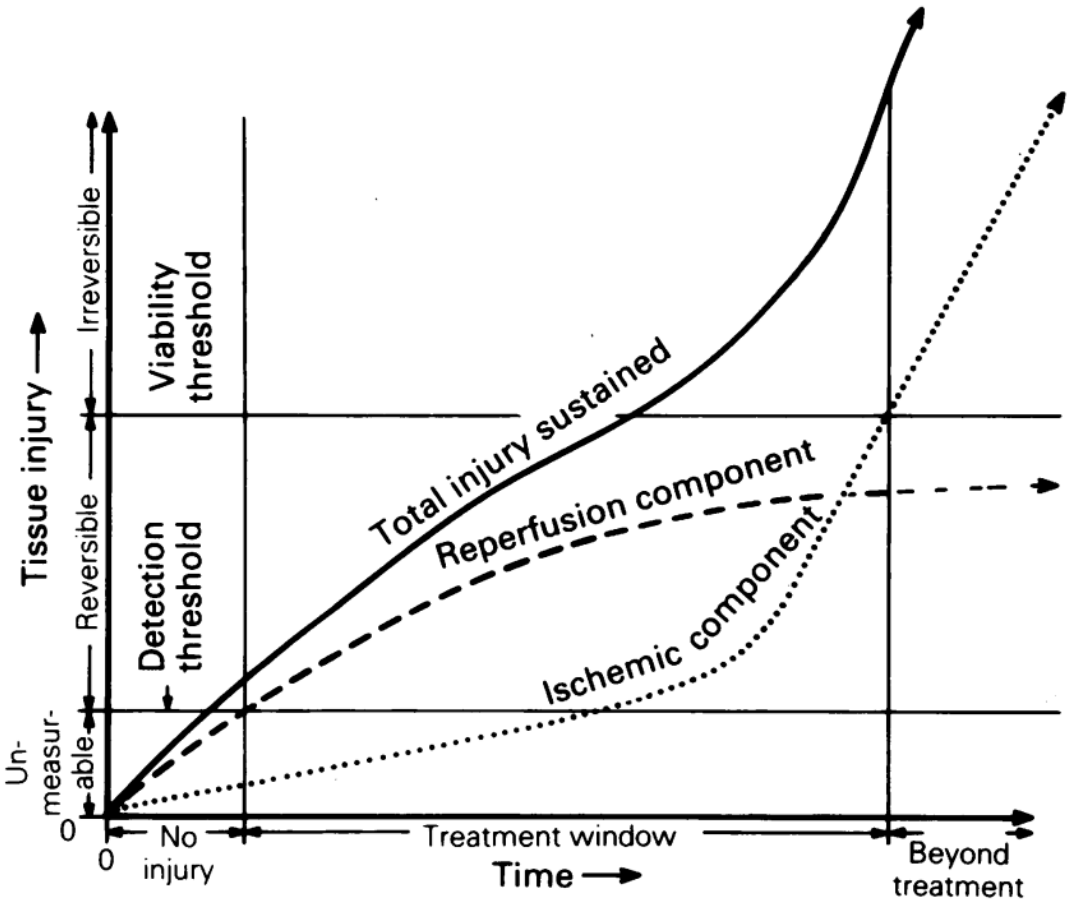


Figure 3 – Ischaemic and reperfusion injury kinetics.

The total injury sustained after an ischaemic event is determined by a combination of ischaemic and reperfusion injury. Ischaemic injury follows an exponential-like trend, and its weight increases dramatically with prolonged ischaemia. Conversely, reperfusion injury follows a logarithmic-like trend and represents the predominant component of total injury in the event of early revascularisation. Image source: Bulkley 1987[22].

patient characteristics, there is virtually always a certain extent of cardiomyocyte necrosis and scar formation after a STEMI. If a large myocardial territory is infarcted, cardiogenic shock and HF can still occur despite effective revascularisation[23].

Interestingly, the final infarct size not only depends on the ischaemic injury. In fact, restoring the blood circulation in an occluded coronary artery, therefore stopping the ischaemic process, causes itself further damage which is defined

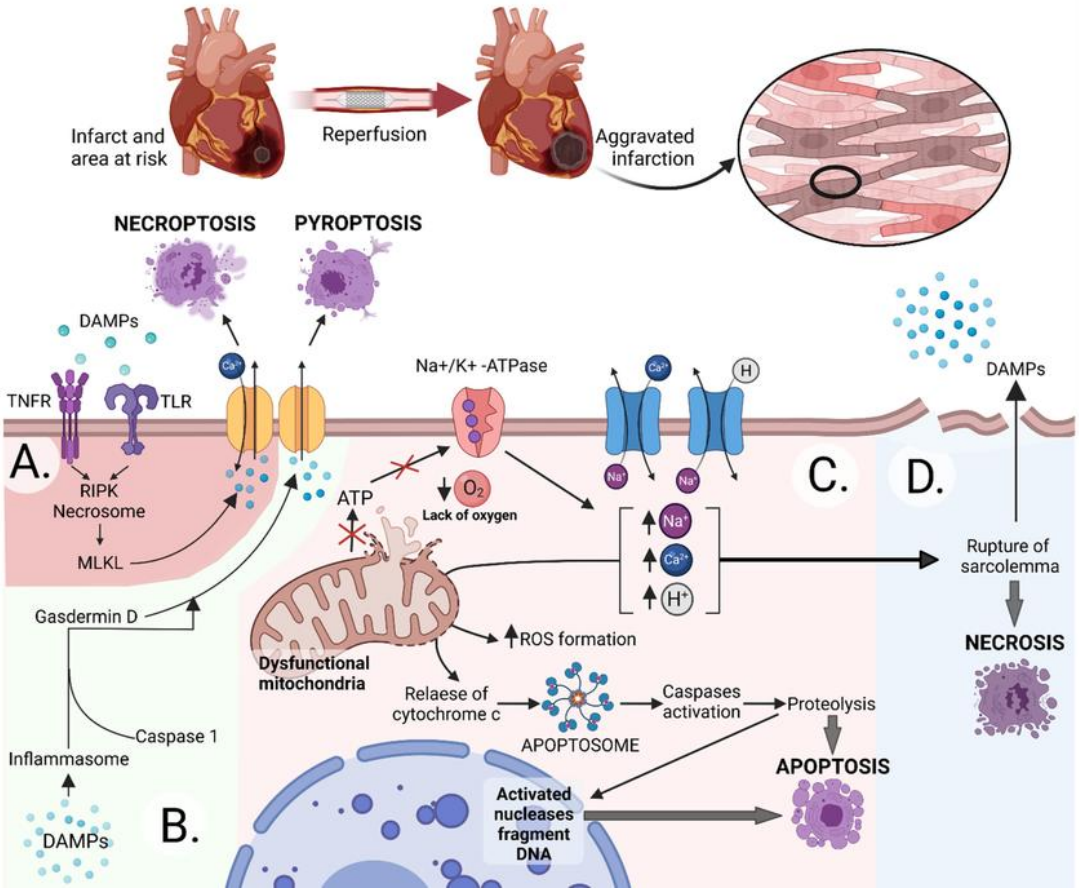


Figure 4 – Main molecular mechanisms of reperfusion injury.

This picture summarised the main molecular processes involved in the reperfusion injury-induced cell death, i.e. necroptosis (A), pyroptosis (B), apoptosis (C), and necrosis (D). Image source: Majka et al. 2021[24].
ATP = Adenosine Triphosphate; DAMPs = Damage-Associated Molecular Patterns; MLKL = Mixed-Lineage Kinase domain-Like protein; RIPK = Receptor-Interacting serine/threonine-Protein Kinase; ROS = Reactive Oxygen Species; TLR = Toll-like Receptor; TNFR = Tumour Necrosis Factor-alpha Receptor.

reperfusion injury (RI) and can be responsible for up to 50% of the final infarct size (**Error! Reference source not found.**)[22,25]. In general, macroscopic manifestations of RI are often in line with the conventional features of inflammation including local pain, swelling, elevated temperature/fever, and redness[26].

