Study protocol

Routine versus selective protamine administration to reduce bleeding in transcatheter aortic valve implantation (ACE-PROTAVI)

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Protocol Synopsis

Title	Routine versus selective protamine administration to reduce bleeding in transcatheter aortic valve implantation
Acronym	ACE-PROTAVI
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Study Hypothesis	Routine administration of protamine reduces vascular and bleeding complications in TAVI patients, compared with selective administration, therefore improving clinical outcomes (reduced morbidity and mortality), but also reducing healthcare costs (by reducing length of stay, additional vascular interventions, and improving post-procedural recovery).
Study Design	Randomised controlled trial in which patients are randomised between routine protamine administration at the end of the procedure, or selective use of protamine if clinical scenario requires it.
Study population	Patients with symptomatic severe aortic stenosis or regurgitation who are accepted by the multidisciplinary heart team for TAVI, based on predicted surgical risk, frailty, and other clinically relevant factors.
Primary objective	Time to haemostasis: elapsed time after sheath removal and first observed and confirmed arterial haemostasis.
Secondary objectives	- Composite rate of all-cause mortality, major and minor bleeding and vascular complications according to VARC-2 criteria at 30-day follow-up - Each individual component of first secondary endpoint. - Need for transfusion for (access-site related) bleeding - Hematoma size by vascular ultrasound - Myocardial infarction - Stroke - Length of stay - Rate of delayed haemostasis failure
Inclusion criteria	Patients undergoing elective trans-femoral TAVI for severe aortic valve stenosis with any commercially available transcatheter heart valve.
Exclusion criteria	- Documented protamine allergy or anaphylaxis - Recent PCI (< 3 months) - Planned arterial access via surgical cut-down - Pregnancy
Intervention	Routine protamine administration at the time of sheath removal after successful deployment of THV

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1 Introduction and rationale

1.1 Lay summary

In patients with diseased aortic valves, catheter-based replacement of the sick valve (TAVI) is a less invasive alternative for surgical valve replacement. TAVI has become the procedure of first choice in patients with increased operative risk. Because the valve is implanted through a catheter from the groin, there is no need for open heart surgery and recovery is much quicker. However, there remains a risk of major or life-threatening bleeding from the access site, especially because patients require blood-thinners (heparin) during the valve implantation. At the end of the procedure, when blood-thinners are no longer required, the effect of heparin can be reversed by protamine injection, but routine use of protamine has not been tested in TAVI patients in a randomised trial. In this study we will evaluate if routine use of protamine reduces the risk of major bleeding and improves outcomes for patients who underwent TAVI. We will compare safety and effectiveness of routine use of protamine in a randomised controlled trial and will compare outcomes after 30 days. The results of this study will help to improve the safety of the TAVI procedure and its outcomes for patient (reduced morbidity and mortality) and society (reduced demand on constrained economic resources).

1.2 Background

Degenerative aortic valve disease is the most prevalent valvular heart disease in the elderly. If left untreated after the appearance of symptoms, expected mortality after two years is almost 50%.[1] In the last 10-15 years, transcatheter aortic valve replacement (TAVI) matured into a less invasive alternative for surgical valve replacement, and landmark randomised clinical trials have demonstrated that TAVI is non-inferior (and maybe superior) to surgical valve replacement across the entire risk spectrum in elderly patients with degenerated tricuspid aortic valve stenosis.[2–8] Despite its successes, TAVI is not a procedure free from complications. Vascular

complications, especially those related to the access site, are one of the most feared and frequent procedure-related complications. Life-threatening or major bleeding Major or life-threatening bleeding not only leads to increased length of stay and increased strain on healthcare resources, but more importantly, they are associated with increased morbidity and mortality. Therefore, prevention of bleeding and selection of the appropriate treatment for vascular complications play a pivotal role to further improve patient outcomes after TAVI.

During the TAVI procedure, heparin anticoagulation is recommended before introduction of the guiding sheath, while maintaining an activated clotting time of >300 s. This recommendation by the American College of Cardiology Foundation/American Association for Thoracic Surgery/Society for Cardiovascular Angiography and Interventions/Society of Thoracic Surgeons (ACCF/AATS/SCAI/STS) expert consensus document on TAVI is based on expert consensus rather than on evidence from RCTs.[9] Heparin reversal by administration of protamine, prior to removal of the sheath and closure of the arteriotomy site, can be considered. However, daily practice varies widely among centres and even operators, from routine protamine administration to almost none or selective administration (in patients at high risk of bleeding).

