PREHOSPITAL PULSE-DOSE GLUCOCORTICOID IN PATIENTS WITH ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION 2 – THE PULSE-MI 2 TRIAL

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1. Abbreviations

AEs	Adverse Events
CAG	Coronary Angiography
CABG	Coronary Artery Bypass Graft
CEC	Clinical Event Committee
ECG	Electrocardiogram
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICU	Intensive Care Unit
LVEF	Left Ventricular Ejection Fraction
PCI	Percutaneous Coronary Intervention
PPJ	Prehospital Patient Journal
PULSE-MI	Prehospital Pulse Glucocorticoid Therapy in Patients with ST-Segment Elevation Myocardial Infarction Trial
RD	Referring Cardiology Fellow Doctor
SAEs	Serious Adverse Events
STEMI	ST-segment elevation myocardial infarction
SUSARs	Suspected Unexpected Serious Adverse Reactions
TIMI	Thrombolysis In Myocardial Infarction

Designation of investigational treatments Prehospital pulse-dose glucocorticoid in patients with ST-segment elevation myocardial infarction (STEMI) transferred for acute coronary angiography
Title of the trial Prehospital pulse-dose glucocorticoid in patients with STEMI – the PULSE-MI 2 trial
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Study Centers Rigshospitalet, Copenhagen University Hospital; Aarhus University Hospital; Odense University Hospital; Aalborg University Hospital
Planned Study Period 2026-2031
Objectives The aim of this nationwide trial is to evaluate the effect of prehospital pulse-dose glucocorticoid on all-cause mortality compared to placebo in patients with STEMI
Methodology Prior to informed consent, patients will be randomized in the prehospital setting in a 1:1 fashion to either pulse-dose glucocorticoid (250 mg methylprednisolone) or placebo. A nationwide, randomized, blinded, placebo-controlled, multicenter trial.
Number of patients A total of 5204 patients with 2602 in the glucocorticoid group and 2602 in the placebo group.
Inclusion Criteria 1. Age ≥18 years including fertile women* 2. Acute onset of chest pain with < 24 hours duration 3. STEMI as characterized on electrocardiogram (ECG) by one of the following: 1) at least two contiguous leads with ST-segment elevation ≥2.5 mm in men < 40 years, ≥2 mm in men ≥40 years, or ≥1.5 mm in women in leads V2-V3 and/or ≥1 mm in the other leads, 2) presumed new left bundle branch block with ≥1 mm concordant ST-segment elevation in leads with a positive QRS complex, or concordant ST-segment depression ≥1 mm in V1-V3, or discordant ST-segment elevation ≥5 mm in leads with a negative QRS complex, 3) Isolated ST depression ≥0.5 mm in leads V1-V3 indicating posterior acute myocardial infarction, 4) ST-segment depression ≥1 mm in eight or more surface leads, coupled with ST-segment elevation in aVR and/or V1 suggesting left main-, or left main equivalent- coronary obstruction

**It is not possible to perform a pregnancy test (HCG urine test) in the prehospital setting. However, methylprednisolone is not contraindicated in pregnant women.*
Exclusion Criteria 1. Suspected other than type I acute myocardial infarction at time of potential randomization 2. Initial presentation with cardiac arrest (out-of-hospital cardiac arrest) 3. Known allergy to glucocorticoid
Primary Outcome All-cause mortality

2. Background

ST-segment elevation myocardial infarction (STEMI) remains a leading cause of global all-cause mortality despite significant advances in reperfusion therapies with timely primary percutaneous coronary intervention (PCI)[1]. One-year mortality after STEMI has decreased to around 8%, yet a substantial number of patients still develop heart failure, which entails high morbidity and an increased risk of cardiovascular mortality[2]. Moreover, high-risk patients with STEMI, such as patients presenting with cardiogenic shock, continues to have a 50% risk of death within the first year[3]. Therefore, while overall mortality after STEMI have decreased, the most critically ill patients continue to have a very poor survival probability, primarily driven by cardiovascular causes such as arrythmia and cardiogenic shock[4].

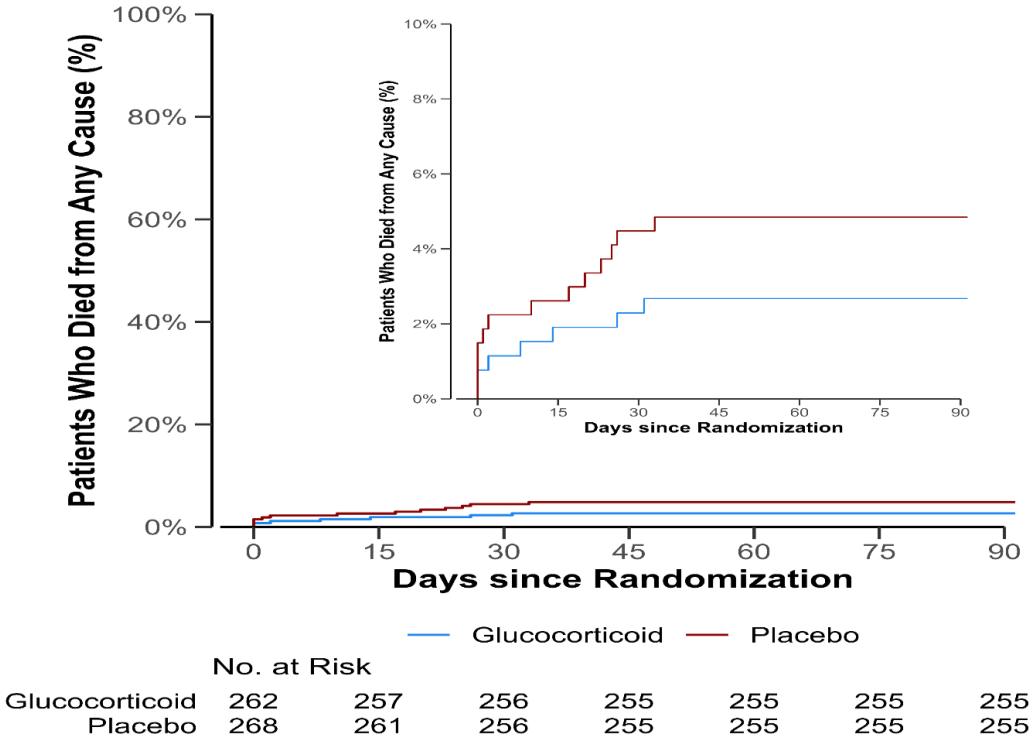
Inflammation is induced immediately after the onset of acute myocardial ischemia and is subsequently exacerbated following reperfusion[5]. Hence, inflammation in the setting of STEMI is a pivotal factor in both acute myocardial ischemia and the subsequent healing of the infarcted myocardium, which is why inflammation *per se* is a feasible and desirable target for improving prognosis in these patients. Nevertheless, inflammation may constitute both favorable and deleterious effects in patients with STEMI[6]. On one hand, the inflammatory response induced after STEMI may serve a protective and healing role favorable for the infarcted myocardium[6]. On the other hand, residual inflammation and in particular the acute burst of inflammation in the early phase of STEMI may contribute to excessive cardiomyocyte death, adverse remodeling, and recurrent major adverse cardiac events[6,7]. When targeting inflammation in STEMI as a therapeutic strategy, it therefore remains crucial to inhibit the deleterious inflammatory response without compromising the protective inflammatory effects.

