Statistical Analysis Plan

Early Kangaroo Mother Care in Gambian Hospitalised Unstable Neonates (eKMC)

Version: 1.1, 24th June 2020

NCT ref: 03555981

Statistical Analysis Plan

1. Administrative information

1.1. Title, registration, versions and revisions

Full title:	Protocol for a randomised trial of early kangaroo mother care compared to standard care on survival of pre-stabilised preterm neonates in The Gambia
Acronym:	Early Kangaroo Mother Care (eKMC)
Local reference:	SCC 1591
ClinTrialsGov code:	ClinicalTrials.gov NCT03555981
Trial protocol version:	4.0 (18th March 2019)
SAP Version:	1.1 (24th June 2020)
SAP revision history:	not applicable
Revision history:	None

1.2. Roles & responsibilities:

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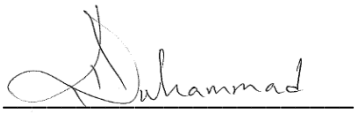
Statistician: Name: **Abdul Khalie Muhammad**

Signature: 

Date: **24th June 2020**

Senior statistician: Name: ___**Simon Cousens**_____

Signature: _  ____

Date: **24th June 2020**

PI: Name: Helen Brotherton

Signature:

Date: 26th June 2020

2. Introduction

2.1. Background / rationale

Neonatal mortality remains unacceptably high and complications of prematurity are the most common, direct, cause of death in children aged under 5y. Nearly half of all preterm neonates die within the first 24h after birth, before post-natal stabilisation has occurred. Focusing on improving hospital care for preterm neonates in the early neonatal period is a global research and public health priority if global neonatal mortality reduction targets are to be met.

Kangaroo mother care (KMC) is recommended as standard care for all hospitalised babies <2000g who are fully stable and have completed the post-natal stabilisation process. It was developed in Colombia in 1978 and has since been adopted in both high-income (HIC) and low-middle income countries (LMIC) as an adjunct to incubator care. KMC is a package of care with the key component being prolonged, continuous skin-to-skin contact between the nearly naked baby and mother/caregiver. This leads to promotion of early and exclusive breastfeeding and early discharge from hospital. There is strong evidence of mortality (36 – 51% reduction) and clinical benefits (reduced nosocomial infection, improved growth, and stability) for KMC in stabilised preterm neonates. However, there is an evidence gap for KMC in neonates who have not completed stabilisation and this is a potentially feasible, game changing intervention for health facility care of the small and sick neonate. There is also an evidence gap with respect to our understanding of how KMC reduces nosocomial and severe infections.

2.2. Hypothesis

Using early continuous skin-to-skin contact in pre-stabilised preterm neonates will improve survival by:

1. Early onset, early impact, stabilisation pathways which improve thermal control and promote cardio-respiratory stabilisation
2. Early onset, late impact causal pathways, including prevention of late-onset infections, gastro-intestinal stability and prevention of apnoea of prematurity.

2.3 Objectives

Objective 1. To assess the effect of early continuous skin-to-skin contact on survival of pre-stabilised preterm neonates <2000g

Objective 2. To assess the effect of early continuous skin-to-skin contact on other important clinical outcomes (growth, late onset infection and duration of hospital stay) for pre-stabilised neonates <2000g

Objective 3. To assess the safety of providing early continuous skin-to-skin contact to pre-stabilised neonates <2000g

Objective 4. To explore early and late mechanistic pathways for the beneficial effects of early continuous skin-to-skin contact compared to standard care in pre-stabilised neonates <2000g

3. Study methods

3.1. Trial design

This individually randomised, controlled, superiority trial compared 2 parallel groups of hospitalised, pre-stabilised neonates <2000g receiving either early continuous skin-to-skin contact (KMC started at <24h since admission) (intervention group) or standard care (KMC started at >24h since admission and once stable) (control group) (ratio 1:1). The intervention was un-blinded to participants and researchers. Primary outcome was all-cause mortality at 28d. The trial was conducted at the neonatal unit of the national teaching hospital (Edward Francis Small Teaching Hospital) in The Gambia from May 2018 to April 2020.

