KEMRI | Wellcome Trust

First Line Antimicrobials in Children with Complicated Severe Acute Malnutrition (FLACSAM)

Statistical Analysis Plan

Version Control	Author(s)	Date
Version 1.0	Created by Sheila Murunga	07 Jan 2020
Version 1.1	Revised by Jay Berkley and Sheila Murunga	13 Feb 2020
Version 1.2	Revised by Jay Berkley and Sheila Murunga	02 Mar 2020
Version 1.3	Revised by Jay Berkley and Sheila Murunga	23 Mar 2020
Version 1.4	Revised by Jay Berkley and Sheila Murunga	01 Apr 2020
Version 1.5	Revised by Jay Berkley and Sheila Murunga	06 Apr 2020
Version 1.6	Revised by Jay Berkley and Sheila Murunga	21 Apr 2020
Version 1.7	Revised by Jay Berkley and Sheila Murunga	18 May 2020
Version 1.8	Revised by Jay Berkley and Sheila Murunga	28 Sep 2020

Version 1.0 to 1.7 of this SAP were developed by the study data manager, statistician & PI.

Version 1.8 of this SAP was revised following review by the Trial Steering Committee and Data & Safety Monitoring Board.

Applies to protocol version: 1.04

1. Signatures

I give my approval for the attached SAP entitled **First Line Antimicrobials in Children with Complicated Severe Acute Malnutrition** dated 24/09/2020

Signature _____ Date _____

Name: Sheila A Murunga, Data Manager/Statistician

Institution: KEMRI Wellcome Trust Research Programme

Signature _____ Date _____

Name Prof Jay Berkley, Chief Investigator

Institution: KEMRI Wellcome Trust Research Programme

Signature _____ Date _____

Name Dr Moses Ngari, statistician

Institution: KEMRI Wellcome Trust Research Programme

Signature _____ Date _____

Name Prof Greg Fegan DSMB statistician

Institution: Swansea Trials Unit, Swansea University

2. Table of Contents

3. Abbreviations

AWARE	**Access, Watch and Reserve**
CRE	Carbapenem resistant Enterobacteriaceae
CRF	Case report form
CONSORT	Consolidated standards of reporting trials
cSAM	Complicated severe acute malnutrition
DSMC	Data, safety and monitoring committee
ESBL	Extended spectrum beta-lactamase
GEE	Generalized estimating equations
GLASS	Global Antimicrobial Resistance Surveillance System
IQR	Interquartile range
IV	Intravenous
ITT	Intention to treat
LFTP	Loss to follow up
LMIC	Low- and middle-income country
MUAC	Mid-upper arm circumference
NG	Nasogastric
PI	Principal investigator
RUTF	Ready-to-use therapeutic food
SAE	Serious adverse event
SAM	Severe acute malnutrition
SAP	Statistical analysis plan
sd	Standard deviation
SOC	Standard of care
TSC	Trial steering committee
WHO	World Health Organisation

4. Background & Rationale

Children with complicated severe acute malnutrition (cSAM) admitted to hospital in sub-Saharan Africa have an inpatient case fatality between 12% and >20% usually because of overwhelming infection, and more die in the first few months after discharge from hospital.[1] Because children with cSAM may not exhibit the usual signs of infection, WHO guidelines recommend routinely giving intravenous broad-spectrum antibiotics.[2] However, this is based on "low quality evidence". Children with cSAM are vulnerable to invasive infections with high-priority pathogens identified by WHO Global Antimicrobial Resistance Surveillance System (GLASS)[3] at admission, in hospital and after discharge. Growing evidence suggests that bacterial resistance is increasing in low- and middle-income (LMIC) countries. However, most antimicrobial susceptibility data are from tertiary centres, intensive care units and predominantly report hospital-acquired infections.[4]

Some hospitals in Africa are already using **ceftriaxone** or other antibiotics In the WHO "Access", "Watch", "Reserve" (AWARE)[5] "Watch" category as a first-line treatment for sick malnourished children rather than **penicillin plus gentamicin** which WHO currently recommends. However, this is not based on evidence of improved outcomes or local data on antimicrobial susceptibility. Of concern is that widespread empiric ceftriaxone use in a context of limited infection control may increase antimicrobial resistance, including extended spectrum beta-lactamase (ESBL) and other classes, affecting health outcomes during hospitalisation and after discharge.

