2022-10-04

MOSAIC Clinical Trial Statistical Analysis Plan Primary Analysis v1.2

Gareth Lingham
Technological University Dublin, gareth.lingham@tudublin.ie

Emmanuel Kobia-Acquah
Technological University Dublin

James Loughman
Technological University Dublin

See next page for additional authors

Statistical Analysis Plan

Myopia Outcome Study of Atropine Treatment in Children (MOSAIC)

Version 1.2

Authors: Gareth Lingham, Emmanuel Kobia-Acquah, James Loughman, Ian Flitcroft

Centre for Eye Research Ireland, Technological University Dublin, Dublin Ireland

Date: 04 October 2022

Background

The Myopia Outcome Study of Atropine Treatment in Children (MOSAIC) is an investigator-led, double-masked randomised controlled trial of nightly atropine 0.01% eye drops compared to nightly placebo eye drops. A previously published protocol paper outlines the rationale, objective and sample size calculation for the study.[1] A total of 250 participants were enrolled in the study and were randomised 2:1 to active treatment and placebo, respectively. This document outlines the plan for analysis of the 24-month outcomes of the MOSAIC.

Objectives

1. Evaluate the efficacy of 0.01% atropine eye drops for the treatment of myopia progression, compared to a placebo eye drop
2. Evaluate the safety and tolerability of 0.01% atropine eye drops

Timepoints

This analysis will include data from the baseline, 12-month, 18-month and 24-month visits. A 6-month visit was planned in the protocol and approximately 30 participants completed this visit; however, due to the COVID lockdown, the 6-month visit was skipped for all remaining participants and will therefore be excluded from this analysis.

STATISTICAL PRINCIPLES

This study will be analysed on an intention-to-treat basis, but a secondary analysis will assess the per-protocol effect of the intervention with adherence to the medication defined as having used more than 75% of of the expected number of eye drop ampoules. This figure is chosen based on the previous Low concentration atropine treatment of myopia (LAMP) study report of the two-year results.

Confidence intervals and p value thresholds will be 95% and 5%, respectively. No direct adjustment for multiplicity will be made – only 2 efficacy outcomes will be tested for and hence there is little multiplicity risk. There is risk of type 1 error when looking at safety and side effect-related outcomes (see below) as multiple testing will occur; however, it is important to not have an overly severe threshold for these types of outcomes as tolerance of the intervention is essential.

OUTCOMES

Efficacy outcomes

- Primary outcome: Change in spherical equivalent from baseline to the 24-month visit.
- Secondary outcome: Change in axial length from baseline to the 24-month visit.

SAFETY AND TOLERABILITY OUTCOMES

Adverse events

- Number of adverse events.

- Number of adverse events deemed possibly, probably or definitely related to the study medication.
- Number of withdrawals related to adverse events.

Patient-reported outcomes

Questions asked on a 4-point scale are:

- How do your eyes feel today?
- Do you feel any itchiness near your eyes?
- Is your vision blurry with your glasses on?
- Do your eyes feel stingy?
- Are your eyes sore when you are in the light?
- Do you find it difficult to read or write?

The proportion of participants reporting each response for each question at each visit up to the 24-month visit will be reported in a table using chi-square tests at each visit for difference in proportion in treatment vs placebo group.

Pupil size and accommodation outcomes

- Change in pupil size from baseline as assessed by pupillometry.
- Change in accommodative amplitude from baseline.
- Change in accommodative facility from baseline.
- Change in accommodative lag from baseline.

Visual outcomes

- Change in distance visual acuity from baseline.
- Change in near visual acuity from baseline.
- Change in stereoacuity from baseline.

STATISTICAL ANALYSIS

All analyses will be conducted using R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Baseline characteristics

Baseline characteristics will be reported in the treatment and placebo groups separately. For ocular variables, the mean of both eyes will be reported for baseline characteristics.

- Approximately normally distributed variables will be summarised with mean and standard deviation (SD) and means compared between treatment and placebo group using an independent samples t-test. Given the relatively large sample size, we expect the t test to be robust to small deviations from a normal distribution due to central limit theorem.
- Skewed continuous variables will be reported using median and interquartile range and medians compared using a Wilcox test.

