Using lactate testing to improve maternal sepsis identification: a multi-country test accuracy study:

LACTate in mATernal sEpsis

Trial registration: ISRCTN12380898

Statistical Analysis Plan

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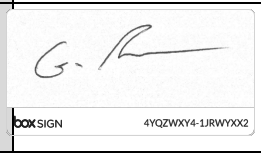
Name of Author:	Caroline Kristunas	Role:	Trial Statistician	Affiliation:	School of Dentistry University of Birmingham
Signature of Author:		Date:	7 Feb 2023		
Name of Reviewer:	Jon Deeks	Role:	Senior Statistician	Affiliation:	Institute of Applied Health Research University of Birmingham
Signature of Reviewer:		Date:	7 Feb 2023		

Name of Chief Investigator:	David Lissauer	Role:	Chief Investigator	Affiliation:	University of Liverpool
Signature of Chief Investigator:		Date:	7 Feb 2023		

This Statistical Analysis Plan has been approved by:

Name of Approver:	Jon Deeks	Role:	Senior Statistician	Affiliation:	Institute of Applied Health Research University of Birmingham
Signature of Approver:		Date:	7 Feb 2023		
Name of Approver:	Girvan Burnside	Role:	Senior Statistician	Affiliation:	Institute of Population Health University of Liverpool
Signature of Approver:		Date:	Feb 10, 2023		

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Protocol version	Updated Sap version no.	Section number changed	Description and reason for change	Date changed

LACTATE

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1 INTRODUCTION

This statistical analysis plan provides guidelines for the final presentation and analysis of the primary and secondary objectives and outcomes for the LACTATE study.

2 BACKGROUND AND RATIONALE

Maternal sepsis can develop in pregnant women and women who have recently experienced pregnancy. Failure to rapidly recognise and treat maternal sepsis is a common factor identified in those women who die (1). In partnership with the WHO, the Global Sepsis Alliance, the UK Sepsis Trust, and other stakeholders, the study team developed the Fluids, Antibiotics, Source identification and control, Transfer and Monitoring (FAST-M) sepsis care bundle specifically for pregnant or recently pregnant mothers in a low resource setting, which should be acted upon in all women with suspected sepsis within one hour. However, there is still considerable uncertainty around the optimal "trigger" used to identify when further action is required by the health care team to prompt FAST-M initiation. A "trigger" should ideally be a sensitive measure that enables reliable and early identification of women who may benefit from the FAST-M bundle use. This needs to be balanced against the risks of an imperfect trigger resulting in false positives, impacting on already overstretched resources and distractions from other clinical priorities. Furthermore, there is the danger of over treatment if FAST-M is administered to patients who do not have sepsis.

Measurement of blood lactate forms a key part of sepsis management and risk stratification in current international guidelines from both National Institute for Health and Care Excellence (NICE) in the United Kingdom (UK) and the Surviving Sepsis Campaign. However, these guidelines were developed for non-pregnant populations in high income countries (2). It would be expected that in the substantially different population of pregnant women, in low-resource settings, that the test may perform differently.

The aim of the LACTATE study is to investigate if a lactate measurement has incremental benefit over conventional maternal vital sign assessment in the diagnosis of sepsis and identification of women at risk of severe morbidity or mortality in low resource settings. We will assess the diagnostic and prognostic accuracy of venous lactate measurement in maternity populations, in low resource settings, which is unknown.

3 OBJECTIVES

The primary objective is to evaluate the diagnostic accuracy of maternal venous lactate measurement in addition to maternal vital signs (at Day 0 and/or Day 1), in maternal sepsis in low-resource health facility settings in Malawi, Uganda and Pakistan.

