Low-dose aspirin for preventing intrauterine growth restriction and pre-eclampsia among pregnant women with sickle cell disease in Nigeria (PIPSICKLE): a randomized controlled trial

Statistical Analysis Plan

SAP Signatures

I give my approval for the attached SAP, "Low-dose aspirin for preventing intrauterine growth restriction and pre-eclampsia in sickle cell pregnancy (PIPSICKLE): a randomized controlled trial," dated 19 December 2024.

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Abbreviations and Definitions

AE	Adverse Event
AE(s)	Adverse Events
CRF	Case Report Form
EGA	Estimated gestational age
Hb SC	Hemoglobin SC
Hb SS	Hemoglobin SS
ITT	Intent to treat
IQR	Interquartile range
IUGR	Intrauterine growth retardation
LDA	Low dose aspirin
SAP	Statistical Analysis Plam
SCD	Sickle cell disease
SD	Standard deviation

1 Introduction

1.1 Preface

Nigeria has the highest number of people living with sickle cell disease (SCD) in the world, amounting to 2-3% of the population(1). Sickle cell disease is a hemoglobinopathy fraught with complications because of vaso-occlusion, thrombosis, and chronic anemia. The red blood cells become abnormal and fragile when deoxygenated, forming polymers and adhering to vascular endothelial tissue with resultant obstruction of the vessels and hemolysis(2,3). These lead to various clinical manifestations, which include splenic sequestration, susceptibility to infections, stroke, bone pain crises, and acute chest syndrome(4). Vasoconstriction from nitric oxide depletion has also been postulated to be a pathophysiological mechanism for some of its adverse effects such as pulmonary hypertension and stroke(5).

Over 50% of females with SCD now achieve pregnancy due to increased survival into adulthood, but they have a high incidence of complications during pregnancy, including preeclampsia (PE) and intrauterine growth restriction (IUGR), infections, acute chest syndrome, and more frequent crises(6–8). Many pregnant women with sickle cell disease are thus more likely to die in pregnancy, especially in low-resource settings like ours(6); pregnancy-related studies in them should, therefore, be a priority. Even when they do not die, a large proportion of pregnant sickle cell patients develop morbidities that can be defined as “maternal near miss,” meaning they would have died but for prompt and appropriate intervention(9).

Previously, we found a reversal in a prostacyclin-thromboxane ratio in pregnant hemoglobin (Hb) SS women(10), a situation that is also found in non-sickle pregnancies with preeclampsia and unexplained IUGR(11). Low-dose aspirin (LDA) has been shown to reduce the incidence of PE and IUGR in high-risk women due to its reduction of vasoconstrictor thromboxane whilst sparing prostacyclin, in effect correcting the ratio(12). It has been found to be safe for use in pregnancy (13) and has been suggested for use in sickle cell pregnant women(14), but all the studies were conducted in non-SCD pregnant women. To the best of our knowledge, there are no studies evaluating the role of low-dose aspirin in the prevention of preeclampsia and IUGR in pregnant SCD women in the scientific literature(15). As women with SCD have such a high incidence of morbidity and mortality in pregnancy, the results of this study could potentially save their lives and that of their babies.

1.2 Scope of the analyses

To determine whether daily administration of 100mg LDA from 12 - 28 weeks of gestation till 36 weeks reduces the risk of IUGR, preeclampsia, perinatal deaths or miscarriages, and other complications (sickling and non-sickling related) in pregnant HbSS women compared with the use of placebo.

2 Study Objectives and Endpoints

2.1 Study Objectives

1) To determine the effect of the use of LDA during pregnancy in Hb SS and Hb SC women on the risk of intrauterine growth restriction (IUGR), perinatal death, or miscarriage (composite outcome).
2) To determine the effect of the use of LDA during pregnancy in Hb SS and Hb SC women on the risk of other maternal complications, including preeclampsia, preterm delivery, number of vaso-occlusive crises, need for blood transfusion, urinary tract infections, respiratory tract infections, acute chest syndrome, retained placenta, placental abruption, and vaginal bleeding.