Although protamine is routinely used in cardiac surgery, the data regarding its safety and efficacy in prevention of bleeding complications in TAVI is scarce. One recent observational study demonstrated in 873 patients that routine administration of protamine significantly reduced bleeding and vascular complications, compared with no protamine (4.1% vs 11.8%), and that there was no increase in ischemic events such as stroke and myocardial infarction (1.8% vs 3.6%). However, the study was performed in a non-randomised, non-matched population with a high risk for selection bias, especially in patients not receiving protamine.[10]

1.3 Study hypothesis and aims

Major bleeding and vascular complications after TAVI are associated with increased morbidity and mortality, and increased demand on restrained healthcare resources. Our hypothesis is that routine protamine administration at the conclusion of the TAVI procedure reduces the risk of major bleeding and vascular complications, without increasing the risk of ischemic events. Our study aims to improve outcomes after TAVI, by demonstrating the efficacy and safety of routine protamine administration in a randomized controlled setting.

1.4 Trial registration

The trial will be registered on Clinicaltrials.gov.

2 Objectives

2.1 Primary objective

The primary objectives of this study are to determine if routine protamine administration, compared with selective protamine administration, reduces the risk of procedural haemostasis failure and reduces the time to haemostasis (TTH): elapsed time after sheath removal and first observed and confirmed arterial haemostasis.

2.2 Secondary objective

The secondary objectives of this study are to determine if reversal of heparin by routine administration of protamine, compared with selective protamine administration reduces:

1) the risk of the composite of all-cause death, major and minor bleeding complications, major and minor vascular complications after 30 days.
2) the risk of each individual component of the first secondary outcome
3) the need for transfusion for access-site related bleeding
4) the hematoma size on vascular ultrasound 3-48h after procedure

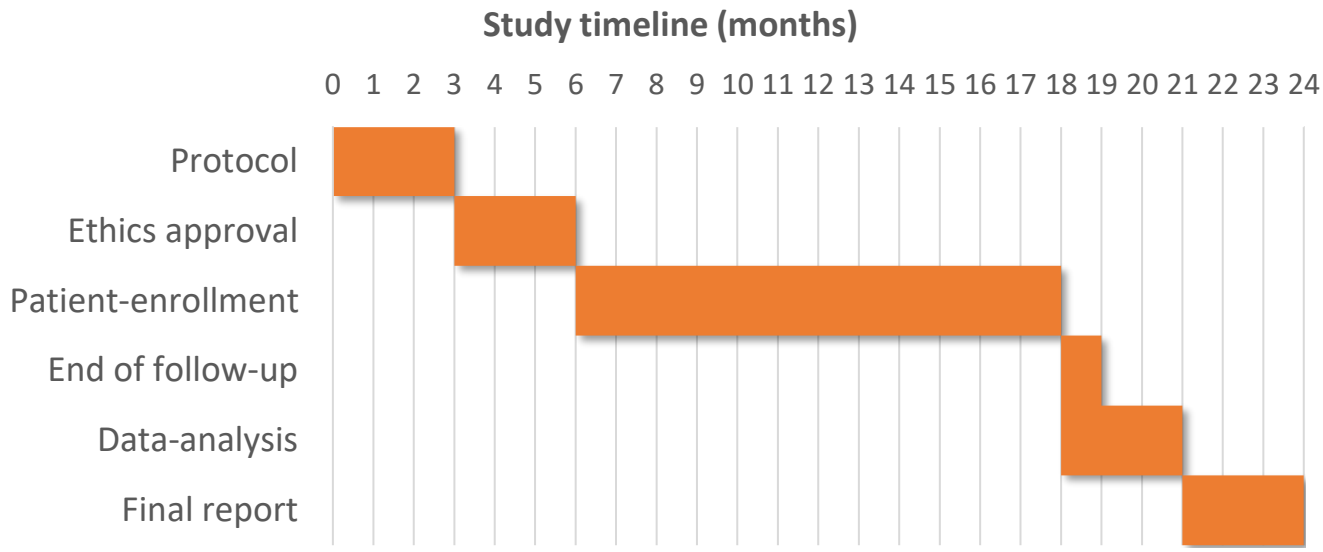
5) the risk of myocardial infarction
6) the risk of stroke
7) length of stay post-procedure
8) the rate of delayed haemostasis failure.

3 Study design

ACE-PROTAVI is a randomised, double blind placebo-controlled trial in patients undergoing TAVI, comparing routine protamine administration versus selective protamine administration.

3.1 Proposed timeline of trial

The expected timeline and study milestones are shown in the Gantt chart below.