Secondary objectives are to:

- evaluate the immediate diagnostic value of lactate testing at Day 0 in addition to maternal vital signs in maternal sepsis at Day 0 (reference standard at Day 0 only);

- assess the short-term predictive value of lactate testing at baseline, in addition to vital signs, for maternal sepsis at 24-hours (24-hour reference standard) in those without sepsis at baseline;
- explore if baseline venous lactate, in addition to vital signs, improves prediction of severe morbidity and mortality from infection;
- explore if the diagnostic accuracy of lactate in addition to maternal vital signs alone, varies by the pre-specified subgroups of pregnancy status (pregnant or post-delivery / post miscarriage / post-abortion), source of sepsis (genital tract or non-genital tract) and condition on admission (stable or critical);
- examine the effect of adjusting the threshold values for lactate assessment on the sensitivity and specificity of the index tests;
- explore the use of an alternative reference standard in which the Sequential Organ Failure Assessment (SOFA) score is modified to use maternity specific ranges for creatinine and platelet concentration.

4 STUDY POPULATION

4.1 STUDY SETTING

The study aims to recruit 500 pregnant or recently pregnant women who will be identified and recruited in low-resource health facility settings from sites in Malawi, Uganda and Pakistan. Sites were identified and selected in collaboration with the PI and ministries of health to ensure that the sites would be able to fulfil the requirements of the protocol including adequate participant numbers, representativeness and equity.

4.2 ELIGIBILITY

Inclusion criteria:

1. Women pregnant or within 6 weeks of the end of the pregnancy.
2. Women with a suspected or confirmed infection requiring in-patient care:
   A. Any suspected or confirmed infection with or without organ-dysfunction.
   B. Any clinical signs suggestive of infection (e.g., fever).
   C. Request for any bodily fluid culture (blood, urine, cerebrospinal fluid, etc.) or swab specimens (nasopharyngeal, oropharyngeal, vaginal, endocervical) for the diagnosis of suspected infection (not routine sampling e.g., routine COVID-19 screening).
   D. Non-prophylactic use of antibiotics or other antimicrobial drugs at admission or during hospital stay.
   E. Any procedure for treatment of a suspected infection (e.g., wound exploration, evacuation of the uterus, laparotomy, etc.).
   F. Any unexplained organ-dysfunction (i.e., organ dysfunction not attributable to an underlying cause).
3. Estimated age ≥ 16 years.
4. Willing to provide a signed (and witnessed, if applicable) informed consent form.

5. Willing to be contacted if necessary.
6. Willing to have additional blood samples taken.

Exclusion criteria:

1. Women In active labour or within 2 hours of delivery are excluded as lactate is expected to be elevated by labour and childbirth.
2. Women with any non-severe, localised, or chronic infection (TB, HIV) or colonization (GBS).
3. Women undergoing only treatment with prophylactic antibiotics (for procedures, GBS).

4.3 SCREENING/RECRUITMENT DATA

The number of patients screened, the number of patients recruited and the number of screened patients not recruited will be summarized overall and by site. The numbers and reasons for non-recruitment will be reported in a table.

4.4 WITHDRAWAL/LOST TO FOLLOW-UP

Participants are free to withdraw from the study at any point, or a participant can be withdrawn by the Investigator (such as if a patient is later found to be ineligible). Some patients may be discharged prior to their Day 1 (24-hour) assessment, in which case they will lack a reference standard at this timepoint and therefore will not be included in the primary analysis. Follow-up until hospital discharge or death is required. The numbers of loss to follow-up and withdrawal over the duration of the study will be reported alongside reasons in a table. A STARD flow diagram will be produced to describe the flow of participants through the study. This will include information on the number (with reasons) of losses to follow-up, withdrawals and participants excluded/ineligible.

Some participants may be transferred to another hospital facility but not discharged. Every effort will be made to collect data about all participants until day 14 (or discharge or death, whichever is sooner) including those transferred.

5 STUDY METHODS

5.1 STUDY DESIGN

LACTATE is a prospective, multi-site, phase III test accuracy study in low-resource health facility settings. Pregnant women (or 6 weeks from end of pregnancy) with a suspected or confirmed infection will be recruited from sites across Malawi, Uganda and Pakistan. They will receive two blood tests at Day 0 (baseline) and Day 1 (22-36 hours). Lactate testing will be conducted, but the results will not be shared with the care teams and will have no influence on the critical care or treatment women receive. Daily (24 (-2 or +12) hours apart) samples will be collected from day 2 to day 14 (or until discharge from hospital or death of the participant), as well as routine key data variables from the health records and physical evaluations of the participants well-being (see Appendix 1).

