IL1-ra polymorphisms and risk of epidural-related maternal fever (EPIFEVER-2): Study protocol for a multicentre, observational mechanistic cohort study.

Amaan Ali,[1] Valentin Weber,[1] Sally J. Thomson,[1] Tom EF Abbott,[2] Rebecca Harmston,[3] Anna L David,[4] Gareth L. Ackland,[1,2] for the EPIFEVER-2 investigators*

1 Barts and The London School of Medicine and Dentistry, Queen Mary University of London London.

2 Translational Medicine and Therapeutics, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London

3 Patient Representative.

4 Elizabeth Garrett Anderson Institute for Women's Health, University College London.

Correspondence to:

Gareth L. Ackland PhD FRCA FFICM FHEA

Professor of Perioperative Medicine,

Translational Medicine and Therapeutics, William Harvey Research Institute,

Barts and The London School of Medicine and Dentistry,

Queen Mary University of London, London EC1M 6BQ, United Kingdom.

E-mail: g.ackland@qmul.ac.uk Tel: +44 207 882 2100

*EPIFEVER-2 investigators:

Royal London Hospital: Amaan Ali, Matt Wikner, James Noblett, Nusrat Usman, Sarah Wray, Holly Blake, Ana Gutierrez del Arroyo, Tom EF Abbott, Valentin Weber, Constantinos Papoutsos, Rebecca Black, Kara Bruce-Hickman, Parvesh Verma, Chris Sadler, Alice Barrett, Eliana Paola Rodriguez Sierra, Charles Thompson, Ayub Khan; *Whipps Cross Hospital:* Laura Fulton, Emma Collins, Tim Martin; *Homerton Hospital:* Tabitha Tanqueray, Rebecca Longbottom, Lisa Canclini, India Noakes, Rachel Frowd, Natasha Kennedy, Angela Nicklin, Eftychia Sousi; *Sheffield University Hospital:* Vicki Wilson, Alison Carey, Beth Lally, Mabel Gertrude, Sally Hoodless, Matt Wilson; *University College London Hospital:* Anna L David, Sarah Weist, Olivia Newth, Morenike Folorunsho, Jihana Ali, Yaa Acheampong, Miriam Bourke, Derek Brunnen, Jennifer Kim, Kei Mak, Pete Odor, Laura Sarmiento, Sarah Ciechanowicz.

Patient Representatives: Rebecca Harmston, Josie Hamper [https://www.barc-research.org/katies-team]

Keywords

Epidural; inflammation; cytokine; outcomes; fever; neonate

Introduction

Epidural-related maternal fever (ERMF) occurs in approximately 15% of women in labour,[1] likely leading to more frequent operative vaginal delivery, caesarean section [2, 3] and intrapartum antibiotic administration due to concerns about bacterial sepsis.[4 5] The neonatal consequences of inflammation and fever during active labour include neurological injury[6] and more frequent exposure to antibiotic treatment. Neonatal exposure to antibiotics is associated with atopic disease in early childhood, which is mechanistically linked to alterations in the neonatal gastrointestinal microbiome.[7] Understanding the mechanisms underlying ERMF may reduce short- and long-term adverse sequelae of epidural analgesia in labour by leading to a more tailored application to intrapartum interventions.

Infectious placental aetiology is absent in the vast majority of women with ERMF[8]; rather, a disordered systemic inflammation has emerged as a putative mechanism for ERMF.[1] The proinflammatory actions of IL-1β, the main form of circulating IL-1, are attenuated by interleukin-1 receptor antagonist (IL-1ra)[9] and prevent IL-1-induced preterm parturition.[10] Administration of recombinant IL-1ra inhibits systemic inflammation following chorioamnionitis.[11] Bupivacaine, which is frequently used as a local anaesthetic agent for epidural analgesia, is also associated with the development of ERMF.[12] Mechanistically, bupivacaine impairs release of interleukin-1 receptor antagonist [IL-1Ra] from circulating leucocytes, suggesting that bupivacaine may increase the risk of ERMF.[1 12]

Two alleles (rs6743376, rs1542176) located upstream of *ILRN*, the gene that encodes IL-1ra[13] increase both transcription and protein levels (the latter log-linearly).[13] The construction of an allele score for IL-1ra using these two alleles has enabled Mendelian randomisation studies to examine the relationship between IL-1ra and clinical outcomes.[13] Mendelian randomisation using allele scores provides evidence for a causal relationship between an exposure variable and an outcome.[14] Because Mendelian randomisation is less

likely to be affected by confounding or reverse causation than conventional observational studies,[15] this approach potentially offers mechanistic insight into the biological impact of IL-1ra on outcomes in active labour.[16]

Mendelian randomisation analysis of UK Biobank data suggests that genetic IL-1ra levels are associated with a reduced risk of an unplanned caesarean section after the onset of labour; this association is disrupted by the use of neuraxial analgesia.[17] In EPIFEVER-2 trial, we hypothesise that the absence of rs6743376 and rs1542176 alleles for IL1-ra, which results in the lowest circulating levels of IL-1ra, are associated with ERMF and/or the administration of intrapartum antibiotics (Figure 1).