2.2 Endpoints

Primary

1. IUGR, perinatal death or miscarriage as a composite outcome

   The occurrence of either IUGR, perinatal death or miscarriage will be the primary outcome. IUGR will be defined as birthweight <10th centile for gestational age on the INTERGROWTH-21st birthweight charts, which is based on a healthy reference cohort from multiple countries, including Kenya(16). IUGR based on the Oken charts from a US national reference(17) maybe presented in sensitivity. A perinatal death refers to the death of a fetus after the age of viability (≥28 weeks' EGA), at delivery, or within the first week of life. A miscarriage refers to pregnancy, loss the death of a fetus before the age of viability (<28 weeks' EGA). All participants will do an ultrasound scan at 20 weeks to estimate the gestational age of the pregnancy. Analysis will be by intention to treat.

Hypothesis:

1. We expect a 50% lower incidence of IUGR, perinatal death or miscarriage in the LDA (intervention) group compared to the placebo (control) group.

Secondary

1. The incidence of preeclampsia. Preeclampsia will be diagnosed based on ISSHP classification(18) if there is development of hypertension (either systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two occasions at least 4 h apart) after 20 weeks gestation plus one of the following: proteinuria, thrombocytopenia, or any multisystem complication. Proteinuria will be defined as a dipstick test with 1+ proteinuria (≥30 mg dl^{-1}) on two occasions at least 4 hours apart, without any evidence of a urinary tract infection, or 2+ proteinuria or more on dipstick, or urinary protein creatinine ration >300 mg/g. Thrombocytopenia will be defined as a platelet count of <100,000 mm^{-3}.
2. The incidence of preterm delivery, defined as delivery at <37 weeks' EGA.
3. The incidence of:
    a. Maternal death
    b. Perinatal death
4. Number of sickle cell crises
5. Need for blood transfusion – identified based on whether the participant received a blood transfusion or not
6. Incidence of complications such as:
    a. Urinary tract infection (UTI),
    b. Lower Respiratory Tract Infection,
    c. Acute chest syndrome,
    d. retained placenta,
    e. placental abruption; and
7. Incidence of potential adverse effects such as:
    a. vaginal bleeding,
    b. epigastric pain, and
    c. heartburn

Analysis will be by intention to treat.

3 Study Methods

3.1 General Study Design and Plan

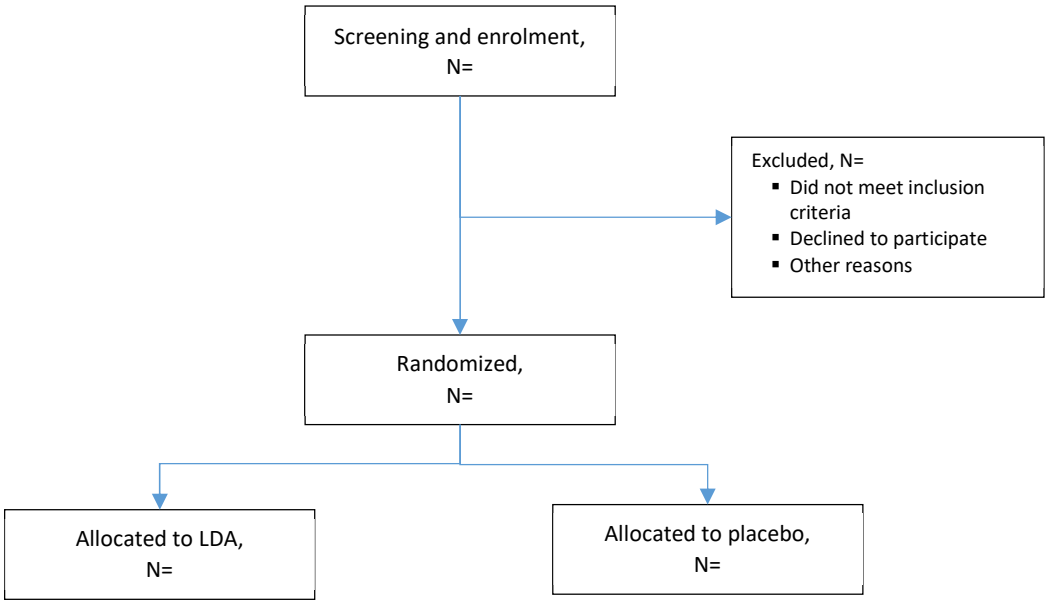
A multicenter clinical trial with a double-blind parallel design, with 476 women allocated in a 1:1 ratio.

Daily administration of 100 mg aspirin tablet. Oral route.

Daily administration of placebo similar in appearance to the aspirin tablet. Oral route.

Participants will be seen in clinic every 4 weeks till 28 weeks' gestation and every 2 weeks until 36 weeks, then weekly until delivery.

