Full Title	Epidural-related maternal fever: personalised genomic medicine to guide labour analgesia.
Short Title	EPIFEVER-2
Sponsor	Queen Mary, University of London *Contact person:* Dr Mays Jawad Research & Development Governance Operations Manager Joint Research Management Office 5 Walden Street London E1 2EF Phone: 020 7882 7275/6574 Email: research.governance@qmul.ac.uk
REC Reference:	
IRAS:	270480
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1. Contents

2. Glossary

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
DMC	Data Monitoring Committee
EC	European Commission
ERMF	Epidural-related maternal fever
GAfREC	Governance Arrangements for NHS Research Ethics Committees
ICF	Informed Consent Form
IL-1Ra	Interleukin-1 receptor antagonist
JRMO	Joint Research Management Office
NHS REC	National Health Service Research Ethics Committee
NHS R&D	National Health Service Research & Development
Participant	An individual who takes part in a clinical trial
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
RCOG	Royal College of Obstetricians and Gynaecologists
REC	Research Ethics Committee
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

3. Signature page

Chief Investigator Agreement

The study, as detailed within this Research Protocol, will be conducted in accordance with the principles of Good Clinical Practice, the UK Policy Framework for Health and Social Care Research, and the Declaration of Helsinki and any other applicable regulations. I agree to take responsibility for the statistical analysis and oversight of this study.

Chief Investigator Name: Gareth Ackland

Signature: 

Date: **5.7.2020**

4. Summary and synopsis

Short title	EPIFEVER-2
Methodology	Observational, mechanistic cohort study.
Objectives / aims	To determine whether polymorphisms in interleukin-1 receptor antagonist gene promotes fever in labouring women receiving epidural analgesia.
Number of participants	637 women
Inclusion and exclusion criteria	Women aged >18 years in active or induced labour.
Statistical methodology and analysis (if applicable)	Incidence of epidural-related maternal fever compared between IL-1ra allele scores.
Study duration	24 months

5. Introduction

5.1. Background

Epidural-related maternal fever (ERMF) occurs in 15-20% of labouring women.(Sultan et al., 2016) We have reported that the local anaesthetic agent used most commonly in epidurals – bupivacaine- reduces caspase-1 activity, resulting in lower release of the anti-pyrogenic cytokine interleukin-1 receptor antagonist (IL-1Ra) from circulating leucocytes.(Del Arroyo et al., 2019) Reduced IL1Ra secretion may promote ERMF since IL-1Ra is a potent anti-pyrogenic cytokine in active labour.(Girard et al., 2010; Kallapur et al., 2009) These data reconcile previous observations where pro-inflammatory cytokines increase during active labour yet did not differ substantially between women who develop fever versus those that do not.(Goetzl et al., 2002).

Impaired IL-1ra release may promote ERMF. IL-1Ra is a key determinant of systemic inflammation in pregnancy IL-1Ra is normally present in the circulation of healthy persons, whereas IL-lα and IL-1β are not typically detectable in the absence of inflammation or autoimmunity.(Witkin et al., 2002) IL-1Ra appears to prevent proinflammatory responses to minor nonpathogenic stimuli. IL-1Ra levels are higher in women.(Lynch et al., 1994) In pregnancy- a proinflammatory state- IL-1β is key for the initiation of infection-related preterm labour and delivery.(Romero and Tartakovsky, 1992) In mice, the injection of IL-1Ra before IL-1β administration prevents preterm birth.(Romero and Tartakovsky, 1992) Similarly, we found IL-1Ra/IL-1β ratio declined 4h after epidural analgesia was instituted, consistent with a role in promoting ERMF.(Del Arroyo et al., 2019)

Genetic determinants of IL-1Ra levels impact on outcomes in pregnancy The gene coding for IL-1ra is polymorphic due to an 86-bp tandem repeat sequence of variable length.(Tarlow et al., 1993) Most individuals are homozygous for IL-1RN*1 (four repeat sequences), which is more common than IL-1RN*1/IL-1RN*2 heterozygotes. Plasma IL-1ra levels are ‘dosedependently’ higher in individuals with increasing number of repeats.(Interleukin 1 Genetics, 2015) For example, median vaginal IL1-Ra levels are ~3-fold higher in women with the IL-1RN*2 variant.(Barton et al., 2003; Jeremias et al., 1999) IL-1Ra gene polymorphisms resulting in lower IL-1Ra/IL-1β ratios are associated with an increased risk of preterm birth.(Nadeau-Vallee et al., 2016)