Molecular pathophysiology

RI is a complex phenomenon resulting from multiple contributors (Figure 4). During myocardial ischaemia, characterised by oxygen deprivation with consequent progressive depletion of adenosine triphosphate (ATP), cellular energy production shifts predominantly toward anaerobic glycolysis, resulting in intracellular acidosis. The activation of the sodium-hydrogen exchanger serves as a compensatory response to restore pH homeostasis, but this results in substantial sodium influx into cardiomyocytes. Elevated intracellular sodium impairs calcium uptake by the endoplasmic reticulum, ultimately eliciting cytosolic calcium overload; this disturbance promotes the generation of reactive oxygen species (ROS) and drives accumulation of long-chain fatty acids within cardiac myocytes[27]. Upon revascularisation, the rapid reintroduction of oxygen and normalisation of pH exacerbate the calcium overload[28]. Reoxygenation leads to a further burst of ROS generation from the dysfunctional electron transport chain in cardiomyocytes, xanthine oxidase in endothelial cells, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in infiltrating immune cells[29]. Together, calcium overload and ROS promote the opening of the mitochondrial permeability transition pore (MPTP), normally closed during ischaemia, where the low pH and abundance of protons

saturates the MPTP calcium binding site[27,30]. MPTP opening uncouples ATP synthesis from oxidative phosphorylation and provokes mitochondrial osmotic swelling and, consequently, mitochondrial dysfunction and cell death[31].

Experimental strategies to prevent/mitigate reperfusion injury

Interventions that minimize ischaemic damage indirectly reduce subsequent RI, and, clinically and mechanistically, differentiating the two components is often challenging. Over the last few decades, remarkable progress has been made to tackle myocardial ischaemia with the invention and optimisation of the pPCI, which has substantially reduced the mortality and morbidity of STEMI. However, in an era where, in ideal conditions, myocardial ischaemia can be promptly addressed and resolved, thus minimising the ischaemic injury, RI still contributes significantly to the overall damage and infarct size, and offsets the benefits of revascularisation. For this reason, RI has become the focus of extensive efforts aimed at preventing or at least mitigating it, a concept often referred to as “cardioprotection”[32].

Ischaemic conditioning

Ischaemic preconditioning (IPC) and postconditioning (IPost) have been investigated in the preclinical setting as capable to target pathways implicated in RI pathophysiology, such as oxidative stress, mitochondrial calcium overload, proapoptotic signalling, and MPTP opening[33,34].

IPC, first explored over 3 decades ago using alternating phases of coronary occlusion and reperfusion, established significant infarct reduction in canine

models[35]. The concept has since evolved, with remote IPC (RIPC). There is scientific evidence that inducing transient ischaemia in other vascular territories — including other coronary arteries[36] or peripheral limbs[37] — may provide protection to the infarcted myocardial territory, likely due to the systemic release of cardioprotective factors such as nitric oxide[38]. The possibility to perform RIPC by repeated sphygmomanometer inflation on a peripheral limb make this approach more feasible than conventional IPC. However, clinical translation would remain challenging, as its implementation is limited to predictable ischaemic events such as elective cardiac surgery or planned PCI. RIPC has been extensively investigated, with discordant results at least partly attributable to protocol heterogeneity, and large, randomized trials have generally failed to demonstrate its efficacy[39-41].

Ischaemic postconditioning (IPost) shares many cytoprotective pathways with IPC[34,42], but offers the pragmatic advantage of being implemented after the onset of ischaemia. Despite the encouraging preclinical data, clinical trials to date have failed to show a robust benefit in myocardial infarct size or clinical outcomes[43-47].

Pharmacological strategies

Coronary vasodilatory medications such as adenosine, nitroglycerin, and sodium nitrite have been investigated as sharing common pathways with ischaemic conditioning, including nitric oxide release and adenosine receptor activation. These medications failed to prove cardioprotective in clinical trials[38,48,49]. On the other hand, another member of the nitrate pharmacological

class, i.e. nicorandil, has been assessed in multiple east-Asian trials for its additional action as potassium channel opener, to prevent calcium overload and consequent RI. Some evidence of efficacy, mainly based on surrogate/mechanistic endpoints and small or single centre studies, has been observed in clinical trials[50-56] and meta-analyses[57,58]. In fact, the Japanese Circulation Society 2018 Guideline on Diagnosis and Treatment of Acute Coronary Syndrome report that “the administration of intravenous nicorandil may be considered for patients undergoing primary PCI within 12 hours after symptom onset to improve coronary microvascular circulation”, with class of recommendation IIb – level of evidence B[59]. However, nicorandil is not mentioned as a potential cardioprotective medication in the European or American guidelines[3,19], possibly due to the lack of definitive clinical outcomes in large, international, high-quality randomised clinical trials.

Cyclosporine-A, an immunosuppressant known for its MPTP opening inhibitory properties, has been extensively tested for its potential cardioprotective effect. Despite the initially promise shown in a small clinical study[60], subsequent large trials failed to demonstrate substantial clinical benefit[61,62]. Likewise, another investigational MPTP opening inhibitor named TRO40303 failed to prove clinical benefit[63].

Similarly, the β-blocker metoprolol, hypothesised to exert cardioprotection through modulation of neutrophil and platelet activity[64], showed initial promise[65,66] that was not confirmed in larger trials[67,68].

The glucagon-like peptide-1 and its analogues exenatide and liraglutide have also been thoroughly investigated, for their putative cardioprotective property of stimulating myocardial glucose uptake during postischemic contractile dysfunction. Despite the initial encouraging results in terms of myocardial salvage and reduction of infarct size and no-reflow phenomenon[69-73], evidence of their efficacy remains equivocal[74].

Systemic hypothermia

Therapeutic hypothermia is one of the most interesting investigational approaches for cardioprotection. It has been reported to protect from RI via multiple mechanisms: macroscopically, addressing the inflammatory response to RI by cooling down the affected organs[75,76]; microscopically, by Akt- and nitric oxide-mediated attenuation of mitochondrial oxidants, reduced ROS formation, preservation of mitochondrial nicotinamide adenine dinucleotide-hydrogen (NADH) levels, and mitigation of the cytosolic and mitochondrial calcium overload[77-80]. In fact, mild systemic hypothermia (32–36°C) is widely recommended in international guidelines for neuroprotection[81], and endorsed as standard care for neonatal hypoxic-ischemic encephalopathy[82], including guidelines from bodies such as the Canadian Paediatric Society[83] and the British Association of Perinatal Medicine[84]. Moreover, in adult critical care, prompt initiation of therapeutic hypothermia (32–36°C) is recommended for comatose survivors of out-of-hospital cardiac arrest to enhance neurological recovery[85,86].

Therefore, not surprisingly, the potential application of hypothermia for cardioprotection has been extensively investigated. Preclinical studies reported that the amount of the ischemic risk zone that advances to necrosis correlates with body temperature at the time of coronary artery occlusion. In animal models, when performed before reperfusion, it has been shown to limit RI and/or the no-reflow phenomenon, to different extents depending on the timing, the temperature delta and the experimental design[87-91].

However, clinical trials have failed to show a clear benefit of systemic hypothermia for cardioprotection during myocardial infarction due to both technical and intrinsic limitations, whether it is performed via intravenous administration of cold fluids, peritoneal lavage, or intravascular cooling catheters[92-100]. In fact, this treatment is challenging to apply in an emergency setting where patient transport is tends to be cumbersome and tolerability is scarce, often requiring sedation and anti-shivering medications. Moreover, despite cooling the whole body, systemic hypothermia often fails to reduce myocardial temperature as this process requires significantly longer time[101,102].

Transcoronary hypothermia

To overcome the limitations of systemic hypothermia, interest has shifted towards transcoronary hypothermia as a means of achieving quicker, targeted hypothermia with less discomfort for patients[90].

The recently published multicentric EURO-ICE trial proposed a clever strategy to deliver selective transcoronary hypothermia during the pPCI. This required a 2-step process with an occlusion phase, where an over-the-wire balloon

(OTWB) was inflated at the level of the coronary occlusion and room temperature normal 0.9% sodium chloride saline solution (NS) was administered through the balloon lumen to achieve a temperature reduction of ~6°C, and a reperfusion phase where the balloon was deflated and cold NS (4°C) was infused and mixed with the blood stream to maintain the desired temperature drop. Despite the ingenious design and the feasibility and safety reported, the EURO-ICE strategy, unfortunately no reduction in infarct size was documented[103].

There were in fact some limitations to this study which might have contributed to its neutrality. These include 1) a complex procedural setup, 2) an average 15-minute delay to reperfusion, 3) crossing the occlusion with an interventional guidewire and the OTWB prior to commencing the infusion, which might have provoked transient recanalisation of the vessel and consequent unprotected reperfusion, and 4) the necessity to switch to 4°C saline after OTWB deflation provoking transient increase in intracoronary (IC) temperature.

STEMI-Cool rationale

An alternative strategy to implement transcoronary cardioprotection was designed by our group and investigated in the “Optimal Restoration of Cardiac Activity-3” (ORCA-3) study. Briefly, the preliminary feasibility and safety of our method to deliver transcoronary cooling and dilution (TCCD) was tested on 10 patients[104]. As compared to EURO-ICE, this protocol implies a simpler setup and no significant extension of the ischaemic time, with 2 additional main advantages: 1) initiating the infusion before crossing the coronary occlusion,

2) combining the putative beneficial effects of cooling and dilution, which by effectively slowing down reperfusion might provide additional cardioprotection[105-107].