- Categorical outcomes will be reported using number and percentage and the proportions compared between treatment and placebo groups using Fisher's exact test for dichotomous outcomes and a Chi-square test for categorical outcomes.

The following characteristics will be compared. Completion of each visit is not technically a baseline characteristics, but is important to compare between groups in a similar manner to the baseline characteristics.

- Non-ocular categorical: Sex, number of myopic parents, ethnicity, eye colour, completion of each study visit
- Non-ocular continuous: age, age of myopia onset, body mass index, outdoor activity, near work activity
- Ocular continuous: spherical equivalent, axial length, distance visual acuity, near visual acuity, intraocular pressure, accommodative amplitude, accommodative facility, accommodative lag, pupil size

Analysis of change in outcomes

Categorical outcomes

Patient-reported outcomes are the only categorical outcome that will be assessed over the study period. The patient reported outcome data will be presented in a table and the proportions of each response in the treatment vs placebo group compared at each visit, separately, using a Chi-square test.

	Atropine 0.01% treatment group	Placebo group	P value (chi-square test)
How do your eyes feel today?	Great (n, %) Good (n, %) Ok (n, %) Bad (n, %)	Great (n, %) Good (n, %) Ok (n, %) Bad (n, %)	p
Do you feel any itchiness near your eyes?	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	p
Is your vision blurry with your glasses on?	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	p
Do your eyes feel stingy?	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	p
Are your eyes sore when you are in the light?	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	p

Do you find it difficult to read or write?	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	Not at all (n, %) Ok (n, %) A little bit (n, %) Very (n, %)	p

Continuous outcomes

Change in outcomes from baseline to 24 months, including efficacy and safety/side-effect outcomes will be analysed first using repeated-measures analysis of variance (ANOVA) and then using linear mixed models.

A two-way repeated measures ANOVA will be used to test whether there is a significant difference in the mean changes in the outcome between the atropine 0.01% and placebo groups. The two factors will be treatment group and visit and an interaction between the two will be used to test for a treatment effect.

Where the two-way repeated measures ANOVA indicates a significant treatment difference, linear mixed models will be used to model and test the differences in the outcome between the treatment and placebo groups at each visit. In linear mixed models, visit and treatment group will be included as fixed effects in the model and an interaction term between the two terms will be included to assess if there is a significant treatment effect at each visit, separately. The baseline value of the outcome variable will also be included as a fixed effect to adjust for the average effect of the baseline value on subsequent progression (e.g. more myopic eyes tend to progress more).

Should any of the non-ocular baseline characteristics be found to be statistically significantly different between the treatment and atropine groups, repeated-measures ANOVA will not be used and the above described linear mixed model will be used instead with the relevant baseline characteristic included as a fixed effect covariate to attempt to adjust for any difference this difference may cause.

The assumptions of the repeated-measures ANOVA and the linear mixed models will be check as follows

Repeated-measures ANOVA:

- Assumes outcome variable is normally distributed – variable distribution will be visually assessed using histograms and a quantile-quantile plot (QQ plot).
- Assumption of sphericity – this will be assessed using Mauchly's test of sphericity and a sphericity correction applied if appropriate.

Linear mixed model:

- Assumes the errors have constant variance (homoscedascity) – this will be visually assessed by plotting the errors (residuals) over the fitted values.
- The errors are independent – this assumption should be met by study design.
- The errors are normally distributed – this will be visually assessed using histograms and QQ plots of the errors.

If any of the above assumptions of a normally distributed outcome, homoscedascity or a normal distribution of the errors are violated this will prompt a look at the impact of data transformation following the procedures described below.

DATA TRANSFORMATION

Data transformation will be used to attempt to transform the outcome distribution so that it more closely resembles a normal distribution. As the outcome data represent the change in the outcome, we will have a mix of positive, negative and zero values. This rules out most common data transformation methods such as log, square root and box-cox transformations. To investigate potential transformation, dummy data were generated that follows a negatively skewed distribution, which is the pattern expected in the primary outcome of change in spherical equivalent – i.e. we expect most people to have a small amount of change, some people to have none and some people to have a lot. Figures below show examples of the randomly generated data and the effect of cube-root and neglog transformations – both transformations that can handle negative, positive and zero values.