5.2. Rationale

Thus, we propose that ERMF is driven by the relative lack of an anti-pyrogenic cytokine that suppresses fever. To establish the clinical relevance of these findings, we propose to employ a genomic approach at the population level to confirm the link between ERMF and IL-1Ra. This complementary approach examines the relationship between genetic variants known to result in higher circulating levels of IL1Ra.(Herder et al., 2017)

5.3. Risks / benefits

There are no study specific risks, as blood samples are obtained as part of routine care.

6. Study objectives

6.1. Primary objective

To demonstrate that single nucleotide polymorphisms in the gene encoding for IL-1Ra are associated with higher risk of ERMF defined as maternal pyrexia (defined as a temperature >38.0 °C once, or 37.5 °C on two occasions ≤2 hours apart) during active labour before delivery. (DelArroyo et al., 2019; Lange et al 2017; Royal College of Obstetricians Green–top Guideline No. 64a).

6.2. Secondary objectives

1. Clinical outcomes for mother and baby.
2. To examine ex-vivo whether local anaesthetics and/or labour analgesics vary in their ability to inhibit IL-1Ra release, and hence ERMF.
3. Post-hoc identification of novel immune-related single nucleotide polymorphisms and/or RNA changes associated with ERMF.

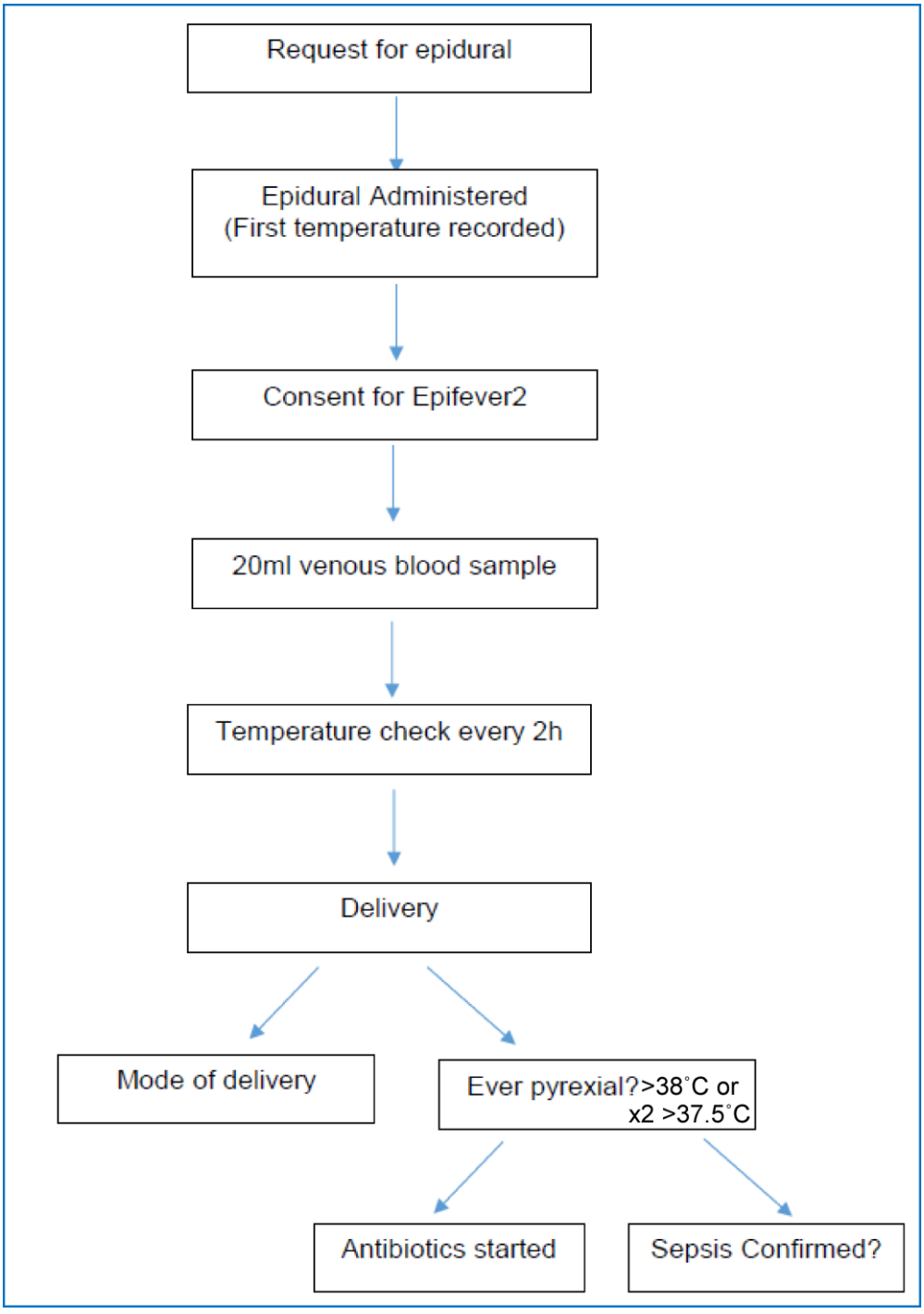
6.3. Primary endpoint

The primary endpoint is maternal temperature >38°C ≥4h after epidural analgesia is commenced and/or prescription of antibiotics during labour before delivery (triggered by RCOG guidelines for two temperatures >37.5°C). Hourly temperature logs kept by midwives as part of routine care for women receiving epidural analgesia.

6.4. Secondary endpoints

Prescription of antibiotics during labour, obstetric intervention, mode of delivery, complications and level of clinical care required for mother and baby will be recorded from electronic and/or drug charts. Laboratory-based measures of genomic and proteomic signatures associated with developing fever after epidural insertion.

6.5. Study Scheme Diagram



7. Subject population

7.1. Inclusion criteria

- ≥18 yr old females of any gestational age,
- Singleton or twin pregnancy,
- requesting an epidural for labour analgesia.

7.2. Exclusion criteria

- Unwilling or unable to give consent
- Refusal of consent for competent participants,
- Inability to understand written and/ or verbal English
- immune/genetic syndromes/mutations,
- microbiologically proven infection prior to epidural insertion,
- established pyrexia
- intrauterine death.

8. Study Design