Figure 1. Flowchart of participant selection



3.2 Inclusion-Exclusion Criteria and General Study Population

Inclusion criteria

- Age 18 years and above
- Singleton foetus
- Women whose genotypes are haemoglobin SS or SC.
- 12 – 28 weeks gestation recruitment, estimated from the last menstrual period or by an early ultrasound scan done before 22 weeks gestation.

Exclusion criteria

- Women with associated medical conditions in pregnancy e.g., HIV infection, diabetes mellitus, hypertension, renal disease, sickle nephropathy
- Hb genotype other than HBSS or HbSC
- Hypersensitivity to aspirin
- A vaso-occlusive crisis in the last 4 weeks

- History of blood transfusion in the last 3 months
- Women who participated in the PIPSICKLE trial during their previous pregnancy

3.3 Randomization and Blinding

At the enrolment visit, any pregnant woman with haemoglobin SS or SC, who meets the eligibility criteria and gives informed consent will be enrolled. Eligible participants will be consecutively enrolled. They will be randomized to one of the two treatment groups. Individual randomization and allocation concealment will be done with the use of a web-based randomization software known as 'Sealed envelope' in a 1:1 ratio in blocks of four. Sealed envelope generated a randomisation code

list, shared only with the unblinded pharmacist by email. Each code is pasted on the appropriate drug kit and the drug kits are sent out to each site by the unblinded pharmacist, who is not in contact with any of the site investigators.

3.4 Study Assessments

Table 1. Schedule of study assessments

Visit	ANC Booking or baseline	20 weeks EGA	Follow up	Delivery	Between delivery and 6 wks pp
Socio-demographic characteristics	X				
Physical exam – maternal	X		X	X	X
Obstetric ultrasound exam		X			
Physical exam – infant				X	X
FBC	X		X	X	
Urine M/C/S	X		X	X	

Analysis Time Windows

We will allow the inclusion of variables collected around the following time windows.

Table 2. Analysis Time Windows

Visit (target day)	Lower bound (days)	Upper bound (days)
Randomization (0)	N/A	N/A
Pregnancy follow-up	N/A	N/A
Delivery	0	+2
Puerparium	N/A	N/A

Description of variables

The key variables used for analysis are described below.

Table 3. Description of variables

Variable	Description
IUGR	Binary variable (0, 1). Refers to birthweight <10th centile for gestational age on the INTERGROWTH-21st birthweight charts(16). If sufficient information is not available to estimate the birthweight percentile using the INTERGROWTH-21st charts which is based on a healthy reference cohort from multiple countries including Ghana, the Oken charts will be used instead(17). The Oken chart is based on a US national reference(17).
Perinatal death	Binary variable (0, 1). Refers to the death of a foetus after the age of viability (≥28 weeks' EGA), at delivery or within the first week of life.
Miscarriage	Binary variable (0, 1). Refers to the death of a foetus before the age of viability (<28 weeks' EGA).
Composite outcome (At least one of IUGR, perinatal death or Miscarriage)	Binary variable (0, 1). Refers to the occurrence of any of IUGR, perinatal death or miscarriage.
Birthweight	Continuous variable, measured in grams, rounded to every 10g
Preeclampsia	Binary variable (0, 1). Based on ISSHP classification(18) as the presence of hypertension after 20 weeks gestation plus one of the following: - proteinuria, - thrombocytopenia, or - any multisystem complication. Hypertension will be defined as either systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two occasions at least 4 h apart. Proteinuria will be defined as a dipstick test with 1+ proteinuria (≥30 mg dl^{-1}) on two occasions at least 4 hours apart, without any evidence of a urinary tract infection. Thrombocytopenia will be defined as a platelet count of <100,000 mm^{-3}. Preeclampsia is a secondary endpoint.
Gestational age at birth	Continuous variable, measured in weeks. The lower limit of acceptable range is 12 weeks. The usual upper limit is 44 weeks, beyond which baby is unlikely to have survived.
Preterm delivery	Binary variable (0, 1). Measured from gestational age at birth and defined as delivery at <37 weeks' EGA ▪ A secondary endpoint
Maternal mortality	Binary variable (0, 1). Defined as any death of a woman during the study (during pregnancy and puerperium) ▪ A secondary endpoint
Haemoglobin F	Categorical variable (<2%, 2 – 10%, >10%), measured in percent. Calculated

Variable	Description
fraction	from continuous HbF fraction ▪ A baseline covariate ▪ Levels of HbF fraction are related to clinical outcomes among people with sickle cell disease(19)
Haemoglobin A2 fraction	Categorical variable (<3.5%, ≥3.5%), measured in percent. Calculated from continuous Hb A2 fraction ▪ A baseline covariate ▪ Levels of HbA2 fraction are suggestive of β thalassemia carrier status(20)
Follow-up duration	Continuous variable, measured in days. Calculated as the difference between the date of randomization to the date of delivery or miscarriage.