Methods

Study design

Multicentre observational mechanistic cohort study. The EPIFEVER-2 study was prospectively registered (ISRCTN99641204) following ethical approval from London Bloomsbury Research Ethics Committee (20/LO/1213).

Setting

Labour wards of at least 4 UK hospitals. Patient recruitment started on 27th April 2021 and is scheduled to last up to 24 months. Recruiting site eligibility criteria include having labour ward services offering epidural labour analgesia and previous participation in research.

Inclusion criteria

Women aged $\geq$ 18 years, with singleton or twin pregnancy in established labour requesting epidural labour analgesia.

Exclusion criteria

Women who are unable to understand written and/or verbal English, immune/genetic syndromes/mutations, microbiologically proven infection, established pyrexia or intrauterine death prior to initiation of epidural analgesia, and concurrent antimicrobial therapy at time of analgesia initiation.

Recruitment

The schedule for enrolment, interventions and assessments is summarised in Figure 2. Strategies to achieve adequate participant recruitment will include local engagement of anaesthetists, midwives, obstetricians, research assistants, and medical students to support screening and recruitment. Recruitment targets will be monitored and communicated to the different sites throughout the study. Potential participants will be screened by a trained researcher at the site having been identified by communication with the attending anaesthetist in antenatal clinic and/or on the labour ward. The patient will be approached prior to, or within four hours of the placement of the epidural catheter before delivery. Written informed consent will be obtained from each participant prior to participation in this study. This process will involve the distribution of a patient information sheet along with the consent form and a verbal explanation of the aims, methods, potential benefits and harms of the trial. Patients who lack capacity to give or refuse informed consent will not be recruited. Eligible patients who do not enter this study will be recorded, including the reason for not enrolling. The duration of study participation will be from epidural catheter insertion until maternal discharge from hospital.

Analgesia

The epidural local anaesthetic agents and/or mixture used are similar across UK participating hospitals. Use of opioid analgesia is permitted before the initiation of epidural analgesia with low-dose bupivacaine (0.1- 0.125%, with fentanyl). Combined spinal-epidural techniques are permitted, according to local centre preference. The standard mode of epidural analgesia maintenance is through continuous infusion, although centres are permitted to use patient-controlled epidural analgesia (PCEA). Continuous real-time monitoring of maternal core body temperature demonstrates that PCEA duration exceeding 6 h is associated with maternal intrapartum fever, similar to that reported for continuous infusions of epidural bupivacaine.[18]

Temperature measurement

During labour, oral temperature correlates most closely with intrauterine temperature and is recommended for routine detection of maternal pyrexia in labour.[19] Each centre will use either Covidien Genius 3 or Welch Allyn Sure Temp Plus digital thermometers (calibration accuracy: ±0.1-0.3ºC)) to record oral temperature at least every 4 h during labour. National Institute for Health and Care Excellence (NICE) guidelines on intrapartum maternal care require women who have had a single temperature >37.5ºC in labour to have a further hourly temperature checks.[20]

Clinical data collection

Clinical characteristics and outcome data will be collected by review of medical records (Supplementary document 1). Temperature logs are kept by midwives as part of routine care for women receiving epidural analgesia, in accord with NICE guidelines.[20, 21] 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 local hospital electronic

and/or paper medical record. The occurrence of a specified clinical outcome will be confirmed by the local principal investigator or an appropriately qualified delegate.

Sample collection and preparation.

A single whole blood sample will be obtained, preferentially via an indwelling intravenous cannula inserted as part of routine care for women receiving epidural analgesia after obtaining informed consent. Recruitment is permitted up to 4 hours after placement of the epidural catheter. In the event that a blood sample cannot be obtained in this manner, participants will be asked to consent to obtaining a sample via venipuncture. The blood samples will be stored in Paxgene DNA tubes (PreAnalytix, Switzerland), which facilitates substantially higher DNA yields compared to saliva samples.[22] The samples will be anonymised through the allocation of a unique identification number so that no patient information will be identifiable. Samples will be stored at -80ºC until the final participant has been recruited, at which point DNA will be extracted for Taqman SNP genotyping[23] by laboratory personnel masked to all clinical details.

