STUDY PROTOCOL

Preserving the endothelial glycocalyx in patients undergoing cardiopulmonary bypass: a prospective randomised interventional pilot study of lidocaine and doxycycline (LiDEG trial) [version 1; peer review: 3 approved with reservations, 1 not approved]

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Abstract

Background: During major surgery, particularly heart surgery, an element of the lining of blood vessels, known as the endothelial glycocalyx (EG), can be damaged. This can lead to swelling, low oxygen levels, kidney failure and other problems, which delay recovery. There are laboratory studies that show lidocaine (a local anaesthetic) and doxycycline (an antibiotic) may help protect this lining. The study agents are widely available, cheap and safe drugs.

Trial design and objective: This is a phase IV, single centre, prospective, unblinded, randomised, parallel-group trial. The objectives of the trial are to investigate the role of doxycycline and lidocaine as potential agents to reduce EG shedding and correlate with early postoperative outcomes.

Methods: 60 adult patients undergoing heart surgery requiring cardiopulmonary bypass (CPB) will be randomly assigned to one of three groups: doxycycline group (oral doxycycline, 200mg preoperatively); lidocaine group (perioperative intravenous lidocaine, 1.5mg/kg bolus at induction followed by 2mg/kg/hr infusion for the duration of surgery); and control group (standard care). The randomisation will be undertaken using a sealed opaque envelope method. The primary outcome will be the relative difference in the biochemical marker of EG injury, syndecan-1, at different timepoints in the intraoperative and early post-operative period. Secondary endpoints include vasopressor requirements, markers of organ

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Approval Status ? X ? ?

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04 Aug 2020	view	view	view	view

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Any reports and responses or comments on the article can be found at the end of the article.

dysfunction (lung, kidney, brain, arrhythmia), coagulation and inflammation.

Discussion: EG injury is ubiquitous in patients undergoing CPB. Maintaining homeostasis of this delicate layer would appear to be a valuable therapeutic target. To date no agents have been shown to be effective in protecting the EG. Our study agents have shown some promise in the preclinical setting and would represent a novel therapeutic approach should they show a protective effect.

Trial registration: Australian New Zealand Clinical Trials Registry, ACTRN12619000621112 (26th April 2019).

Keywords

Endothelial glycocalyx, lidocaine, doxycycline, cardiopulmonary bypass, anaesthesia

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Competing interests: No competing interests were disclosed.

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Introduction

Heart surgery is known to have harmful effects on the lining of blood vessels called the “endothelial glycocalyx” (EG)^{1,2}. The EG is a proteoglycan coating on the luminal side of blood vessels, and is made up of a dynamic layer of polysaccharides, the composition of which is balanced under normal physiological conditions by vascular shear forces and continued biosynthesis. Under normal conditions, the EG provides protection that helps preserve barrier function, modulate inflammation and house anticoagulant factors¹. The EG is also important for normal vascular functions, including fluid and protein extravasation, coagulation/haemostasis and microvascular blood flow.

Damage to the EG results in vascular permeability, white blood cell adhesion, changes in coagulation and ultimately organ oedema and dysfunction³, making it a viable causal mechanism for these outcomes noted in post-operative cardiac patients. Supporting this, there is good evidence that the EG is rapidly shed under both inflammatory and ischaemic conditions⁴. Rehm and colleagues also detected a component of the EG, syndecan-1, in the plasma of patients undergoing procedures requiring local systemic ischemia, including total circulatory arrest and aortic cross clamping, which indicates that shedding has occurred⁵. Strategies to protect the EG are thus a viable clinical target in order to reduce adverse outcomes. Currently no measures are taken in routine practice specifically to protect the EG.

One potential strategy is the use of doxycycline, a broad-spectrum matrix metalloprotease inhibitor that reduces metalloprotease activity known to be involved in the EG shedding process⁴. Research trials have demonstrated that administration of doxycycline reduces EG shedding in rat mesentery vasculature⁴.