Glucocorticoids are crucial in regulation of the systemic inflammatory response and may therefore be beneficial in limiting myocardial injury after STEMI[8]. If given in high doses, glucocorticoids mediate two different mechanisms: the genomic effect mediated by glucocorticoid receptor occupation, gene transcription, and translation within the cells which is induced within hours and the non-genomic effect, which is induced rapidly (<15 minutes) after administration via plasma membrane bound receptors and independent of cytosolic receptor occupation and genomic regulation[9]. Some of the proposed nongenomic effects of glucocorticoids on the cardiovascular system include decreased vascular inflammation and reduced infarct size by activating the endothelial nitric oxide synthase, cardioprotection through membrane stabilization through nitric oxide production, and increasing the contractility of the vascular smooth muscle cells[9]. Taken altogether, the non-genomic

actions secure that the anti-inflammatory effect is initiated quickly to protect the myocardium immediately whereas, in addition, the genomic actions protect the tissue in the subsequent period.

The use of glucocorticoid in relation to ischemia has therefore been investigated in experimental, in-vivo, and clinical studies in the 1970's and 1980's. In experimental animal studies, glucocorticoids showed beneficial effects on hemodynamics and infarct size following ischemia, however most studies were performed in non-reperfused animals[10–12]. Albeit this, clinical studies in patients with STEMI have shown conflicting results[10–12]. However, these studies performed in patients with STEMI were before the era of primary PCI[10], and since the implementation of PCI, the prognosis in these patients has improved significantly[1]. Therefore, we conducted a phase-II randomized, placebo-controlled, clinical trial (PULSE-MI) investigating the effect of prehospital pulse-dose glucocorticoid on final infarct size in patients with STEMI transferred for acute coronary angiogram (CAG)[13]. The trial was conducted between November 2022 and October 2023, and in 742 patients with STEMI randomized in the prehospital setting, pulse-dose glucocorticoid deemed safe and showed acute beneficial effects on left ventricular ejection fraction (LVEF), infarct size, and microvascular obstruction[14]. Moreover, there was a trend towards a lower incidence of both all-cause mortality (7 (3%) vs 13 (5%)) at three months in patients treated with glucocorticoid compared to placebo (Figure 1)[14].

Figure 1. All-cause mortality in patients included in PULSE-MI



As this was a phase II trial, the trial was not powered to detect differences in clinical outcomes. Therefore, it remains to be proven whether prehospital pulse-dose glucocorticoid in patients with STEMI transferred for acute CAG will translate into reduced mortality after STEMI.

3. Hypothesis and Aim

In patients with STEMI, single-pulse-dose glucocorticoid (250 mg methylprednisolone) administrated in the prehospital setting reduces mortality after STEMI. Thus, the aim of this prospective, randomized trial is to evaluate the effect of prehospital pulse-dose glucocorticoid in patients with STEMI.

4. Clinical Relevance

A Continued aim to improve the prognosis after STEMI is pivotal as it remains one of the leading causes of death worldwide. The main cause of death in patients with STEMI is cardiovascular due to myocardial damage leading to either arrythmia or fatal heart failure. Thus, inflammation serves as a key factor, and inhibiting the acute, deleterious inflammatory response without compromising the subsequent protective inflammatory response may be crucial to improve the prognosis in patients with STEMI. The proof-of-concept trial (PULSE-MI) showed acute beneficial effects of prehospital glucocorticoid in patients with STEMI, and patients treated with glucocorticoid had numerically lower incidence of all-cause mortality at three months[14]. As this was a phase-II trial and not powered to detect differences in clinical outcomes, this present and adequately powered trial addresses whether prehospital pulse-dose glucocorticoid improves survival in patients with STEMI.

5. Trial Setup

5.1 Trial Design

This multicenter trial is an investigator-initiated, 1:1 randomized, blinded, placebo-controlled clinical trial to investigate whether pulse-dose glucocorticoid (250 mg methylprednisolone) in the prehospital setting reduces all-cause mortality in patients with STEMI. The trial will be chaired at the Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Denmark. Other sites include Odense University Hospital, Aarhus University Hospital, and Aalborg University Hospital.

5.2 Target Patient Population and Number of Patients

The target patient population is male and female patients aged 18 years and over with documented evidence of STEMI, eligible planned for primary PCI with symptom onset within 24 hours. As done in the previous PULSE-MI trial[13], randomization will be managed by an ambulance staff in the pre-hospital setting and patients are included by the referring doctor (on-call cardiology fellow doctor (RD)).

A total of 5204 patients with STEMI (2602 patients treated with intravenous infusion of glucocorticoid (250 mg methylprednisolone) and 2602 patients with intravenous placebo infusion) will be included for the primary endpoint of all-cause mortality.

5.3 Trial Setting

Patients with STEMI will be included in the prehospital setting throughout all regions of Denmark prior to acute CAG at Rigshospitalet, Aarhus University Hospital, Odense University Hospital, or Aalborg University Hospital if eligible to participate in the study. Eligibility will be carefully screened by the RD at Rigshospitalet, Aarhus University Hospital, Odense University Hospital, or Aalborg University Hospital.

5.4 Estimated Study Duration

We expect the trial to begin once the trial has been approved by the ethical and legal authorities (estimated start/medio 2026). The trial will run in all four regions covering 6.0 million Danish citizens as of Q3 2025[15] and perform approximately 4000 acute CAG procedures per year. An inclusion is planned to be completed in four years, and the follow-up period will be completed after five years. All patients will be followed for a minimum of one year. Following completion of the trial, the hospital records will be accessed up to 10 years to obtain information on clinical events.

Planned time schedule:
Start of trial: start/medio 2026
End of trial: start/medio 2031

5.5 Inclusion and Exclusion Criteria

Inclusion criteria for recruitment are listed below and must be fulfilled for the patient to be randomized:

1. Age ≥18 years including fertile women*
2. Acute onset of chest pain with < 24 hours duration
3. STEMI as characterized on electrocardiogram (ECG) by one of the following[16]:

   1) at least two contiguous leads with ST-segment elevation ≥2.5 mm in men < 40 years, ≥2 mm in men ≥40 years, or ≥1.5 mm in women in leads V_2-V_3 and/or ≥1 mm in the other leads,

   2) presumed new left bundle branch block with ≥1 mm concordant ST-segment elevation in leads with a positive QRS complex, or concordant ST-segment depression ≥1 mm in V_1-V_3, or discordant ST-segment elevation ≥5 mm in leads with a negative QRS complex

   3) Isolated ST depression ≥0.5 mm in leads V_1-V_3 indicating posterior acute myocardial infarction (AMI)

   4) ST-segment depression ≥1 mm in eight or more surface leads, coupled with ST-segment elevation in aVR and/or V_1 suggesting left main-, or left main equivalent-coronary obstruction

Exclusion criteria for recruitment are listed below and none of the criteria must be fulfilled for a patient to be eligible:

1. Suspected other type I acute myocardial infarction at time of potential randomization[17]
2. Initial presentation with cardiac arrest (out of hospital cardiac arrest)
3. Known allergy to glucocorticoid

6. Trial Progress and Conduct

6.1 Screening and Inclusion

As in the PULSE-MI trial[13], screening, inclusion, and registration will be done in the ambulance in the prehospital setting. The including RD will carefully check the eligibility of the patient to participate in the trial. The including RD is continuously in touch with the ambulance staff in the ambulance by telephone from the time of screening until randomization has been done. The including RD has all the necessary information about the patient including previous medical history, actual medications, and clinical status. Clinical status includes patients' general condition, blood pressure, heart rate, and ECG changes. Due to the acute nature of STEMI and the trial design, the qualifying ECG will be

* It is not possible to perform a pregnancy test (HCG urine test) in the prehospital setting. However, methylprednisolone is not contraindicated in pregnant women.

performed in the prehospital settings as normal procedure. These data will be used for eligibility evaluation by the including doctor (RD). The RD that is responsible for inclusion of the patient via telephone must ensure that there are no contraindications for administration of glucocorticoids as part of the eligibility check. This information is available via the patient charts that can be accessed by the RD. If the including RD find the patient eligible to participate in the study, the including RD will ask the ambulance staff to give the study medicine to the patient in the prehospital setting.