3.1.1. Eligibility criteria

Inclusion criteria:

- New admission to the neonatal unit at EFSTH during the study period
- Admission weight less than 2000g
- Age 1 – 24h at time screening begins
- Alive at time of enrolment
- Singleton or completed twin birth admission
- Written informed consent provided by a parent/caregiver willing to provide the intervention

Exclusion criteria:

- Congenital malformation which is incompatible with life or requires immediate surgical correction
- Severe jaundice needing immediate management
- Seizures
- Clinically stable as assessed over a pre-defined period of cardio-respiratory monitoring
- Severely unstable as assessed over a pre-defined period of cardio-respiratory monitoring
- Completed triplet admission
- Mother and/or neonate already enrolled into another MRCG study at time of hospital admission

- No study bed available

3.1.2. Intervention procedures

Participants in the intervention arm commenced the continuous skin-to-skin contact aspect of KMC within 24h after admission, aiming for >18h/day. It was provided by the first available caregiver (mother, father or other relative) in the trial area of the study site at the same time as any other medical or nursing care required by the neonate (E.g. oxygen, intravenous fluids, antibiotics, gastric feeds). Clear stopping criteria were in place for when to stop KMC due to clinical reasons. Skin-to-skin contact was re-commenced when stability criteria were met, as per the control arm.

3.1.3. Control procedures

Participants were managed in an incubator or under a radiant heater, naked except for a woollen hat and nappy or wrapped in a cloth. The parent/caregiver could touch, hold, and feed the neonate as per standard practice but KMC was not provided until stability criteria were met and at greater than 24h since hospital admission. Participants received intermittent KMC in the trial area and continuous KMC was started after transfer to the adjacent KMC unit.

3.1.4. Procedures for both arms

A pragmatic study design was used with a standardised preterm/LBW clinical management protocol based on current standard care. All management was provided by study site staff in collaboration with research clinicians with compliance to the management protocol monitored daily.

3.2 Randomisation & allocation

An independent statistician generated a randomisation sequence using VBA (Visual Basic Application) within an Access database to produce two random number tables with stratification by admission weight categories (<1200g; ≥1200g). Random permuted blocks of varying sizes were used with 1:1 allocation. Allocation concealment was performed with sequentially numbered, opaque, sealed envelopes prepared by an independent researcher and accessible to study team only. Following collection of baseline data, the study nurse opened the next numbered envelope for the correct weight category. The participant identifier, date and time were recorded on the outside of the envelope prior to opening, to identify any subversion of allocation sequence. Twins were allocated to the same arm, according to the first eligible twin's weight.

3.3 Sample size

A total of 392 participants (1:1 ratio) were required to detect a 30% relative reduction in the primary outcome (power 80%, alpha=0.05). This is based on an expected mortality rate of 48%, which was adjusted from observed rates of 56% to account for 15% mortality reduction due to trial implementation. Loss to follow up rates were low (<2%) due to the restricted geographical area, co-ordination of follow-up with routine appointments and re-imbursement of travel expenses.

3.4 Statistical interim analyses and stopping guidance

An un-blinded interim analysis was conducted by the DSMB in December 2019, once 50% of the intended sample size (n=196) were recruited and followed-up. The interim analysis included consideration of primary and secondary outcomes with the exception of intestinal carriage of ESBL-producing K*lebsiella pneumoniae*. Stopping rules for efficacy were pre-defined by the DSMB using the Haybittle-Peto rule and there was no adjustment of significance levels following the interim analysis. The DSMB recommended that the trial continue with no protocol changes.

3.5 Timing of final analysis

Data cleaning and data locking were performed once the final participant recruited during the study period completed follow up. All outcomes will be analysed at the same time, with the exception of secondary outcome "Intestinal carriage of ESBL *Klebsiella pneumoniae"* which may be performed at a later date, dependent on funding.

The statistical analysis plan will be added to the study protocol at clinicaltrials.gov before closure of the database and before any analyses are conducted.

3.6 Timing of outcome assessments

Outcomes are assessed according to the following schedule:

Timing	Outcome
24h after study participation	• Cardio-respiratory stability (Mean adjusted SCRIP score) • Hypothermia (Prevalence of participants with temperature <36.5°C)
Daily whilst admitted to neonatal unit Weekly (day 7, 14, 21, 28) whilst admitted to KMC unit Ad-hoc reviews in event of clinical deterioration	• Time from start of study procedures to death (hours) • Incidence of clinically suspected infection from 3 – 28 days of age or latest follow-up
Day of discharge	• Exclusive breastfeeding • Duration of admission
Age 28 +/- 5 days	• Survival status (alive / died) • Weight gain (mean weight gain in g/day from baseline) • Prevalence of participants with intestinal carriage of ESBL-Klebsiella pneumoniae

4. Statistical principles

4.1 Confidence intervals (CIs) & P values

We consider P values <0.05 as statistically significant evidence of a difference between arms for all pre-specified outcomes. Results will be presented with 95% Cis.