Alternatively, lidocaine is an amide local anaesthetic that has a broad range of applications, including as part of adenosine/lidocaine/magnesium (ALM), a cardiac protective agent. As a combination, ALM is implicated in maintaining endothelium function and vascular permeability and leads to the restoration of EG after shedding⁶. The primary mechanism within ALM is not yet known, however lidocaine alone has been shown to improve coagulopathies, have vasoactive effects, be a free radical scavenger and have anti-inflammatory properties and is a coronary vasodilator⁶. Therefore, it is suspected that lidocaine alone may be responsible for the EG-related effects of ALM. In a porcine study, an intraoperative lidocaine infusion significantly reduced the shedding of the EG in a lung transplant model⁷.

This research trial aims to investigate EG shedding as a cause of adverse outcomes and evaluate the use of both doxycycline and lidocaine as agents to reduce EG shedding and thereby improve early postoperative outcomes.

Objectives

1. To evaluate the EG shedding in plasma and coronary sinus effluent at key time points in cardiopulmonary bypass surgery.

2. To evaluate the relative reduction in EG shedding with doxycycline and lidocaine as individual agents.
3. To correlate any reduction in EG shedding to early postoperative clinical outcomes.

We hypothesize that doxycycline and/ or lidocaine will reduce endothelial glycocalyx shedding in patients during cardiopulmonary bypass surgery.

Protocol

This is protocol version 2.2.

Design

This trial is a phase IV, single centre, prospective, unblinded, randomised, parallel-group trial consisting of three equal treatment arms (1:1:1): control group, doxycycline group, and lidocaine group. There will be 20 patients in each arm.

Study setting

This is a single centre pilot trial that will take place at the Fiona Stanley Hospital (FSH; Perth, WA, Australia), which is a large academic hospital.

Eligibility criteria

All patients undergoing cardiopulmonary bypass at Fiona Stanley Hospital who are ≥ 18 years old and provide written informed consent will be eligible to participate in the trial.

Patients will be excluded for the following reasons: patient refusal, allergy to trial drug(s), undergoing heart and lung transplant, doxycycline and/or lidocaine contraindicated in patient management, and history of chronic alcohol abuse.

Patients will be recruited via medical personnel in cardiothoracic and anaesthetic clinics and on the ward when applicable.

Written informed consent will be obtained at recruitment (*Extended data*⁸). This consent will be obtained by trained research nurses, anaesthetists or cardiothoracic surgery staff. Information sheets and consent forms will only be available in English.

Participants are able to withdraw at any stage of the trial. Samples specifically collected for the trial will be removed and disposed of if requested by the patient. Samples will not be entered to a biorepository.

Outside of initial recruitment, and the possibility of ingesting a single tablet (see Intervention section), participant responsibilities are minimal, and retention should therefore be high with recruited patients.

Outcomes

The primary outcome is the relative difference in the biochemical marker of EG injury, syndecan-1, at different timepoints in the intraoperative and early post-operative period.

Secondary outcomes include:

- *Vasopressor/inotropic requirements:* requirement and agent when leaving theatre (low cut off threshold of 5mcg/minute Noradrenaline); requirement at 6 hours into ICU stay
- *Markers of organ function:* kidney – Δ creatinine, (Including subgroup analysis for pre-existing renal failure and might need to subgroup into existing and not pre-existing renal failure, requirement of RRT); oxygen requirement – $\text{PaO}_2/\text{FiO}_2$ ratio.
- *Inflammation:* white cell count, c-reactive protein, cytokine levels (including tumour necrosis factor α and interleukin (IL)-2, IL-4, IL-6, IL-10, and IL-17 and interferon- γ)
- *Bleeding:* blood products usage, ROTEM, prolonged coagulopathy
- *Patient-based:* length of ICU stay and pain severity
- *Unplanned return to theatre*
- Postoperative delirium
- Arrhythmia (presence or absence of atrial fibrillation at 30 days)

Sample size

We plan to recruit 60 patients randomised into three groups of 20 (see [Figure 1](#)). All recruits will be at FSH. It is difficult to do accurate power calculations as this is an exploratory pilot study, but using non-interventional human data and a published pig model^{2,7}, with data only available for lidocaine, syndecan-1 levels peak at the end of surgery with a mean of approx. 50 pg/mL (but widely distributed), a pig model showed levels reduced by over 50% with lidocaine exposure. Therefore, if we take a mean of 50, with a standard deviation of 25 and anticipate a reduction by 50%, alpha error 0.05 and power of 80%, we require 16 in each group. Since these are very crude

figures, we hope to enrol 20 patients per group to increase our chance of seeing meaningful data.

