Sonothrombolysis in Patients with an ST-segment Elevation Myocardial Infarction

A prospective single-arm study

Author(s): Kevin R. Bainey and Harald Becher

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Study Coordinating Group: ABACUS
Mazankowski Alberta Heart Institute
University of Alberta
Edmonton, Canada

T6G 2B7

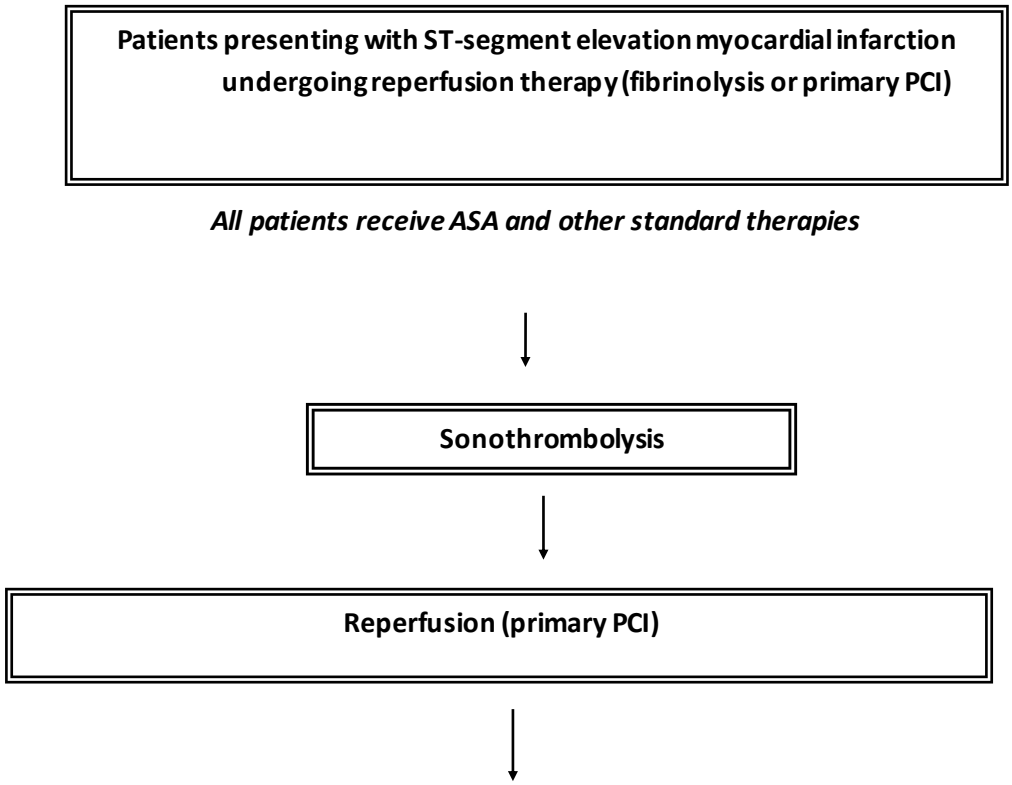
STUDY SYNOPSIS

Title	Sonothrombolysis in Patients with ST-segment Elevation Myocardial Infarction (STEMI)
Investigator(s)/center/study location	Investigator-initiated prospective, single-center, single-arm study
Study Objectives	To assess the efficacy and safety of sonothrombolysis in the acute management of STEMI undergoing reperfusion therapy with primary percutaneous coronary intervention [PPCI].
Study Design	The sonothrombolysis in STEMI study will be an investigator-initiated prospective, single center, single-arm study of sonothrombolysis performed before coronary reperfusion with PPCI.
Study population ***Inclusion criteria*:**	Patients presenting with STEMI within 6 hours of symptom onset and: 1. Are expected to receive reperfusion therapy with primary PCI 2. Have a high-risk STEMI ECG defined as: • ≥2mm ST-segment elevation in 2 anterior or lateral leads; or • ≥2 mm ST-segment elevation in 2 inferior leads coupled with ST-segment depression in 2 contiguous anterior leads for a total ST-segment deviation of ≥4 mm 3. Age ≥30 years. 4. Adequate apical and/or parasternal images by echocardiography.
Exclusion criteria:	1. Isolated inferior STEMI without anterior ST-segment depression 2. Previous coronary bypass surgery