Evidence for policy is also lacking for the use of **metronidazole** in severely malnourished children. The WHO guidelines recommend *"Metronidazole 7.5 mg/kg every 8 hours for 7 days may be given in addition to broad-spectrum antibiotics; however, the efficacy of this treatment has not been established in clinical trials."*[2] Metronidazole acts against anaerobic bacteria, small bowel bacterial overgrowth, *Clostridium difficile* colitis and Giardia. Small cohort studies of metronidazole usage suggest there could be benefits for nutritional recovery.[6] However, metronidazole can cause nausea and anorexia, potentially impairing recovery from malnutrition.

The FLACSAM trial is examining whether mortality and other outcomes are improved by allocating children to two alternatives to the currently recommended standard of care (SOC):

- **A strategy of starting empiric intravenous antimicrobials with ceftriaxone** (the currently recommended second-line treatment) **compared to SOC** starting with intravenous penicillin plus gentamicin (open-label) *
- **Routine oral metronidazole** for 7 days **compared to placebo** representing SOC of no routine metronidazole (double-blind) **

* First-line IV antimicrobial regimes are given for a default of 7 days. Procedures are as per WHO guidelines and detailed in a FLACSAM trial SOP: if a child does not respond after at least 2 days or deteriorates, intravenous antimicrobial treatment is escalated or refined; if a child improves after at least 2 days, they may switch to oral amoxycillin to complete a total of 7 days of antibiotics and may be discharged to complete this treatment at home.

** oral metronidazole/placebo is given whilst in hospital for 7 days. If a child is discharged before 7 days, the remaining doses are provided to complete 7 days at home.

5. Objectives

5.1 Primary Objective

To determine the efficacy of allocation to two randomised interventions on 90-day mortality compared to current SOC amongst children with cSAM.

5.2 Secondary objectives

To evaluate safety, effects on duration of hospitalisation, antimicrobial usage, antimicrobial resistance carriage and growth of two randomised interventions compared to current SOC amongst children with cSAM.

6. Study Methods

6.1 Trial design

FLACSAM investigates two separate antimicrobial interventions in a 2x2 factorial design randomised controlled clinical trial. Children are screened, enrolled and randomised at admission to hospital based on the standard WHO criteria for cSAM.

Randomisation 1 is open label is starting intravenous (IV) treatment with ceftriaxone vs benzyl penicillin plus gentamicin (SOC). These antimicrobials are given for a minimum of 48 hours and default duration of 7 days. IV antibiotic treatment may be upscaled for deterioration or non-response or downscaled to oral amoxicillin to complete 7 days treatment, including at home if discharged before 7 days.

Randomisation 2 is double blind oral metronidazole vs oral matched placebo given for 7 days, including at home if discharged before 7 days.

Follow up is for 90 days with scheduled assessments at discharge from hospital, day 14, day 45 and day 90.

6.1.1 Screening

Children admitted to Kilifi County Hospital, Coast General Hospital, Mbagathi Hospital and Mbale Regional Referral Hospital were screened using modified WHO criteria for Severe Acute Malnutrition (SAM), inclusion and exclusion criteria:

6.1.2 Inclusion Criteria

- Age 2 months to 13 years inclusive
- Admitted to hospital
- Severe malnutrition defined as: Kwashiorkor at any age*; or Weight-for-length Z score <-3 for children aged 2 to 59 months*; or MUAC <11.5cm for children aged 6 to 59 months*; or BMI-for-age Z score <-3 for children aged 5 to 13 years*; or MUAC <11.0cm for children aged 2 to 5 months**. [7]
- Eligible to start first-line intravenous antibiotics according to WHO guidelines.
- Planning to remain within the hospital catchment area and willing to come for specified visits during the 90 day follow up period
- Informed consent provided by the parent or guardian