4 Study population

4.1 Population

The study population will consist of patients with symptomatic severe aortic stenosis or regurgitation that are accepted in multidisciplinary heart team for TAVI, based on predicted surgical risk, frailty, and other clinically relevant factors.

Patients will be recruited from the Alfred Hospital, Cabrini Hospital and Epworth Hospital, all located in Melbourne, Australia.

4.2 Inclusion Criteria

Participants eligible for inclusion in this study are:

- aged > 18 years,
- undergoing elective trans-femoral TAVI with any commercially available transcatheter heart valve.

4.3 Exclusion Criteria

Participants **not** eligible for inclusion in this study are:

- Documented protamine allergy or anaphylaxis
- Recent PCI (< 3 months)
- Planned arterial access via surgical cut-down
- Pregnancy

4.4 Sample size

Sample size calculation for a 25% reduction of baseline 13±10 min of TTH after sheath removal, with >80% power and a 2-sided a of 0.05 leads, and a drop-out of 15% to a minimum of 400 enrolled patients.

The baseline TTH is based on several published studies: a randomised trial comparing proglide and prostar devices after large-bore arteriotomy (mean TTH was 9.8±17 minutes after ProGlide

and 13±19 minutes after ProStar)[11] and a prospective registry of the Manta device (mean TTH 2.4 minutes)[12].

5 Treatment of subjects

5.1 Investigational product/treatment

The investigational treatment will be one dose of protamine IV in a 0.6-1.0 ratio of 1mg per 100IU of heparin.

5.2 Additional study treatment

No additional study treatment will be given for the purpose of this trial.

6 Investigational product

6.1 Name and description of investigational product

Protamine Sulfate - a highly cationic peptide that binds to either heparin or low molecular weight heparin (LMWH) to form a stable ion pair, which does not have anticoagulant activity. Protamine is used to counteract the anticoagulant effect of heparin before surgery; after renal dialysis; after open-heart surgery; if excessive bleeding occurs and when an overdose has inadvertently been given. Protamine is registered in Australia (reg. number: AUST R 27971) and distributed by Sanofi Aventis Australia. The product is a sterile, pyrogen-free, clear, colourless 1% solution of Protamine Sulfate (Salmine) in Sodium Chloride Intravenous Infusion B.P. (0.9% w/v) adjusted to a pH of 2.5 to 3.5 and supplied in 5 mL glass ampoules.

6.2 Summary of findings from non-clinical and clinical studies

Protamine is a positively charged peptide consisting of approximately 32 amino acids, and neutralises the effect of heparin through electrostatic binding between the cationic arginine

groups of protamine and the anionic heparin in a 1:1 ratio. This creates a neutral protamine-heparin salt aggregate, and the binding of protamine to heparin dissociates the anti-thrombin/heparin complex, leading to the recovery of the original anti-thrombin activity.[13–16]

Protamine has a rapid onset of action: a heparin-neutralising effect within 5 min for unfractioned heparin, and a relatively short half-life of about 10 min in healthy volunteers.[17] Clearance of low-molecular-weight heparin by protamine depends on the molecular weight of heparin, with small heparin fragments being more difficult to neutralise than larger molecules.[18]

Whilst protamine primarily neutralises heparin, protamine has anticoagulant properties that are attributed to an interaction with platelet function, interference with coagulation factors, and stimulation of clot breakdown. First, protamine reduces platelet activity and aggregation, but the underlying mechanism is multifactorial.[19–21] Second, protamine may interfere with coagulation factors involved in clot formation, especially in the case of higher protamine doses. In vitro experiments showed that protamine mediates the down-regulation of thrombin generation, leading to a reduced conversion of fibrinogen to fibrin, reduces the rate of factor V activation by thrombin and factor Xa, and decreases factor VII activation by tissue factor thrombin activity.[21–24] Third, the decreased thrombin concentrations associated with protamine administration may also potentiate fibrinolysis.[25]

6.3 Summary of know potential risks

Protamine administration is associated with immunological and inflammatory alterations, and may induce an anaphylactic response with hypotension, bradycardia, pulmonary vasoconstriction, and allergy, as the most frequently reported side-effects. A systematic review of prospective and retrospective studies reporting serious anaphylactic reactions caused by protamine administration revealed an incidence of adverse reactions varying from 0.19% to

0.69%.[26] Patient risk factors for an anaphylactic response include treatment of diabetes mellitus with protamine-containing insulin and allergies for fish proteins.[27]

Infusion of protamine is frequently associated with a transient drop in blood pressure and a rise in pulmonary arterial pressure. Vascular reactivity to protamine is most likely mediated by increased mechanical stretch-induced intracellular endothelial calcium release, and nitric oxide production.[28,29] This transient systemic hypotension and pulmonary hypertension reverses without treatment.