In the prehospital setting, the ambulance staff will do the following:

1. Include the patient in the study following eligibility check by the RD
2. Randomize the patient by picking a random box from a storage in the ambulance containing the study medicine (glucocorticoid or placebo). All boxes are identical, and the ambulance staff is therefore blinded when randomizing
3. Register the patient in an electronic database, REDCap (described in section 6.5)

All patients, or the legally designated representative must personally sign and date the informed consent form (ICF) as soon as possible following the acute treatment of the patient (primary PCI/acute coronary artery bypass graft (CABG)) (described in detail in section 9.2). If patients remain unable to provide informed consent five days following admission, the nearest relative to the patient will also sign the ICF on behalf of the patient as soon as possible. Informed consent from the patient will subsequently be obtained as soon as possible. The procedure of informed consent and ethical considerations are described thoroughly in section 9.2 and 9.2.1, respectively.

6.2 Randomization

Randomization of the patient will take place in the prehospital setting. Patients who satisfy all the eligibility requirements will be randomized. Randomization is done by the staff in the ambulance. Patients will be randomized in a 1:1 ratio to either infusion of glucocorticoid (250 mg methylprednisolone) or placebo that are placed in identical boxes. The ambulance staff randomizes the patient by picking a random box stored in the ambulance delivered by the pharmacy, including glucocorticoid or placebo, and administrates (over a period of 5 minutes) the content as a bolus infusion. After infusion of the study intervention, the ambulance will bring the box to the hospital alongside with ECGs obtained in the ambulance in case of need for emergency unblinding (described in detail in section 6.4). All boxes are identical and has a unique box serial number. Once a patient has been randomized (i.e. a box is opened), the patient is considered included in the trial. The ambulance staff will ensure the following:

1. Annotate the number of the study medicine box in the prehospital journal (PPJ)
2. Take a picture of the study medicine box in the PPJ
3. Bring the study medicine box to the hospital
4. Register the patient by scanning QR-code on study medicine box

The hospital staff and investigators will always have access to the PPJ of the patients. All RD's and ambulance staff have relevant knowledge of the study from the handing out of the study protocol, protocol summaries, and pocket cards as well as via e-mail and on-going project recaps. In case of any technical issues (i.e. register the patient with QR-code), one of the investigators will be available by telephone.

6.3 Study Medicine and Placebo

Patients will be allocated to either infusion of glucocorticoid (250 mg methylprednisolone) or placebo that are placed in identical boxes. Each box will contain either methylprednisolone or placebo and each box will be numbered by the pharmacy. Administration of the study medicine will not prolong the transportation or any aspect of the standard treatment of the patient. The patients will be treated according to standard procedures in the ambulance. Primary PCI and antithrombotic regimens will be performed according to standard procedures.

The numbers on the identical boxes are unique, and the pharmacy has the allocation list. The active study medicine is 2 x 125 mg/2 mL Solu-Medrol, a total of 250 mg/4 mL, which comes as a sterile powder with preservative free isotonic NaCl as diluent. The medicine takes 30 to 60 seconds to mix and needs to be used within 48 hours when opened. The placebo will be 0.9% NaCl in 4 mL ampoules. The infusion of both study medicine and placebo will be done over a period of 5 minutes during transportation.

Following preparation of the study medicine boxes and before initiation of the trial, the boxes will be shipped to all the critical care unit stations in the respective regions. All units are manned 24 hours 7 days a week. To ensure that all ambulances always have study medicine boxes in all ambulance, the ambulance staff will pick up a new study medicine box from the receiving hospital personal following transfer of a randomized patient.

6.4 Blinding

The pharmacy of the Capital Region of Denmark will pack the boxes and label the study medicine and placebo ampoules. The randomization will be allocated by the pharmacy and their independent

statistician using a random number generator. The pharmacy will keep the allocation list and will not be involved otherwise in the trial. Sponsor and investigators of the trial keep an allocation list locked at in a safety cabinet in case of emergency unblinding. Sponsor and investigators are responsible for any emergency unblinding. Moreover, investigators can unblind the study medication without prior contact to the sponsor. According to the ICH-GCP guideline 4.3.1, the investigators are responsible for all trial-related medical decisions and may unblind immediately without restrictions.

The pharmacy is not able to make matching placebo ampoules as Solu-Medrol does not come in a premixed ampoule and needs to be mixed before use in the ambulance. The treating ambulance staff is therefore unblinded after randomization, however, the ambulance staff will not take any further part in the trial nor the treatment of the patient once arrived at the hospital.

6.5 Registration

All ambulance staff have access to the registration form in REDCap. The registration form can be accessed through a QR-code on the study medicine box or through an internet browser. When a patient is registered, the patient will be allocated a patient identification number by a computer-generated code. The identification number will be used to identify the patient throughout the whole study. The identification number consists of three digits assigned in sequential order as patients are screened (001, 002, 003, etc.). All patients considered for acute CAG due to suspected STEMI are screened for eligibility will be registered in an electronic database, REDCap. In the registration form, the ambulance staff will annotate the following information about the patient: 1) civil registration number of the patient, 2) the number of the study medicine box, 3) time of study medicine intervention, and 4) if the study medicine was given according to the protocol (yes/no), and if not, why the study medicine was not given. The staff in the ambulance will sign the registration form following inclusion.

6.6 Dosage Adjustments

The dose of the study medicine will be fixed. In case of any clinical signs of an allergic reaction or severe side effects are suspected, the infusion will be terminated immediately. No dosage adjustments are allowed.

6.7 Discontinuation of Study Participation

A patient can withdraw from the study at any time, or when medically necessary, as judged by the investigator. The information about withdraw at any time from the trial is emphasized to the patient.

6.8 Concomitant Procedures

6.8.1 Acute CAG and Primary PCI Procedure

The patient is admitted to the hospital because of STEMI with symptom onset less than 24 hours before contact. The acute CAG and primary PCI will be performed as usual clinical practice. The patient is pretreated with aspirin (300 mg) and 10.000 units of heparin in the prehospital setting by the ambulance staff as soon as the including RD has set the diagnosis of STEMI and referred the patient to acute CAG. These procedures are of standard clinical practice and in accordance with guidelines. Primary PCI is prepared and performed as per guidelines the catheterization laboratory. Immediately after primary PCI, a loading dose of either 60 mg prasugrel or 180 mg ticagrelor is given per os. Clopidogrel 600 mg can be chosen alternatively at the discretion of the treating physician (e.g. high bleeding risk, ongoing anticoagulation therapy).