This is an observational mechanistic cohort study in which the development of fever after epidural analgesia and/or antibiotic prescription will be correlated with an allele score generated by the presence/absence of polymorphisms in the IL-1Ra gene. The primary clinical outcome is masked to genomic analyses. 637 women in active labour or commencing induction of labour will be studied. Participants will be selected for the study from antenatal clinics and/or following admission to labour ward. Participants will be informed about the study by their obstetric and/or anaesthetic team. No vulnerable groups will be included. Duration of participation will be from epidural insertion until birth of the baby.

9. Study procedures

9.1. Screening procedures

Pregnant women scheduled for hospital birth will be recruited from Barts Health NHS Trust clinical sites by their surgical team. First approach to the patient will be made by a member of the direct clinical care team. For example, they can identify and provide the PIS to the patient, after which a trained member of the research team can approach with patient with the ICF. After identification of eligibility, a trained member of the research team will seek patients and provide patient information sheets. Potential participants will be screened to determine whether patients meet inclusion and exclusion criteria (see eligibility above).

9.2. Informed Consent Procedures

Written informed consent will be obtained from research participants by an appropriately trained researcher in a face to face setting either at Barts Health NHS Trust clinical sites or the William Harvey Research Institute. This may occur in antenatal clinic, or on labour ward at the time of request for epidural analgesia.

9.3. Blinding and procedures to minimise bias

Both primary and secondary outcomes will be analysed by individuals masked to temperature or antibiotic prescription by women during labour.

9.4. Participant recruitment schedule

Potential participants will be given the PIS for at least 1 week before deciding whether they wish to participate. If they do, they will then be consented pre-surgery by a trained member of the research team.

9.5. Criteria for Discontinuation

Patient removal of consent is the only criteria for discontinuing the study.

9.6. Procedure for Collecting Data including Case Report Forms (CRFs) and storage

All samples to be processed in the laboratory will be linked anonymised with a unique identification number (UIN). All data will be stored in password restricted computer facilities in the William Harvey Research Centre at QMUL and hard paper copy in the study master file under the custody of the CI. Research data and samples will be stored for 20 years in accordance in accordance with the Research Governance Framework and Barts Health NHS Trust Policy.