6.4 Description of justification of route of administration and dosage

A comprehensive review on dosing of protamine suggests that protamine administration based on the initial heparin dose should target a ratio below 1:1 in order to prevent protamine-related coagulopathy and bleeding, but the exact ratio is not elaborated and may vary between 0.6 and 1.0 based on the initial heparin dose.[21]

In this study the final dosage of protamine will be at the operator's discretion but should not exceed total heparin dose.

6.5 Preparation and labelling of investigational medicinal product

Two 10cc syringes, one with protamine (10mg/mL) and one placebo (10mL NaCl 0.9%) will be prepared by either the anaesthetics team or the nursing staff. Dependent on outcome of randomisation, one syringe will be labelled with 'A' and contains protamine, and the other will be labelled 'B' and contains placebo, or vice versa. This will be documented, and primary operators will be blinded to the true contents of syringe A and B. (See also 7.3)

7 Methods

7.1 Study parameters/endpoints

Primary and secondary endpoints are mentioned in paragraph 2.1 and 2.2, and full definitions will be described in chapter 13.

7.2 Randomisation, blinding and treatment allocation

Randomisation forms will be generated by computer-generated code, numbered, and sealed in opaque envelopes, that conceals the treatment designation. Stickers with ‘A’ and ‘B’ will also be provided in the envelope.

For patients randomised to protamine syringe A will contain 100mg (10mg/mL) protamine-sulphate. Syringe B will contain 10mL 0.9% NaCl. For patients randomised to placebo, syringe A will contain 10mL 0.9% NaCl, and syringe B will contain 100mg (10mg/mL) protamine-sulphate.

7.3 Study procedures

The patient will be consented for the study prior to the TAVI procedure. After successful deployment of the THV, the patient will be randomised, as described in paragraph 7.2.

Prior to removal of the large sheath, ACT will be assessed. Then syringe A will be injected and sheath-removal/arteriotomy closure per operator’s discretion will be performed. If haemostasis is achieved, time between sheath removal and confirmed arterial haemostasis will be recorded.

If after 10 minutes no haemostasis is achieved, syringe B may be injected per operator’s request. This preserves the blinding of primary operators but ensures if ongoing bleeding issues that protamine is injected to improve haemostasis (as would in routine management). The need for syringe B will be documented on the study registration form. A sticker on arterial access-site dressing will be applied, with a notification if compression on the ward is required, so delayed haemostasis failure can be documented. Femoral ultrasound will be performed 3-48h afterwards

to assess haematoma size and vascular complications. Time to discharge will be documented. Any occurrence of secondary endpoints during in-hospital stay will be documented.

7.4 Data assessment

Baseline data will be registered on an electronic case report form. After signature of informed consent baseline demographical data, medical and cardiovascular history, cardiovascular risk factors, concomitant drug use, laboratory data, and echocardiographic parameters will be collected.

7.4.1 Clinical data

Data that will be collected include, but are not limited to:

- Demographical status:
    - Age, sex, ethnicity, weight, length, BMI
    - Timing of TAVI (elective/in-patient)
- Cardiovascular risk factors
    - Family history of cardiovascular disease
    - Diabetes mellitus
    - Hypertension
    - Smoking status
- Cardiovascular history
    - Coronary artery disease (single vessel, multivessel), date of last event, revascularisation (surgical or percutaneous), prior myocardial infarction
    - Prior cardiac surgery (coronary bypass grafting, valvular repair or replacement, prior endocarditis)

- Atrial tachyarrhythmia's (atrial fibrillation/flutter)
- Prior cerebrovascular event (ischemic or haemorrhagic stroke, or TIA)
- Peripheral artery disease, with or without intervention (percutaneous, bypassing, amputation)

- Medical history
  - Renal function (creatinine, GFR, on dialysis)
  - Chronic (obstructive) pulmonary disease
  - Coagulopathy/bleeding diathesis
- Concomittant drug use:
  - Anti-platelet therapy (Aspirin, clopidogrel, prasugrel, ticagrelor)
  - Anticoagulation therapy (Vitamin K antagonist, direct oral anticoagulants)
  - Beta-blockers, calcium channel blockers, angiotensin-converting enzyme – inhibitors or angiotensin receptor blockers
  - Diuretics
  - Other cardiovascular drugs

7.4.2 Laboratory data

Data that will be collected include, but are not limited to:

- Full blood count, renal function, liver function test, coagulation profile (INR, aPTT, etc.), C-reactive protein.