	3. Cardiogenic shock 4. Known or suspected hypersensitivity to ultrasound contrast agent used for the study 5. Life expectancy of less than two months or terminally ill. 6. Known bleeding diathesis or contraindication to glycoprotein 2b/3a inhibitors, anticoagulants, or aspirin 7. Known large right to left intracardiac shunts.
Evaluation criteria	**Primary Outcome** **1.** Spontaneous reperfusion as assessed by a pre PCI ECG complete ST-segment resolution (>50%) (immediately prior to angiogram) and by pre PCI TIMI 2-3 flow on diagnostic angiogram. **Secondary Outcomes** 1. Complete (>50%) ST-segment resolution at 30 minutes post PCI as assessed by the worst lead on electrocardiogram (ECG core lab). 2. Left ventricular ejection fraction (LVEF) by echocardiography (ECHO) (Simpson method) assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction. 3. Wall motion score index (WMSI) by ECHO assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction. 4. Microvascular perfusion score index (MPSI) by ECHO assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction.
Treatment	Sonothrombolysis: Echocardiographic imaging with intermittent diagnostic high mechanical index (MI) using a commercially available echocardiography scanner (Epiq,Philips Healthcare) to the myocardium during an intravenous 5% Definity infusion (Lantheus Medical) over a maximum 30 minute treatment period (started prior to PCI)

Statistical Considerations	**Efficacy Analysis**: This is an observational study to reproduce findings of a previous study which showed angiographic spontaneous reperfusion rates of 80% with sonothrombolysis prior to PCI (i.e. pre PCI TIMI 2-3 flow). If we can demonstrate a patency rate > 60%, a multicenter study will be arranged.
Sample Size	15 patients
Duration of Follow-Up	Minimum of 90 +/- 7 days

1.0 OVERVIEW OF STUDY DESIGN



Primary Outcome

1. Spontaneous reperfusion as assessed by a pre PCI ECG complete ST-segment resolution (>50%) (immediately prior to angiogram) and by pre PCI TIMI 2-3 flow on diagnostic angiogram..

Secondary Outcomes

1. Complete (>50%) ST-segment resolution at 30 minutes post PCI as assessed by the worst lead on electrocardiogram (ECG core lab).
2. Left ventricular ejection fraction (LVEF) by echocardiography (ECHO) (Simpson method) assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction.
3. Wall motion score index (WMSI) by ECHO assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction.
4. Microvascular perfusion score index (MPSI) by ECHO assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction.

2.0 INTRODUCTION AND RATIONALE

In Canada, more than 40,000 patients are hospitalized with an acute coronary syndrome (ACS) each year of which one-third suffer an ST-segment elevation myocardial infarction (STEMI) (1,2). As such, STEMI remains a significant public health concern given the substantial morbidity and mortality associated with the condition (3-7). Prompt reperfusion therapy has been shown to reduce mortality, infarct size and improve left ventricular function (8-13).

However, it has been recognized that reperfusion itself may result in adverse events. Abrupt reperfusion therapy can lead to myocardial stunning, ventricular arrhythmias and microvascular dysfunction as a result of distal coronary embolization and myocardial inflammation. This injury pattern inflicted upon the myocardium has been termed reperfusion injury (14). The accumulating effects result in myocyte necrosis, myocardial stunning and impaired infarct healing contributing to microvascular dysfunction and subsequent adverse clinical events in STEMI (15-19). The bedside electrocardiogram is a simple non-invasive tool that can be used to assess microvascular perfusion as defined by the degree of ST-segment resolution following the administration of reperfusion therapy (20).