5.2 INDEX TESTS

The three index tests being evaluated are:

1) Vital signs at Day 0 (see Table 1)
2) Vital signs at Day 0 + lactate at Day 0
3) Vital signs at Day 0 and Day 1 + lactate at Day 0 and Day 1

The vital signs will indicate sepsis if one or more of the thresholds listed in Table 1 are exceeded. A lactate >2mmol/L will indicate sepsis. For tests combining vital signs and lactate, both will need to indicate sepsis to give a positive test result. For the test combining Day 0 and Day 1 measurements, a positive test will require the vital signs and lactate measurements to indicate sepsis at either Day 0 or Day 1.

Table 1: Vital sign threshold values

Vital sign	Threshold
Respiratory Rate	>25 per minute
Heart rate	>120 beats per minute
Systolic Blood Pressure	< 90mmHg
Urine output	Less than 0.5ml/kg/hour
Mental state	Altered state

5.3 REFERENCE STANDARDS

The reference standard for diagnosis of maternal sepsis is defined by Sepsis-3 criteria (3): women with both (i) suspected maternal infection and (ii) organ dysfunction, which will be identified by a SOFA score ≥2. Maternal sepsis will be assessed at Day 0 (sample 1) and Day 1 (sample 2) (22-36 hours after sample 1 is taken). Women will be diagnosed as having sepsis if they meet the criteria at either time point. If a woman dies due to infection prior to the 24-hour repeat sample, this death will be assumed to have been preceded by sepsis and will also be attributed as a sepsis related death. There is the potential for some women to be discharged prior to their Day 1 assessment. If this occurs, they will lack a reference standard at this timepoint and so will not be included in the primary analysis. However, these women with early discharge will be included in a sensitivity analysis which assumes that they were discharged because they were well and therefore did not have sepsis.

For the secondary outcome exploring if Day 0 venous lactate in addition to vital signs, improves prediction of severe morbidity and mortality from infection, the reference standard is the WHO “severe maternal outcomes” (4) which comprises of maternal death and organ specific complications (“near misses”) (see Appendix 2).

5.4 OUTCOME DEFINITIONS

The primary outcomes are the absolute differences in sensitivity and specificity between pairs of index tests: vital signs (Day 0) vs vital signs (Day 0) + lactate (Day 0), and vital signs (Day 0) + lactate (Day 0) vs vital signs (Day 0 and Day 1) + lactate (Day 0 and Day 1).

Secondary outcomes include:

- The absolute differences in sensitivity and specificity between vital signs (Day 0) alone and vital signs (Day 0) + lactate (Day 0) for maternal sepsis assessed at Day 0 only;
- The predictive value of vital signs (Day 0) alone compared to that of vital signs (Day 0) + lactate (Day 0) in those without sepsis at baseline, for maternal sepsis assessed at Day 1 only;
- The predictive value of vital signs (Day 0) alone compared to that of vital signs (Day 0) + lactate (Day 0) for the occurrence of severe morbidity and mortality from infection (WHO “severe maternal outcomes”) (4), which is assessed daily until day 14 or discharge or death, if sooner;
- The sensitivity and specificity of vital signs (Day 0) + lactate (Day 0) and vital signs (Day 0 and Day 1) + lactate (Day 0 and Day 1) by: pregnancy status (pregnant or post-delivery/post miscarriage/post-abortion), source of sepsis (genital tract or non-genital tract) and condition on admission (stable or critical);
- The sensitivity and specificity of the primary index test comparisons including those women discharged early, assuming that they did not have sepsis;
- In an exploratory analysis, the sensitivity and specificity of each index test will be estimated based on different threshold values for lactate;
- To explore the use of an alternative reference standard, in which the SOFA score is modified to use maternity specific ranges for creatinine and platelet concentration, an independent expert review group will examine all cases with discordant results to adjudicate based on clinical features, clinical management, response to treatment and outcomes, if the case fitted the WHO definition of maternal sepsis. The pattern of discrepant results will be investigated descriptively.