Previous experience in our department shows a recruitment rate to any clinical trial at approx. 20% of all eligible patients.

Randomization and blinding

This trial aims to recruit 60 patients. Patients will be screened for eligibility in the preoperative clinic or inpatients seen on the ward. Informed consent will be sought from eligible patients. This will be undertaken by anaesthetic staff or research nursing staff. No additional visits will be required by the participants. Participants will be randomised via blocked number draw and assigned to one arm of the study. Patients will undergo randomisation in groups of three, i.e. at the time of recruitment each patient will be randomised to one of the three trial arms with the process repeating with each subsequent three patients. Randomisation will be conducted by opaque envelope method in a 1:1:1 ratio. The envelopes will be sequentially numbered, and each patient will be assigned to the next envelope. The sequencing will be performed externally to the investigators by the hospital pharmacy (clinical trials) unit.

Clinicians and researchers will not be blinded to allocations; however, laboratory staff will not be aware of the allocations and statistical analysis will be performed on the data by researchers on anonymised data blinded to the allocations. Emergency unblinding therefore will not be an issue.

Intervention

Drugs will be supplied by the South Metropolitan Service Pharmacy Department. All treatments will take place in FSH perioperative department. Treatment conditions will be as follows:

1. *Doxycycline group:* preoperative doxycycline 200mg to be administered at least two hours before patients go to theatre; in addition, patients are to be sitting upright

Figure 1. Flow diagram for participant recruitment and treatment.

when taking a doxycycline tablet with appropriate amount of water.

2. *Lidocaine group*: perioperative lidocaine infusion (1.5 mg/kg bolus given at induction of anaesthesia followed by 2 mg/kg/hr infusion until leaving the operating theatre).
3. *Control group*: standard care

The doxycycline group will be administered their treatment in the ward by ward nursing staff. The lidocaine group will be administered by the attending anaesthetist using the BD Alaris PK syringe pump (CareFusion, Switzerland) using a preprogrammed protocol of 1.5mg/kg bolus delivered over five minutes followed by 2mg/kg/hr. Outside of the study drugs, concomitant care will be at the discretion of the primary anaesthetist. This is highly standardised in FSH; all patients receive high dose fentanyl, propofol and vecuronium at induction, and blood pressure support with noradrenaline and glyceryl trinitrate. Dobutamine is the inotrope of choice in FSH. Anaesthesia is maintained using sevoflurane and is titrated to Bispectral Index. Patients are anticoagulated with heparin with target activated clotting time of 400 seconds or greater for the duration of cardiopulmonary bypass and reversed with a suitable dose of protamine. All patients receive 2 grams of tranexamic acid at induction.

Data collection

Trial specific blood samples will be collected intra-operatively and post operation (Table 1).

Blood samples will be collected for processing of plasma and storage for batch analysis by specifically appointed trial staff in anaesthesia and ICU. All samples, with the exception of syndecan-1 will be obtained from the existing hospital computer systems as appropriate. Syndecan- 1 and cytokines

associated with EG injury will be quantified by ELISA using commercially available kits. ROTEM after protamine will be performed for all patients.

All other data will be extracted from the electronic medical record including ICU records. Telephone contact will be established with the participants if required.

Assessment of safety and adherence

A Data Safety Monitoring Board (DSMB) has been set up specifically for this trial. It consists of a multidisciplinary group to review, at regular intervals, accumulating trial data, in order to monitor the progress of this clinical trial. Their role is to provide advice on safety and/or trial conduct issues by making recommendations to the investigators, on whether to continue, modify or stop this trial for safety or ethical reasons. The DSMB consists of three suitable members with no vested interest into the outcome of this trial who have relevant scientific and medical expertise.

Doxycycline and lidocaine are widely available and are used frequently in the both the community and hospital setting with a well-established therapeutic and safety profile. Furthermore, lidocaine is used often in the setting of cardiac surgery for its class 1b antiarrhythmic and analgesic properties.

Any suspected adverse effects, including allergic reactions, due to the study drugs will be reported as an adverse event and investigated appropriately.