7.4.3 Echocardiographic data

Data that will be collected include, but are not limited to:

- LV dimensions
- LV function

- Aortic valve function (mean pressure gradient, peak gradient, aortic valve area, doppler velocity index)
- Mitral valvular function

7.4.4 Follow-up data

Final study follow-up will be scheduled after 30-days post-procedure to assess occurrence of any of secondary endpoints. In case of occurrence of an event, source documents will be collected for central adjudication. Patients and their general practitioners will be encouraged to report any occurring cardiac problems, hospitalisations or adverse reactions to coordinating research professionals or their cardiologist.

7.5 Adjudication of outcomes events

An adjudication endpoint committee will be formed to evaluate all reported clinical events in a blinded manner. The EAC members are experienced clinicians with a track record in cardiovascular research. Source documentation will be provided to the EAC and adjudication will be performed according to predefined definitions documented in the endpoint adjudication charter. If necessary, additional adjudication by a specialist physician (cardiologist, neurologist) will be practiced. See paragraph 12 for the definitions used for adjudication of clinical endpoints.

7.6 Withdrawal of individual subjects

Patients may withdraw from the study prior to randomisation. The trial is conducted according to the intention to treat principle and aims on full follow up for all patients randomised. Withdrawn consent or lost to FU is only applicable to patients who refuse to have their data collected during follow up or a phone call at the end of the trial.

7.7 Premature termination of the study

Premature termination of the study will be on behalf of the recommendations made by the DSMB (for more information see 8.5).

7.8 Feasibility of recruitment schedule

Participants will be recruited from 3 Australian hospitals. Each hospital performs between 100-200 TAVI's annually, and even if only 50% of potential subjects were recruited, enrolment of 250 patients will be completed over a 12 month period. Since the risks and additional burden for participants are low, it is hoped that recruitment will be complete well before that time. Another unique advantage in recruitment is the pre-existing collaboration of all participating hospitals in the ACE-registry, and in addition, each centre is familiar with participation in (large scale) clinical trials.

8 Safety

8.1 Temporal halt for reasons of subjects safety

The study will be suspended if there is sufficient ground to expect that continuation of the study will jeopardize subject safety. This decision will be made by the steering committee, based on advice of the DSMB.

8.2 Safety monitoring

Based on the wide experience with the investigational product no additional diagnostic tests (i.e. blood testing) to assess pharmacodynamics or pharmacokinetic profile are used.

During the trial, participating sites will be monitored to ensure completeness of patient records, accuracy of entries on the electronic case report forms, adherence to the protocol and handling of study medication, in accordance to the guidelines for good clinical practice.

8.3 (Serious) Adverse events and suspected, unexpected severe adverse reactions

The associate investigator will compile a list and summary of adverse events (AE) on a monthly basis which will be reviewed by the principal investigators. An AE will be defined as any untoward medical occurrence in a trial participant administered a medicinal product which may not necessarily have a causal relationship to the intervention. Adverse events in each group will be reported as part of the secondary outcomes. Serious adverse events (SAEs) or serious adverse reactions (SARs) will be defined as those suspected of causing death, danger to life, admission to hospital, prolongation of hospitalisation, absence from productive activity or resulting in increased investigational or treatment costs as defined by the Therapeutic goods administration (TGA). Suspected unexpected serious adverse reactions (SUSAR) will be defined as an unexpected reaction that results in death, is life threatening, requires prolongation of hospitalisation or persistent significant disability. Significant safety issues (SSI) will be defined as AE, SAE, SAR or SUSARs that could adversely affect the safety of participants, impact on the safety of continuing the trial or which lead to halting or termination of the trial.

Reporting of adverse events will be in accordance with the Alfred Hospital and Alfred Hospital Ethics committee safety monitoring and reporting requirements. Namely that SAEs/SARs and SUSARs which are deemed related to the study by the principal investigators will be reported to Alfred Health (Governance) using the Alfred SAE report form. Additionally, unexpected or possibly related events which do not meet the definition of an SAE which suggest a greater risk of harm for participants than previously reported will be emailed to research@alfred.org.au. SAEs and SUSARs will also be reported to the TGA. SSIs will be reported to the HREC using the Safety report form as well as any urgent safety measures (USM) put in place to eliminate any immediate hazards to participants. These will be reported within 72 hours as per the Alfred Hospital ethics committee guidelines.