5.5 SAMPLE SIZE

We aim to recruit 500 women to the study in total. In computing sample sizes, we estimated that an increase of 20% in sensitivity would be both feasible and clinically important. This equates to detecting an additional sepsis case for every 5 women who have sepsis. Assuming a prevalence of 40%, defining statistical significance at the 5% level and ensuring 90% power, up to 346 participants in total would ensure we are appropriately powered across a range of baseline sensitivities between 30 and 60% when vital signs alone are used. It is important to also estimate specificity to detect any important increase in false positives. With 200 patients without sepsis, the specificity of each test will be estimated with 95% confidence interval (CI) width of 15 percentage points for a specificity of 75% and width less than 10 percentage points for a specificity of 90%. This is adequate precision to allow important changes to be estimated. Allowing for loss to follow-up and missing / laboratory results, we consider an initial sample size of 500 as appropriate to allow the study to have adequate power to test these hypotheses. This number of cases will be feasible to collect within 12 months, based on current infection rate data from all sites.

6 STATISTICAL PRINCIPLES

6.1 LEVELS OF CONFIDENCE AND P-VALUES

All confidence intervals presented will be 95% and two-sided. All applicable statistical tests will be two-sided, with a p-value of ≤0.05 considered to be statistically significant.

6.2 ADJUSTMENT FOR MULTIPLICITY

No adjustments will be made for multiple testing.

6.3 ADHERENCE AND PROTOCOL DEVIATIONS

Numbers and percentages by maternal sepsis status will be tabulated for the protocol deviations and violations.

7 ANALYSIS METHODS

7.1 DESCRIPTIVE STATISTICS

The demographic and clinical characteristics of women with and without maternal sepsis will be described. Continuous data will be summarised using mean and standard deviation if data are normally distributed, and median and interquartile range if data are skewed. Categorical data will be summarised using frequencies and percentages. The number of women with missing data will also be tabulated.

7.2 INTERIM ANALYSIS

Interim analyses will be conducted at 6 months during the trial recruitment. The analyses will include a description of key demographic and clinical characteristics of women with and without maternal sepsis and the estimate of prevalence of sepsis. Based on the estimated prevalence, the sample size requirements will be revisited to assess whether the target sample size needs to be adjusted. The completeness of variables used for the index and reference tests will be assessed, along with the quality of the data collection. The number and characteristics of the women missing a Day 1 assessment will also be assessed. The TOC Subgroup will consider these results, accruals, as well as any data from external trials to make an ongoing judgement on recommendations to be made according to their terms of reference.

7.3 FINAL ANALYSIS

Primary analysis

We will estimate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the three index tests to detect sepsis assessed at Day 0 and Day 1. The 95% CIs for the estimates will be calculated using the Wilson method for proportions. To compare the accuracy of sepsis diagnosis by vital signs alone versus vital signs + lactate, we will fit separate generalized estimating equation (GEE) models for women with and without sepsis to estimate absolute differences in sensitivity and specificity, respectively. This approach exploits the paired nature of the data. We will compute absolute differences

in sensitivities and specificities from the GEE models using a post-estimation procedure with CIs computed using the delta method.

Secondary analyses

We will assess (a) index tests based on Day 0 only against Day 0 reference standard to determine immediate diagnostic value; (b) index tests based on Day 0 only against Day 1 reference standard in those without sepsis at baseline to determine short-term predictive value; and (c) index tests based on Day 0 only with occurrence of infection related "severe maternal outcome" (near miss or maternal mortality) (4) prior to hospital discharge to determine predictive value for severe morbidity or mortality (5) assuming that those women discharged prior to the Day 1 assessment did not have sepsis. Sensitivity, specificity, PPV, and NPV will be calculated for each test. In addition, for each of the analyses above, we will estimate absolute differences in sensitivity and specificity of vital signs alone versus vital signs (Day 0) + lactate (Day 0).