4.2 Multiplicity

No adjustments for multiple statistical tests will be made.

4.3 Analysis populations

Analysis of primary and secondary outcomes will be conducted on an intention-to-treat basis for all enrolled participants. A sensitivity analysis will be performed excluding any participants

who were recruited in error and did not meet the eligibility criteria as stated in the most recent trial protocol.

4.4 Adherence and protocol deviations

4.4.1 Adherence to the intervention

Adherence to the intervention was monitored by direct observation of time a participant spent in KMC position and was manually recorded on paper CRFS by trial personnel. The following variables were recorded: date and time of first KMC contact; relationship of person providing KMC to participant; frequency and duration of each KMC session; number of neonates receiving KMC during each session and reason for stopping KMC session. The raw data were manually inputted to Excel and the total daily dose (from start of study procedures) of KMC received was automatically calculated before being reconciled with the trial database for every in-patient day. The average daily dose of in-patient KMC per duration of admission was automatically calculated in REDCap for each participant.

4.4.2 Definition of protocol deviation

A major protocol deviation is defined as a departure from the Trial Protocol or ICH-GCP standards which had an impact on the conduct of the study, the credibility of the data or safety of participants. This includes recruitment of an ineligible neonate, recruitment without consent or use of impartial witnesses; participants in the control arm receiving the intervention inappropriately or any deviation that resulted in a Serious Adverse Event (SAE, see section 6.5). Major deviations were reported to the sponsors and SCC / ethics committees within 5 working days, as per MRCG SOPs.

All other deviations from the study protocol or GCP standards were considered to be minor and were reported to the monitors on a monthly basis and the ethics committees on an annual basis. Only major protocol deviations will be summarised and reported in any publication of trial results.

5. Study population

5.1 Screening data

The total number of neonatal admissions and proportion of those weighing ≤2000g was prospectively recorded and will be presented as part of a separate survival analysis comparing mortality before and during the trial. Clinically eligible neonates who were not recruited will be compared with recruited neonates to compare general characteristics (age at admission, sex, referral status (inborn/outborn), stability at first screening) and outcomes (in-hospital mortality).

5.2 Eligibility

Eligibility was assessed in all neonates with referral weight ≤2000g as soon as possible after admission and once aged >1h old. Weight was confirmed using calibrated SECA™ 757 digital weighing scale and source documents were checked for age and other study involvement. All potentially eligible neonates aged <24h underwent an examination with cardio-respiratory stability assessed over 10-minutes using Nonin™ 2500A pulse oximeter.

Inclusion criteria:

- New admission of singleton or twin
- Weight <2000g as per study scale
- Aged 1 - 24h old when screening begins
- Mother or other caregiver available and willing to provide intervention

Exclusion criteria:

- Triplets who were all admitted to study site
- Congenital malformation not compatible with life or needing immediate surgical intervention
- Severe jaundice
- Seizures
- Stable as assessed during cardio-respiratory screening
- Severely unstable as assessed during cardio-respiratory screening or died during screening
- No study bed available
- Neonates/mothers enrolled in another research study
- No written informed consent from parent or caregiver within 24h of admission

5.3 Recruitment

The number and flow of subjects through screening, randomisation, allocation, follow-up, and analysis will be documented, as per CONSORT 2010 guidelines. Reasons for exclusion, withdrawal and non-analysis will be described (Fig.1).