* standard WHO criteria; ** modified WHO criterion

6.1.3 Exclusion Criteria

- Known allergy or contraindication to penicillin, gentamicin, ceftriaxone or metronidazole
- A specific and documented clinical indication for another class of antibiotic
- Previously enrolled in this study

6.2 Randomisation

Two independent blocked randomisation lists of random block sizes of 2, 4 and 8 in a 1:1 ratio using STATA v15, each with a different seed. Randomised allocation codes were linked to study numbers. Trial allocation was stored in opaque envelopes until use, then used in numerical order for pre-assigned study ID numbers at each site.

6.3 Sample size

Sample size for mortality is based a minimum of 16% mortality in the control group (based on surveillance at Kenyan sites) and target effect size of a hazard ratio of 0.67 to 0.72 for the primary outcome of mortality which would be clinically important. Loss to follow up (LTFU) was assumed to be 6% up to 90 days. A sample size of 1000 per randomly allocated group gave >90% power at a two-sided alpha of 0.05 for hazard ratios of up to 0.67 and power of >80% for hazards ratios up to 0.72.[8]

The sample size for growth is based on detecting a mean change in MUAC from baseline of 1.9cm (sd 1.3) vs 1.6cm (sd 1.1) at 90 days. A sample size of 1,000 per allocated group, allowing for estimated mortality and loss to follow up described above, gives >90% power to detect this difference (accounting for unequal variances).

Because of early discontinuation of enrolment at N=1,872 due to the COVID-19 pandemic, power for the actual numbers recruited will be retrospectively assessed using the event rate in each of the two SOC groups, effect sizes given above, and actual LTFU.

6.4 Framework

Separate superiority hypothesis testing for mortality during 90 days will be conducted for each of the two randomisations (A vs. non-A and B vs. non-B) assuming no interaction. Each cell of the factorial structure will be enumerated. A test for interaction between the two randomisations will be performed.

6.5 Statistical interim analyses and stopping guidance

Two interim unblinded analyses were conducted by the DSMC in closed session after 154 and 772 children were enrolled. A recommendation to the sponsor to discontinue recruitment in all patients or in selected subgroups, may be made by the TSC on advice from the DSMC if the data provide proof beyond reasonable doubt that one of the treatment arms is better in terms of the primary outcome or safety, guided by the Haybittle-Peto criteria. Interim analyses were not revealed to the study statistician, principal investigator (PI) or other team members.

6.6 Timing of final analysis

Final analysis will be conducted on all outcomes collectively, after database lock.

6.7 Timing of outcome assessments

The primary outcome of mortality will be assessed from enrolment to 90 days later. Secondary outcome assessments also include mortality and SAEs in hospital during the index admission, after discharge from the index admission, within the first 7 days and within the first 30 days.

Anthropometric assessments will be at enrolment and at days 14 (+/-3) days, 45 (+/-7) days, and 90 (+/-14) days. Suspected toxicity will be assessed during the first 7 days.

7. Statistical Principles

7.1 Confidence intervals and P values

95% confidence intervals will be reported. A *P* value greater than or equal to 0.05 ($P \geq 0.05$) will indicate the null hypothesis is not rejected, unless accounting for multiplicity is specified elsewhere in this document. P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

7.2 Adherence and protocol deviations

Adherence will be assessed using prospectively collected daily antimicrobial records during the trial. Oral metronidazole/placebo provided to complete a 7-day total course will be assumed to have been received for those discharged before 7 days. Switching antibiotics is anticipated and not regarded as a protocol deviation. Findings will be presented as laid out in section 9.3.3.