Further to the satisfactory safety and feasibility profile observed in ORCA-3, we designed the randomised pilot study STEMI-Cool to assess the recruitment rate, feasibility and safety of TCCD, explore mechanistic outcome measures including index of microvascular resistance (IMR), biomarkers of inflammation before infusion and at 24 hours, and cardiac magnetic resonance (CMR) of microvascular obstruction (MVO), myocardial salvage and infarct size at 1-7 days[108].

Methods

Study design

The STEMI-Cool Randomised Pilot Trial is a single-centre, registry-based randomised controlled trial (RRCT) with a pragmatic design (ClinicalTrials.gov NCT06128993). The study was embedded within the ongoing Heart-ACS project (www.heartacs.net; ClinicalTrials.gov NCT04218344), a registry of patients with ACSs at the host institution. All patients enrolled in STEMI-Cool were simultaneously included in the registry, which served as the main platform for capturing clinical and laboratory outcomes, as well as follow-up at 6 weeks, 6 months, and 1 year. Importantly, this design avoided the need for additional visits, since follow-up was aligned with the Heart-ACS protocol.

The trial was conceived as a pilot study, with primary objectives focused on recruitment rate, feasibility, and safety, rather than efficacy. A pragmatic design was chosen; therefore, eligibility criteria were deliberately broad and non-restrictive, ensuring that the patient population reflected real-world clinical practice (Table 1). This inclusive approach extended to patients who were unconscious at the time of presentation — such as those with cardiac arrest or severe cardiogenic shock — who were also considered eligible.

Sixty patients would be recruited and randomised in a 1:1 ratio, allocating patients either to the control arm, which consisted of standard-of-care (SOC) pPCI alone, or to the intervention arm, which combined pPCI with TCCD.

Inclusion criteria
Clinical STEMI (or equivalents including new LBBB)
Within 12 hours of index symptom onset
TIMI 0-1 flow in target artery
Exclusion criteria
Age <18 years
History of severe asthma
Pregnancy
Severe concomitant disease or conditions with a life expectancy of less than one year

Table 1 – Eligibility criteria.

A pragmatic design has been favoured by keeping essential eligibility criteria only to foster the generalisability of our findings. Severe asthma was an exclusion as a contraindication to adenosine, used for the invasive haemodynamic assessments.
LBBB = Left Bundle Branch Block; STEMI = ST-segment Elevation Myocardial Infarction; TIMI = Thrombolysis in Myocardial Infarction.

Study procedure

In all patients, pPCI was performed using a combined pressure and thermistor wire (Coroventis™ Pressure Wire X, Abbott Vascular), which has tip stiffness comparable to standard guidewires and is routinely used in clinical practice. Alternatively, the Coroventis™ could be parked in the proximal vessel and the lesion subsequently crossed with a standard interventional guidewire. The Coroventis™ wire was essential for continuous IC temperature monitoring and had to be positioned before starting the NS infusion. In case of wire failure, it was replaced according to standard interventional techniques.

Patients in the control arm (n=30) underwent pPCI according to standard local practice without infusion. Patients randomised to the intervention group (n=30) received IC infusion of room-temperature NS through the guiding catheter during the pPCI. The infusion was started immediately prior to crossing the coronary occlusion and delivered from a pressure bag, via an additional 3-way

tap connected to the interventional manifold. The pressure bag was inflated to the pressure needed to achieve a target IC cooling effect of 6–8°C below the baseline coronary temperature, as displayed on the thermistor readings.

The infusion was continued until 10 minutes after restoration of TIMI 2 flow or higher, with only brief pauses to allow aortic pressure assessment (as coronary pressure was continuously displayed via the CoroFlow‡ console). A maximum of 750 ml of NS was infused. No significant delay to reperfusion was anticipated with this simple setup.

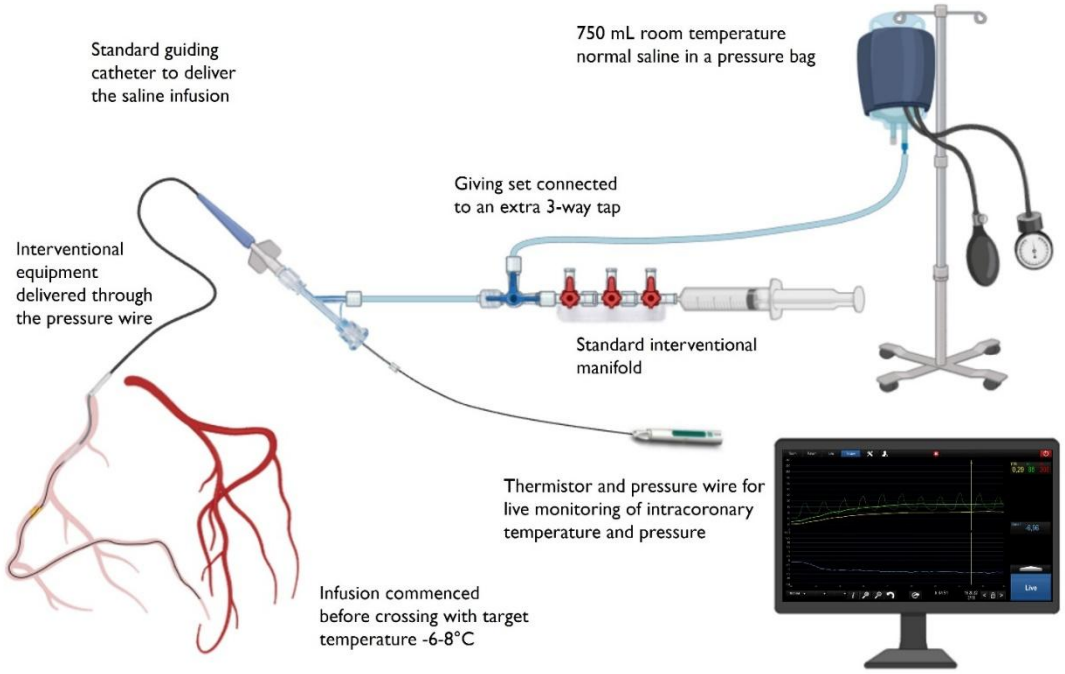


Figure 5 – Procedural equipment setup.

The setup consists of an extra 3-way tap connected in series to the interventional manifold, with a saline bag attached to the distal tap and pressurised to achieve a target temperature differential (ΔT) of -6 to -8°C. A Coroventis™ Pressure Wire X, is inserted through the O-ring and is advanced via the guiding catheter across the lesion/thrombus. IC temperature is continuously monitored on the CoroFlow‡ console, and the Coroventis™ can be used as the guidewire for the entire procedure (adapted from Carulli et al. 2024[108] and modified using BioRender.com).
IC = Intracoronary.

Haemodynamic assessments

After PCI completion, a comprehensive invasive physiological study was conducted in all patients. Measurements included fractional flow reserve (FFR), resting full-cycle ratio (RFR), coronary flow reserve (CFR), resistive reserve ratio (RRR), and IMR, using the Coroventis™ wire and CoroFlow‡ console. Body temperature was measured before and after PCI in both arms to confirm that the intervention did not cause any significant systemic hypothermia.

Given the complexities of IC temperature measurement, the study also investigated potential strategies to eliminate the need for a thermistor wire in future trials. Specifically, a potential relationship was investigated between the patient's blood pressure and the infusion pressure needed in the pressure bag to achieve a consistent and predictable degree of IC cooling across patients. This would allow integration of the method into standard PCI workflows using conventional equipment only, and elimination of the thermistor wire from the protocol.

In a subgroup of up to 10 intervention patients, additional haemodynamic assessments were planned: after the PCI, the infusion would be briefly recommenced, and boluses of cold (4°C) NS would be injected in the infusion stream. The thermistor readings would then be used to calculate the haemodilution constant ratio, a parameter we designed to assess how much the blood is diluted during TCCD and investigate the correlation of haemodilution with other clinical and mechanistic outcomes (Figure 6).