8.4 Annual safety report

In addition to the expedited reporting of SUSARs, the primary investigators will submit, once a year throughout the clinical trial, a safety report to the accredited ethical committee and other competent authorities.

- The minimum information to report will include:
- Patient initials and study number.
- Nature of the event
- Commencement and cessation of the event.
- The coordinating principal investigator or co-investigator's opinion of the relationship between the study intervention and the event (not related, possibly related and probably related).
- Whether treatment was required for the event and what treatment was administered.

8.5 Study termination

The study may be terminated at any time at the request of a regulatory authority, with proper and timely notification of all parties concerned. The local or lead HREC will be informed promptly and the lead investigator will supply reason(s) for the termination or suspension. Otherwise, the study is considered terminated upon completion of all patient treatments and evaluations.

8.6 Data monitoring safety board (DSMB)

The independent DSMB will consist of five members with expertise in trial methodology, cardiovascular disease, and biostatistics.

8.6.1. Monitoring for effectiveness

The DSMB is responsible for monitoring greater than expected efficacy, using adjudicated endpoints events. The DSMB will undertake an interim analysis after 100 patients have been

enrolled in each arm of the study. The study will be stopped if there is a significant difference in the primary outcome measure between the two arms ($p<0.001$) at the interim analysis.

8.6.2 Monitoring for safety

The DSMB may recommend termination of the study for any safety concern that is felt to outweigh potential benefits. The DSMB will review safety outcomes, including (S)AE, with special attention to potential side-effects of protamine.

9 Statistical analysis

9.1 Baseline characteristics, primary and secondary outcomes

Summary statistics, including mean and standard deviation or median and interquartile range, will be calculated for all baseline characteristics by treatment arm.

Outcome analysis will be based on the intention-to-treat principle. The intention-to-treat analysis will include all randomised subjects and all events during the time from randomisation to the trial termination. The trial termination will be fixed by the DSMB as described above.

9.2 Interim analysis

Interim analyses will be performed when 100 patients are enrolled in each arm of the study. (also see paragraph 8.5).

9.3 Co-enrollment

Study participants may be enrolled in any trial which does not affect adherence to the ACE-PROTAVI protocol. Approval for co-enrolment in other randomised or interventional trials will be granted by the steering committee on a case-by-case basis.

10 Ethical considerations

10.1 Regulation statement

The study will be conducted according to the current Declaration of Helsinki, the Good Clinical Practice guidelines and according to the local guidelines, regulations and acts. Documented approval of appropriate Ethics Committee will be obtained prior to start of the study.

10.2 Recruitment and consent

All relevant information on the study will be summarized in an integrated subject information sheet and informed consent form. The study is explained by the investigator or designee based on this document prior to entry to the study. Subject will have enough time to decide if he/she wants to participate, to ask questions and will be informed about the right to withdraw from the study at any time without having to provide reason. The informed consent will be revised when new important information comes available. No study-specific procedures will be conducted prior to receiving informed consent.

10.3 Human Research Ethics Committee review

The protocol for this project will be reviewed by a lead human research ethics committee (HREC). Documentation of the approval of the protocol and the consent documents will be provided to the coordinating centre before the study may begin. The content and format of the standard information statements and consent forms will be adapted if necessary, to comply with local HREC guidelines and requirements. During the trial, any amendment or modification to the study protocol or consent documents will be notified to the HREC by the principal investigator and only implemented on receipt of HREC approval, unless the change is necessary to eliminate an immediate hazard to patients, in which case the HREC will be informed as soon as possible. The principal investigator will also be responsible for submitting progress reports, adverse event reports, and any other required documentation to the HREC in accordance with their guidelines. Copies of the same documents will also be kept with the study investigator files.

11 Administrative aspects, monitoring and publication

11.1 Handling of storage and data documents

Relevant data and documents will be electronically captured in a dedicated add-on to the existing ACE database. Data collection will be done by study personnel in participating sites. Space for storage of records is provided. All databases are password protected. All hard copies and electronic records will be kept for 15 years after conclusion of the study.

Data and safety monitoring will be performed every six months by the DSMB committee independent of the executive committee.

11.2 Monitoring and quality assurance

This trial will be managed according to the principles of risk-based monitoring. A monitoring plan will be developed to ensure site management according to the local regulations. Data collection procedures are reviewed as well as identification and documentation of source data. Principal investigator oversight, task delegation and training will be discussed, documented and monitored for the duration of the trial. Sites will be monitored to ensure that the data is authentic, accurate and complete, the safety and rights of subjects are protected, the study is conducted according to protocol and that all other study agreement, good clinical practice and applicable local regulations are met.