6.8.2 Acute Coronary Artery Bypass Graft (CAGB)

Approximately 4% of patients transferred for acute CAG with STEMI will undergo acute CABG as judged by the treating PCI operator and thoracic surgeons[14]. This is part of the standard procedure.

6.8.3 Follow-Up Medical Treatment

The follow-up medical treatment will be completely in accordance with international guidelines and local standard operating procedures. Dual antiplatelet therapy with aspirin 75 mg once daily and prasugrel 10 mg or 5 mg (age above 75 or body weight under 60 kg) b.i.d., or ticagrelor 90 mg will be given for 12 months. Deviation from this is allowed at the discretion of the PCI operator.

6.8.4 Follow-Up

All randomized patients will be followed for events for 10 years after inclusion. All patients will be followed for at least one year. Follow-up will be done by hospitals files and national registries. No safety event recording and annual safety reports will be done in the time between completion of the trial (5 years) and the 10-year follow-up assessment.

7. Endpoints

Primary and secondary endpoints are listed in Table 1.

Table 1. PULSE-MI 2 endpoints	
	Intervention with glucocorticoid vs. placebo
Primary endpoint	All-cause mortality
Secondary endpoints	Cardiovascular mortality
	Spontaneous myocardial infarction
	Admission for heart failure
	All-cause mortality or admission for heart failure
	Cardiovascular mortality or admission for heart failure
	Recurrent non-fatal cardiovascular events (spontaneous myocardial infarction and admission for heart failure) with death treated as a terminal event

Exploratory endpoints:

1. Urgent revascularization
2. Target vessel revascularization
3. Target lesion revascularization
4. Any repeat revascularization
5. Definitive stent thrombosis
6. Peak troponin-T within 24 hours of STEMI
7. Malignant ventricular arrhythmia during admission
8. Admission to intensive unit during admission
9. Implantation of implantable cardioverter defibrillator (ICD)

7.1 Endpoint Definition

All endpoints are defined according to previous definitions (please see Appendix A for endpoint definitions)[17–19].

7.2 Endpoint Recording

All endpoints will be identified using national registries and patient files, in which all deaths and hospital referrals are reported. All clinical endpoints are defined in Appendix A. Violations of the protocol will be monitored continuously. All clinical endpoints will be adjudicated by an independent clinical events committee (CEC) who will review all relevant medical records. The CEC will consist of at least two cardiologists, and the members will be selected before initiation of the trial. No members of the CEC participate in recruitment or data collection or have access to any information regarding treatment allocation. A list of the proposed CEC members is given in Appendix B.

7.3 Monitoring

Monitoring of the study will be done by a local Good Clinical Practice (GCP) units. The trial will be conducted in compliance with the trial protocol, the Helsinki Declaration, the GCP guidelines (ICH-GCP), and European and national laws. The sponsor of the trial allows investigators and institutions involved in the trial to perform trial-related monitoring, audit, and inspection from the authorities including direct access to the data and documents related to the trial.

The first monitoring meeting will be held after inclusion of 100 patients. The following meetings will be held as needed. A full monitoring plan will be elaborated and composed before initiation of the trial by the local GCP unit.

8. Ethical Assessment

The patient will be informed and treated according to the international guidelines (ICH-GCP) and protected under the General Data Protection Regulation (GDPR) including the processing of personal data (data protection law) and the Danish Health Act. As to the need for fast transportation and acute treatment, the main ethical issue that could arise from this trial is that informed consent cannot be obtained prior to intervention and treatment with primary PCI due to the acute setting of the trial. Study intervention before informed consent is done to secure initiation of the study treatment as soon as possible due to the need of acute intervention and treatment in STEMI and vulnerable patients. Moreover, the ability of the patient to provide informed consent in the prehospital setting is questionable due to vulnerability, chest pain, and acute setting. This implies the following:

- Recruitment and randomization in the prehospital setting will be performed by the ambulance staff

- The including doctor is the RD. The including RD is continuously in touch with the ambulance staff in the ambulance by telephone from the time of screening until randomization has been done
- The patient will provide informed consent as soon as possible following the acute management of the patient (primary PCI/acute CABG), once the patient is stable
- The process of informed consent is described thorough in section 9.2. The process follows article 35 of Clinical Trial Regulation EU No 536/2014 and Law of Clinical Trials, §3
- The justification for providing informed consent following intervention due to the emergency situation is described thorough in the section 9.2.1

9. Ethical Considerations

9.1 Ethical Conduct of the Study

The ethics committee will approve the study protocol and ICF before the initiation of the trial. The study protocol will be registered at the Danish Data Protection Agency. Good Clinical Practice will be observed throughout the whole trial and appropriate international, European, and national legislations and guidelines will be respected. The Data Safety Monitoring Board (DSMB) will monitor the study by assessing the safety and efficacy of interventions during the trial and the overall conduct of the clinical trial. The responsibilities of the DSMB will be described thorough in the DSMB charter. The trial will be conducted in compliance with the published trial protocol, the Helsinki Declaration, the GCP guidelines (ICH-GCP), and national laws.

Due to the acute nature of STEMI, vulnerability, and inability of the patient to provide informed consent in the prehospital setting, intervention will occur in the prehospital setting prior to obtaining informed consent. Patients referred to primary PCI are in an acute, vulnerable, and painful situation during transportation to the hospital, therefore, we find it acceptable to include and initiate the intervention in the prehospital setting before provision of informed consent. The study medicine (glucocorticoid (250 mg methylprednisolone)) is a well-known and described drug used in several acute settings and is therefore considered safe without any harm/risks to the patient. This was tested in the PULSE-MI trial and there were no differences in terms of adverse and serious adverse events in patients treated with glucocorticoid compared with placebo[14]. Additionally, time from medical contact to intervention and treatment is pivotal to secure the best possible prognosis of patients with

STEMI. Thus, intervention in the prehospital setting without informed consent ensure no delay of the urgent treatment of the patient's medical condition.

9.2 Informed Consent

Up to 90-95% of patients with STEMI will be stable, without chest pain, and able to provide informed consent as soon as possible following the acute treatment with primary PCI. Following the acute phase, a study nurse or one of the investigators will provide detailed and thorough information about the study and obtain informed consent from the patient (signature of the ICF). Signature of ICF is designed and encrypted in REDCap and will be signed in REDCap following informed consent. The patient will provide informed consent as soon as possible following the acute initial treatment (primary PCI), once the patient is stable. The right to withdraw informed consent at any time will be emphasized to the patient. If the patient does not wish to participate in the study, the data collection of the patient will be stopped immediately. Data will be kept and used unless the patient requests to have all data deleted.

In the remaining 5-10% patients with STEMI unable to provide informed consent directly following primary PCI, consent will be obtained by the legally designated representative who will sign the ICF. The legally designated representative consent will be followed by informed consent and signature of the ICF by the patient, once the patient is stable and able to provide informed consent. If the patient remains unable to provide informed consent five days following admission, the closest relative to the patient and legally designated representative will provide consent on behalf of the patient and sign the ICF as soon as possible. The patient will subsequently provide informed consent as soon as possible. In cases of death prior to informed consent, the legally designated representative will provide informed consent as soon as possible. The closest relative will be informed about participation in the trial including the use of the patient's data in relation to the study and informed consent will be obtained. Further, the closest relative will be informed of the patient's right to discontinue from the study at any time. In case of any objection or unwillingness towards participation in the trial, the patient will then be excluded from the study. However, medical records of the patient following death informed consent will be checked for causes of death to ensure no relation to the study medicine.