We originally planned to validate this measurement by collecting a small blood sample from the distal vessel during infusion with a microcatheter, to measure the exact haematocrit during infusion. However, this step was later deemed

Steward-Hamilton equation *Poiseuille's law*

$$Q = \frac{V(T_b - T_i)k_1k_2}{\int_{t_1}^{t_2}\Delta T dt} \qquad Q = \frac{\pi R^4 \Delta P}{8l\eta}$$

$$Q = \frac{V(T_b - T_i)k_1k_2}{\int_{t_1}^{t_2}\Delta T dt} = \frac{\pi R^4 \Delta P}{8l\eta} \qquad \eta k_1 = \frac{\pi R^4 \Delta P \int_{t_1}^{t_2}\Delta T dt}{8V(T_b - T_i)k_2 l} = H$$

$$\frac{H_1}{H_2} = \frac{(T_b - T_i)_2\ \Delta P_1 \left(\int_{t_1}^{t_2}\Delta T dt\right)_1}{(T_b - T_i)_1\ \Delta P_2 \left(\int_{t_1}^{t_2}\Delta T dt\right)_2}$$

Figure 6 – Relative haemodilution formula.

Both Stewart-Hamilton equation[109] and Poiseuille's law[110] allow for calculation of blood flow (Q). The first serves to measure cardiac output in the context of thermodilution and the parameters included are V = injected volume, Tb = blood temperature, Ti = temperature of the injected solution, k1 = density constant, k2 = calibration constant, t = time, $\int_{t1}^{t2}\Delta \boldsymbol{T}\boldsymbol{dt}$ = area under the extrapolated dilution curve; the second is the general fluid dynamics law, where R = vessel radius, ΔP = pressure drop, l = conduit length, η = viscosity. These 2 equations have been combined to obtain a new parameter, k1 η = H (haemodilution constant), which should reflect the density and viscosity of the fluid. The ratio between H1 = haemodilution constant measured during saline infusion, after stenting (by injecting 2.5 mL 4 °C saline into room temperature saline), and H2 = haemodilution constant measured after saline infusion and stenting (by injecting room temperature saline into the blood stream), will change according to the extent of dilution during saline infusion and will therefore represent a surrogate parameter to estimate it. V, k2 , R and l are the same for H1 and H2 and can be cancelled out; ΔP is approximated to distal coronary pressure assuming downstream pressure ≈ 0, and $\int_{t1}^{t2}\Delta \boldsymbol{T}\boldsymbol{dt}$ is approximated to the mean transit time of the injected volume. Image source: Carulli et al. 2024[108].

too complex and not necessary for the formula validation, and was therefore not performed.

Laboratory assessments

Routine laboratory parameters (C-reactive protein, high-sensitivity Troponin, brain natriuretic peptide and creatinine) were collected and analysed as per standard STEMI patient management at our organisation, and their results stored in the eCRF of the Heart-ACS registry. A further 60 ml arterial blood sample was drawn from the sheath at the start of pPCI (day-0), immediately centrifuged, and plasma/serum stored at –80°C for additional test in the context of the Heart-ACS registry and/or STEMI-Cool.

In a subpopulation of 25 patients (14 intervention, 11 control), an additional 30 ml venous blood sample was obtained ~24 hours after pPCI (day-1) and processed in the same way. The paired day-0 and day-1 blood samples were planned to be used to measure a panel of biomarkers implicated in ACSs and RI, including interleukins (IL-1 β, IL-6, IL-10), tumor necrosis factor α, lactate dehydrogenase (LDH), heart-type fatty acid binding protein (H-FABP), and the novel biomarker dipeptidyl peptidase 3 (DPP-3). Prior studies have highlighted their relevance in RI pathophysiology and prognosis[111-117]. In particular, DPP-3 has been shown to predict cardiogenic shock and mortality in ACSs with high accuracy, suggesting potential clinical utility[113]. LDH and H-FABP have also been observed to correlate with RI severity[114-117]. STEMI-Cool therefore provided a unique opportunity to evaluate these biomarkers in the setting of

TCCD, and possibly detect differences in their trends between the treatment and control arms.

Imaging

A routine echocardiogram was performed at 24–48h post-pPCI to assess left ventricular ejection fraction (LVEF), wall motion score index (WMSI), and global longitudinal strain (GLS). Where available, data from a follow-up echocardiogram were also collected.

A Cardiac magnetic resonance (CMR) substudy was performed in a subpopulation of up to 20 patients (10 per arm) between day 1–7. We originally planned to secure additional funds for a further CMR at 6 months, but this was not possible. CMR imaging was performed using a 1.5 Tesla scanner (Siemens Healthineers, Erlangen, Germany), 1-7 days after pPCI. The imaging protocol included standard cine, T2-weighted, short tau inversion recovery (STIR), early gadolinium enhancement (EGE), and late gadolinium enhancement (LGE) sequences. Full short-axis stacks were acquired for T2-weighted, STIR, EGE, and LGE imaging, covering the entire left ventricle from base to apex.

Gadolinium-based contrast agent was administered intravenously at a standard dose of 0.1 mmol/kg body weight. EGE images were acquired approximately 1–2 minutes post-injection, while LGE imaging was performed 10–15 minutes after contrast administration using a phase-sensitive inversion recovery (PSIR) sequence. Inversion time was adjusted to null normal myocardium.

Image analysis was performed using dedicated post-processing software (cvi42® release 6.0.3 – 4064, Circle Cardiovascular Imaging). The presence, pattern, and distribution of edema (T2 and STIR), MVO (EGE), and fibrosis or necrosis (LGE) were assessed qualitatively and quantitatively according to established criteria. Although the study was open-label, CMR analysis was conducted by a blinded operator.

Statistical analysis

Statistical analysis was performed with RStudio 2022.12.0+353. All tests were two-tailed and significance level (α) was set at 0.05. Continuous variables were assessed for normality using visual inspection and the Shapiro–Wilk test. The t-Student test was used where data were normally distributed or the Wilcoxon rank-sum test where non-normally distributed. When ties were present in non-parametric data, approximate *p*-values were reported using the normal approximation. Categorical variables were analysed using the chi-squared (χ^2) test or the Fisher exact test where >20% of expected cell counts were less than 5. Data are shown as mean ± standard deviation or median (interquartile range) depending on their statistical distribution.

To investigate the relationship between the patient's blood pressure and pressure needed in the pressure bag to obtain the desired temperature reduction (–6-8°C) or inflation pressure (IP), simple linear regression models were constructed. In each model, IP was the dependent variable and either mean, systolic, or diastolic blood pressure (DBP) was used as the predictor. The strength of association was assessed using the coefficient of

determination (R^2), *p*-values for the regression coefficients, and standard diagnostic plots. Models were compared based on the proportion of observations with prediction errors ≥20 and ≥50%, defined as: prediction error = $\left|(\text{predicted} - \text{observed})/\text{observed}\right|$. Correlation between variables was also assessed using Pearson's correlation coefficient, with 95% confidence intervals and hypothesis testing for correlation ≠0.

Study endpoints

STEMI-Cool was conceived as a pilot trial, and therefore its primary endpoints focused on feasibility rather than efficacy. These were:

- Recruitment rate: number of patients enrolled per month.
- Feasibility: this was initially conceived as the proportion of cases in which NS infusion was maintained for at least 5 minutes after achieving TIMI 2 flow or higher without substantial interruption. However, we decided to change the definition of feasibility to the proportion of cases in which NS infusion was initiated before crossing, regardless of infusion duration. This aligns with our hypothesis that the potential beneficial effects of TCCD are mainly exerted by protecting reperfusion at the time of outset.
- Safety: adverse events assessed according to CTCAE v5.0.

A comprehensive list of the secondary outcomes of the study, including procedural, inpatient, and outpatient clinical and mechanistic outcomes, can be consulted in Table 2.

Procedural
IMR, CFR, FFR, RFR, RRR values 10 min after completion of PCI
IC temperature change, infusion rate and volume delivered during the infusion
Chest pain during study infusion
ECG changes during study infusion
Heart rhythm changes during study infusion
Myocardial blush grade 10 min after completion of PCI
Final TIMI flow grade 10 min after completion of PCI
ST segment resolution 10 min after completion of PCI
Inpatient
ST segment resolution 1 h after completion of PCI
Routine laboratory biomarkers
Inflammation biomarkers
Hospital length of stay
Heart Rhythm disturbance from baseline to 12 h
Haemodynamic compromise from baseline to 12 h
Echocardiography for LVEF, WMSI, and GLS at 24-48 h
CMR for initial MSI and infarct size at 1-7 days
Outpatient
All-cause mortality and hospitalization for heart failure at 6 weeks, 6 months and 12 months
Hospitalization for heart failure at 6 weeks, 6 months and 12 months
Composite of all-cause mortality and hospitalization for heart failure at 6 weeks, 6 months and 12 months
Cardiovascular mortality at 6 weeks, 6 months and 12 months
Echocardiography for LVEF, WMSI, and GLS at 6 months
CMR for area at risk and infarct size at 6 months

Table 2– Secondary study outcomes.