Multiple mechanisms to reduce infarct size have been undertaken with limited success and inconsistent results(21). While transthoracic high mechanical index (MI) impulses from a diagnostic ultrasound (DUS) transducer have been utilized to diagnose microvascular obstruction and detect myocardial perfusion during a continuous microbubble infusion(22-26), the microbubble cavitation induced by these high MI impulses(27) creates shear forces that are capable of dissolving epicardial and microvascular thrombi in animal models of acute STEMI (28-30). These same high MI impulses, when applied to the microvasculature, also induce nitric oxide release (31), which may further augment microvascular flow. Termed sonothrombolysis, these effects have been demonstrated and verified in animal models, however the utility of DUS in humans has not been well studied during the contemporary management of STEMI, where emergent reperfusion (primary percutaneous coronary intervention [PPCI] or fibrinolysis) is routinely employed with such rapidity that only brief applications of ultrasound may be possible prior to interventional therapies. Recently in a small PPCI STEMI randomized trial (N=20 treated with sonothrobolysis), high MI with DUS (continuous microbubble infusion) (sonothrombolysis) was associated with improved patency rates of the infarct vessel prior to PCI. Specifically, the infarct related artery was found to be open in 80% with sonothrombolysis compared to patients with standard of care prior to PCI (~20%). If these results can be reproduced with another ultrasound system, a large clinical trial may be warranted (32). No serious adverse events were reported in this study and in a small previous study involving five patients (33). However, in another study vasoconstriction in the culprit coronary artery was observed in three out of six patients during coronary angiography (34). In this study the pulse duration of the ultrasound scanner was prolonged in order to expose the intracoronary thrombus and the ultrasound contrast to a higher power. One patient withdrew from the study and was lost during follow up (but still alive according to the Dutch national registry), the other two patients had no long term side effects. The authors stress the higher ultrasound power used in this trial (20 μs instead of 5 μs which is transmitted in diagnostic ultrasound) and explain the findings by a "summative effect of

myocardial ischemia, reperfusion, damage and long-pulse-duration sonoporation on endothelial damage, all leading to calcium overload. It was concluded that new trials should proceed with short (5 μs) pulse duration. Therefore we will use the pulse duration of 5 μs which is clinically used for diagnostic ultrasound in the proposed pilot study.

The purpose of this study was to examine what effect adding emergent DUS guided high MI impulses (sonothrrombolysis), applied both before PPCI during an intravenous commercially available microbubble infusion (5% Definity, Lantheus Medical Imaging, Inc., North Billerica, Massachusetts), have on spontaneous reperfusion (i.e. pre PCI coronary artery patency rates), microvascular obstruction, left ventricular function and infarct size in patients presenting with their first STEMI. Accordingly, given the potential impact of utilizing this practical application for attenuating reperfusion injury, we propose a prospective, single center, single-arm study of pre-procedural sonothrombolysis as an adjuvant to contemporary therapy for ST-segment elevation myocardial infarction receiving PPCI.

3.0 STUDY HYPOTHESIS

In adult patients with high-risk ST-segment elevation myocardial infarction (STEMI) planned for reperfusion therapy (primary PCI), the use of sonothrombolysis will enhance spontaneous reperfusion (i.e. pre-PCI ST-resolution and pre-PCI epicardial patency). We expect that >60% of the patients will have an open infarct vessel at the beginning of coronary angiography.

4.0 STUDY OBJECTIVES

4.1. Primary Objective

To determine whether the use of sonothrombolysis along with standard therapy in patients with STEMI undergoing reperfusion therapy (primary PCI) is resulting in a higher rate of spontaneous reperfusion (i.e. pre-PCI ST-resolution and pre-PCI epicardial patency) compared to standard therapy alone.

4.2. Secondary Objective

To determine whether the use of sonothrombolysis along with standard therapy in patients with STEMI undergoing reperfusion therapy (primary PCI) is resulting in better microvascular function than what is reported for standard therapy alone.

4.3. Tertiary Objective

To determine whether the use of sonothrombolysis along with standard therapy in patients with STEMI undergoing reperfusion therapy (primary PCI) is resulting in improved left ventricular function compared to what is reported for standard therapy alone.

5.0 STUDY DESIGN

5.1. Study Design

This clinical study is a prospective single-center, single-arm investigation of sonothrombolysis in adult patients presenting with high-risk STEMI within 6 hours of the onset of clinical symptoms and receiving reperfusion therapy with PPCI. All patients will receive standard therapy according to the current American College of Cardiology / American Heart Association (ACC/AHA) or European Society of Cardiology (ESC) STEMI and PCI guidelines (35).