7.3 Analysis populations

As an intention-to-treat analysis, all randomised participants, regardless of compliance or protocol violations (unless withdrawn early after randomisation for mistaken eligibility – not being treated for cSAM) will be analysed to the time of withdrawal, LTFU, death or completion of 90 days follow up for analyses of mortality, SAEs, antimicrobial resistance carriage and growth.

8. Trial Population

8.1 Screening data

The total number screened will be described in a CONSORT diagram.

8.2 Eligibility

Numbers eligible and reasons for non-eligibility will be described in a CONSORT diagram.

8.3 Recruitment

The number enrolled will be described in a CONSORT diagram. Numbers at each site will be tabulated and rate of recruitment by randomisation graphed.

8.4 Withdrawal/follow-up

Participants who withdraw (with reasons) and those lost to follow up (LTFU) will be enumerated and median (IQR) days to withdrawal/LTFU calculated and presented overall and by randomisation. Differences between randomised groups will be tested by Cox proportional hazards modelling.

8.5 Baseline patient characteristics

Clinical, demographic and laboratory characteristics at enrolment will be described by allocated groups in a table. Continuous data will be summarised: n, mean and standard deviation for variables with parametric distribution (normal distribution) or median and Interquartile range (IQR) for variables with non-parametric distribution. Categorical data will be summarized using n and proportions (%). Missing data will be reported. Differences between allocated groups will not be compared using statistical tests as they are assumed to occur at random.

9. Analysis

9.1 Primary endpoint

Mortality during 90 days

9.2 Secondary endpoints

Mortality

- During index admission
- Post-discharge from the index admission
- First 7 days (intended duration of study treatment)
- First 30 days (mortality temporally associated with the admission event)

SAEs

- All SAEs
- SAEs deemed causally related to the investigational products
- SAEs resulting in readmission

Toxicity and tolerability

- Grade 4 toxicity events whilst receiving investigational products
- Tolerability:
  - Number of days experiencing potential side effects not necessarily meeting criteria for an SAE or grade 4 toxicity that may be associated with metronidazole usage: vomiting, diarrhoea, NG tube in-situ and convulsions whilst in hospital during the first 7 days
  - Number of days before first starting secondary feeds (F100, RUTF) as a marker of appetite and nutritional stabilisation (age ≥6 months only)

Duration of hospitalisation

- Number of days in hospital during the index admission among children discharged alive
- Number of days during the index admission and re-admissions to study hospitals

Antimicrobial usage

- Number of days receiving each of the study antimicrobials during the first 7 days and total during the index admission
- Reasons for discontinuing study antimicrobials
- Number of days receiving any IV antibiotics during the first 7 days
- Number of days receiving any IV antibiotics during the index admission
- Number of days receiving non-investigational IV antimicrobials during the first 7 days
- Number of days receiving non-investigational IV antimicrobials during the index admission
- Number of days receiving specific classes of antibiotics and agents in the WHO AWARE categories during the index admission

Faecal carriage of antimicrobial resistance

- Change in the prevalence of extended spectrum beta-lactamase (ESBL) and carbapenem resistant Enterobacteriaceae (CRE) between:
  - Enrolment and discharge
  - Discharge and day 45
  - Discharge and day 90

Growth

- Changes from enrolment to day 14, 45 and day 90 in:
  - Mid-upper arm circumference (MUAC) cm
  - Length-for-age z score
  - Weight-for-age z score if non-oedematous at enrolment and age ≤10 years (WHO standards are available up to 10 years of age)
  - Weight-for-length z score if non-oedematous and length ≥45cm at enrolment (WHO standards/references are available for 45 to 200cm length/height) if age <5 years
  - BMI-for-age z score if age ≥5 years

9.3 Analysis methods

9.3.1 Primary endpoint

Mortality during 90 days from enrolment will be assessed using Cox proportional hazards regression separately for the two randomisations. Time-varying effects (proportional hazards assumption) will be investigated and if found, addressed using a time interaction variable or a parametric survival analysis such as the Weibull distribution. Participant who were withdrawal/LTFU will be included up to the last day they were known to be alive. If withdrawal/LTFU varies between randomised groups, analysis weighted for observed observation time within allocated groups will be conducted as a sensitivity analysis. A sensitivity analysis will examine site as a random effect. Each cell of the factorial structure will be enumerated.

