ASD-Probiotic R&D ID:17/0148

The efficacy of the multi-strain probiotic, Vivomixx, on behaviour and gastrointestinal symptoms in children with autism spectrum disorder (ASD)

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Statistical Analysis Plan

Principal Analysis

The principal analysis will look for between phase differences in outcome measures assuming no order of treatment effects, which will be investigated in the secondary analysis. The outcome variables consider are:

1. Change from baseline ATEC scores **(Interval Data: between groups): PRIMARY OUTCOME MEASURE**

Secondary measures

1. Change from baseline ABC scores **(Interval Data: between groups)**
2. Gastrointestinal events: frequency of abdominal pain, gaseousness, diarrhoea, constipation, pain on stooling, and difficulty swallowing. **(Interval Data: between groups)**
3. Bristol Stool Scale: proportion of phase participants with type 4 stool samples after active treatment. **(Dichotomous data: between groups)**
4. Clinical Global Impression **(Interval Data: between groups)**
5. Treatment Effectiveness Score **(Interval Data: between groups)**
6. Correlations: ATEC vs constipation frequency on GI History; ATEC vs diarrhoea frequency on GI History; ATEC vs ATEC components (i.e. Health/Physical/Behaviour scores); ATEC vs Clinical Global Impression. **(Interval Data: Correlation)**

All between groups interval data will be subject to the following protocol for statistical analysis.

1. Calculation of mean, standard error and median and 25-75 quartiles for both phases.
2. Kolmogorov-Smirnov test to assess normality in the between phase differences in primary and secondary outcome measure with interval measurements.
3. If there is no statistically significant (alpha = 0.05) evidence of non-normality in the mean differences then a paired t-test[1] will be used to assess statistical significance.
4. If there is statistically significant (alpha = 0.05) evidence of non-normality in the mean differences, then a Wilcoxon-Signed-rank-test will be used to assess statistical significance.

All between groups dichotomous data will be assessed for statistical significance by calculating the Relative Risk and corresponding 95% confidence intervals for exposure to the active agent. Corresponding p-values can, if desired, be calculated from the 95% Confidence Intervals; however, 95% Confidence Intervals that do not contain the value 1 are statistically significant at alpha = 0.05.

The correlations will be measured using Pearson's R unless the assumptions for Pearson's R are not met: if these are not met, then Spearman's Rho will be used. The relevant assumptions for Pearson's R are:

1. Absence of outliers: i.e. no outliers for each variable more than ±3.29 Standard deviations from the mean.
2. Normality of variables: both variables will be assessed for normality using a Kolmogorov-Smirnov test.
3. Linearity: the relationship follows a straight-line relationship

[1] Note: assumption for paired-t-test is that the differences between the pairs are values are normally distributed, not that the values in each phase need to be normally distributed. The test does not assume homoscedasticity: i.e. that the standard deviations of the two groups are the same, so we will not test for this.

4. Homoscedasticity: how the datapoints are similarly distributed about a straight-line linear regression does not vary as either variables varies (technically the Standard error does not change significantly).

Assumptions three and four can be assessed visually using a scatter plot with a linear regression line added, and this can be generated by SPSS.

Secondary Analysis

Safety Analysis

The number of adverse events will be recorded that occurred to participants whilst on the active agent and placebo phases. The results will be presented both descriptively and assessed for a statistical difference in risk. To do this, the Relative Risk (RR) of adverse event as due to exposure to the active agent will be calculated with corresponding 95% confidence intervals.

Adherence and Deviation from Protocol Analysis

Similarly, adherence will be analysed descriptively for both the placebo and active agent phases, with totals for longer washout, withdrawal, lost to follow-up, did not reach full dose recorded for each phase. The total number of deviations will be summed for each phase, and the Relative Risk (RR) of deviation due to exposure to the active agent calculated with corresponding 95% confidence intervals.

Order of Treatment Effects

This analysis will replicate the between groups principal analysis; however, it will compare outcomes between the placebo/active and the active/placebo groups of participants rather than between the placebo arm and the active agent arm. Where dichotomous between groups data is considered, the Risk of exposure to the active agent in the first phase will be calculated with 95% confidence intervals.

The principal analysis of the primary outcome measure will be performed on the Placebo-first group vs Placebo-second group, and between the Active-first group vs the Active-second group to assess for an order effect.

Order of Treatment group demographics

The demographics of the placebo/active and the active/placebo groups of participants, will be analysed and compared for significant differences. The characteristics that will be analysed are age, gender, baseline ATEC total score and additional diagnoses.