Construction of allele score

We will use an established scoring system using the IL-1ra polymorphisms that are independently associated with circulating IL-1ra concentration from genome-wide association studies.[13, 24] Since the IL-1ra polymorphisms rs6743376 and rs1542176 are not correlated with each other, the linear associations between the allele score constructed by the Interleukin 1 Genetics Consortium and IL-1ra concentration mean that it is biologically highly unlikely that the score reflects a pathway other than IL1 signalling.[13]

Blinding and procedures to minimise bias

Genotyping and allele scores will be compiled by individuals masked to clinical data. Researchers compiling the clinical data from the medical records are also blinded to the genotyping and allelic scores.

Data monitoring and protocol compliance

Local research staff will be responsible for the completion of the REDCAP case report forms at site level throughout the study. The electronic case report form will be hosted on a secure Queen Mary University of London database. The chief investigator will ensure that the study is conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice 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 case report forms, source documents and other study files. Audit reports will be kept confidential. Protocol deviations will be monitored and feedback given to centres at monthly online meetings.

Primary outcome

The primary outcome is a composite of maternal temperature >38°C after initiation of epidural analgesia or prescription of intrapartum antibiotics before delivery (for two maternal temperature readings >37.5°C, measured at least one hour apart [per NICE guidelines on intrapartum care]).[20, 21] The primary outcome will be assessed using information from the medical records of participants following their discharge from the labour ward.

Secondary outcome

Secondary outcomes will be assessed using information abstracted from the medical record following discharge of study participants from the labour ward.

1. Maternal temperature >38°C after initiation of epidural analgesia.
2. Intrapartum antibiotic administration.
3. Mode of delivery (vaginal/operative vaginal/Caesarean delivery (including urgency category))
4. Blood culture sampling after the initiation of epidural analgesia but before delivery.
5. Maternal and/or neonatal requirement for critical care.
6. Maternal duration of hospital stay.

Exposure of interest

The exposure of interest is the allele score for IL-1ra constructed using two rs6743376 and rs1542176 alleles, which cumulatively increase both *IL1RN* mRNA expression and log-linear soluble IL-1ra concentration.[13] By constructing an allele score for IL-1ra using these two alleles, Mendelian randomisation analysis can be used to examine the relation between IL-1ra single nucleotide polymorphisms and the primary outcome, as has been adopted in studies focussed on rheumatoid arthritis and cardiometabolic disease.[13] Because Mendelian randomisation is less likely to be affected by confounding or reverse causation than conventional observational studies,[15] this approach offers mechanistic insight into the biological impact of IL-1ra on outcomes in active labour.[16]

Sample size

The initial sample size was based on data from the IL-1Ra Consortium score, which was constructed from an analysis of the relationship between IL-1Ra polymorphisms and tissue/circulating levels of IL-1Ra protein.[13] Our previous analysis of over 250,000 women from the UK Biobank showed that approximately 11% of UK white women have a zero genetic (allele) score for IL-1Ra (i.e., the lowest genetically determined amount of IL1-ra).[17] The overall incidence of ERMF in EPIFEVER was 13.4%.[25] Our original sample size estimate was based on the single outcome of ERMF- (temperature > 38ºC), assuming that women with 0-1 allele scores are more likely to develop temperature > 38ºC (approximately 22%) compared with with IL-1Ra allele scores >1. Based on our more recent Mendelian randomisation study of unscheduled Caesarean deliveries after the onset of labour, we found that that women with a zero-allele score were at greater risk of early obstetric intervention, and hence surmise that this group may be more likely to develop ERMF and/or require intrapartum antibiotic treatment, compared to women with higher IL-1Ra secretion (allele score ≥1). For ERMF alone, at least 637 women will be required to detect an absolute difference in the frequency of women with a temperature >38ºC of approximately 13%, assuming 9% incidence in ERMF for women with allele scores ≥1 (α=0.05; 1-β=0.2). Since the NICE guideline on management of suspected sepsis during labour[21] suggests that more women are likely to receive antibiotics having registered two separate temperatures >37.5ºC earlier in labour, before progressing to reach the ERMF-defined pyrexia threshold of 38ºC, we also catered for this possibility by estimating the sample size for the composite outcome. If the true relative risk of meeting the primary outcome for women with a zero allele score is 2, 61 women with a zero allele score versus 488 women with allele score ≥1 are sufficient to be able to reject the null hypothesis at α=0.05 (power 0.9, assuming an overall intrapartum

antibiotic prescription rate of 20% and an 8-fold ratio of women with allele scores ≥1 versus zero). There are no stopping criteria before 637 women are enrolled.