The Vital Heart Response Program (VHR) facilitates treatment of patients with STEMI within the pre-hospital emergency medical services environment as well as within emergency departments within the Edmonton region via a STEMI network. After completion of initial clinical assessment and documentation of a 12-lead with diagnostic ST-elevation, patients will be approached for participation.

5.2. *Intervention (sonothrombolysis)*

Patients will immediately receive an intravenous infusion of commercially available ultrasound contrast agent (5% Definity, Lantheus Medical Imaging, Inc., North Billerica, Massachusetts) 1.3 mL diluted in 50 ml saline and infused running at 2 to 5 ml/min. After starting the infusion, myocardial contrast echocardiography will be performed using a commercially available echocardiography scanner (Epiq, Philips) with a diagnostic ultrasound transducer (X5-1) and a preset for myocardial perfusion imaging. 4-, 2- und 3-chamber views will be recorded to document the size of the perfusion defect according to method published by Porter et al (27). The focus will be set at the mitral valve level for all studies. Loops of 15 cardiac cycles will be recorded using low mechanical index (MI) ultrasound with a 'flash' delivered after the second cardiac cycle of the loop. The flash is a short impulse of high MI ultrasound* which is transmitted to destroy the ultrasound contrast in the myocardium and then to assess the replenishment of myocardial contrast. These recordings will also be used to assess regional wall motion as well as the LV volumes and ejection fraction.

**** Mechanical index (MI) is the unit to measure the ultrasound energy transmitted by the ultrasound transducer. High MI (>0.8) ultrasound imaging is the standard for echocardiographic imaging without contrast agents. In contrast echocardiography, however, high MI leads to destruction of the microbubbles in the contrast agent. Therefore contrast imaging has to be performed with very low MI (<0.2), fortunately there are very sensitive postprocessing tools that can still detect the specific signals from contrast agent at very low MI. In myocardial perfusion imaging, high MI ultrasound is applied only briefly to clear the myocardium from the contrast agent and to assess the time it takes for the blood from the aorta to reach the microvessels. In diagnostic imaging this is performed several times in multiple imaging planes in order to detect myocardial thickening and to assess myocardial perfusion. In this pilot trial we are using the same techniques. The ultrasound scanning time will be in the range of that of echocardiographic imaging in clinical practice. The total***

amount of the ultrasound contrast agent Definity® will be similar to what is clinically used in stress echocardiography and not exceed the maximum dose which is approved for clinical use.

Immediately after the diagnostic ultrasound, the therapeutic ultrasound will start using the same transducer by applying multiple high mechanical index (MI) ultrasound impulses (HMI). The HMI are the same as those which are used for assessment of myocardial perfusion in diagnostic ultrasound. These pulses will be applied in the apical 4-, 2-, and 3-chamber views to the apical windows that contained the risk area. The intervals between HMI impulses will vary from 5 to 15 s depending on the time required for myocardial contrast replenishment.

The following measurements will be performed on the recordings obtained before sonothrombolysis, after completion of sonothrombolysis, 72h or prior to hospital discharge and 3 months after sonothrombolysis:

1. Wall motion score index (WMSI) will be computed by analyzing wall thickening in all 3 contrast-enhanced apical windows using a 17-segment model as recommended in the 2015 American Society of Echocardiography Recommendations for Chamber Quantification.

2. Biplane measurements of LVEF by the the Simpson method

3. Microvascular perfusion score index (MPSI) within the same 17-segment model used for assessment of regional wall motion using a scoring system of 1 for myocardial contrast replenishment within 4 s of the applied HMI impulse; a score of 2 (mildly reduced) when complete replenishment within the risk area is delayed longer than 4 s after the HMI impulse; or a score of 3, which was defined as virtually no replenishment of myocardial contrast over 10 s after the MI impulse. The score index will be computed as total score divided by total number of segments analyzed. Attenuated basal segments will not be included in the calculation.