9.7. Follow-up Procedures

There only follow-up procedures after the blood sample is obtained will be to record the way the baby was delivered and whether the mother/baby needed to be treated with antibiotics.

9.8. Subject withdrawal

The participant will be informed that their involvement is completely voluntary and that they may stop participation at any point throughout the study without having to give a reason. Data will be retained for analysis from all participants after the point of consent and recruitment unless the participant specifically withdraws consent for their data to be used.

9.9. Schedule of Assessment

Event/Visit	Screening	Antenatal clinic	Labour
Inclusion/exclusion	x		
Informed consent		x	x
Medical history	x	x	x
Note review			x
Blood sample			x

9.10. End of Study Definition

The end of study is defined as the point when the last participant delivers her baby.

9.11. Subject withdrawal

All study participants are free to withdraw from the study at any time. All participant data will be included in the final analysis, unless a participant specifically asks for their data not to be included.

9.12. Data collection and follow-up for withdrawn subjects

Patients that withdraw consent or drop out before surgery will be replaced. The withdrawal will be documented in the CRF and medical records. Participants are not obliged to give the reason for withdrawing their consent, but we will attempt to ascertain trends (where possible) relating to study procedures in case this necessitates a protocol amendment.

10. Statistical considerations

10.1. Sample size

Based on the IL-1Ra Consortium score (Interleukin 1 Genetics, 2015), women with low IL-1Ra secretion associated with an allele score 0 comprise ~12% of the population. We hypothesise that these women are more likely to sustain ERMF or be prescribed antibiotics for >2 temperatures >37.5°C over a two-hour period, compared to women with higher IL-1Ra secretion (allele score ≥1). The overall incidence of ERMF in EPIFEVER was 13.4% (probably an underestimate with broader inclusion criteria and lower temperature threshold for antibiotic use, as per RCOG guidelines). If individuals with an allele score 0 are more likely to develop ERMF (~22%), compared with women with IL-1Ra allele scores ≥1, at least 637 women will be

required to detect an absolute difference in primary outcome of ~13%, assuming 9% incidence in women with allele scores ≥1 (α=0.05; 1-β=0.2).

10.2. Method of analysis

For the primary clinical outcome, the relative incidence of the primary clinical endpoint will be compared between allele scores 0 versus ≥1 and across individual allele scores 0-4 inclusive. Significance will be set at $p<0.05$. For the laboratory measures in a subset of women in hospitals local to WHRI, ex-vivo IL-1Ra functionality in freshly obtained leucocytes will be assessed by flow cytometry and/or protein/gene transcription assays. For these studies, at least 26 samples are required to detect ≥25% increase in IL-1Ra levels from control-treated samples for within-subject comparisons (α=0.05; 1-β=0.9).

Ethics

10.3. Annual safety report

This protocol and any subsequent amendments, along with any accompanying material provided to the patient in addition to any advertising material will be submitted by the Investigator to an Independent Research Ethics Committee. Written Approval from the Committee must be obtained and subsequently submitted to the JRMO to obtain Declaration of Sponsorships and approval.

11. Public involvement

The grant funding this work was written in conjunction with The Barts Health patient and public advisory group supporting Barts Research Centre for Women's Health Research Unit- Katie's Team- who have provided views on the study from a mixed group of more than 50 women with varied personal experiences of pregnancy and pregnancy complications, as well as family members, partners and carers attending. To ensure that the full benefit of patient and public involvement in this study, a co-applicant Rebecca Harmston has kindly agreed to be involved in the steering group and provide continuity of advice in partnership with Katie's Team (from whom Josie Hamper has kindly agreed to contribute). The design of the protocol has benefited from Rebecca's review and Josie's input into PIS and consent forms. The study will hugely benefit from Rebecca's experience in NIHR PPI involvement. Feedback from Josie and Katie's team has helped inform the content and design of patient information sheets, including disseminating information about the proposed study. This has been done in accordance with INVOLVE guidelines on public and patients' engagement.