4 Sample Size

Assuming an incidence of intrauterine growth restriction (IUGR) of 20% in sickle cell disease(21) and expecting a 50% IUGR reduction with the use of LDA as detected by Bujold et al(22), we calculated that 198 women per group would be required to have a 90% power of detecting a decrease in IUGR at the 5% significance level, with a total of 396, using the formula for proportions in superiority parallel trial(23). Allowing for 20% attrition, this would become 476 in total.

5 General Analysis Considerations

5.1 Timing of Analyses

The final analysis will be performed on the final unblinded dataset, after data cleaning is completed and database is locked.

5.2 Analysis Populations

5.2.1 Intention to Treat (ITT) population

- The intention to treat population refers to all subjects who were randomised. Following the intention-to-treat principle, patients will be analysed according to the treatment they were assigned to at randomization.

5.3 Covariates and Subgroups

The following table is a list of covariates to be presented in Table 1, and to be explored for inclusion in models.

Table shell 1. Participant characteristics

Variables	LDA	Placebo	Overall
Age			
Mean (SD)			
Median [IQR]			
Age Categories (years)			
<20			
20 - <30			
30 - <40			
40 and above			
Haemoglobin genotype			
Hb SC			
Hb SS			
Fetal hemoglobin fraction			
<2%			
2 – 10%			
>10%			
Hemoglobin A2 fraction			
<3.5%			
≥3.5%			
Hemoglobin concentration			
Mean (SD)			
Median [IQR]			
Primip			
Yes			
No			
Gestational age at enrollment			
Mean (SD)			
Median [IQR]			
Body weight			
Mean (SD)			
Median [IQR]			
Educational_attainment			

Variables	LDA	Placebo	Overall
No formal education			
Completed Primary			
Completed Secondary			
Completed Tertiary			
Currently in School			
Postgraduate			
Married			
No			
Yes			

Values are n(%) for categorical variables and mean (SD) or median (min, max) for continuous variables. The decision to report the mean (SD) or median (min, max) may be guided by the distribution of the variable based on the Shapiro-Wilk test

5.4 Missing Data

No imputation of endpoints will be done in the main analysis. Throughout, the denominator for each analysis will be reported to be transparent wherever there is missing data. For outcomes missing in <5% of those for whom it is expected, missing data will be ignored as imputation is unlikely to improve the precision or prevent bias(24). This approach is known as a *complete-case intention-to-treat* approach(25).

For outcomes missing in ≥5% of participants for whom it is expected, supplementary tables comparing baseline characteristics in those with and without the missing outcome will be reported. In addition, multiple imputation or inverse probability of treatment weighting will be considered, and results compared with the complete case analysis.

5.5 Multiple Testing

There is one primary endpoint in our analysis, and no adjustment for multiple testing will be done(26). Thus, our analysis's alpha level for statistical inference will be 0.05, and all p-values will be presented to the third decimal place.

6 Summary of Study Data

Baseline covariates will be summarized and stratified by intervention arm to describe the population . Continuous variables will be summarized using the following descriptive statistics, n (non-missing sample size), mean, standard deviation (SD), medians, interquartile ranges (IQR), minimum and maximum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. In general, all summary tables will be structured with a column for the overall population. Summary tables will also be presented for each treatment and will be annotated with the total population size relevant to that table/treatment. The number of missing observations will be presented in the footnote of each table.

6.1 Subject Disposition

The following CRFs will be used to determine which participants reached the following stages.

Visit (target day)	**CRF**
Baseline (0)	Enrollment
20 wks	Obstetric ultrasound report
Follow-up	Visit
Delivery	Delivery
Puerperium	Postpartum Visit

6.2 Protocol Deviations

Given that analysis will be by ITT, no specific protocol deviations will impact the approach to analysis. The summary statistics will be produced in accordance with section 5 (General Analysis Considerations).