Both the actual and potential risk to patients from both agents used in this trial is considered intermediate.

Clinicians administering treatments and taking samples will be given an education session and supervised by investigators initially.

Table 1. Sample collection time points and measures taken at each time point.

	T1	T2	T3	T4	T5	T6
	Induction (pre-operative bloods)	Pre cross clamp	3 min post cross clamp	Post protamine	ICU arrival	ICU
White cell count	✓	✗	✗	✗	✗	✓
Haemoglobin	✓	✗	✗	✗	✗	✓
Creatinine	✓	✗	✗	✗	✗	✓
Troponin	✓	✗	✗	✗	✗	✓
Syndecan-1 & cytokines associated with EG injury	Plasma	Coronary sinus & Plasma	Coronary sinus & Plasma	Plasma	Plasma	Plasma
ROTEM	✓	✗	✗	✓	✗	✗

Data analysis and management

Data analysis. Data will be analysed using GraphPad statistical software version 8.4.2 (Prism Software, San Diego, USA).

We will perform interim analysis after four patients from each group (i.e. 12 patients) and at the halfway point (30 patients) and re look at power analysis if indicated by any early signals or lack of. Patients will be randomised by block number draw whereby participants are randomly assigned to one of the three trial arms in 20 rounds of three, so as to ensure balance across conditions and effective interim analysis. We will perform per protocol and intention to treat analysis on the data. Given the nature of the clinician delivered interventions, we anticipate that very few patients will crossover or be lost to follow-up, therefore the analyses should line up closely.

The principal investigators have a strong working knowledge of medical statistics; however appropriate statistical specialist input will be sought and utilised for this study. Prior studies have shown non normal distribution of syndecan-1 level distribution between participants required logarithmic transformation to use parametric testing². We will we therefore scrutinise the data using the D'Agostino & Pearson Omnibus test and suitable parametric or non-parametric statistical tests will be used on the data. Results will be expressed accordingly.

Continuous variables and binary data will be scrutinised using appropriate tests, i.e. chi-squared test for binary outcomes, and Student's T-test for continuous outcomes.

Data management. Information will be stored and analysed on password protected hospital computers at FSH for 15 years. Participants will be anonymised. Demographics, type of surgery etc. and treatment arm will be recorded with the recorded results. All data will be included if possible. Any missing or excluded data will be clearly outlined and explained in resulting manuscript.

All principal investigators will have access to cleaned data sets.

Monitoring

The principal investigators, will permit trial-related monitoring, audits, and regulatory inspections, providing direct access to source data/documents. This may include, but not limited to, the DSMB, review by external sponsors, Human Research Ethics Committees and institutional governance review bodies.

Formal systematic auditing and monitoring will be undertaken by the authors under the supervision of the DSMB at regular time points (i.e. after 12 & 30 participants and at the end of the data collection). This study will be undertaken by experienced researchers. If there is evidence of harm at any timepoint the trial will be suspended and investigated by the DSMB and may be prematurely terminated at any timepoint. Being a pilot, the trial will not be analysed for futility at the interim stages.

The principal investigators state that the trial will be conducted in compliance with the protocol, Good Clinical Practice and the application regulatory requirements. Researchers will regularly liaise with clinicians to further ensure protocol adherence.

Data will be systematically collected by experienced researchers. Of note, there is no financial incentive (or any other form of incentive) for the results to show superiority or non-inferiority in this pilot study.

Ethical considerations

This project has been reviewed and approved by the health research ethics committee (HREC) of the South Metropolitan Health Service, Western Australia (ref RGS-1190). No atypical ethical or consent issues are associated with this pilot study. Participant Information and Consent Forms will be provided to all participants and are available as *Extended data*⁸.

Any modifications with a significant impact on the study conduct, patient safety, design, sample size will require a formal protocol amendment. Significant changes considered necessary by principal investigators will require approval from South Metropolitan HREC and relevant health authorities before being instigated. Administrative changes and minor corrections deemed necessary by the principal investigators will be documented and the HREC notified.

Patients enrolled in the trial are covered by FSH hospital indemnity. Due to the short duration of the intervention and the pharmacokinetics of the study agents, post-trial care is not predicted to deviate from standard of care but will be covered by FSH indemnity if required.