11.3 Amendments

All substantial amendments will be approved by the ethics committee.

11.4 Annual progress report

The investigator will submit progress of the trial annually to the ethics committee including number of included subjects and completion of follow-up, SAE, possible amendments and problems.

12 Risk analysis

No structured risk analysis including mechanism of action, pharmacokinetic considerations and management of effect is described in this protocol as protamine is a registered product.

13 Endpoint definitions

13.1 Primary outcome

The primary endpoint is described in chapter 2.1. Procedural haemostasis failure will be defined as failure to achieve haemostasis at the arteriotomy site leading to alternative treatment (e.g. fem-stop device, or adjunctive endovascular ballooning/stenting). Time to haemostasis will be measured in the time (minutes) elapsed after large sheath removal and first observed and confirmed arterial haemostasis.

13.2 Secondary outcomes

The secondary endpoints are described in chapter 2.2.

13.2.1 Composite of all-cause death, major and minor bleeding and vascular complications

This endpoint is a composite of all-cause death, major and minor bleeding and vascular complications according to modified VARC-2 criteria.[30] The definitions of each component will be described below.

13.2.2 All-cause death

All-cause death is defined as any death occurring in the study period (30-days post-procedure).

13.2.3 Major bleeding complications

Bleeding complications are defined according to modified VARC-2 criteria:

- Life-threatening or disabling bleeding
    - Fatal bleeding (BARC type 5)
    - Bleeding in a critical organ, such as intracranial, intraspinal, intraocular, or pericardial necessitating pericardiocentesis, or intramuscular with compartment syndrome (BARC type 3b and 3c)
    - Bleeding causing hypovolaemic shock or severe hypotension requiring vasopressors or surgery (BARC type 3b)
    - Overt source of bleeding with drop in haemoglobin ≥5 g/dl or whole blood or packed red blood cells (RBCs) transfusion ≥4 units (BARC type 3b)
- Major bleeding (BARC type 3a)
    - Overt bleeding either associated with a drop in the haemoglobin level of at least 3.0 g/dl or requiring transfusion of two or three units of whole blood/RBC, or causing hospitalization or permanent injury, or requiring surgery and does not meet criteria of life-threatening or disabling bleeding

13.2.4 Minor bleeding complications

Bleeding complications are defined according to modified VARC-2 criteria:

- Minor bleeding (BARC type 2 or 3a, depending on the severity)
    - Any bleeding worthy of clinical mention (e.g. access site haematoma) that does not qualify as life-threatening, disabling, or major

13.2.5 Major vascular complications

Vascular complications are defined according to modified VARC-2 criteria:

- Access site or access-related vascular injury (dissection, stenosis, perforation, rupture, arterio-venous fistula, pseudoaneurysm, haematoma, irreversible nerve injury, compartment syndrome, percutaneous closure device failure) leading to death, life-threatening or major bleedings, visceral ischemia, or neurological impairment.
- Distal embolization (non-cerebral) from a vascular source requiring surgery or resulting in amputation or irreversible end-organ damage.
- The use of unplanned endovascular or surgical intervention associated with death, major bleeding, visceral ischaemia or neurological impairment.
- Any new ipsilateral lower extremity ischemia documented by patient symptoms, physical exam, and/or decreased or absent blood flow on lower extremity angiogram.
- Surgery for access site-related nerve injury.
- Permanent access site-related nerve injury.

13.2.6 Minor vascular complications

Vascular complications are defined according to modified VARC-2 criteria:

- Access site or access-related vascular injury (dissection, stenosis, perforation, rupture, arterio-venous fistula, pseudoaneurysms, haematomas, percutaneous closure device failure) not leading to death, life-threatening or major bleeding, visceral ischemia, or neurological impairment.
- Distal embolization treated with embolectomy and/or thrombectomy and not resulting in amputation or irreversible end-organ damage.
- Any unplanned endovascular stenting or unplanned surgical intervention not meeting the criteria for a major vascular complication.
- Vascular repair or the need for vascular repair (via surgery, ultrasound-guided compression, transcatheter embolization, or stent-graft).

13.2.7 Need for transfusion for (access-site related) bleeding

Any bleeding requiring transfusion of 1 or more units of whole blood/RBC.

13.2.8 Hematoma size of vascular ultrasound

Measurement of haematoma size on routine vascular ultrasound performed 3-48h after the procedure. Haematoma size will be defined by maximal dimensions measured.