4. Global longitudinal strain

Table.1 Diagnostic and therapeutic procedures

Baseline diagnosis of STEMI (pre-reperfusion)		
day 1	12-lead electrocardiogram-baseline	in ER
day 1	Diagnostic Ultrasound - baseline	start in ER or cath lab Myocardial Contrast Echo (MCE) LV ejection fraction WMSI Global longitudinal strain
day 1	Sonothrombolysis A	start immediately after MCE prior to reperfusion

day 1	12-lead electrocardiogram-immediately prior to PPCI	in cath lab
Reperfusion (PPCI)		
day 1	Sonothrombolysis B	start immediately after reperfusion to complete a total of 30 min same method as before reperfusion
day 1	12-lead electrocardiogram-30 min post PCI	in CCU
day 1	Diagnostic ultrasound – post intervention	Myocardial Contrast Echo LV ejection fraction WMSI Global longitudinal strain
day3/discharge	Diagnostic ultrasound	Myocardial Contrast Echo LV ejection fraction WMSI Global longitudinal strain
day3/discharge	12-lead electrocardiogram-discharge	in CCU/Ward
3 months	Diagnostic ultrasound	Myocardial Contrast Echo LV ejection fraction WMSI Longitudinal strain

5.3. Study periods

Follow-up visit is scheduled at 90 (+/-7) days. The maximal observational period for a randomized patient would be 90 (+/- 7) days. The study end date will be at 90 days after the last patient. At this time, it is expected that the majority of patients will have complete follow-up at 90 days. All patients must be followed for 90 days or until death prior to completion of study period.

6. STUDY POPULATION

Adult patients with STEMI diagnosed through the VHR Program will be reviewed for treatment. For patients presenting direct to the cardiac catheterization laboratory patients will be assessed by the study team prior to treatment. Fifteen (15) patients will be enrolled once inclusion / exclusion criteria are fulfilled and informed consent is obtained.

6.1. Inclusion Criteria

Patients presenting with STEMI within 6 hours of symptom onset and:

1. Are expected to receive reperfusion therapy with primary PCI
2. Have a high-risk STEMI ECG defined as:
    - ≥2mm ST-segment elevation in 2 anterior or lateral leads; or

- ≥2 mm ST-segment elevation in 2 inferior leads coupled with ST-segment depression in 2 contiguous anterior leads for a total ST-segment deviation of ≥4mm

3. Age ≥30 years.

4. Adequate apical and/or parasternal images by echocardiography

6.2. Exclusion Criteria

1. Isolated inferior STEMI without anterior ST-segment depression
2. Previous coronary bypass surgery
3. Cardiogenic shock
4. Known or suspected hypersensitivity to ultrasound contrast agent used for the study
5. Life expectancy of less than two months or terminally ill.
6. Known bleeding diathesis or contraindication to glycoprotein 2b/3a inhibitors, anticoagulants, or aspirin
7. Known large right to left intracardiac shunts.

7.0 STUDY OUTCOME EVENTS

7.1 Primary Outcome

Spontaneous reperfusion as assessed by pre PCI ECG complete ST-segment resolution (>50%) (immediately prior to angiogram) and pre PCI TIMI 2-3 flow on the diagnostic angiogram.

7.2 Secondary Outcomes

1. Complete ST-segment resolution (≥50%) at 30 minutes post PCI as assessed by the worst lead on electrocardiogram
2. Left ventricular function as assessed by left ventricular ejection fraction (LVEF) by echocardiography (ECHO) (Simpson method) assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction.
3. Left ventricular function as assessed by wall motion score index (WMSI) by ECHO assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction.
4. Left ventricular function as assessed by microvascular perfusion score index (MPSI) by ECHO assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction.
5. Left ventricular function as assessed by global longitudinal strain by ECHO assessed on day 1 (pre and post reperfusion), day 3±2 (discharge) and 90±2 days after infarction.