CFR = Coronary Flow Reserve; CMR = Cardiac Magnetic Resonance; FFR = Fractional Flow Reserve; GLS= Global Longitudinal Strain; hs-TnT = high sensitivity Troponin T; IC = Intracoronary; IMR = Index of Microvascular Resistance; LVEF = Left Ventricular Ejection Fraction; PCI = Percutaneous Coronary Intervention; RFR = Resting Full-cycle Ratio; RRR = Resistive Reserve Ratio; TIMI = Thrombolysis In Myocardial Infarction; WMSI = Wall Motion Score Index.

Results

As the 1-year follow-up was still ongoing when this manuscript was written, the results reported here are limited to the 6-week follow-up.

Patient population

One hundred and thirty-seven patients were screened and 60 were initially enrolled. One patient (SOC) was later taken off study due to consent form-related issues. Baseline patient characteristics are summarised in Table 3.

Patient population (n)	59
Male sex (n)	46 (78%)
Age (y)	63±13
BMI (kg/m^2)	28±4.50
History of CAD (n)	16 (27%)
Hypertension (n)	24 (41%)
Diabetes mellitus (n)	14 (24%)
Dyslipidaemia (n)	22 (37%)
History of tabagism (n)	23 (39%)
Previous MI same territory (n)	4 (7%)
Antiplatelet/Anticoagulant therapy (n)	12 (20%)
Lipid lowering therapy (n)	21 (36%)
Culprit LAD (n)	22 (37%)
Culprit LCx (n)	10 (6%)
Culprit RCA (n)	27 (46%)
Proximal vessel lesion (n)	29 (49%)
Out-of-hospital cardiac arrest (n)	7 (12%)
Cardiogenic shock (n)	6 (10%)

Table 3 – Baseline patient characteristics.

Data are presented as number (percentage) or mean ± standard deviation.
BMI = Body Mass Index; CAD = Coronary Artery Disease; LAD = Left Anterior Descending (artery); LCx = Left Circumflex (artery); RCA = Right Coronary Artery.

Procedural data

In the treatment arm, an average of 549±229 mL were infused over 11.0±4.6 minutes at an infusion rate of 61±29 mL/min. The proximal coronary temperature was reduced by 6.37±1.22°C, but there was no significant effect on the postprocedural systemic temperature reduction: -0.10 (-0.30 – 0.00)°C in the treatment arm vs ±0.00 (-0.30 – 0.325)°C in the SOC arm, p=0.722.

The average preprocedural delay was 2.0 (0.9 – 3.0) min ranging from 0 to 5.0 min. There was no delay when the thermistor wire was successfully prepared during concomitant pre-PCI steps such as angiogram of the contralateral vessels or review of the angiographic pictures, while the main reasons for prolonged delays included issues with the pressure wire setup and cases where switching to a standard guidewire was necessary due to failure to cross the occlusion with the pressure wire.

Primary outcomes

Recruitment rate and feasibility

Patients were recruited between 1 November 2023 and 14 February 2025, for a total of 14.5 months. After addressing the initial challenges associated with the study setup, from March 2024 the recruitment rate stabilised at 4.8 patients/month in a single centre (Figure 7).

Our method was generally feasible: we were able to commence TCCD before crossing in 24/30 (80%) treatment arm patients. TCCD was not possible in 2/30 (7%) cases: in 1 patient due to connectivity issues with the Coroventis™

wire, and in 1 patient due to the exhaustion of the saline bag before being able to cross. In the remaining 4 patients, TCCD was established after crossing due to a challenging lesion, or haemodynamic instability of the patient requiring immediate restoration of the blood flow. In 4/28 (14%) patients the infusion was interrupted prematurely upon the occurrence of ventricular fibrillation (VF, 3 patients) or transient ST elevation (1 patient).

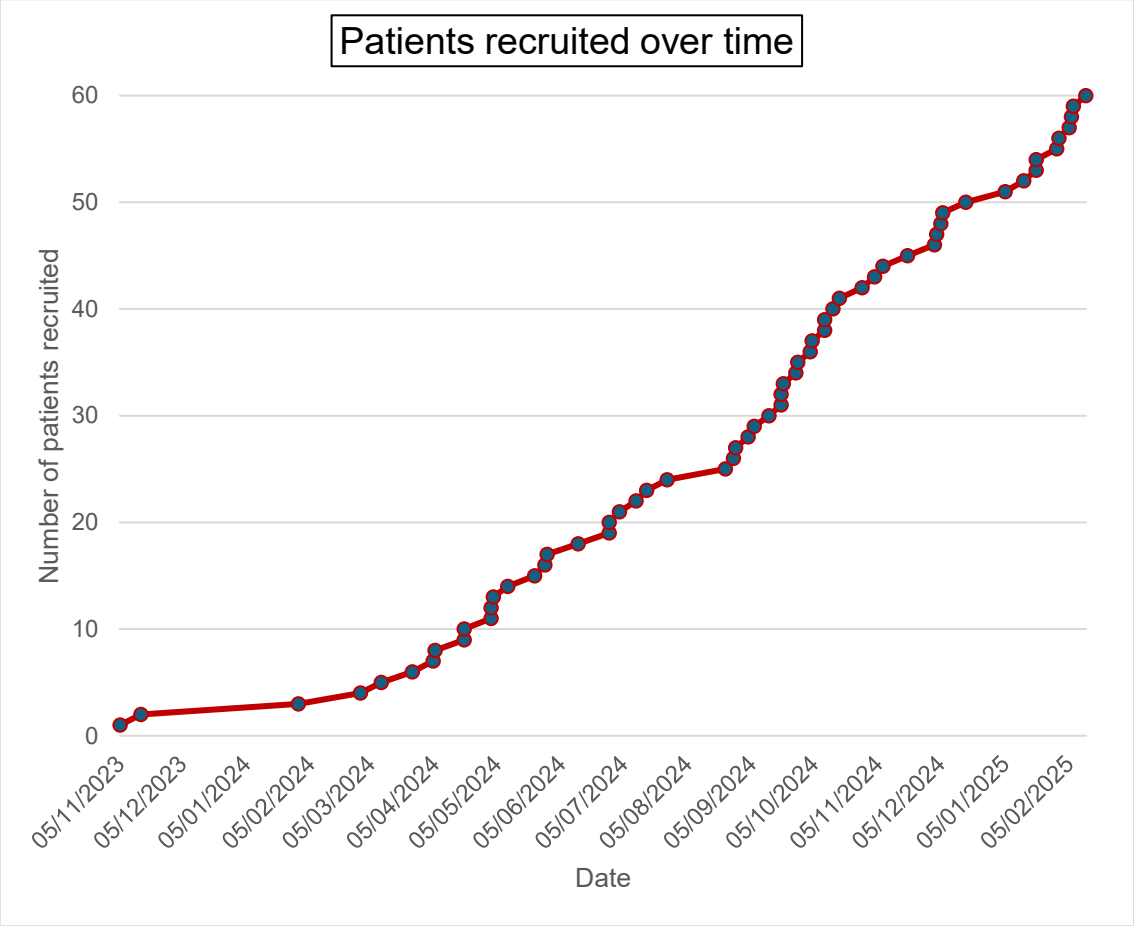


Figure 7 – Recruitment rate.

Steady-state recruitment rate was 4.8/month in a single centre.

Safety

All patients survived the procedure. Six out of 59 patients (10%) died before discharge. Five of these patients were already in cardiogenic shock at the time of enrolment, 3 of whom had experienced a VF arrest. The remaining patient was a 93-year-old woman for whom PCI was unsuccessful. There was no significant difference in mortality between the treatment and SOC arms (2 vs 4 patients, p=0.370).

In terms of adverse events, we found a significantly higher rate of VF episodes in the treatment arm vs the SOC arm (10 vs 2 patients, p=0.011). All VF episodes were promptly defibrillated. Three patients (1 treatment, 2 SOC) were admitted to the intensive care unit (ITU) for cardiogenic shock and 1 of these patients subsequently died (SOC). Of the 10 VF events observed in the treatment arm, 9 occurred during transcoronary infusion, one of which before and all the others after crossing the occlusion. In the remaining treatment arm patient, the VF episode occurred just after the randomisation and before any treatment-specific interventions, with no further episodes during the transcoronary infusion. In 8/10 patients, the infusion was either not stopped or only suspended, with no further episodes documented upon resumption, while in 2/10 it was stopped as per operator decision. In the 2 SOC arm patients, the VF events occurred before crossing.