Statistical analysis

Mean (SD) or median values (interquartile range) will be presented, unless stated otherwise. For the primary and secondary outcomes, a chi-squared test will establish whether there was independence between allele scores. Fisher's exact test (two-tailed) for post hoc analysis of each chi-squared test will be undertaken to derive exact (two-sided) *P*-values.[26] Odds ratio (95% confidence intervals (95%CI)) are presented for post hoc testing. Missing data will not be replaced by imputation. Significance will be set at $P<0.05$. A full data analysis plan will be published online at https://www.qmul.ac.uk/ccpmg/sops--saps/statistical-analysis-plans-saps/.

Safety monitoring

There are no study specific risks, as blood samples are obtained as part of routine care, thus no safety monitoring is planned.

Patient and public involvement

The grant funding for this work was written in conjunction with Katie's Team, who are Barts Health patient and public advisory group supporting the Barts Research Centre for Women's Health Research Unit. They 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. To ensure the full benefit of patient and public involvement in this study, 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, Barts Research Centre for Women's Health (from whom Josie Hamper has kindly agreed to contribute). The design of the protocol has benefited from Ms. Harmston's review and Ms. Hamper's input into patient information and consent forms. Feedback from Ms. Hamper and Katie's Team has helped inform the content and design of patient information sheets, including disseminating information about the proposed study. Patient-public involvement has been undertaken in accordance with guidelines from the NIHR Centre for Engagement and Dissemination on public and patients' engagement.

Ethics and dissemination

EPIFEVER-2 has been approved by the UK National Research Ethics Service. All participating centres have full ethical approval. Any additional recruiting sites joining the trial will require full ethical approval prior to participation. Data arising from this research will be made available to the scientific community in a responsible and timely manner. A detailed scientific report will be submitted to a widely accessible scientific journal on behalf of the EPIFEVER-2 investigators. Requests for data sharing will be considered in accordance with the data sharing policy of the sponsor Queen Mary University of London.

Discussion

EPIFEVER-2 will be the largest prospective study of epidural-related maternal fever conducted to date. UK NICE guidelines on management of labour and suspected sepsis during labour has necessitated a re-evaluation of the relationship between epidural analgesia and fever,[21 20] because women who receive antibiotics (having registered two separate temperatures >37.5ºC) are likely to receive obstetric interventions before progressing to reach an ERMF-defined pyrexia of 38ºC. By accounting for this possible confounder using a composite outcome, our genetic approach is designed to shed further light on the mechanism of ERMF.

Mendelian randomisation exploits genetic variations that are associated with an exposure of interest to assess their possible causal relationship with outcomes by reducing bias from confounding, including reverse causation. Three key assumptions must be met to perform Mendelian randomisation analysis. First, the genetic variant (typically a single-nucleotide polymorphism) is associated with the exposure of interest, in this case IL1-RA. Second, the genetic variant cannot be associated with other confounders. Third, the genetic variant influences the outcome only through the exposure of interest. In the case of IL1-ra, there is a consistent and reproducible relationship between allele score, *IL1RN* mRNA expression, and cytokine levels. The two alleles (rs6743376, rs1542176) that encode IL-1ra11 independently increase both *IL1RN* mRNA expression and soluble IL-1ra concentration in a log-linear, “dose–response” manner in two tissues.[13] Moreover, a previous Mendelian randomisation analysis using the same allele score approach as our study accurately predicts an anti-inflammatory effect on biomarkers that is concordant with the effects of a recombinant human interleukin 1 receptor antagonist protein.[13] We will adhere to the recently published STROBE-MR guidelines (Strengthening the Reporting of Observational Studies in Epidemiology-Mendelian Randomisation.[27]

Our study design is strengthened by genetic analyses being undertaken by personnel masked to all clinical data. Although the study design benefits from minimising many limitations associated with observational databases, the sample size is informed using UK Biobank genetic data derived from just two racial backgrounds, with more than 93% of women with British or Irish white race.[17] We cannot exclude that IL-1 signaling by IL-1Ra influences the quality of analgesia, but we will be able to rule out that IL-1Ra signaling impacts upon potential confounders such as duration of labour.[28] Induction of labour will likely be common in labouring women requesting epidural analgesia, so a planned subgroup analysis will assess whether there is any relationship between induction of labour and the study exposure of interest and/or outcomes.

In summary, EPIFEVER-2 will provide the largest contemporary data on the development of ERMF using tightly defined inclusion criteria. A finding that a lower number of alleles for the IL-1Ra gene predispose women to ERMF and more complicated labour will help design future studies that objectively refine intrapartum antibiotic use and/or optimal analgesic choice to reduce obstetric interventions. This approach has the potential to help obstetricians, midwives and anaesthetists personalise optimal labour management.