7.3 Safety Outcomes**:**

1. Allergic reaction to Definity®
2. Vasospasm in culprit coronary artery
3. **Adverse events (see appendix)**

8. DEFINITIONS OF STUDY OUTCOMES

8. 1 ST-segment Resolution

ECGs will be evaluated centrally at the ECG core laboratories (Canadian VIGOUR Centre, Edmonton, Canada) without knowledge of treatment, procedural results, or clinical outcomes. ST-E will be measured at the J point with magnified calipers to the nearest 0.05 mV. ST-E sums (ST-E) will be calculated as follows: for anterior infarction, the sum of ST-E in V1 to V6, I, and aVL; for inferior infarction, the sum of ST-E in leads II, III, aVF, V5, and V6. ST-segment depression will also be measured at the J point by similar methods. ST-segment deviation sums (ST-D) will be calculated by adding the sum of ST-segment depression measured in reciprocal leads to _ST-E. Leads II, III, and aVF will be considered potential reciprocal leads for anterior infarctions, and leads V1 through V4 will be considered potential reciprocal leads for inferior infarctions.
The resolution of ST-segment elevation (worst lead) prior to PCI and at 30 minutes post PCI will be classified according to the number of patients achieving complete ST-segment resolution (>50%) versus no ST-segment resolution (≤50%). The resolution of ST-segment elevation (worst lead) at discharge will be calculated using the discharge ECG using the above criteria.

8.2 Spontaneous Reperfusion

8.2.1 Angiographic spontaneous reperfusion

Angiographic spontaneous reperfusion is defined as TIMI flow grade 2-3 of the infarct related artery before PCI (first contrast injection) (37).
Other definitions of spontaneous reperfusion (will be collected but not included as the primary endpoint)

8.2.2. Electrocardiographic (ECG) spontaneous reperfusion

ECG spontaneous reperfusion is defined as those patients who achieved complete (>50%) ST-segment resolution on the 12-lead ECG (worst lead) performed immediately prior to the coronary angiogram in patients undergoing primary PCI (37).

8.2.3 Echocardiographic spontaneous reperfusion

Echocardiographic spontaneous reperfusion is defined as score 1 or 2 in the infarct segments and/or recovery of systolic function during follow-up

9. STATISTICAL AND ANALYTICAL METHODS

The primary efficacy analysis will include 15 patients in the study regardless of treatment they actually received in accordance to the intention-to-treat (ITT) principle. A per protocol analysis will be performed on those patients who actually received sonothrombolysis. For the primary outcome, 12-lead electrocardiograms will be collected prior to PPCI and epicardial patency will be assessed at the time of the diagnostic angiogram. For the secondary endpoints, myocardial perfusion will be assessed based on the 12-lead ECG 30 minutes post PCI for complete ST-segment resolution. Myocardial contrast echocardiography will be performed using a commercially available echocardiography scanner (Epiq, Philips) with a diagnostic ultrasound transducer (X5-1) and a preset for myocardial perfusion imaging. 4-, 2- und 3-chamber views.

10. TRIAL ORGANIZATION

This study will be conducted in Edmonton through the Vital Heart Response Program and the Alberta Cardiovascular and Stroke center (ABACUS). The principle investigator, co-investigators and research coordinator will meet regularly (monthly) and will address the day-to-day operations of the trial.

11. DATA MANAGEMENT

Data will be managed at Alberta Cardiovascular and Stroke center (ABACUS) located at the University of Alberta Hospital Edmonton, Canada.

11.1. Case Report Forms

Case report forms (CRF) will be designed and managed by the principle investigators involved in the research study. Key demographic and procedural data along with outcome and adverse events will be collected. The CRF must be signed by the investigator or designate at study completion. Signing of the 'Study completion – Investigator's statement CRF' is considered to be the final authorization of the CRFs.

11.2. Data Quality Control

All data will be reviewed to ensure completeness and accuracy of information (CRF). Any concerns will be immediately addressed with modifications made accordingly.