The informing doctor/study nurse will ensure:

1. Each patient is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study,

2. The patient is aware of the right to discontinue from the study at any time,
3. The patient is given the opportunity to ask questions about the study.

9.2.1 In the Emergency Situation

The inherent acute nature of the study necessitates circumvention of the usual requirements of a 24-hour time for consideration, as it has previously been done in comparable acute studies e.g. PULSE-MI[14], ATLANTIC[20], EUROMAX[21], and CONDI-2/EPIC-PPCI[22]. This approach is reasonable because the intervention is without any known risk and because the intervention has potential benefit for the individual patient.

Up to 60% of patients with STEMI have reperfusion in the prehospital setting due to effective antithrombotic treatment with heparin and aspirin. Thus, myocardial damage and inflammation occurs immediately after myocardial ischemia and is further exacerbated following reperfusion. To effectively alleviate the acute inflammation and myocardial injury, randomization and intervention is to be done in the prehospital setting. Hence, intervention in the ambulance before reperfusion is needed to initiate the potentially beneficial and immediate nongenomic effects and subsequent protective genomic actions of pulse-dose glucocorticoid as soon as possible. Moreover, as demonstrated in previous trials, the potential effect of a treatment seems to be more pronounced if the treatment is initiated early after the onset of STEMI. In addition to reperfusion induced inflammation, ischemia itself, immediately after occlusion of the artery, induces inflammation. Hence, initiation of the intervention is needed as close to the debut of symptoms as possible to inhibit the inflammation adequately and effectively in relation to STEMI. Thus, by performing intervention in the prehospital setting, we expect that participation in the trial will have the potential to produce a direct clinically relevant benefit for the patient resulting in a measurable health-related improvement alleviating the suffering and potentially improving the health of the patient and the prognosis of the medical condition. Moreover, the trial relates directly to the patient's medical condition and it is not possible to obtain prior informed consent from the patient and to supply prior proper information about the trial due to the acute nature of STEMI. Therefore, the trial is of such nature that it may be conducted solely in emergency situations.

Patients with STEMI are unable to provide prior informed consent and receive prior information on the clinical trial, as STEMI is a sudden, life-threatening, and serious medical condition requiring urgent treatment. Most of patients with STEMI treated with primary PCI have less than 30 minutes of transportation to the hospital. Therefore, it is not possible within the therapeutic window

to supply all prior information about the trial and obtain prior informed consent. The including RD is continuously in touch with the ambulance staff in the ambulance by telephone from the time of screening until randomization has been done. The including RD has all necessary information about the patient through medical records to ensure that the patient does not have any objections towards participating in the trial. In addition, the trial and intervention pose minimal risk and burden on the patient in comparison with standard treatment of the patient's condition, as glucocorticoid is a well-known and thoroughly investigated drug of minimal risk if administrated as a single dose administration. Moreover, the PULSE-MI trial showed no harm of glucocorticoid compared with placebo[14].

Most of patients with STEMI will be able to provide informed consent and receive adequate information about the trial as soon as possible following the acute treatment. Continuation of participation in the trial will therefore be ensured with adequate information and informed consent as soon as possible following the acute treatment.

The Principal Investigator(s) will ensure:

1. Each patient will give informed consent based on the ICF as soon as possible following the acute treatment (primary PCI/acute CABG) including signature of the ICF.
2. In patients who are unable to provide informed consent as soon as possible following the primary PCI procedure, informed consent will be obtained by the legally designated representative including signature of the ICF followed by informed consent from the patient as soon as possible.
3. In patients who remain unable to provide informed consent five days following admission, the legally designated representative and nearest relative will provide informed consent and signature of the ICF on behalf of the patient. Informed consent from the patient will subsequently be obtained as soon as possible.
4. In patients who do not wish to participate in the study, the data collection of the patient will be stopped immediately.
5. In cases of death prior to informed consent, the legally designated representative will provide informed consent as soon as possible. The closest relative will be informed about participation in the study including the use of the patient's data in relation to the study and provide informed consent. In case of any objection or unwillingness towards participation in the study, the patient will then be excluded from the study.
6. All signed ICFs are encrypted and kept in REDCap.

9.3 Benefits and Risks

The expectation of the trial is that administration of pulse-dose glucocorticoid (250 mg methylprednisolone) in the prehospital setting will reduce death in patients with STEMI. The patients included in the study will have a closer medical control due to participation, however, there is no guarantee that the individual participants achieve any benefits from participating in the trial. The study will not delay or interfere with standard therapeutic or diagnostic procedures.

Based on the experience from the PULSE-MI trial and the findings hereof, glucocorticoid is easy to administrate, has an acute effect, and implementation of glucocorticoid administration in the prehospital setting is highly feasible to be a part of the routine clinical care of patients with STEMI[13,14,23]. The feasibility of the trial is underlined by all patients in the PULSE-MI trial (742 patients) being included within 11 months and only 4% of eligible patients transferred to Rigshospitalet with STEMI were not included in the trial[14]. The study medicine (glucocorticoid) is approved and recommended in other medical conditions and is expected to be of minimal risk to patients as a single dose. If successful, the increased knowledge on the therapeutic beneficial potential of glucocorticoid will 1) increase scientific knowledge, and 2) improve clinical outcomes in patients with STEMI by reducing the number of deaths after STEMI. The information gathered from this trial may therefore help future patients and change future clinical guidelines.

10. Statistics

A detailed statistical analysis plan is given in Appendix C done by a statistician and the principal investigators.

10.1 Data Management

The data management work up and statistical analyses will be performed at Rigshospitalet, Copenhagen University Hospital, Denmark.

10.2 Power Calculations and Sample Size

The median follow-up of the trial is expected to be 3 years with a maximum follow-up of 5 years and minimum follow-up of 1 year. The primary outcome will be all-cause mortality. As an estimate based on findings from the PULSE-MI trial[14] and the DANAMI-3 trial[24], the estimated event rate of in the placebo arm is 9% during follow-up. Glucocorticoid is expected to reduce all-cause mortality corresponding to a hazard ratio of 0.77. To demonstrate the reduction in the primary outcome with an 80%

power at a 5% significance level, 2602 patients in each treatment arm is needed. The primary analyses will be intention to treat principle.

After inclusion of 50% of the planned sample size, a blinded evaluation of the total number of events will be conducted annually to assess the need for potential sample size adjustment. No interim analysis will be done.

10.3 Analysis populations

10.3.1 Intention-to-treat (ITT)

The intention-to-treat (ITT) analysis population will include all randomized patients who provided consent for use.

10.3.2 Additional key analysis

A differentiation between a classic type I myocardial infarction with plaque rupture and thrombosis formation and a type II myocardial infarction with other reasons for ST-segment elevation (e.g. takotsubo, myocarditis, pericarditis, spontaneous coronary artery dissection, coronary embolism, coronary spasm, anemia, etc.[17]) can only be made after inclusion and acute CAG. Any patient with type II myocardial infarction or a patient who does not fulfill all inclusion and no exclusion criteria will be excluded from the additional analysis. Hence, the additional analysis will only include patients with STEMI and type I myocardial infarction[17].