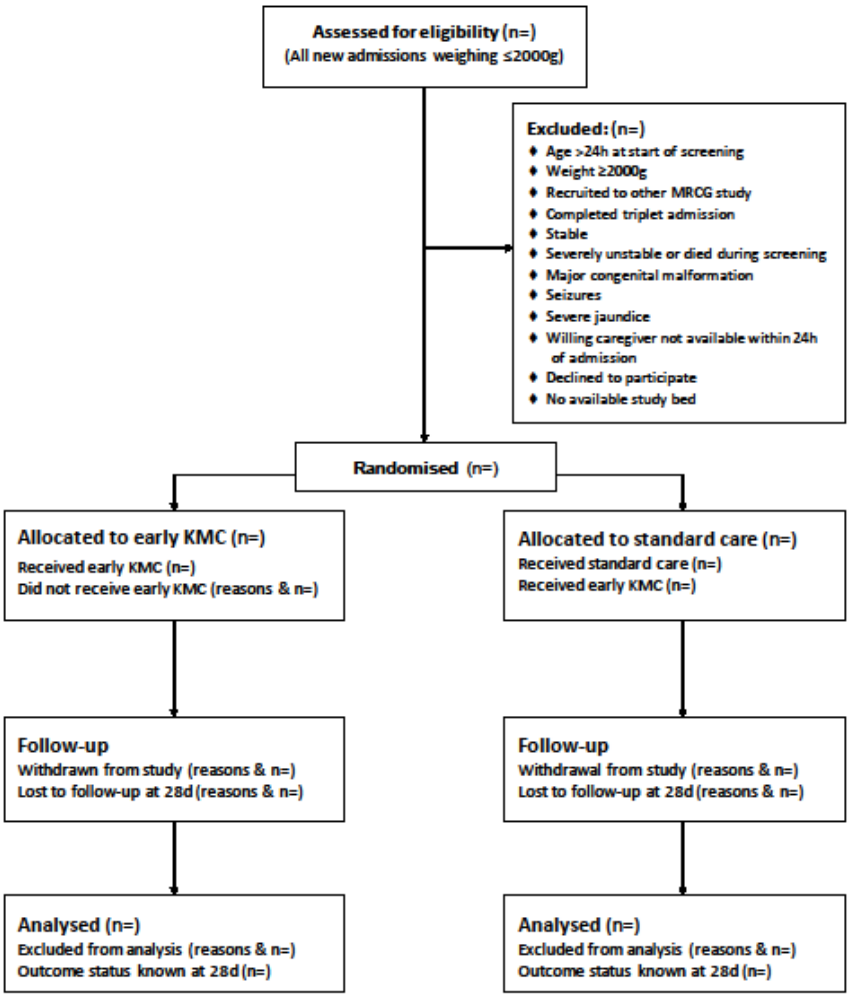


Figure 1. Trial flow diagram, as per CONSORT guidelines 2010

5.4 Withdrawal / follow-up

The number and proportion of participants who permanently withdrew from the study will be described with reasons for withdrawal. The proportion of participants who complete follow-up at 28+/-5 days will be described. Participants who are permanently withdrawn or lost to follow-up will be included in the analysis using data collected up-to the point that they are lost or withdrawn. An exception to this is if a participant withdraws and requests for no data to be used during analysis.

5.5 Baseline patient characteristics

Baseline socio-demographic and clinical characteristics of the neonate, mother and perinatal period will be summarised for each arm by number and proportion (categorical variables) and either means with standard deviations or medians and interquartile ranges, as appropriate (continuous variables). This will include comparison of treatments received prior to enrolment. Statistical tests of differences in baseline characteristics will not be performed.

5.6 Adherence to the Standardised Preterm Management Protocol

Compliance to the standardised preterm/LBW management protocol was monitored daily by the field team and key indicators of standard hospital management were recorded as concomitant medications (E.g. antibiotic therapy, provision of caffeine citrate or aminophylline to prevent apnoea of prematurity). Indicators will be compared between arms from time of study enrolment to discharge or death and will include receipt of key management and investigations. Characteristics will be descriptively summarised with categorical data presented using counts and percentages and continuous data presented using number of patients, mean, median, standard deviation, minimum, maximum and IQR, as appropriate. Data will be presented in the form of a table with arm-specific and total data. Statistical tests comparing concomitant treatments between both arms will be conducted, using Fishers exact tests for categorical variables and two sample *t* test for continuous variables to calculate p values with 95% CIs.

5.7 Adherence to the intervention

A descriptive analysis of KMC provided to both arms will be conducted. The following will be calculated as indicators of adherence and presented in a table format: mean chronological age at first KMC contact; mean time since admission at first KMC contact; mean daily dose of KMC (h/day) for first 7 days since enrolment; mean daily dose of inpatient KMC, per number of days admitted. The proportion of patients transferred from the neonatal unit to the KMC unit for each arm will be described with mean (and standard deviations) chronological ages at admission. Individual patient data on the actual daily dose of in-patient KMC, up-to 28 days of age, will be represented for both control and intervention arms using heat maps and box plots. This will provide a visual representation of the intervention provided over the study period. The number and proportion of participants in the intervention arm who met KMC stopping criteria for clinical reasons will be described with reporting of reasons and mean age at time of stopping.