12. Data management

An electronic CRF will be used to collect data at site level. On data collection times illustrated in section 5, research staff will be responsible for the completion of the electronic CRF throughout the life cycle of the study. The electronic CRF will be hosted on a secure, custom-designed study Queen Mary University of London bespoke clinical study database. The CI will ensure that the study is conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Policy Framework and the Medicines for Human Use (Clinical Study) Regulations 2004, and all subsequent amendments, Trust and sponsor policies and procedures and any subsequent amendments. In addition, sponsor auditors and Competent Authority inspectors will be allowed access to CRFs, source documents and other study files to evaluate the study. Audit reports will be kept confidential.

11.1. Source data

Hourly temperature logs kept by midwives as part of routine care for women receiving epidural analgesia. Prescription of antibiotics during labour, obstetric intervention, mode of delivery, complications and level of clinical care required for mother and baby will be recorded from electronic and/or drug charts. Laboratory-based measures of genomic and proteomic signatures associated with developing fever after epidural insertion.

11.2. Confidentiality

The CI has a responsibility to ensure that patient confidentiality is protected and maintained. They must also ensure that participant identities are protected from any unauthorized parties. Information with regards to study patients will be kept confidential and managed in accordance with the GDPR and Data Protection Act 2018, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and REC Approval. The PI as well as the study team must adhere to these parameters to ensure that the patient's identity is protected at every stage of their participation within the study. To ensure this is done accordingly, each patient, at time of consent must be allocated a unique screening number by either the PI or a member of the study team before undergoing any screening procedures. The patient's initials (the first letter of their first name and the first letter of their last name)

should be used as a means of linked-anonymizing parameters. This information should be kept on a screening log, which should be updated accordingly throughout the study. Once the patient has completed screening procedures and is enrolled onto the study, the patient will be allocated a randomization number. No identifiable information will be collected from the subjects. The CI is the 'Custodian' of the data and maintains access to the data. No patient identifiable details will be transferred outside the EU. Subjects maintain their right to revoke their authorization for the use of their PID. The patients will be linked anonymised with regards to any future publications relating to this study.

11.3. Record retention and archiving

At the end of the study, as defined by GCP all documentation should be stored by each individual site's archiving facility, for a minimum of 20 years or the maximum period required by the Institution in which the study will be conducted, whichever is longer. The Sponsor should be contacted prior to destruction. A 'close out' visit will be conducted where all study documentation will be prepared for archiving by that site. Records will be retained at each individual site. All records relating to the study should be stored together, including the Investigator Site File (ISF) and CRF. It is the responsibility of the PI to ensure a full set of records is collated and documented. In addition, source documentation should be retained, as per local policy, for the duration of the archiving period.

12. Laboratories

12.1. Central and local laboratories *Error! Bookmark not defined.*

Laboratory samples will be processed in the lab of Gareth Ackland, Translational Medicine & Therapeutics, William Harvey Research Institute, John Vane Science Centre, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ.

12.2. Sample preparation and collection

After providing informed consent, 20ml of blood will be taken from a cannula inserted as part of routine care for women receiving epidural analgesia. In the unlikely event of blood not being obtained in this manner, lower arm venepuncture will be undertaken by an anaesthetic doctor or nurse trained in phlebotomy and first aid within the research team. This will occur only once. The blood samples will be linked anonymised so that no patient information will be identifiable through the allocation of a unique identification number and processed at the William Harvey Research Institute at Charterhouse Square by the research team.

12.3. Sample receipt/chain of custody/accountability

Handling of the samples upon arrival at the local and central laboratory will be documented. All samples will be logged upon receipt and the laboratory will ensure that the physical integrity of these samples have not been compromised in transit. If compromise has occurred, the trial coordinating team, as well as the Sponsor, will be informed of this. Upon receipt of samples, laboratory staff will ensure that all samples are accounted for as per the labelling.

12.4. Sample storage and transfer

All blood samples will be pseudo-anonymised. Samples collected at each participating site will be labelled with the participant's corresponding study ID, and kept in a hospital freezer until batched processing. The samples will be routinely collected and transferred to WHRI. The full sample, collection, labelling, logging and transfer procedure will be documented in the study laboratory log. The study coordinating team will provide sites with a Standard Operating Procedure (SOP) on sample collection, processing, and storage. The samples will not be destroyed if a patient withdraws from the study unless they specifically request so. If the patient requests for the samples to be destroyed the Tissue Custodian, (CI) will inform the

lab who will ensure the samples are destructed as per the Human Tissue Act. This will be documented in the TMF and ISF of the participating site

12.5. Laboratory procedures

Single nucleotide polymorphisms will be assessed by polymerase chain reaction. In a subset of women, we will also compare the effect of different local anaesthetic agents on the ability of white blood cells to secrete IL-1Ra using RNA sequencing and flow cytometry.