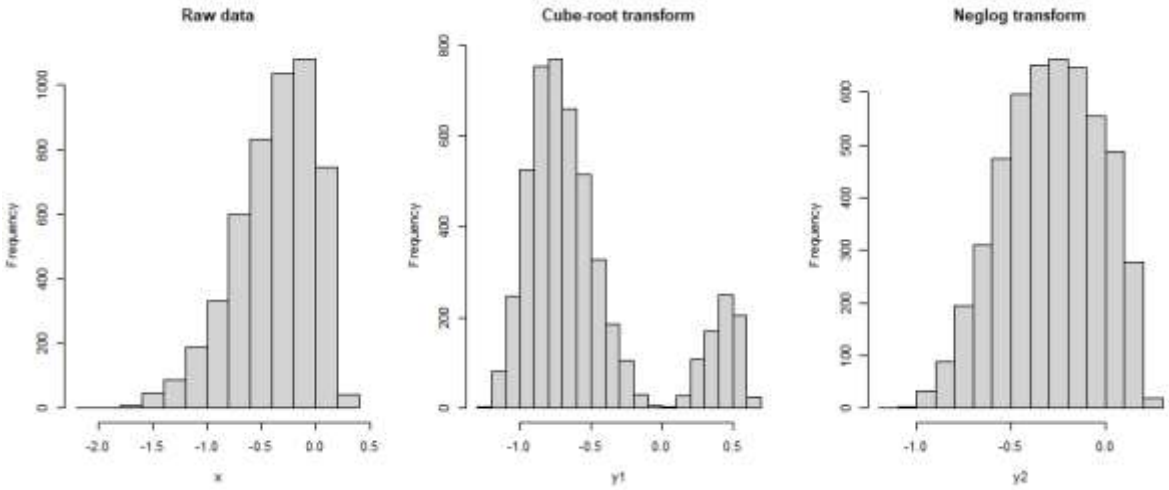

Figure 1 Histograms showing distribution of skewed, randomly generated data and distributions of the same data after cube-root and neglog transformations.

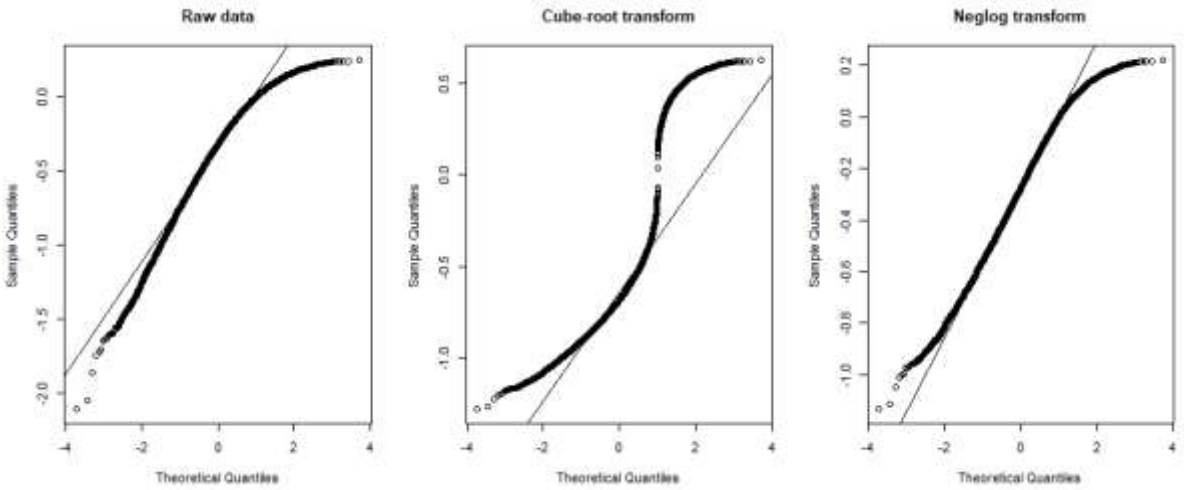

Figure 2 Quantile-quantile plots showing distribution of skewed, randomly generated data and distributions of the same data after cube-root and neglog transformations.