Further periprocedural arrhythmias included atrial fibrillation/tachycardia (2 treatment, 0 SOC), complete heart block (0 treatment, 1 SOC), ventricular

bigeminy with or without couplets or triplets (0 treatment, 3 SOC), non-sustained VT (0 treatment, 1 SOC), reperfusion rhythm (1 treatment, 0 SOC).

One patient felt cold during TCCD and required a blanket. Two patients in the treatment arm experienced transient headache and confusion, or felt generally unwell with transient blurred vision, respectively, but no brain imaging findings were documented. One patient in the treatment arm developed transient chest pain towards the end of the infusion. One patient in the treatment arm developed transient pulmonary oedema which however did not require admission to the ITU.

Inpatient adverse events included transient hypotension (1 treatment, 2 SOC), acute HF/pulmonary oedema (2 treatment, 1 SOC), acute kidney injury (4 treatment, 4 SOC), left ventricular thrombus (1 treatment, 3 SOC).

Two patients in the treatment arm experienced recurrent chest pain. One of these patients was admitted with inferior STEMI and significant left anterior descending artery (LAD) bystander disease and, 2 days after the pPCI, developed anterior ST elevation requiring urgent bystander disease PCI. One patient in the treatment arm (anterior STEMI) experienced recurrent asystolic events necessitating provisional external pacemaker insertion. Six patients experienced episodes of non-sustained ventricular tachycardia (VT, 2 treatment, 4 SOC), 1 of which (treatment arm) was temporarily transferred to the ITU for 24 h where the arrhythmias were effectively managed with amiodarone and metoprolol, and 1 (SOC) underwent further PCI to bystander disease due to the high burden of non-sustained VT.

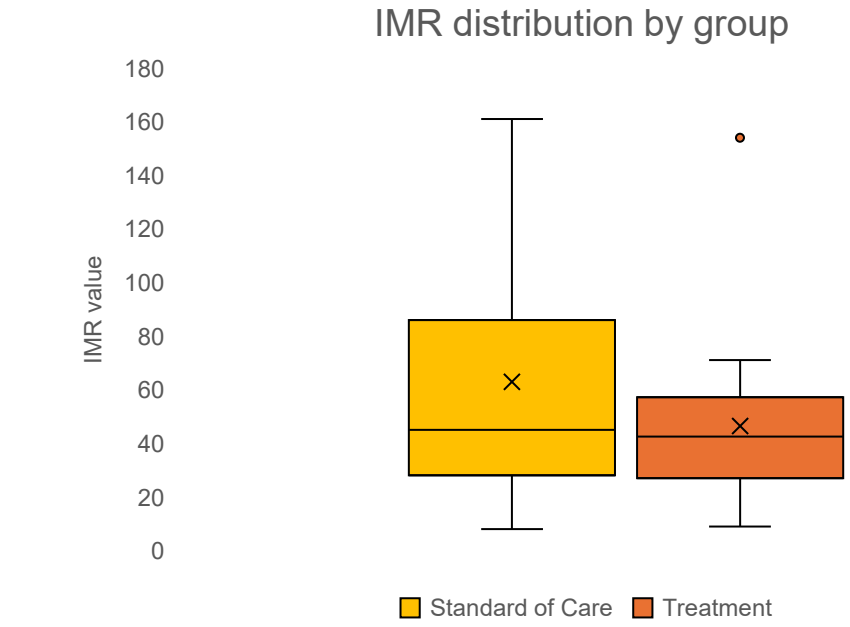
At the 6-week follow-up, 12/53 (23% excluding the inpatient deaths) patients complained of further chest pain episodes (6 treatment, 6 SOC) and 7/53 (13%) experienced marked fatigue or exertional shortness of breath (3 treatment, 4 SOC).

Clinical and mechanistic outcomes

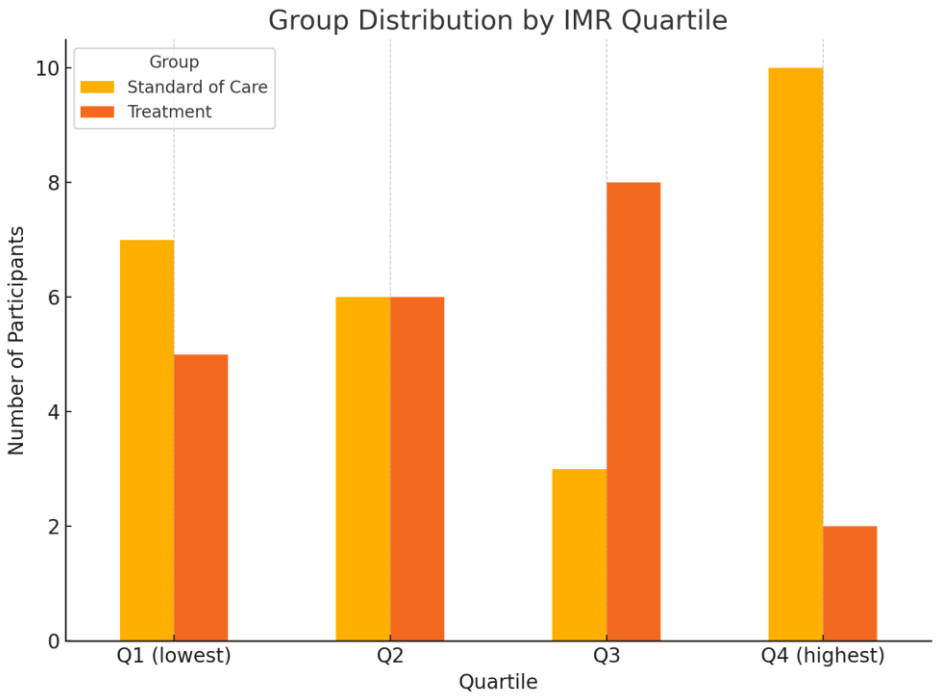
Coronary physiology

There was no significant difference between the treatment and SOC arms in median postprocedural resting full cycle ratio (RFR, 0,94 (0.90-0.98) vs 0.96 (0.93-0,99) respectively, *p*=0.336), fractional flow reserve (FFR, 0.96 (0.94-0.99) vs 0,97 (0.94-0.98) respectively, *p*=0.458), coronary flow reserve (CFR, 1.3 (1.0-1.4) vs 1.2 (1.0-1.8) respectively, *p*=0.458), and IMR (43 (28-58) vs 43 (27-83) respectively, *p*=0.398). Particularly, the 4th quartile was dominated by SOC patients (2 vs 10 respectively) while the 3rd quartile was dominated by treatment patients (8 vs 3 respectively). As a result, the data distributions are skewed and there is a more appreciable difference in the mean values (46±30 vs 63±44 respectively) as noticeable in Figure 8 A and B.

Concerning the haemodilution constant ratio, only 3 measurements were successfully recorded due to the difficulty of obtaining transit time readings when injecting 4°C saline boluses into the NS stream during TCCD. This number of readings was deemed insufficient to establish a reliable correlation with the haematocrit during TCCD.



(A)



(B)

Figure 8 – IMR distribution by group (A) and group distribution by IMR quartile (B).

There was a higher prevalence of SOC patients in the 4th quartile and a higher prevalence of treatment patients in the 3rd quartile, but no statistically significant difference between the 2 groups (p=0.398). In (A) means are represented by a cross and medians by a horizontal line. IMR=Index of Microvascular Resistance; SOC = Standard Of Care.

Postprocedural ST-segment elevation

There was no significant difference between the treatment and SOC arm in the median ST-segment elevation at baseline (2.0 (1.5-4.0) vs 2.5 (1.5-4.5) respectively, p=0.802), on reperfusion (2.5 (2.0-4.0) vs 3.0 (1.5-6.5) respectively, p=0.455), 10 min post procedure (1.0 (0.5-3.0) vs 2.0 (1.0-2.5) respectively, p=0.163) and 1 hour post procedure (1.0 (0.0-2.0) vs 1.3 (0.5-2.0) respectively, p=0.156). However, there was a trend in favour of the treatment arm in the 10 min and 1 hour post procedure ST-segment elevation.

TIMI flow and blush grade

There was a trend in favour of the treatment arm, with no significant statistical difference, for the number of cases where the final TIMI flow grade was 3 (26/30 vs 20/29 for treatment and SOC arms respectively, *p*=0.101). There was no statistically significant difference for the number of cases where the blush grade was assessable and equal to 3 (12/28 vs 10/28 for treatment and SOC arms respectively, *p*=0.584).