6.3 Concurrent Illnesses and Medical Conditions

Summary statistics of concurrent illnesses and medical conditions will be produced in accordance with section 5 (General Analysis Considerations).

6.4 Treatment Compliance

Treatment compliance was assessed using the remaining pill count and diary records. Each participant's average compliance rate will be estimated thus:

$$\frac{Number\ of\ pills\ absent\ from\ returned\ regimen\ sachet}{Number\ of\ pills\ ever\ dispensed\ to\ participant}\%$$

The summary statistics will be produced in accordance with section 9.

7 Efficacy Analyses

7.1 Primary Efficacy Analysis – a composite of IUGR, perinatal death or miscarriage

The main analysis will be conducted in the ITT population. The frequency of the primary endpoint will be presented as N and percent of the total study population and by treatment group (LDA vs. placebo). The frequency of the components of the composite endpoint will also be considered.

Log-binomial regression models will be used to evaluate the effect of LDA (vs. placebo) on the occurrence of the composite endpoint, and risk ratios and confidence intervals will be presented. All components of the composite endpoint are included as secondary endpoints, and their analysis is described in 7.2 below. In some cases, the log-binomial models may not converge, and log-Poisson models, which provide consistent but not fully efficient estimates of the relative risk, and its confidence intervals will be used(27).

Baseline covariates that are strongly prognostic of the outcome or imbalanced in the treatment arms may be included in the model if their inclusion improves the precision of the estimates(28,29). Baseline covariates identified from previous studies to be strongly prognostic of the outcome or its components include: maternal age(30–32), hemoglobin genotype(8,33), parity(31), body weight (34–36), educational attainment(37), marital status(35), preeclampsia(31,35), and hemoglobin(38). It is important to note that much of this evidence is from studies in the general population rather than among people with sickle cell disease – and this may impact the extent to which these factors may be prognostic in this study population. The estimates will be regarded as improved if the distance between the upper and lower confidence limits reduces by >10%.

Cluster-robust standard errors from the sandwich package will be employed, clustered on sites.

Table shell 2: Primary composite outcome and components

Outcome	Level	LDA, n(%)	Placebo, n(%)	Risk ratio	Confidence intervals	P-value
IUGR, perinatal death or miscarriage	At least one					
	None					
IUGR	Yes					
	No					
Miscarriage	Yes					

Outcome	Level	LDA, n(%)	Placebo, n(%)	Risk ratio	Confidence intervals	P-value
Perinatal death	No					
	Yes					
	No					

7.2 Secondary Efficacy Analyses

7.2.1 Important secondary endpoints

Preeclampsia is an important secondary endpoint in this study. This analysis will be conducted in the ITT population.

The frequency of occurrence of preeclampsia will be presented as N and percent of the total study population and by treatment group. To obtain the relative risk and 95% CI of the occurrence of the secondary endpoints, log-binomial regression models will be used. If the log-binomial model does not converge, the log-Poisson models will be used instead(27). The inclusion of covariates will be considered in the same manner as for the primary endpoint.

The time-to-occurrence of preeclampsia will be compared by treatment using Kaplan-Meier curves, with a p-value from Log-rank test reported.

7.2.2 Other Secondary Endpoints

This analysis will be conducted in the ITT population. The relevant endpoints include preterm delivery, maternal death, perinatal death, need for blood transfusion, and the incidence of complications such as UTI.

The frequency of occurrence of the categorical endpoints will be presented as N and percent of the total study population and by treatment group.

To obtain the relative risk and 95% CI of the occurrence of the secondary endpoints, log-binomial regression models will be used. If the log-binomial model does not converge, the log-Poisson models will be used instead(27). The inclusion of covariates will be considered in the same manner as for the primary endpoint.

Table shell 3: Secondary outcomes

Outcome	Level	LDA, n(%)	Placebo, n(%)	Risk ratio	Confidence intervals	P-value
Preeclampsia	Yes					
	No					
Preterm delivery	Yes					
	No					
Maternal death	Yes					
	No					
Need for blood transfusion	Yes					
	No					
Incidence of UTI	Yes					
	No					
Pneumonia	Yes					
	No					
Sepsis	Yes					
	No					
Stillbirth	Yes					
	No					

7.2.3 Number of crises

The number of crises is a count variable and will be analyzed in the ITT population. Crises of the same type that occur within 28 days of one another will be regarded as a single event in line with the clinical convention in Nigeria(39).