It can be seen that the cube-root transformation does not perform well, probably because of the large number of values that fall below 1 – the cube-root of a value <1 is a larger value hence this pushes values away from 0 and closer to 1. The neglog transformation performs pretty well and will generally be favoured for transformations. We will, however, check the transformation performance using the real data and may use an alternative transformation should it be shown to do a better job of representing a normal distribution.

Data transformation has its own down-sides, not least of which is a loss of interpretability. Thus, we will only report results using transformed data if it can be shown to substantially improve the results of our model. That is, does running the model with the transformed data substantially change the p values obtained with the untransformed data. If not, then we will report the raw data results.

AD HOC ANALYSIS

As an ad hoc analysis, we will investigate whether the effect of being assigned to the treatment vs placebo group is different between participants of different eye colours. Eye colour has been graded and will be grouped into blue, hazel and brown eye colours. Additional interaction terms between treatment group and eye colour group and visit and eye colour will be included in a linear mixed model to assess whether the effect of treatment on progression was different between eye colour groups and whether progression across visits was different between eye colour groups.

MISSING DATA

We expect participants to have dropped out during the course of the study and hence for some data to be missing. In the first instance, we do not plan to impute missing data. However, we will impute missing data using multiple imputation in the following scenarios:

1. Number of participants missing data is significantly different between the placebo and treatment groups
2. Participants with missing data were progressing faster than their peers prior to withdrawing i.e. mean/median spherical equivalent or axial elongation was significantly different between those who did and did not subsequently withdraw from the trial.

APPENDICES

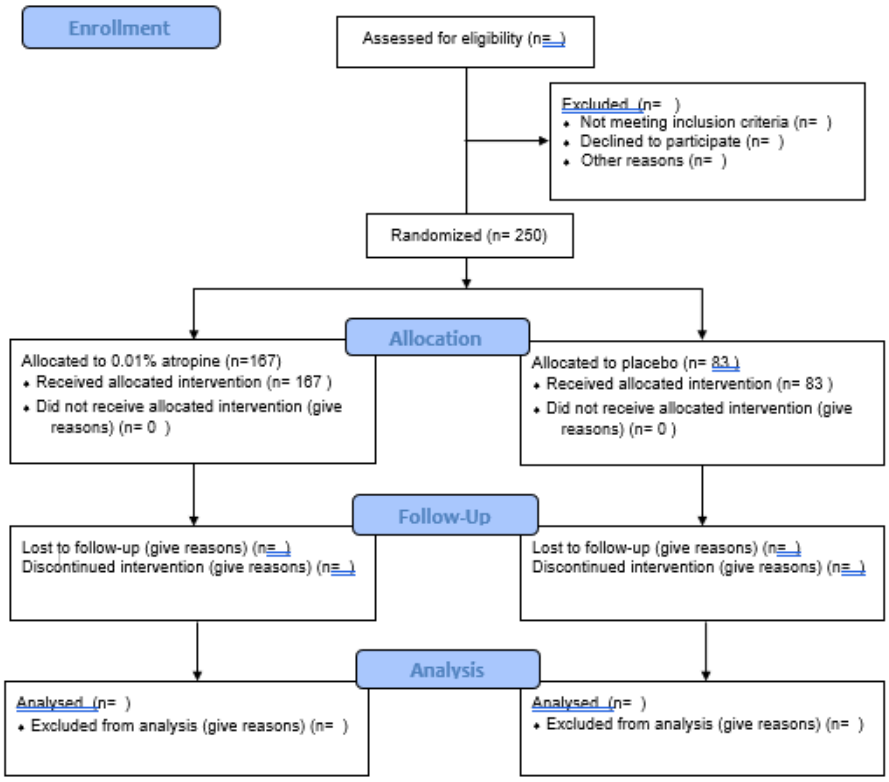

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) of the study.

Figure 3 Flow chart showing enrolment and follow-up data