Laboratory assessments

There was no significant difference between the treatment and SOC arms in postprocedural brain-type natriuretic protein (BNP, 182 (93-278) ng/L vs 235 (159-313) ng/L respectively, *p*=0.661), C-reactive protein (CRP, 3 (2-7) mg/L vs 3 (1-7) mg/L respectively, *p*=0.416), and troponin I peak (39025 (11967-55120) ng/mL vs 35699 (17191-70301) ng/mL respectively, *p*=0.519).

With regard to the additional panel of biomarkers as mentioned in the Methods section, we have submitted an integrative grant application to support the day-0 and day-1 blood analysis which if successful will allow these measurements.

Imaging

There was no significant difference between the treatment and SOC arms in echocardiographic parameters collected at 24-48 hours from admission: left ventricular ejection fraction (LVEF, 49±10 vs 48±9%, respectively, *p*=0.504), wall motion score index (WMSI, 1.25 (1.19-1.50) vs 1.38 (1.19-1.50) respectively, *p*=0.391), and global longitudinal strain (GLS, 12.5 (9.0-17.7) vs 12.7 (11.7-14.6) respectively, *p*=0.705).

Eight treatment and 7 SOC patients underwent CMR scan 1-7 days after the admission. Between these small groups there was no statistically significant difference in terms of presence of MVO (2/8 vs 4/7 respectively, *p*=0.464) and area at risk as % of left ventricular mass (23.69±11.01% vs 22.70±14.79% respectively, *p*=0.871). However, there was a trend in favour of the treatment arm for infarct size as % of left ventricular mass (11.73±10.40% vs 17.74±12.39% respectively, *p*=0.240).

Length of stay and readmission

There was no significant difference between the treatment and SOC arms in length of stay (3 (3-5) vs 4 (3-11) respectively, *p*=0.188). At 6-week follow-up, unplanned hospital readmissions were 8/53 (15%) excluding inpatient deaths with no significant difference between the two groups (5 vs 3 respectively,

p=0.478). Specifically, 1 patient (treatment) was readmitted for dizziness and discharged shortly after, 5 patients (3 vs 2 respectively) were readmitted for chest pain, 1 of which (SOC) underwent repeated angiogram with no further intervention, 1 patient (treatment) with history of non-compliance with pharmacotherapy was readmitted for new inferior STEMI, and one patient (SOC) was readmitted for HF.

Predictive model for pressure bag inflation to achieve target temperature

As part of our efforts to eliminate the need for a thermistor wire to monitor the IC temperature, we recorded the patient pre-infusion blood pressure and IP

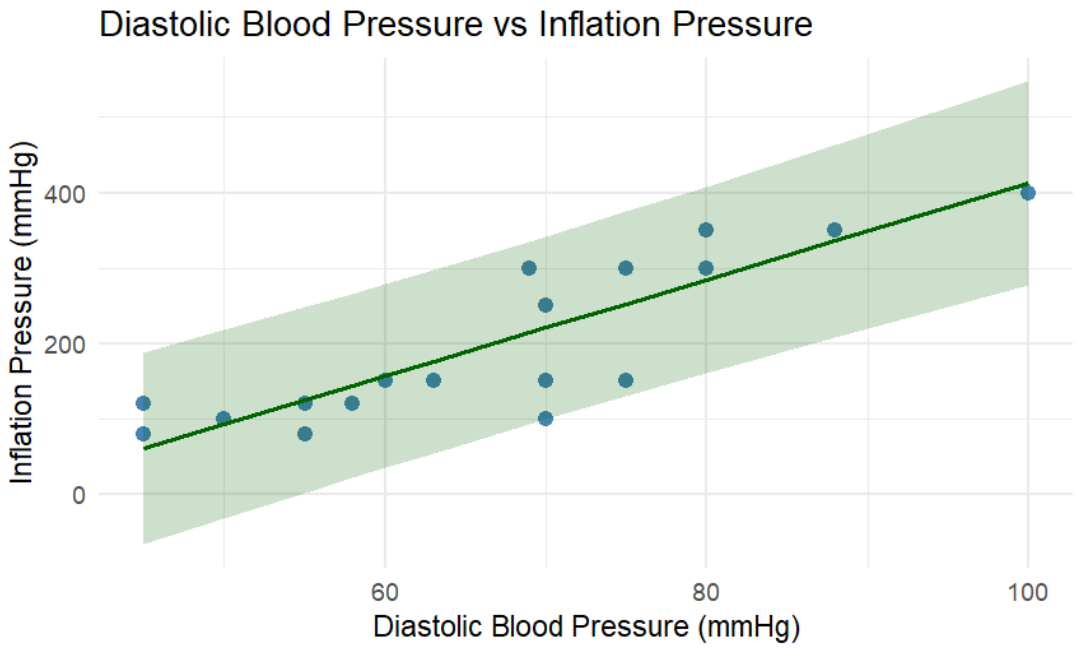


Figure 9 – Linear regression model to correlate DBP to IP.

This model serves to predict the IP needed to achieve an IC temperature of –6-8°C, based on the patient's DBP. The band represents the prediction interval where 95% of future points are expected to fall.
DBP=Diastolic Blood Pressure; IC=Intracoronary; IP=Inflation Pressure.

needed to obtain the desired temperature reduction (–6-8°C). DBP exhibited the strongest correlation with the IP needed (R^2=0.75, p<0.001) and predicts it with the formula: IP=DBPx4.27–120 mmHg (Figure 9). With this model, the probability of over/underestimating the IP by 20% is 40%, and the probability of over/underestimating the IP by ≥50% is 15%.

Discussion

Primary outcomes

Preventing or at least limiting RI following pPCI is an unmet medical need that researchers have been trying to address for decades without success. Several strategies with a solid scientific rationale have been tested, but none has shown convincing evidence to date.

STEMI-Cool proposes a simple strategy specifically designed to overcome the limitations of similar transcoronary cooling trials, mainly by 1) initiating the transcoronary infusion before crossing the occlusion with the wire, therefore preventing any potential unprotected reperfusion, 2) not requiring complex setup nor significant preprocedural delay, thus not prolonging the ischaemic time, 3) leveraging the combined effect of cooling and haemodilution.

Sixty patients were recruited in 14.5 months in a single centre (including one drop out) at a steady-state rate of 4.8 patients/month, which is satisfactory in terms of recruitment rate. Moreover, most of the interventional cardiologists at our centre were engaged and could easily run the protocol with the verbal assistance of trained team member. Cardioprotection before crossing the occlusion was also feasible in 80% of cases. Four (13%) patients received TCCD after occlusion crossing and only 2/30 (7%) treatment patients did not receive any TCCD, due to failure to connect the pressure wire or infusion exhaustion before being able to cross, respectively. Specifically, the second of these scenarios prompted a small change in the study protocol to establish

TCCD only immediately before crossing, which prevented further similar episodes. Furthermore, eliminating the thermistor wire from the protocol would also eliminate the risk of connectivity issues.

In terms of safety, there were no significant differences in mortality, admissions to ITU, or hospitalisations for HF the two patient groups. However, there was an excess of intraprocedural VF episodes in the treatment arm. VF is a relatively common complication of STEMI and data from previous studies report an incidence of intraprocedural VT/VF at 3.1-4.3%[118,119]. In our SOC arm, the 2 VF cases occurred before reperfusion, while the majority of the VF cases (8 out of 10) in the treatment arm occurred during reperfusion and IC infusion. This finding is not easy to interpret: although reperfusion arrhythmias are historically known to indicate effective reperfusion, they may also be related to reperfusion injury itself[120]. Moreover, reperfusion VT/VF are associated with higher risk of need for cardiopulmonary resuscitation, orotracheal intubation and worse 90-day mortality[118], although 1-year adverse outcomes seem to be similar to patients without these arrhythmias[119]. Lastly, there is a series of known triggers or predisposing factors for VF, including excess catheter manipulation, utilisation of high osmolar ionic contrast agents, new ischaemic events, and reperfusion itself, with an inverse relationship between the duration of the ischaemia and the likelihood of malignant ventricular arrhythmias[120-122].

A few considerations need to be made with regard to the VF episodes occurred in our treatment arm: 1) all the VF episodes were successfully defibrillated, 2) in 8/10 cases the infusion was restarted without further VF episodes, 3) 1/10

treatment arm patient with intraprocedural VF was admitted to the ITU for cardiogenic shock vs 2/2 in the SOC arm.

There was no significant difference in the time from symptom onset in patient with vs without VF (2.9 (2.5-5-1) h vs 3.0 (2.5-4.9) h respectively, *p*=760) and the VF episodes were not coronary artery-specific (5 when infusing through the right coronary artery = RCA and 7 when infusing through the left main stem = LMS), however there was a significantly higher proportion of proximal lesions in the VF group vs no VF (10/12 vs 28/47 respectively, *p*=0.008).