Comparisons between randomly allocated groups will be presented as hazards ratios, incidence rate ratios and rate differences. Survival will be visualised using Kaplan-Meier survival plots.

Causes of death adjudicated by an endpoint review panel will be tabulated by allocated groups.

9.3.2 Subgroup analyses

Prespecified subgroup analysis for mortality within 90 days within Cox proportional hazards regression, testing for interaction using likelihood ratio tests and presented in a Forrest plot:

- Age groups (3 factor levels: <6m, 6 to 59m, 60m or more)
- Sites (4 factor levels)
- Kwashiorkor (oedematous malnutrition: binary)
- HIV status (4 factor levels: infected, exposed, negative, untested)
- Elevated risk of death at admission (binary), defined as having 2 or more of the following features (derived from the CHAIN cohort study):
  - Respiratory distress (subcostal indrawing/head nodding/nasal flaring/deep breathing);
  - Hypoxia (SaO_2 <90%, central cyanosis, or requires oxygen);
  - Impaired circulation (limb temperature gradient or capillary refill time >3 seconds);
  - Tachycardia (heart rate>180 bpm);
  - Impaired consciousness (AVPU < “A”);
  - Hypoglycaemia (glucose <3mmol/l);
  - Severe anaemia (haemoglobin <7 g/dl);

- Abnormal leucocyte count (<4 or >=17.5 x10^9/l);
- Axillary temperature (<36 or >38.5˚C);
- MUAC <11cm

- Prior admission to hospital (binary)
- Receipt of antibiotics in the 24 hours prior to enrolment (factor 3 levels: Y, N, unknown)
- Enrolment rectal swab ESBL status (factor 3 levels: negative, positive, not done)

9.3.3 Secondary endpoints

Mortality

Incidence of death during defined time periods using single event Cox proportional hazards regression, with results presented as hazards ratios, incidence rate ratios and rate differences:

- First 7 days (intended first line treatment duration)
- First 30 days (mortality temporarily associated with an acute event)

The proportion of children enrolled who died during index admission, compared using a Chi squared test and a time to inpatient death will be compared using a competing risk (vs. discharge) regression model using the Fine-Gray method.

SAEs

Multiple event Cox proportional hazards regression models, with results presented as hazards ratios, incidence rate ratios and rate differences:

- Incidence of all SAEs
- Incidence of SAEs judged to be 'probably' or 'definitely' caused by investigational products
- Incidence of SAEs occurring after discharge from the index hospital admission
- Incidence of SAEs involving renal impairment

Toxicity and side effects

Grade 4 toxicity events will be compared between allocated groups using Fisher's exact tests.

The number of days during up to the first 8 calendar days of the index hospital admission with each of the following clinical features that may be associated with metronidazole usage, assessed using (zero inflated if needed) negative binomial or Poisson regression comparing counts (days with these may be discontinuous): Diarrhoea, Vomiting, NG tube in situ.

The number of continuous days before first starting secondary feeds (F100, RUTF) as a marker of appetite and nutritional stabilisation (age 6 months and over only) will be assessed by rank sum test.

Duration of hospitalisation.

Time to discharge will be described in median (IQR) days and compared using a competitive risk (vs. death) Cox proportional hazards regression model using the Fine-Gray method. Length of hospital stays including readmissions to study hospitals will be described in median (IQR) days and compared using a rank sum test. The number of readmissions will be compared between allocated groups using Poisson regression or similar.

Antimicrobial usage

Graphs of daily receipt of study IV antimicrobials and other IV antimicrobials by each of the 2x2 randomised groups for the first 8 calendar days will be presented with and total days exposure to the randomly allocated antimicrobials.