13.2.9 Myocardial infarction

Myocardial infarction is defined according to modified VARC-2 criteria:[30]

- Peri-procedural MI (≤72 h after the index procedure)
    - New ischaemic symptoms (e.g. chest pain or shortness of breath), or new ischaemic signs (e.g. ventricular arrhythmias, new or worsening heart failure, new ST-segment changes, haemodynamic instability, new pathological Q-waves in at least two contiguous leads, imaging evidence of new loss of viable myocardium or new wall motion abnormality)

      AND
    - Elevated cardiac biomarkers within 72 h after the index procedure, consisting of at least one sample post-procedure with a peak value exceeding 15× as the upper reference limit for troponin or 5× for CK-MB. If cardiac biomarkers are increased at baseline (>99th percentile), a further increase in at least 50% post-procedure is required AND the peak value must exceed the previously stated limit
- Spontaneous MI (>72 h after the index procedure)
    - Any one of the following criteria:

- Detection of rise and/or fall of cardiac biomarkers (preferably troponin) with at least one value above the 99th percentile URL, together with the evidence of myocardial ischaemia with at least one of the following:
- Symptoms of ischaemia
- ECG changes indicative of new ischaemia [new ST-T changes or new left bundle branch block (LBBB)]
- New pathological Q-waves in at least two contiguous leads
- Imaging evidence of a new loss of viable myocardium or new wall motion abnormality
- Sudden, unexpected cardiac death, involving cardiac arrest, often with symptoms suggestive of myocardial ischaemia, and accompanied by presumably new ST elevation, or new LBBB, and/or evidence of fresh thrombus by coronary angiography and/or at autopsy, but death occurring before blood samples could be obtained, or at a time before the appearance of cardiac biomarkers in the blood.
- Pathological findings of an acute myocardial infarction

13.2.10 Stroke

Stroke complications are defined according to modified VARC-2 criteria:[30]

- Diagnostic criteria
    - Acute episode of a focal or global neurological deficit with at least one of the following: change in the level of consciousness, hemiplegia, hemiparesis, numbness, or sensory loss affecting one side of the body, dysphasia or aphasia, hemianopia, amaurosis fugax, or other neurological signs or symptoms consistent with stroke

    - Stroke: duration of a focal or global neurological deficit ≥24 h; OR <24 h if available neuroimaging documents a new haemorrhage or infarct; OR the neurological deficit results in death
    - TIA: duration of a focal or global neurological deficit <24 h, any variable neuroimaging does not demonstrate a new haemorrhage or infarct
    - No other readily identifiable non-stroke cause for the clinical presentation (e.g. brain tumour, trauma, infection, hypoglycaemia, peripheral lesion, pharmacological influences), to be determined by or in conjunction with the designated neurologist.
    - Confirmation of the diagnosis by at least one of the following
        - Neurologist or neurosurgical specialist
        - Neuroimaging procedure (CT scan or brain MRI), but stroke may be diagnosed on clinical grounds alone
- Stroke classification
    - Ischaemic: an acute episode of focal cerebral, spinal, or retinal dysfunction caused by infarction of the central nervous system tissue
    - Haemorrhagic: an acute episode of focal or global cerebral or spinal dysfunction caused by intraparenchymal, intraventricular, or subarachnoid haemorrhage
    - A stroke may be classified as undetermined if there is insufficient information to allow categorization as ischaemic or haemorrhagic
- Stroke definitions
    - Disabling stroke: an mRS score of 2 or more at 90 days and an increase in at least one mRS category from an individual's pre-stroke baseline
    - Non-disabling stroke: an mRS score of <2 at 90 days or one that does not result in an increase in at least one mRS category from an individual's pre-stroke baseline

13.2.11 Post-procedural length of stay

Post-procedural length of stay will be measured in the time (days) from procedure to discharge.

13.2.12 Delayed haemostasis failure

The occurrence of bleeding requiring prolonged manual compression or alternative interventions (fem-stop device, endovascular or surgical repair) after initial haemostasis was achieved and patient is no longer in the cathlab.

13.3 Safety outcomes

In addition to the rate of stroke and myocardial infarction, allergic reaction or anaphylaxis to protamine will be evaluated according to the definitions stated below.

13.3.1 Allergic reaction/Anaphylaxis.

Anaphylaxis is defined according to the modified clinical criteria in the NIAID definition of anaphylaxis[31]

Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING

   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   b. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):
    a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
    b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
    c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)
    d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)
3. Reduced BP after exposure to known allergen for that patient (minutes to several hours):
    a. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP
    b. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

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