Dissemination

It is intended that the results of this pilot study will be published and disseminated in an international journal. A minimum time will be taken to compile results after recruitment of the final participant. Deidentified data will be accessible with the final manuscript.

Study status

The trial started on 1st May 2020 and is currently recruiting patients. The trial is anticipated to finish on the 31st January 2021.

Data availability

Underlying data

No data are associated with this article.

Extended data

Open Science Framework: LiDEG, <https://doi.org/10.17605/OSF.IO/Y2F8C>⁸.

This project contains the following extended data:

- Informed consent form

Reporting guidelines

Open Science Framework: SPIRIT checklist for 'Preserving the endothelial glycocalyx in patients undergoing cardiopulmonary

bypass: a prospective randomised interventional pilot study of lidocaine and doxycycline (LiDEG trial)', <https://doi.org/10.17605/OSF.IO/Y2F8C>⁸.

Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

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Version 1

Reviewer Report 02 February 2021

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David M Baron

Department of Anaesthesia, Intensive Care Medicine and Pain Medicine, Medical University of Vienna, Vienna, Austria

In the submitted study protocol, the authors describe their plan to conduct an unblinded, randomized trial to explore the effects of lidocaine and doxycycline on preserving the integrity of the endothelial glycocalyx in patients undergoing surgery requiring cardiopulmonary bypass. The authors plan to investigate a total of 60 patients separated into three groups: 20 patients will receive doxycycline, 20 patients will receive lidocaine, and 20 patients will serve as control. The primary outcome is the relative difference in syndecan-1 levels, reflecting the damage to the endothelial glycocalyx.

1. The term "pilot study" seems inappropriate since the study does not aim to test feasibility of patient recruitment, data collection, or other points of the protocol in preparation for a larger study.
2. Since the sample size calculation was based on a study including 12 pigs, interim power calculation would be rational to avoid underpowering.
3. Please provide details on the decisions for the specific dosages of doxycycline and lidocaine.
4. Please provide details on the definition of secondary outcomes and observation periods. Are you planning on using the KDIGO criteria for the categorization of acute kidney injury? Are you planning to monitor urine output as well? Since serum creatinine is a slow marker in detecting renal dysfunction, only comparing levels before induction of anesthesia with levels at admission to the ICU (see Table 1) seems insufficient. How do you define postoperative delirium?
5. Please provide further details on the planned statistical analyses. Which groups will be compared? Are you planning to compare all groups using, for example, ANOVA? Are you planning to compare each intervention group separately to the control group with unpaired samples t-tests?

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

No

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Blood transfusion, transplantation.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 25 January 2021

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Jong Wook Song

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This trial aims to investigate the effect of lidocaine and doxycycline on reduction of EG shedding in cardiac surgery.

1. In my opinion, assumptions for the sample size calculation seems to be too optimistic. As the authors described, and previous studies showed that syndecan-1 concentration varies wildly. The authors assumed very large treatment effect (50%). The calculation seems to be based on comparison between two groups. Taken together, this trial is likely underpowered.
2. The investigators plan to sample coronary sinus effluent. It may provide heart-specific information. I think the investigators should describe strategy for cardioplegia administration. I'm afraid that warm blood administration before aortic de-clamping wash-out most of syndecan-1 from coronary circulation.
3. Secondary outcome measures should be described in detail for their definition, time frame

and assessment method. I think KDIGO or AKIN criteria for AKI may be more appropriate to assess kidney injury rather than delta-creatinine.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Anesthesiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 18 January 2021

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Antonio Nenna

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Sample size calculation should be verified after ad-interim analysis. Current sample size, considering the published details of the manuscript, seems low.

Details of the primary and secondary endpoints (i.e. definitions of endpoints) should be required. Fold-increase rather than crude numbers might be an option.

Considering baseline data, preoperative pharmacological treatments should be considered as they might lead to significant variability (i.e. statins, steroids, DMARDs,...). Inclusion/exclusion criteria should be revised accordingly to reduce variability.

Considering operative data, including patients with a single procedure (i.e. CABG) is generally performed to provide reliable results in this scenario. Please consider this issue; inclusion /

exclusion criteria should be revised accordingly to reduce variability.