10.4 Statistical Analysis

All analysis will be performed in the ITT population. The primary outcome is defined as the occurrence of all-cause mortality. The primary analysis will be a comparison of the rate of mortality between the glucocorticoid group and placebo group. Hazard ratios and 95% confidence intervals will be calculated using Cox proportional hazard model and probability of survival will be displayed using the Kaplan-Meier methodology. The assumptions for the Cox proportional hazard model will be assessed and if there is clear non-proportionality, hazard ratios will be presented separately for the relevant time periods. A two-tailed P-value <0.05 will be considered statistically significant.

Secondary outcomes will be handled as time-to-event outcomes. The composite endpoints 1. all-cause mortality and admission for heart failure and 2. cardiovascular mortality and admission for heart failure will also be handled as binary outcomes. Kaplan-Meier curves for each treatment group will be calculated, graphically displayed, and compared using either the log-rank test or competing

risk models. Competing risk models may be used to account for the competing risk of all-cause mortality. Further, Cox proportional hazard models will be calculated to assess differences in the time to event between groups. Analysis of cumulative number of events is described in Appendix C.
All endpoints will also be assessed in an additional key analysis in patients with type I myocardial infarction (please see section 10.3.2 for the description of the population).

10.4.1 Missing Data

Missing values for the primary endpoint will be minimal to none, as survival status will be cross-checked with regulatory registries where all deaths in the countries are recorded and linked to a unique personal identifier.

10.3 Pre-specified Subgroup Analyses

The primary endpoint will be compared between treatment groups in pre-specified subgroup analyses: Age, sex, left ventricular ejection fraction <45% at arrival, anterior infarction, multivessel disease (>50% angiographic stenosis in non-culprit artery), symptom to intervention <6 hours, and thrombolysis in myocardial infarction grade 0-1 before percutaneous coronary intervention.

11. Data Storing and Handling of Personal Data

All trial data will be stored pseudo-anonymously for 25 years. Data of the included patients with STEMI will be stored at an electronic database, REDCap, where data will be blinded, and patients will be identified by a study identification number only. Study participation will be annotated in the medical record of the patient.

12. Assessments of Safety and Harm

Mortality after STEMI has decreased after implementation of primary PCI and drug-eluting stents (DES). Primary PCI is performed daily at all participating hospitals, and the risks associated with the procedure is well known. STEMI is often complicated by a myocardial dysfunction leading to heart failure and arrhythmias during the acute phase. However, mechanical complications such as free wall rupture, ventricular septal rupture, and papillary muscle rupture may also occur in the setting of STEMI[10]. These complications are well known, and, as part of standard care, an echocardiography is performed in all patients admitted with STEMI to assess potential mechanical complications and the function of the left and right ventricle. AEs/SAEs potentially related to the intervention of the study

will include: death, infection, gastroduodenal ulcer, hypertension, fluid retention resulting in peripheral edema, anaphylaxis, and skin complications such as acne, atrophy, purpura, and wound healing complications. The half-life of methylprednisolone is 12 hours, and the drug is considered eliminated after 5 half-times. Therefore, AEs will be recorded for the first three days following intervention.

13.1 Adverse Events (AEs)

AEs are considered any untoward medical occurrence in the patient to whom intervention (glucocorticoid) has been administrated and which does not necessarily have a causal relationship with this treatment. The PULSE-MI trial showed no risk of glucocorticoid administration regarding adverse events. Therefore, the AE that will be recorded is infection. C-reactive protein, a known acute-phase reactant, increases as a direct result of STEMI. Therefore, this can not be considerd sign of infection per se and infection as an AE will be defined as as evident signs of infection such as fever, fatigue and/or delirium accompanied by positive blood or urine screening.

13.2 Serious Adverse Events (SAEs)

The principal investigator and other investigators will report all SAEs to Sponsor within 24 hours of awareness. All SAEs are any toward medical occurrence that at any dose requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, results in a congenital anomaly or birth defect, is life-threatening, or results in death.

All SAEs occurring from the time the study medicine has been administrated to one week after the administration of the study medicine will be reported by completing the SAE form within 24 hours after the investigator becomes aware that the event meets the protocol definition of an SAE. All SAEs will be reported in the encrypted electronic database, REDCap.

Hospitalization and prolongation of hospitalization is defined as presentation at hospital for an urgent, unscheduled emergency department visit or hospital admission (>24 hours).

13.3 Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is defined as a suspected, serious adverse reaction, the nature, severity, or outcome of which is not consistent with the reference safety information. *'Unexpected'* reactions are defined using the summary for product characteristics (SPC) for Solu-Medrol[25]. Sponsor of the trial will be registered in the EudraVigilance system prior to initiation of the trial and is responsible of reporting any SUSARs to the EudraVigilance system and the ethics committee as soon as possible, at latest 7

days after awareness of event if life-threatening. If the SUSAR is not life-threating the event will be reported at latest 15 days after awareness of the event.

14. Finance and Insurance

This study is investigator-initiated, and the study will be funded by external foundations for medical research. The study has received funding from Hjerteforeningen (8.8 mio.) and Sundhedsdonationer (6.0 mio). The preliminary budget for the study is estimated at approximately 18 million DKK, and funding applications are being submitted on an ongoing basis.

All patients participating in the trial are insured by the Patient Compensation Association and the Danish patient insurance.

15. Publication

All results (positive, negative, or inconclusive) will be published in international peer-reviewed journals. The data will also be presented at national and international congresses. No participants will be identified in any reports or publications. All analyses will be conducted by the investigators of the trial and a statistician. The trial will not be unblinded until acceptance from the steering committee. The publication policy of the trial data will be uploaded to the Clinical Trials Information System (CTIS) database within one year after end of trial in accordance with Clinical Trial Regulation 536/2014 Annex IV.

The authorship order: Dr. Jasmine Melissa Madsen will be first author, Dr. Ben Elezi will be second author and Professor Thomas Engstrøm will be last author. The following authorships will be granted in according to ICMJE guidelines. However, all co-investigators will be offered authorship.

16. Annual Safety Report

Throughout the whole study, annual safety reports will be uploaded to the CTIS database every year. Annual safety reports will not be sent in the period between trial completion and the 10 years follow up.

Appendix A (End-Point Definitions)

The clinical event committee (CEC) will review and adjudicate all occurrences of the clinical end-points according to the 2017 Cardiovascular and Stroke Endpoint Definitions for Clinical Trials[19] and Standardized End Point Definitions for Coronary Intervention Trials: The Academic Research Consortium-2 Consensus Document[18]:

1. Mortality: Cardiovascular and non-cardiovascular
2. Admission for heart failure
3. Spontaneous myocardial infarction
4. Revascularization
5. Malignant ventricular arrhythmia

Mortality will to the extent possible be classified according to underlying disease. The CEC will classify the mortality as cardiovascular or non-cardiovascular death.

Cardiovascular mortality

Mortality considered as cardiovascular unless it is clearly attributable to another cause and thus includes:

- Death due to proximate cardiac cause (e.g. myocardial infarction, cardiac tamponade, worsening heart failure).
- Death caused by non-coronary vascular conditions such as neurological events, pulmonary embolism, ruptured aortic aneurysm, dissecting aneurysm, or other vascular disease.
- All procedure-related deaths, including those related to a complication of the procedure or treatment for a complication of the procedure.
- Sudden or unwitnessed death.
- Death of unknown cause.