12. FEASIBILITY AND LIMITATIONS

Considering the patients admitted with STEMI during the last year, recruitment of 15 participants within 1 year appears to be feasible. An Epiq/Philips ultrasound scanner will be available for almost 24/7 in ABACUS and can be moved within minutes into ER or the cath laboratory. However, a sonographer or fellow to perform the ultrasound imaging may not always

be available when a suitable patient with STEMI arrives. In order to avoid delays in the standard treatment of the myocardial infarct, the sonographer or fellow on call will receive a telephone call from the attendings in the cath lab immediately after they were notified by the ambulance that a patient with STEMI is approaching. That will give the echo fellow or sonographer enough time to be ready for ultrasound imaging with contrast, when the patient arrives. If the echo personnel arrive too late, no sonothrombolysis will be performed.

The current protocol mandates contemporary treatment for patients with STEMI undergoing reperfusion therapy. We do advocate therapy according to the current consensus guidelines to promote universal care (38, 39).

13. ETHICAL AND REGULATORY STANDARDS

13.1. Good Clinical Practice

The study will be conducted in accordance with both the Tri-Council Policy Statement (40) and Good Clinical Practice Guidelines (41).

13.2. Informed Consent of the Patient

Patients who meet all inclusion criteria and none of the exclusion criteria are deemed eligible. Before being enrolled into the clinical study, the patient must provide written consent to participate in the study after the nature, scope and possible consequences of the clinical study have been explained both orally and in writing.

13.3. Approval of the Study Protocol

Before the start of the study, the study protocol and the informed consent form used at the site and other appropriate documents must be submitted to and approved by the local Ethics Committee or Institutional Review Board (REB) and the appropriate regulatory authorities in accordance with local legal requirements.

13.4. Maintenance of Records

The Investigator agrees to obtain a correctly completed informed consent form for each patient included in the study. The investigator will maintain a personal list of patient numbers and patient names to enable records to be found at a later date.

The Investigator must maintain all study records, patient files and other source data for the maximum period of time permitted by the hospital, institution or private practice. However national regulations should be taken into account, the longest time having to be considered.

14. STUDY MONITORING

14.1. Responsibilities of the investigator(s)

The Investigator(s) undertake(s) to perform the study in accordance with Good Clinical Practice (41).

The Investigator is required to ensure compliance with respect to the invasive strategy schedule, visit schedule and procedures required by the protocol. The Investigator agrees to provide all information requested in the CRF in an accurate and legible manner according to instructions provided.

14.2. The use and completion of case report forms (CRFs)

It is the responsibility of the Investigator to prepare and maintain adequate and accurate CRFs. All CRFs should be completed in their entirety in a neat, legible manner to ensure accurate interpretation of data.

15. ADMINISTRATIVE RULES

15.1. Curriculum vitae

An updated copy of the curriculum vitae for each Investigator and co-Investigator will be provided to the principle investigator prior to the beginning of the study.

15.2. Confidentiality agreement

All goods, materials, information (oral or written) and unpublished documentation provided to the Investigators, inclusive of this protocol and the patient case report forms are the exclusive property of the ABACUS.

They may not be given or disclosed by the Investigator or by any person within his authority either in part or in totality to any unauthorized person without the prior written formal consent of the PI.

It is specified that the submission of this protocol and other necessary documentation to the Ethics Research Committee is expressly permitted, the Ethics Committee members having the same obligation of confidentiality.

The Investigator shall consider as confidential and shall take all necessary measures to ensure that there is no breach of confidentiality in respect of all information accumulated, acquired or deduced in the course of the trial, other than that information to be disclosed by law.

15.3. Record retention in investigating center(s)

The Investigator must maintain all study records, patient files and other source data for the maximum period of time permitted by the institution.

16. OWNERSHIP OF DATA AND USE OF THE STUDY RESULTS

The investigators of the study have ownership of all data and results collected during this study. Full publication rights of the study data solely reside with the investigators.

17. PUBLICATIONS

All study presentations and/or publication of the results will be based on clean, checked and validated data in order to ensure the accuracy of the results. All analyses for publication will be provided by ABACUS. The responsibility for presentations and/or publications belongs to the investigators. The final content of the manuscript is the responsibility of the investigators. Publication of the main findings of this study will be authored based on the contributions of the individuals to the overall study.