Is the rationale for, and objectives of, the study clearly described?

Partly

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cardiac surgery.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 05 August 2020

<https://doi.org/10.5256/f1000research.27010.r68613>

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Eero Pesonen

Department of Anesthesiology and Intensive Care and Pain Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Main Considerations

The aim of the intended RCT is to investigate the efficacy of p.o. doxycycline and i.v. lidocaine in reducing endothelial degradation (measured as plasma syndecan-1) in heart surgery with CPB. The focus of the RCT is important.

The study is already recruiting patients. I am deeply worried of the statistical power of the RCT. I am afraid that it will be underpowered. 1) I suggest revised power calculation after recruitment of 30 patients. 2) To reduce variability, I suggest to exclude certain types of cardiac surgery. Furthermore, I suggest to exclude patients with such medications which may directly influence on the CPB-induced inflammatory reaction, and patients with bleeding diathesis. While these measures reduce variability, they may increase potential intervention effect and thus study power.

Above-mentioned measures can be applied although the patient recruitment has already started.

I do not understand why the authors do not conduct the RCT as double-blind. It would be easy to do and would substantially improve the quality of the study. While the patient recruitment has already started, this modification probably should not be done anymore.

Otherwise, I believe that the modifications suggested below can be applied although the patient recruitment has already started. However, an approval of the ethics committee may be required.

Australian New Zealand clinical trials registry

There are numerous and significant discrepancies between the present manuscript and the registry. These should be corrected either in the registry or the manuscript to correspond the actual conduct of the RCT.

Inclusion and exclusion criteria

In terms of surgery with CPB, only transplantations will be excluded. Consequently, patients undergoing surgery of the aortic arch (requiring deep hypothermia with/without circulatory arrest or selective cerebral perfusion), descending aorta (spinal cord ischemia) and redo congenital defects (often complex and unpredictable surgery) will be recruited. This will result significant variability of the surgical insult. I suggest to exclude these types of surgery. Most probably, the number of these patients would be low anyway.

Furthermore, I suggest to exclude patients with preoperative or perioperative corticosteroids or immunomodulatory drugs or any other drugs with significant anti-inflammatory action. Perioperative corticosteroids should not be allowed.

Patients with bleeding diathesis should be excluded.

Primary Outcome

According to the registry, syndecan-1 concentration will be expressed as a fold-increase of baseline (pre-induction) level. This should be indicated also in the manuscript.

I support the use of fold-increase to manage the vast inter-individual variation of absolute syndecan-1 plasma concentrations. However, even fold-increase is not without problems. If the preoperative level is small, it will yield high fold-increase even for small absolute increase and vice versa. Also fold-increase can result in large variation. I suggest to calculate the primary outcome both as fold-increase and as absolute concentrations. If so, this should be indicated in the manuscript. This is justified because of vast inter-individual variation of absolute syndecan-1 plasma concentrations.

Secondary Outcomes

The secondary outcomes (how and when they are measured and calculated?) should be defined unequivocally.

Vasopressor/inotropic requirements: Will some vasopressor and inotropic score be calculated? There are formulas to calculate equivalent doses? (Later in the description of patient care: How realistic it is that only noradrenaline and dobutamine will be used?)

Markers of organ function: Delta-creatinine, during how many postoperative days? P/F-ratio, at what time points or when?

Bleeding: Blood products usage, the length of the postoperative observation period? Prolonged coagulopathy, definition?

ROTEM, which parameters will be used?

Pain severity, how do you measure?

Postoperative delirium, definition?

Sample size

I assume that the sample size was based on unpaired t-test? The test applied for the sample size calculation should be indicated. To be precise, the power calculation should be based on repeated measures ANOVA. Because there is paucity of applicable literature, in my opinion, unpaired t-test is justified. After all, power calculation is always only estimation.

It should be clearly indicated, that the power calculation applies only between the control group and one of the intervention groups (doxycycline or lidocaine). The assumed effect does not apply for comparison between the intervention groups.