Non-cardiovascular mortality

Any death in which the primary cause of death is clearly related to another condition (e.g. infection, cancer, suicide, accident, pulmonary, hepatobiliary, gastrointestinal, renal, overdose, neurological (excluding stroke and or cardiovascular hemorrhage of central nervous system).

Admission for heart failure is presentation of the patient for an urgent, unscheduled clinic/office/emergency department visit or hospital admission (>24 hours), with a primary diagnosis of heart failure (HF) after discharge of STEMI hospitalization,

OR

Initial STEMI admission is prolonged due to heart failure as characterized below

AND

where the patient exhibits new or worsening symptoms of HF on presentation, AND

has objective evidence of new or worsening HF*, AND

receives initiation or intensification of treatment specifically for HF.

*Objective evidence consists of at least 2 physical examination findings OR at least 1 physical examination finding and at least 1 laboratory criterion (chest x-ray or BNP) of new or worsening HF on presentation.

Table 1. Value definition heart failure

Values	Value definition
New or worsening symptoms of HF	Dyspnea Decreased exercise tolerance Fatigue Volume overload
Objective evidence of new or worsening HF	*Either* at least one on physical exanimation: Peripheral edema Increasing abdominal distention or ascites Pulmonary rales/ crackles/crepitations Increased jugular venous pressure and/ or hepatojugular reflux S3 gallop Clinically significant or rapid weight gain *Or* at least on laboratory criterion: Biomarker increases BNP/ NT-pro BNP (> upper reference limit). In patients with chronically

	elevated natriuretic peptides, a significant increase above baseline is required. Radiological evidence of pulmonary congestion: imaging findings consistent with increased intravascular blood volume in the lungs.
Receives initiation or intensification of treatment specifically for HF	Augmentation of oral diuretic therapy Intravenous diuretic, inotrope, or vasodilator therapy Mechanical or surgical intervention including any LV assist device and mechanical fluid removal

Spontaneous myocardial infarction is defined according to current guidelines[16,17]. The definition of spontaneous myocardial infarction is:

1. Detection of rise and/or fall of cardiac biomarkers (preferably troponin) with at least 1 value above the 99th percentile URL, together with the evidence of myocardial ischemia with at least 1 of the following:

(i) Symptoms of ischemia,

(ii) ECG changes indicative of new ischemia (new ST-T changes or new left bundle branch block),

(iii) new pathological Q-waves in at least 2 contiguous leads,

(iv) imaging evidence of a new loss of viable myocardium or new wall motion abnormality,

(v) Identification of an intracoronary thrombus by angiography or autopsy.

2. Sudden, unexpected cardiac death or aborted cardiac death, involving cardiac arrest, often with symptoms suggestive of myocardial ischemia, but death occurring before blood samples could be obtained, or at a time before the appearance of cardiac biomarkers in the blood and accompanied by at least 1 of the following:

i) Presumably new ST-segment elevation,

ii) New left bundle branch block,

iii) Evidence of fresh thrombus by coronary angiography and/or at autopsy

3. Pathological findings of a myocardial infarction

Revascularization will be grouped and defined accordingly:

- *Urgent revascularization:* Revascularization during admission for acute coronary syndrome which covers patients with STEMI, non-ST-segment elevation myocardial infarction, and unstable angina[16].
- *Target vessel revascularization:* Revascularization will be classified according to the culprit lesion of index STEMI. Definitions are:
    - Target vessel: the entire culprit vessel including side branches.
    - Target vessel revascularization: any repeat PCI or surgical bypass of any segment of the target vessel including the target lesion.
- *Target lesion revascularization:* Revascularization will be classified according to the culprit lesion of index STEMI as target/non-target lesion/vessel. Definitions are as allow:
    - Target lesion: treated culprit segment including the 5-mm margin proximal and distal to the stent.
    - Target lesion revascularization: repeat PCI on the target lesion or bypass surgery of the target vessel performed for restenosis or the complication of the target lesion.
- *Any repeat revascularization:* Any PCI or surgical coronary intervention.

Malignant ventricular arrhythmia is any ventricular arrhythmia with sustained ventricular tachycardia or ventricular fibrillation leading to acute medical treatment or acute cardioversion.

Appendix B (the PULSE-MI 2 committee members and investigators)

Steering committee:
Jacob Lønborg, MD, PhD, DMSc (Principal Investigator)
Jasmine Melissa Marquard, MD (Co-Principal Investigator)
Thomas Engstrøm, MD, PhD, DMSc (Sponsor, Co-Principal Investigator))
Ben Elezi, MD (Sub-investigator)
Sub-Investigators:
Ben Elezi, MD
Laust Obling, MD, PhD
Helle Collatz Christensen, MD, PhD
Fredrik Folke, MD, PhD
Christian Juhl Terkelsen, MD, PhD, DMSc (Principal Investigator, Aarhus)
Lars Wiuff Andersen, MD, MPH, PhD, DMSc
Ashkan Eftekhari, MD, PhD (Principal Investigator, Aalborg)
Martin Rostgaard-Knudsen, MD
Lisette Okkels Jensen, MD, PhD (Principal Investigator, Odense)
Søren Mikkelsen, MD, PhD
Statistician:
Thomas Scheike
Writing committee:
According to publication charter (TBD)
Clinical event committee (CEC):
Kasper Iversen (Suggested)
David Erlinge (Suggested)
Felix Böhm (Suggested)
Data Safety Monitoring Board (DSMB):
Lars Køber, chair (suggested)
Nico Pijls (suggested)
Steen Dalby Kristensen (suggested)
Christian Torp (suggested)
Data manager:
Emil Fosbøl (Unblinded data (independent))
Rasmus Paulin Beske (Data handling of blinded data only)

Appendix B (Statistical Analysis Plan)

Statistical Analysis Plan

Prehospital Pulse-Dose Glucocorticoid in Patients with ST-Segment Elevation Myocardial Infarction – PULSE-MI 2

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Preamble

This statistical analysis plan represents an overview of the statistical methods, which will be used for the main analyses in the PULSE-MI 2 trial. The main analyses of the primary and secondary outcomes of the PULSE-MI 2 trial will be performed according to this statistical analysis plan. Additional statistical analyses for predefined exploratory endpoints will be specified in separate protocols and are not intended to be included in main publication.

Aim

The aim of this prospective, randomized PULSE-MI 2 trial is to evaluate the effect of pre-hospital pulse-dose glucocorticoid in patients with STEMI.

Methodology

This trial is an investigator-initiated, 1:1 randomized, blinded, multicenter, placebo-controlled clinical trial to investigate whether pulse-dose glucocorticoid (250 mg methylprednisolone) in the pre-hospital setting reduces mortality in patients with STEMI.

Inclusion criteria

1. Age ≥18 years including fertile women*
2. Acute onset of chest pain with < 24 hours duration
3. STEMI as characterized on electrocardiogram (ECG) by one of the following[26]:
    a. at least two contiguous leads with ST-segment elevation ≥2.5 mm in men < 40 years, ≥2 mm in men ≥40 years, or ≥1.5 mm in women in leads V2-V3 and/or ≥1 mm in the other leads,
    b. presumed new left bundle branch block with ≥1 mm concordant ST-segment elevation in leads with a positive QRS complex, or concordant ST-segment depression ≥1 mm in V1-V3, or discordant ST-segment elevation ≥5 mm in leads with a negative QRS complex
    c. Isolated ST depression ≥0.5 mm in leads V1-V3 and ST-segment elevation (≥0.5 mm) in posterior chest wall leads V7-V9 indicating posterior acute myocardial infarction (AMI)
    d. ST-segment depression ≥1 mm in eight or more surface leads, coupled with ST-segment elevation in aVR and/or V1 suggesting left main-, or left main equivalent- coronary obstruction

* It is not possible to perform a pregnancy test (HCG urine test) in the pre-hospital setting. However, methylprednisolone is not contraindicated in pregnant women.