Pre-specified subgroup analyses will include pregnancy status (pregnant or postnatal) and illness severity to explore if the test accuracy of lactate in addition to vital signs alone varies by these subgroups.

Exploratory receiver operating characteristic (ROC) curve analyses will examine the effect of adjusting the threshold value for lactate on the sensitivity and specificity of the index tests.

We will assess a modified SOFA score incorporating maternity specific ranges for creatinine and platelet concentration as an alternative reference standard. Following the recommendations of Bowyer et al (5), based on the findings of Larsson et al. (6), the serum creatinine cut off for the scores of 0, 1 or 2 have been adjusted to <90µmol/L, 90-120µmol/L or greater than 120µmol/L respectively. Based on a mean platelet count decrease of 17% during pregnancy (7), we will propose t modified cut offs for the scores of 0, 1, 2, 3 and 4 respectively (e.g. >=125, 83-124, 42-82, 17-41 and <17). This preliminary assessment, conducted after the main analysis, will follow a methodological approach we have used for test accuracy studies in tuberculosis and other diseases to compare reference standards (8). The approach involves considering women for whom the alternative reference standard gives discordant results compared to the SOFA score, and will involve two parts: (a) examination by an independent expert review group of all cases with discordant results to adjudicate based on clinical features, clinical management, response to treatment and outcomes, determining if the case fitted the WHO definition of maternal sepsis; (b) an investigation of the patterns in the discrepant results.

7.4 HARMS

No adverse events (AEs) are expected to occur due to participation in this study. This is primarily an observational study where an additional blood sample (at two timepoints) are taken by the clinical team to monitor the woman's well-being. There are no other study interventions, which have the potential to create an untoward medical occurrence (AE).

7.5 STATISTICAL SOFTWARE

Statistical analyses will be undertaken using Stata version 17.0 (Stata, College Station, Texas).

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Appendix 1: Table of assessments

ASSESSMENT		DAYS IN STUDY													
	Screening	Week 1							Week 2						
Face to face	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
Study participation															
Eligibility	X														
Informed consent	X														
Eligibility confirmed	X														
Baseline data collection	X														
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medications and fluids	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Escalation of care	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Maternal near-miss criteria assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Sample															
Lactate sample 1 Day 0 (blinded)	X														
Lactate sample 2 Day 1 (22-36 hours) (blinded)		X													
Study completion															
Study completion form															X

Appendix 2: Maternal "near misses"

Status
Brain conditions
Unconscious for more than 12 hours
Stroke
Status epilepticus or uncontrolled fits
Clotting and bleeding
Failure to form clots (clinically absence of clotting from an IV site or suture after 7 minutes, or bedside clotting test taking more than 7 minutes)
Transfusion of more than 2 units of blood
Platelets of less than 50*10^3/µL
Heart conditions
Cardiac arrest
Cardiopulmonary resuscitation
Shock (persistent systolic blood pressure less than 80mmHg with pulse more than 120 beats per minute)
Use of continuous vasoactive drugs e.g. adrenaline infusion
Kidney conditions
Oliguria (urine output less than 30ml/h for 4 hours or less than 400ml/24 hours) not responsive to fluids
Creatinine greater than 300 µmol/L or 3.5mg/dL
Dialysis
Liver conditions
Bilirubin more than 100 µmol/L or 6.0 mg/dL
Lung conditions
Cyanosis
Gasping
Respiratory rate more than 40 or less than 6 breaths per minute
Low oxygen saturations of less than 90%% for more than 1 hour
Need for ventilation (not due to anaesthesia)
Uterus conditions
Laparotomy other than for caesarean section or ectopic pregnancy
Hysterectomy due to infection or haemorrhage
Uterine rupture