The estimated reduction of 50% is high and possibly unrealistic. Furthermore, the assumed SD is significantly lower than in the syndecan-1 results of the reference #2. I am afraid that the power calculation is too optimistic. On the other hand, the sample size is now calculated for actual syndecan-1 plasma concentrations. To reduce the effect of large inter-individual variation in plasma syndecan-1 concentrations, the authors will calculate fold-increase (i.e. multiples of the respective baseline values), which I fully support. It might be better to conduct the power calculation also for fold-increase values. The authors should screen the literature if they could find applicable data. In my opinion, it would be justified to choose the power calculation (absolute concentrations or fold-increases), which yields lower patient number.

Finally, I suggest a revised power calculation as an interim analysis (see below).

Blinding

I do not understand why the authors do not conduct the study as double-blind? It would have been easy to do with placebo tablets and saline syringes with non-transparent foil. In all groups, the patient would receive a tablet (either doxycycline or placebo) and infusion (either lidocaine or saline). While doxycycline and lidocaine are well-known drugs, there should be no ethical concerns, either. Double-blind design would improve the study substantially.

Protamine Dose

Does the study site not have a more or less established protocol for protamine dosing (initial and potential additional doses) in relation to heparin dosing? If there is, it should be defined in the manuscript.

Blood Samples

I assume that the systemic blood samples as a function of time will be drawn from the arterial cannula? This should be clearly indicated. This is especially important, if the authors will calculate trans-coronary concentration differences between the sinus coronarius and concomitant arterial samples. If the systemic samples will be taken from the central venous line (I assume not), such trans-coronary differences cannot be calculated.

The placement of the sinus coronarius catheter should be described in detail. Especially, verification of the correct placement is important. I refer to a previous publication where sinus coronaries catheter placement is verified rigorously¹.

Table 1

The place of the systemic samples (presumably arterial) should be indicated. The expression “3 min post cross clamp” can be understood as 3 minutes after the placement of the arterial clamp. I suggest to replace it with the expression “3 min post de-clamp”.

Interim Analysis

Usually, an interim analysis is conducted for two reasons: 1) to detect that the study hypothesis is already evident and there is no reason to continue patient recruitment; 2) to detect that the study effect is so small that it is evident that even after full recruitment there would not be statistically significant difference between the study groups. Again, there would not be any reason to continue patient recruitment. These goals are not realistic with small patient numbers of 12 (the first interim analysis) and 30 (the second interim analysis). I suggest that the primary goal of an interim analysis would not be estimation if patient recruitment should or should not be continued.

Instead, I suggest to conduct an interim analysis after 30 patients to evaluate statistical power of the study by using the study effect (i.e. the mean difference between the intervention and control groups) and SDs at the time of analysis. If power calculation necessitates extra patients, the authors should decide *a priori*, how many extra patients it would be realistic to recruit. If the result of the power calculation exceeds this patient number, the RCT should be terminated and published as a negative study result. Otherwise, the patient number should be enlarged according the revised power calculation. Both interventions (doxycycline and lidocaine) should be calculated separately. It might occur, that one intervention would be terminated but the other would be continued with a large patient number. In the end, the authors would have an RCT with adequate power.

In theory, it is possible that there will be statistically significant difference ($p < 0.05$) between the study groups after 30 patients. I find it highly unlikely but it is theoretically possible. In such a case, the patient recruitment should be terminated and the RCT published with a positive result.

I admit, that also my suggestion carries a risk a premature stopping of the study. This is, however, lower than in "conventional" interim analysis, because the study power should not be weight against the intended patient number (n=20/group) but against the enlarged patient number.

In any case, the authors should have serious re-consideration of the present power of the study.

Withdrawals

As the authors point out, there probably will not be many (or even a single) withdrawals or dropouts in the study. Still, the authors should be prepared to replace such patients, especially when the power calculation even with full recruitment is questionable. I suggest the following statement: "Patients whose primary outcome cannot be assessed will be replaced retaining the original allocation to the study groups."

Per Protocol

I do not believe that per protocol analysis will be needed. Still, it can be included in the study protocol.

References

1. Pesonen E, Passov A, Salminen US, Ilmakunnas M, et al.: Heparin Binding Protein in Adult Heart Surgery. *Ann Thorac Surg.* **107** (4): 1154-1159 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Inflammation, coagulation and related phenomena (including also the endothelial glycocalyx) in cardiac surgery, solid organ transplantations and sepsis.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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