12.6. End of study

The samples will be used for this study only and will be destroyed according to the Human Tissue Authority's Code of Practice once the final analyses have been performed.

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13. Safety reporting

13.1. Serious breaches

The Sponsor of the Clinical Study is responsible for notifying the research ethics committee in writing of any serious breach of the study protocol or the principles of GCP. A 'serious breach', is a breach which is likely to effect to a significant degree:

- The safety or physical or mental integrity of the subjects of the study; or
- The scientific value of the study.

The CI is responsible for reporting any serious breaches to the Sponsor (JRMO) **within 24 hours**. The Sponsor will notify and report to the REC within 7 working days of becoming aware of the serious breach."

13.2. General definitions

An Adverse Event (AE) is any untoward medical occurrence in a subject to whom a medicinal product or procedure has been administered, including occurrences which are not necessarily caused by or related to that product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of an IMP, whether or not related to the IMP. Only patient discomfort during standing up will be recorded as an AE and assessed accordingly.

13.3. Study specific adverse events

There are no adverse events related to this observational study. If venepuncture is necessary (rather than obtaining blood directly from the large bore cannula placed as part of routine care for an epidural), bruising can usually be prevented by applying pressure once the needle/cannula has been removed. Risk of infections from venepuncture for a short time frame are minimal and will be reduced by using sterile techniques (cleaning the area with alcohol, wearing gloves, and covering the insertion point with a plaster once the process is finished). The procedure will be performed by an anaesthetist or trained staff following the WHO guidelines (best practice in phlebotomy, 2010).The risks to participants donating synovial and epithelial tissue during surgery, is the same as the risks of the surgery itself.

14. Monitoring and auditing

14.1. Quality control and quality assurance

The Sponsor or delegate retains the right to audit any study, study site or central facility. In addition, any part of the study may be audited by the funders where applicable. Monitoring will involve a review of the ISF as well as 10% of source data verification. This will involve direct access to patient notes, which will include the review of consent forms and other relevant investigational reports. Missing data will be sought, unless confirmed as not available. A summary of all monitoring activity for this study will be provided to the Sponsor at least every six months. All sites will undergo an on-site site initiation visit. On site monitoring visits will then occur within six months or 10 patients, whichever is sooner as per monitoring plan. At the end of the study all sites will undergo an on-site close out visit.

14.2. Audit and Inspection

This study may be audited by representatives from the coordinating centre and the Sponsor or its delegate. The investigator and institution will be informed of the audit outcome. Investigators are obliged to cooperate in any audit allowing the auditor direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor to discuss any findings or issues. An audit may occur at any time during or after completion of the study. Inspections may be carried out by the Competent Authority at any time and the investigator should notify the Sponsor immediately if there are any such plans for an inspection.

15. Study committees

There will a study committee for this study comprising at least one lay member, an obstetrician and an obstetric anaesthetist not directly involved in the study. The committee will take on the responsibility for:

- ensuring that views of the participants are taken into consideration,
- advising on the trial protocol,
- advising on changes in the protocol based on considerations of feasibility and practicability,
- assist in resolving problems brought to them by the research team
- monitor the progress of the trial and adherence to protocol
- consider new information of relevance from other sources,
- consider and act on the recommendations of sponsor and/or REC,
- review trial reports, statistical analysis plan, protocol papers and papers for publication.

16. Finance and funding

This study is supported by a project grant from the Obstetric Anaesthetists Association, administered by the National Academy of Academic Anaesthesia. There are no participant study payments or travel expenses available for this study.

17. Insurance and indemnity

The insurance that Queen Mary has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm. Dr Gareth Ackland (Queen Mary University of London) is the CI. Queen Mary University of London is also sponsoring and insuring the study.

18. Dissemination of research findings

This is an investigator-led study sponsored by the CI's substantive employer, Queen Mary University of London. The data collected will not be used to license/register any pharmaceuticals. Authorship of the final manuscript(s), interim publications, or abstracts will be decided according to active participation in the design, accrual of eligible patients and statistical analysis. Contributing/participating investigators) will be acknowledged in the final manuscript.

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