Exclusion criteria

1. Suspected other than type I acute myocardial infarction at the time of potential randomization[27]

2. Initial presentation with cardiac arrest (out of hospital cardiac arrest)
3. Known allergy to glucocorticoid

Randomization

Randomization of the patient will take place in the pre-hospital setting. Patients who satisfy all the eligibility requirements will be randomized. Randomization is done by the staff in the ambulance. Patients will be randomized in a 1:1 ratio to either infusion of glucocorticoid (250 mg methylprednisolone) or placebo that are placed in identical boxes. The ambulance staff randomizes the patient by picking a random box stored in the ambulance delivered by the pharmacy, including glucocorticoid or placebo, and administrates (over a period of 5 minutes) the content as a bolus infusion. Following infusion of the study intervention, the ambulance will bring the box to the hospital alongside with ECGs obtained in the ambulance in case of emergency unblinding. All boxes are identical and has a unique box serial number. Once a patient has been randomized, the patient is considered included in the trial. The pharmacy of the Capital Region of Denmark will pack inbox and label the study medicine and placebo ampoules. The randomization will be allocated by the pharmacy and their independent statistician using a random number generator.

Endpoints

Table 1. PULSE-MI-2 endpoints

	Intervention with glucocorticoid vs. placebo
Primary endpoint	All-Cause mortality
Secondary endpoints	Cardiovascular mortality
	Spontaneous myocardial infarction
	Admission for heart failure
	All-cause mortality and admission for heart failure
	Cardiovascular mortality and admission for heart failure
	Recurrent non-fatal cardiovascular events (spontaneous myocardial infarction and admission for heart failure) with death as a terminal event

Sample size

The median follow-up of the trial is expected to be 3 years with a maximum follow-up of 5 years and minimum follow-up of 1 year. The primary outcome is all-cause mortality. As an estimate based on

findings from the PULSE-MI trial[28] and the DANAMI-3 trial[24], the estimated event rate in the placebo arm is 9% during follow-up. Glucocorticoid is expected to reduce all-cause mortality corresponding to a hazard ratio of 0.77. To demonstrate this reduction in the primary outcome with an 80% power at a 5% significance level, 2602 patients in each treatment arm is needed. The primary analyses will be intention to treat principle.

After inclusion of 50% of the planned sample size, a blinded evaluation of the total number of events will be conducted annually to assess the need for potential sample size adjustment. No interim analysis will be done.

General statistical considerations

The endpoints will be analyzed as intention-to-treat principle. A prespecified additional key analysis will also be done due to the following:

A differentiation between a classic type I myocardial infarction with plaque rupture and thrombosis formation and a type II myocardial infarction with other reasons for ST-segment elevation (e.g. takotsubo, myocarditis, pericarditis, spontaneous coronary artery dissection, coronary embolism, coronary spasm, anemia, etc.[27]) can only be made after inclusion and acute coronary angiogram. Any patient with type II myocardial infarction or who does not fulfill any other inclusion or exclusion criteria will be excluded from the additional analysis but included in the intention-to-treat analysis. Approximately 15% of the included patients are expected to have other reasons for ST-segment elevation[28].

Thus, endpoints will be assessed in the following populations:

The intention-to-treat (ITT) analysis population will include all randomized patients.

The additional analysis will include patients with STEMI and type I myocardial infarction[27].

In general, differences between groups in time-to-event endpoints will be assessed with the log-rank test. Survival probabilities will be displayed using Kaplan-Meier methodology. Hazard ratios between groups will be calculated using a Cox proportional hazard model. Differences between group means/medians will be assessed with parametric or non-parametric statistics. The Chi-square analysis or Fisher's exact test will be used to test differences between proportions. All statistical tests and/or confidence intervals, as appropriate, will be performed at α=0.05 (2-sided), except for those specified

otherwise. A two-tailed P-value <0.05 is considered statistically significant. All endpoints will also be assessed in the additional key analysis only including patients with type I myocardial infarction.

Data safety monitoring

The data safety monitoring board (DSMB) should meet at least once when the first 25% of the patients have been included. Also, the primary endpoint for these patients much be adjudicated by the event committee. All additional patients included at the time point of full event adjudication will also be included in the safety analysis. The DSMB will receive data on the primary endpoint from the data manger. The DSMB may ask for additional data and events at the discretion of the DSMB. The DSMB will 1) evaluating the quality of ongoing study conduct including accrual rate, adherence to protocol, accuracy and completeness of data capture and 2) assessing safety and efficacy data. The DSMB develops consensus on its list of recommendations including that relating to whether the trial should continue While no formal statistical stopping boundaries are mandated, the DSMB will consider early termination for safety, efficacy, or futility based on magnitude, consistency, and clinical plausibility of observed effects. A recommendation of stopping the study is at the discretion of the DSMB. The DSMB may ask the study team to provide additional data (baseline and endpoints ect.) before the report is finalized. The DSMB Chair will formalize the recommendations in secure email or formal with the DSMB's final recommendations to the principal investigator within 2 weeks of the meeting. Details on the DSMB is described in the DSMB Charter.

Analysis of primary endpoint

The primary endpoint all-cause mortality will be analyzed using Cox proportional hazards analysis after testing that the assumption of proportional hazards is met. The primary analysis will be an un-adjusted analysis of treatment effect. Predefined subgroup analyses will be performed for: Age, sex, left ventricular ejection fraction <45% at arrival, anterior infarction, multivessel disease (>50% angiographic stenosis in non-culprit artery), symptom to intervention <6 hours, and thrombolysis in myocardial infarction grade 0-1 before percutaneous coronary intervention. A Forest plot of hazard ratios for the primary endpoint and 95%-confidence intervals resulting from univariate comparisons between the treatment groups will be shown for these subgroups.

Analyses of secondary endpoints

The secondary composite endpoints will be analyzed using Cox proportional hazards analysis after testing that the assumption of proportional hazards is met. This will be done as unadjusted analyses of treatment effect and presented as hazard ratio and corresponding confidence intervals. Data will be presented in Kaplan Meier plot when appropriate. For secondary endpoints not involving all-cause mortality, the endpoints may also be analyzed considering all-cause death as a competing risk.

Recurrent non-fatal cardiovascular events (spontaneous myocardial infarction and hospitalization for heart failure) with death as a terminal event will be analyzed using a cox-type model for the mean cumulative function according to Gosh and Lin.

Missing data

All centers will be encouraged to obtain as complete data as possible and optimized with 100% monitoring. Missing values for the primary endpoint will be minimal to none, as survival status will be cross-checked with regulatory registries where all deaths in the countries are recorded and linked to a unique personal identifier. Imputations of missing data for the primary endpoint will not be performed. In the event of loss to follow-up patients will be censored at the last date at which respective patient data was recorded.

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