We initially suspected that VF might be triggered by vigorous injection/high flow rate in the coronary circulation. Bolus IC injections, which are commonly delivered vigorously and therefore at higher pressure, have been described to be associated with VF: for example, Shah et al. reported 3 cases of VF triggered by adenosine boluses performed during FFR measurement as an alternative to the standard IC infusion[123]. The exact flow rate of our infusion was impossible to measure with the simple equipment we utilised. Moreover, pauses to the infusion occurred frequently when exchanging equipment or injecting contrast, and their overall impact on its effective duration was difficult to record reliably. However, we estimated an average flow rate of 61±29 mL/min and a maximum of 115 mL/min. For this reason, as part of the measures taken to tackle the apparent VF excess in the treatment arm, from patient n°47 we decided to revert to the conventional giving set we were originally using (inner diameter 3 mm), which had been replaced from patient n°8 with blood giving set of larger calibre (4 mm). However, we found no

statistically significant difference between these two giving sets in terms of VF rate (7/19 vs 3/11 with the blood vs standard giving set respectively, *p*=0.59).

Another possibility is that NS might affect the electrical properties of the myocardium, especially at a higher flow rate, and the utilisation of NS as IC perfusate might have facilitated the occurrence reperfusion VF in our study. Romanelli et al. have compared IC bolus injections of NS solution vs 5% dextrose and lactate Ringer's (Hartmann's) solution in patients with normal coronaries and found that the QT dispersion was increased by >40 msec in 26% of patients during IC NS infusion, compared to only 3% of patients with IC Hartmann's solution and 5% dextrose infusions[124]. Similarly, Kim et al. reported a significant prolongation of repolarisation and increase in T wave amplitude when administering NS, and no effect with 5% dextrose water, in patients with variant angina[125].

Of note, in the original proof-of-concept study ORCA-3 Hartmann's solution was used with the rationale that its chemical properties and acidic pH (5.0–7.0) would exert an additional cardioprotective effect[104]. In fact, there is evidence that preventing rapid correction of pH during reperfusion might delay the MPTP opening providing additional time for cytoprotective survival kinases to be activated[126]. Moreover, slowing the normalization of pH in the first minutes of reperfusion is known to reduce infarct size in animal models[127,128]. The choice to use NS in STEMI-Cool, which is also acidic with pH 5-5.5, was made to align with the most relevant clinical studies in the field where no important safety signals were documented[93,103]. However, in CHILL-MI the NS was administered peripherally and in EURO-ICE the transcoronary infusion

was slower (20 mL/min) and initially performed through an OTWB in the context of the occlusion phase of the protocol. It is therefore possible that NS increases the myocardial susceptibility to malignant arrhythmias when infused at higher rates and mixed with the blood at the time of reperfusion.

Mechanistic outcomes

Although STEMI-Cool was not powered to prove the efficacy of TCCD, several mechanistic outcomes were included in the study protocol. The main aims were to capture potential efficacy trends, which would enable power calculation in future trials, explore the mechanism of action of TCCD, and shed light on possible haemodynamic, clinical, and laboratory implications.

As anticipated, no mechanistic outcome showed a statistically significant benefit of TCCD. However, most of the outcomes trended in favour of the treatment arm and no outcome clearly trended against it. The most remarkable positive trend obtained was the numerically smaller early infarct size (11.73±10.40% vs 17.74±12.39%), which corresponds to a 34% reduction in infarct size in the treatment arm as compared to the SOC arm. Only 15 patients underwent CMR, and this might be the reason why the numerical difference between the two groups was not statistically significant (p=0.240). Of note, we performed a subgroup analysis to look for differences in these two groups. We found trends favouring the treatment arm in terms of smaller IMR (28 (19.5-43) vs 52 (42-82), p=0.138), lesser ST-segment elevation at 1 hour (0.5 (0.0-1.625) vs 2 (0.5-2.25), p=0.171), and higher proportion of final TIMI 3 flow (7/8 vs 3/7, p=0.119). It is not clear if these differences were responsible for the

smaller mean infarct size, or else were the means through which TCCD exerted its putative therapeutic effect, as similar trends can generally be found in the whole patient population. However, the fact that the area at risk was similar (23.69±11.01% vs 22.70±14.79%) suggests that the infarct size without treatment would have been the same in the two groups, and the study intervention might have been responsible for the salvage of an extra portion of vulnerable myocardium.

Limitations

This study had some limitations to acknowledge. Due to its small sample size, it was underpowered to provide statistically significant efficacy data, especially due to the limited number of patients who underwent CMR. Furthermore, we were unable to perform a CMR scan at six months, so we could only measure the initial infarct size, which can change significantly by the six-month scan. The broadly inclusive eligibility criteria allowed the recruitment of a heterogeneous STEMI population with lesions affecting different coronary vessels and territories of different sizes, and with different clinical presentation (from mild chest pain to out-of-hospital cardiac arrest). This way we demonstrated the feasibility of TCCD in a broad range of patients and clinical settings, but further limited our ability to detect efficacy by not selecting the patient population where this treatment is likely to be more beneficial, i.e. large infarcts. This is reflected by a mean infarct size of 17.74±12.39% in our control population, which is smaller than that observed in EURO-ICE (21.6±12.2%), where only proximal-mid LAD occlusions were included. Moreover, the open-

label design might have introduced bias in the patient perception of symptoms especially during the follow-up, where this might have affected the reported symptoms and readmissions.

Moving on to a multicentre randomised clinical trial

The purpose of the STEMI-Cool pilot was to pave the way for a future multicentre trial by proving recruitability, feasibility, and safety of the protocol, and collecting useful preliminary data.

We involved several interventional cardiologists at our centre who did not participate directly in the study design, but were able to run the protocol with directions from a trained team member and with an excellent grade of acceptance. However, to further enhance the possibilities of widespread applicability, we have been striving to simplify the method and specifically eliminate the need for thermistor wire monitoring. In fact, we found a significant linear correlation between DBP and IP required to obtain the desired temperature reduction (–6-8°C). which would permit to perform TCCD with acceptable accuracy without the need for a thermistor wire. A proposed simplification of the formula could be IP=DBPx4–100 mmHg, which would be a reasonable approximation especially for DBP values around 80 mmHg, and improve its ease of use.

In terms of patient population, we believe that patients with STEMI affecting any main coronary vessel should be included, which would improve the recruitment rate as compared to clinical trials involving a single vessel only, and make the results more generalisable. However, the infarcted territory

needs to be sufficiently large to allow the detection of a significant reduction in infarct size. Therefore we would exclude lesions beyond mid-level in the main vessels (LAD, RCA, left circumflex artery = LCx). Additionally, to ensure that we are only selecting patients with no reperfusion prior to TCCD, we would exclude TIMI 1 lesions, where reperfusion might be happening already to some extent.

In terms of time from symptom onset, we extended the cut-off to 12 hours as opposed to the 6 hours classically adopted in other trials. This is based on the notion that, while ischaemic injury becomes more important towards the end of the treatment window, RI continues to increase over time[22]. Therefore, we believe that TCCD might still be beneficial at this stage. Moreover, recruiting very early STEMIs (<2 hours) with minimal myocardial damage also carries the risk of not being able to detect an infarct size reduction as compared to standard practice, but excluding these patients would be impractical. A timeframe of 0-12 hours would align with the current ESC guidelines, which recommend reperfusion therapy in all patients with a working diagnosis of STEMI and symptoms of ischaemia of ≤12 hours duration[3].

Lastly, we would revert to using Hartmann's solution instead of NS, which was safe in our proof-of-concept study[104].

In summary, our proposed eligibility criteria for a multicentre clinical trial powered for efficacy would be as follows; 1) STEMI with symptom onset < 12 hours; 2) angiographic evidence of acute proximal or mid-vessel occlusion of

the LAD, LCx, or RCA; 3) coronary lesion with TIMI 0 flow; and 4) life expectancy >1 year.

Conclusions

The STEMI-Cool pilot has been run successfully in terms of recruitment rate, feasibility, and safety. Experience from the study has provided the means to further simplify the method for potential widespread applicability, should it prove effective, by eliminating the need for a thermistor wire and by calculating the IP needed with a simple formula. The encouraging trends in mechanistic outcomes suggest that this cardioprotective strategy has the potential to prove effective in a large multicentre trial, which will be the next investigational step.

Acknowledgments

This work was financially supported by the Royal Brompton and Harefield Hospitals Charity.

I am deeply grateful to Dr Miles Dalby (Harefield Hospital, Guy's and St Thomas' NHS Foundation Trust) for starting this project, mentoring me during my work in the UK, and helping me rise in my clinical and scientific career.

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