Authors' contributions

Study design/planning: VW/AA/SJT/TEFA/ALD/GLA

Writing paper: VW/AA/SJT/TEFA/ALD/GLA

Declaration of competing interests

GLA is an editor for British Journal of Anaesthesia and has undertaken consultancy work for GlaxoSmithKline. TA has performed consultancy work for MSD, outside the submitted work; is an editor of the British Journal of Anaesthesia and is a member of the editorial board of BJA Open. ALD has performed consultancy work for Esperare Foundation, outside the submitted work. There are no other relationships or activities that could appear to have influenced the submitted work.

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Figures

Figure 1. Study hypothesis.

This hypothetical graph summarises the basis of the EPIFEVER-2 study. Genetically-predicted mean plasma IL1-ra levels are shown by red datapoints.[13] Grey bars indicate predicted rate of epidural-related maternal fever. Both of these variables are plotted against allele score [blue text in abscissa]. The allele score for IL-1ra is constructed by adding together the number of alleles present for rs6743376 and rs1542176 alleles.

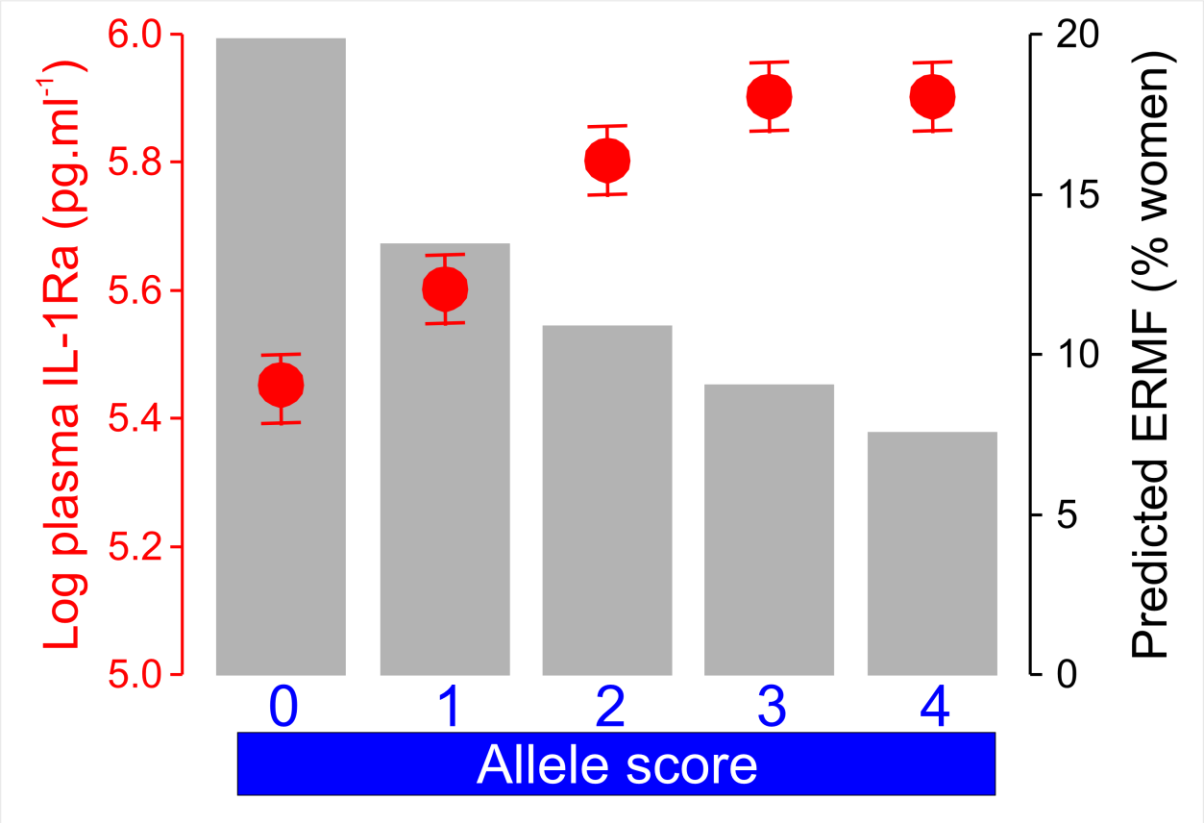

Figure 2. Study design.

Primary outcome in red box; secondary outcomes in green box.

NICE- National Institute for Clinical Excellence.