Among participants allocated to penicillin + gentamicin and receiving the other randomised IV treatment will be documented per day for the first 8 calendar days:

- Among all participants randomised to pen + gent: proportion per day who received an IV cephalosporin: proportion (95% CI) - descriptive
- Among participants randomised to pen + gent who died in hospital within the first 8 calendar days: proportion who received an IV cephalosporin at any time: proportion (95% CI) - descriptive
- Among all participants randomised to pen + gent, the association between faecal carriage of ESBL at enrolment and switching to or adding any IV cephalosporin: Chi squared test and logistic regression

Among participants allocated to ceftriaxone, the proportion changing to penicillin + gentamicin during the first 8 calendar days without continuing an IV cephalosporin: proportion (95% CI) – descriptive.

Number of days receiving metronidazole/placebo in the metronidazole and placebo allocated groups, compared using a rank sum test.

For all randomised allocations, reasons for discontinuing before 8 calendar days (encompassing seven 24h dosing periods) will be listed: table n, proportion (95% CI).

Randomly allocated groups (2x2) will be compared for the following outcomes:

- IV antimicrobial retreatment rate within the first 30 days: rate ratio
- Changing to from IV antibiotics to oral amoxycillin (including to be discharged with) within the first 8 calendar days: proportion: Chi squared test
- Stopping the allocated metronidazole/placebo treatment before eight calendar days: proportion: Chi squared test
- Number of days receiving any IV antibiotics during the entire index admission: rank sum test
- Number of days receiving any IV cephalosporin during the index admission: rank sum test
- Number of days receiving IV carbapenems during the index admission: rank sum test
- Number of days receiving "Access", "Watch" and "Reserve" groups of antimicrobials during the index admission: rank sum tests
- Proportion of participants receiving specific classes of antimicrobials (binary) by Chi squared test and total days exposure: rank sum test:
  A. 'Anti-pseudomonal' cephalosporins (cefepime, ceftazidime, amikacin or fluoroquinolones
  B. Carbapenems (meropenem, imipenem)
  C. Others: clindamycin, (flu)cloxacillin, chloramphenicol, co-amoxiclav

Carriage of antimicrobial resistance

The proportions of children who acquire carriage of ESBL and CRE resistance between enrolment and discharge (negative at enrolment and positive at discharge) will be compared between 2x2 allocated groups using a Chi-square test. The proportions losing ESBL and CRE resistance carriage between discharge and days 45 and 90 (positive at discharge and negative at those visits) will be compared between allocated 2x2 groups using a Chi-square test.

Growth

Changes in MUAC, weight-for-age, weight-for-length and height-for-age z-scores from enrolment values among survivors will be graphed and compared over time using generalized estimating equations (GEE) as a global test of difference between allocated groups. If global differences are found, an independent sample t-test will be performed to compare changes from enrolment for each scheduled visit. Tests for an interaction between the randomised allocation and site will be performed. A sensitivity analysis will examine site as a random effect. The proportion of children

meeting WHO criteria for severe acute malnutrition and moderate acute malnutrition at days 45 and 90 will be compared using a Chi squared test.

9.4 Missing data

Missing data will be minimized through source data review during and after the data collection, and quality control phase and database validation.

Anthropometry

Missing length/height values will be expected from participants who have chronic illness such as congenital abnormality or are too sick for their length/height measurement to be taken. Height/length at discharge may be used in place of admission values when accurate measurement at admission was precluded. At follow up, missing anthropometric data are expected in participants who missed their visits or confirmed their vital status by phone only.

Demographic, clinical, social and laboratory variables

These variables will be assumed missing not at random and a “missing” category will be added, or in the case of HIV status, a “not tested” category.

9.5 Harms

SAEs and grade 4 toxicities will be tabulated by allocated groups, including severity, type, diagnosis, final classification and outcome. Comparisons between allocated groups will be done using multiple event Cox proportional hazards regression models, with results presented as hazards ratios, incidence rate ratios and rate differences.

9.6 Statistical software

Analysis will use STATA version 15 and R version 4.0.2.

10. References