18. POTENTIAL IMPACT OF STUDY

Once completed, this will provide prospective data on the efficacy and safety of sonothrombolysis which we believe will lead to a randomized clinical trial of contemporary PCI STEMI patients (treated with PPCI or fibrinolysis) aimed at assessing the efficacy of sonothrombolysis as an adjuvant therapy to improve microvascular function.

19. REFERENCES

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APPENDIX

Adverse Events

An **Adverse Event** (AE) is defined as any untoward medical occurrence, including an exacerbation of a pre-existing condition, in a patient in a clinical investigation who received a pharmaceutical product. The event does not necessarily have to have a causal relationship with this treatment.

A **Serious Adverse Event** (SAE) is defined as any AE which results in death, is immediately life-threatening, results in persistent or significant disability / incapacity, requires or prolongs patient hospitalisation, is a congenital anomaly / birth defect, or is to be deemed serious for any other reason representing a significant hazard, which is comparable to the aforementioned criteria.

Adverse events occurring during the course of the clinical trial (i.e. from signing the informed consent) will be collected, documented and reported by the investigator.

Reporting of Serious Adverse Events (SAE)s:

- On an SAE form:

For the following events an SAE form (in addition to entering the SAE data in the CRF) will need to be completed and mailed for expedited reporting:

  - Serious and treatment-related SAEs
  - Serious, not treatment-related and not mentioned on the 'list of STEMI-related events'

For each SAE, the investigator will provide the onset, end, treatment required, outcome, seriousness and action taken with the investigational drug. The investigator will also determine the relationship of the investigational drug to all SAEs.

The basis for judging the causal relationship between the investigational product and the SAE is described below.

Causal Relationship*	
Yes	There is a reasonable causal relationship between the study drug administered and the AE.
No	There is no reasonable causal relationship between the study drug administered and the AE.

*Medical judgement should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge,

confounding factors such as concomitant medication, concomitant diseases and relevant history.

These completed SAE forms need to be mailed within 24 hours (1 workday) to the co-investigators after the Investigator (or any member of the study team) has become aware of the event. Timelines are the same for initial reports and for follow-up reports. The investigator and the coinvestigators will assess the treatment relationship, the category classification, listedness and seriousness of reported SAE cases. Expedited reporting of serious adverse events, e.g. suspected unexpected serious adverse reactions (SUSARs), will be done according to local regulatory requirements within the required timelines.

- In the eCRF:

Any SAE, whether or not considered related to the study medication, and whether or not the study medication has been administered, must be reported immediately in the eCRF.

Reporting of Non-Serious Adverse Events:

Non-serious events need to be recorded in the CRF

THE FOLLOWING ADVERSE EVENTS ARE EXPECTED AS DISEASE-RELATED EVENTS (I.E. RELATED TO ACUTE MYOCARDIAL INFARCTION):

Arrhythmias

All arrhythmias occurring later than 3 hours after fibrinolysis
All arrhythmias in patients who did not receive fibrinolysis

Ischaemia and symptoms of coronary artery disease

Angina pectoris (stable)
Angina pectoris (unstable)
Back pain (of cardiac origin)
Cardiac enzymes (abnormal)
Cardiac markers (abnormal)
Chest pain
Electrocardiogram abnormalities
Myocardial ischaemia
Myocardial reinfarction
Recurrent myocardial ischaemia
ST-elevation
Substernal chest pain
Substernal pain

Artery disorders

Aortic dissection
Coronary artery dissection
Coronary artery disorder
Coronary artery thrombosis
Coronary occlusion
Vascular anomaly

Cardiac failure

Acute pulmonary oedema
Cardiogenic shock
Congestive heart failure
Cor pulmonale
Heart failure
Left heart failure
Pulmonary oedema
Haemodynamic and circulatory shock

Pericardium disorders

Pericardial effusion
Pericarditis

Other cardiac disorders

Acute mitral regurgitation
Acute ventricular septum defect
Cardiac rupture
Cardiomyopathy
Electro-mechanical dissociation

Other events

Deep thrombophlebitis
Hypertension
Livedo reticularis
Peripheral oedema
Peripheral vascular disorder
Syncope

Thrombocytosis
Vascular disorder
Venous thrombosis