6. Analysis of outcomes

6.1 Outcome definitions

Primary outcome:
All-cause mortality at 28d

Secondary outcomes:
1. Time from intervention/control procedures starting to death (days & hrs)
2. Mean cardio-respiratory stability at 24h of intervention (aSCRIP score)
3. Prevalence of hypothermia (T<36.5°C) at 24h of intervention
4. Mean daily weight gain (g/day) at 28d
5. Proportion of infants exclusively breastfeeding at discharge
6. Mean duration of hospital admission (days & hours)
7. Incidence of clinically suspected infection after 3 days and by 28 days or latest follow up
8. Prevalence of neonatal intestinal carriage of ESBL-Klebsiella pneumoniae at 28d

6.2 Analysis methods

Primary & secondary outcome analysis

Primary outcome:
The number of subjects who met criteria for the primary outcome will be calculated for each arm and generalised estimating equations used to calculate intervention efficacy. This will be presented as a relative effect (E.g. risk ratio) and absolute effect (risk difference) with a measure of precision (E.g. 95% CI). The results will also be expressed as the number needed to treat for benefit. Kaplan-Meier survival curves will also be used to present the risk ratio and risk differences visually for both arms.

Secondary outcomes:
Analysis of secondary outcomes will be performed according to type of data (mean, proportion, number, incidence) and using either number of subjects or person time as the denominator, as appropriate. Binary variables will be compared between arms using generalised estimating equations. Continuous variables will be compared using random effects models. The appropriate effect size will be presented. Survival analysis of the time to death within first 28 days after birth will be performed using cox regression with frailty to account for twins. Random effects models will be used to account for multiple episodes for the same participant (E.g. infection). A 95% CI will be presented for the treatment effect for all outcomes.

Adjustments for potential confounders / covariates:
The key covariates which are known to independently predict neonatal mortality (primary outcome) are: Admission weight, gestational age, and twin status. Two analyses will be performed for all outcomes:

Primary analysis: Adjustment for covariates (weight category, gestational age, and twin status) will be performed regardless of whether there are differences in baseline covariates between arms. Linear mixed effects models will be used for continuous data. Generalised estimating equations will be used for binary data. Both analyses will be reported but the unadjusted analysis is considered the primary analysis.

Exploratory analysis: No adjustment for covariates

Subgroup analysis

Subgroup analysis for all outcomes will be performed for infants according to: birth weight categories ; singleton or twin status. The relative measures of effect within each of these subgroups will be estimated (with 95% CIs) and a test of interaction performed and reported.

Sensitivity analysis

A sensitivity analysis will be performed excluding any participants recruited in error as per most recent protocol definitions and eligibility criteria.

6.3 Missing data

The amount of missing data is expected to be low (<5%) and a complete case analysis will be conducted.

6.4 Additional analyses

6.4.1. Effect of intervention on cardiorespiratory stability

A detailed analysis of cardio-respiratory stability will be done for the first 24h of study participation. This will include but is not restricted to a descriptive analysis of: average heart rate; average oxygen saturation; proportion of time spent with abnormal heart rate or oxygen saturation over first day of study participation. Differences between arms will be analysed using Fishers exact tests for categorical variables and two sample *t* test for continuous variables to calculate p values with 95% CI. This may be reported with the primary analysis results or as a secondary analysis.

6.5 Harms

Adverse events and SAEs will be listed and defined with reference to standardised criteria where appropriate. The methods used for data collection and attribution of events will be described.

The number and proportion of participants with an SAE (life-threatening event; risk of disability; re-admission to hospital or prolonged hospital stay >28d) will be presented for both arms. Death will not be included as this is the primary outcome of the trial.

A detailed analysis of blood glucose levels over the first 24h of study participation will be performed as a proxy indicator of disturbance to the IV fluid administration. This will involve number and proportion of participants with hypoglycaemia or hyperglycaemia at defined time points as well as mean glucose level at baseline, 12h and 24h. The significance level will be calculated using chi squared or Fisher's exact tests for categorical variables. Continuous variables will be compared using Student's t-test or nonparametric tests, when appropriate. The appropriate effect size will be presented, using risk ratios for binary outcomes and difference between means for continuous data.

6.6 Statistical software

STATA Version 16 will be used for all analyses with the exception of generation of heat maps to report intervention adherence, which will be done in R version 3.6.3.

References:

Gates S, Brocklehurst P. How should randomised trials including multiple pregnancies be analysed? BJOG. 2004 Mar;111(3):213-9.