For the total study population and each treatment group, N, mean, standard deviation (SD), median, interquartile range, minimum and maximum will summarize count variables as appropriate based on its distribution and other statistical considerations.

To obtain the rate ratio and 95% CI of the number of crises, Poisson regression models or its variant will be used with the duration of intervention (the number of weeks between study enrolment and end of pregnancy) as the offset term.

Table shell 4: Sickle cell crises

Outcome	Value	LDA	Placebo	IRR	Confidence intervals	P-value
Number of sickle cell crises	Mean (SD)					
Follow-up time, weeks	Mean (SD)					
Types of sickle cell crisis						
Vaso-occlusive	Mean (SD)					
Hemolytic	Mean (SD)					
Sequestration	Mean (SD)					
Acute Chest Syndrome (ACS)	Mean (SD)					
Severe anemia	Mean (SD)					
Others	Mean (SD)					

8 Safety Analyses

8.1 Adverse events

This analysis will be conducted in the ITT population. The number and proportion of participants with adverse events that occur in up to 0.5% of the study population will be presented by the study arm. Adverse events categorized by the treating physician to be related to the treatment will also be listed by the study arm, regardless of their frequency. These will include, but are not limited to, vaginal bleeding, epigastric pain, and heartburn.

Table shell 5. Adverse events

Adverse events	LDA, n(%)	Placebo, n(%)	Overall
Total			
Listed			

8.2 Serious Adverse Events (SAEs)

A Serious Adverse Event (SAE) is defined as an event which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
    - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug.
    - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission.
    - social reasons and respite care in the absence of any deterioration in the patient's general condition.

- is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

The number and proportion of participants who experience SAEs will be analysed and presented. The appropriate grading of severity of the SAEs will also be presented in counts and proportion. A listing of SAEs experienced will be included per participant.

If any serious adverse events occur in >5% of individuals who receive either intervention, the time to occurrence of the adverse event will be summarized using median (IQR) and graphically with Kaplan-Meier curves.

Table shell 6. Serious adverse events – summary

SAE	LDA, n(%)	Placebo, n(%)	Overall
Total			
Listed			

Table shell 7. Serious adverse events – list

record_id	SAE	Relationship	Comments including action taken

8.3 Prior and Concurrent Medications

The proportion of participants who have used aspirin in the preceding month will be estimated.

9 Sub-group Analyses

The efficacy analyses of LDA on occurrence of the composite outcome (at least one of IUGR, miscarriage, and perinatal death) will be repeated in the following subgroups:

a) Concurrent medications – hydroxyurea
b) Hemoglobin genotype – Hb SS vs. Hb SC
c) Body mass index at ANC enrolment

Subgroup analyses will only be considered if the minimum number of participants in each subgroup is at least 30 people.

10 Reporting Conventions

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.

11 Quality Assurance of Statistical Programming

A second review statistician will independently check the code and output for the entire analyses. To ensure fidelity of results, another statistician will review the analysis based on the codes supplied and by using another statistical software. Stata version 18 (StataCorp, USA) and R-Studio statistical software will be the main statistical software for the analyses. High quality code that is understandable, and reproducible will be produced. Generous annotation and explanations of steps will be performed. Data specifications such as the population of analysis, date and time included, and the description of desired output from analysis will be included.

12 Summary of Changes to the Protocol and/or SAP

12.1 Adjustments of Statistical Analysis Plan from Protocol

The SAP in the protocol has been revised extensively in this current SAP. Major highlights of the revision include:

i. This SAP provides more detail on the statistical analysis approach than provided for in the protocol.

ii. The preferred binomial model for the primary outcome in the protocol was the logistic regression. We now opt for the log-binomial model as this yields risk ratios which are easier to interpret than the odds ratios from logistic regression(40).

iii. The protocol specified that analysis would be by multivariate regression.

   a. Multivariate regression is a valid approach for analysing clinical trials with multiple outcomes, as it accounts for any correlation between the outcomes(41,42). We however opt to analyse the multiple components of the composite outcome as a single outcome – this approach is simpler, easier to understand, and could increase the number of events in our study.

   b. The term “multivariate” is often wrongly used interchangeably with “multivariable”(43). We now remove the term “multivariate”, and instead explain our approach to selecting covariates that we may adjust for in our model.

12.2 Adjustments to analysis after finalizing the Statistical Analysis Plan

None. If any adjustment will be made, standard protocols will be adhered to.

13 References

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14 Listing of Tables and Figures