Statistical analysis plan (SAP)

Probiotics to treat inflammatory depression – a randomized controlled trial

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Key Abbreviations

CRP	C-reactive protein
MDD	Major depressive disorder
CBT	Cognitive behavioral therapy
BMI	Body mass index

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1. Introduction

Alterations of the gut microbiota by consumption of specific functional foods, e.g. probiotic bacteria may be possible strategies to treat a subtype of depression associated with low-grade inflammation. We will now test the antidepressant effects of add-on probiotics (a specific strain of the genus *Lactobacillus*). In order to maximize the likelihood that we target a subpopulation of depressed individuals who would benefit the most from this treatment, we will enrich the sample using CRP and body mass index (BMI).

This statistical analysis plan (SAP) will give more detailed descriptions of the endpoints in the study and the corresponding analyses.

2. Study design

The project started in 2018 and was completed in September 2023. The study intervention was specific probiotic lactobacilli or placebo added to ongoing treatment with antidepressant medication or Cognitive Behavioral Therapy (CBT). 75 subjects are randomized to placebo or probiotics, and 71 completed the whole study. To assure a balanced subject recruitment, block randomisation within strata (ongoing treatment with CBT, antidepressant or both) was performed. Blood and feces sampling and symptom rating scales were completed at baseline, at weeks 4 and 8 (end of study). Biomarkers (from baseline, week 4 and week 8) in blood and feces will be analyzed after the study. Subjects are recruited from outpatient or inpatient settings, Psychiatry Skåne or primary care clinics in Skåne, Sweden. Subjects are recruited via clinical referrals, but also via ads.

2.1 Sample size calculation

We are planning a study of a continuous response variable from independent control and experimental subjects with 1 control(s) per experimental subject. In a previous study, the response within each subject group was normally distributed with standard deviation of 6 on MADRS score [1]. If the true difference in the experimental and control means is 4, we will need to study 36 experimental subjects and 36 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0,8. The Type I error probability associated with this test of this null hypothesis is 0.05.

3. Aims and objectives

The main aim of the study, which is also the focus of this SAP, is to test if**:**

i) Specific probiotic lactobacilli (added to stabilized ongoing treatment) is efficacious in treating depressive symptoms in individuals with low-grade inflammation, defined using high sensitivity C-reactive protein (hs-CRP) and BMI.

4. Outcomes

4.1 Clinical outcome measures

Primary outcomes are i) absolute change in Montgomery-Åsberg Depression Rating Scale (MADRS-M) score and ii) absolute change in "inflammatory depressive symptoms", from baseline to the two follow-up assessments. "Inflammatory depressive symptoms" is defined as a total composite score of the following items from the Patient Health Questionnaire-9 (PHQ-9): item 3 (sleep problems), item 4 (lack of energy), and item 5 (appetite disturbance) [2]. Secondary outcomes are changes in the following self-rating scales: Generalized Anxiety Disorder-7 (GAD-7), Gastrointestinal Symptom Rating Scale for Irritable Bowel Syndrome (GSRS-IBS), the Dimensional Anhedonia Rating Scale, Insomnia Severity Index, Fatigue Severity Index, and the Digit Symbol Coding Test. Symptom ratings are done at baseline, week 4 and week 8,

4.2 Safety outcomes
- Adverse events: are reported monitored at each clinic visit.
- Concomitant medications: Usage of medications during study period will be recorded.

5. Populations to be analyzed

The primary analysis is on the Intention-to-treat (ITT) population which includes all randomized study subjects, with at least one post-baseline visit with symptom ratings.

We will also, as a sensitivity analysis, do a per protocol (PP) analysis which includes all randomised study subjects completing the whole study period (complete cases). Subjects with protocol deviations defined as severe, other factors or events that may interfere with the effect and outcome of the intervention are excluded from this analysis. According to preliminary examinations, 7 subjects will be excluded from the analysis; causes for exclusion are covid-19 infection during the study (n=1), prolonged respiratory tract infection during the study requiring NSAID over several

weeks (n=1), taking double daily dose of the study product (n=1), long delay between baseline visit and start of intervention (n=1), poor compliance (n=1, see section 7. Compliance) and change of medication that can interfere with effect and outcome of the intervention (n=2, sertraline and levaxin).

6. Analyses

All analyses will be performed using the Statistical Package for the Social Sciences for (SPSS version 28 or 29, IBM, Armonk, NY, USA) and SAS Enterprise Guide 8.3 for Windows (SAS Institute Inc., Cary, NC, USA). All variables will assessed for normal distribution and in case of non-normality, according to histogram or Q-Q-plot. All outcomes will be presented using descriptive statistics; normally distributed data by the mean and standard deviation (SD) and skewed distributions by the median and interquartile range (IQR). Binary and categorical variables will be presented using counts and percentages. Transformation of data might be needed to meet the assumptions of the statistical methods.

The outcome is the absolute difference before treatment and week 8 of the outcome measures defined above. We will use a linear mixed model with repeated measures.

In our model, the treatment groups, the time points (baseline, week 4 and week 8) and the interaction between the groups and time points will be fixed effects, while the patients will be set as random effects. Pre-treatment scores of outcome measures will be used as a covariate and by use of the parsimony measure Akaike Information Criterion (AIC) we will assess which structure covariance best fits the model [3]. Lower scores indicate a better-fitting model.

7. Compliance

Compliance will be monitored through a diary, counting of returned tablets, and self-reported compliance by repeated inquiry. The diary will be handed out to the study subjects at the baseline visit and collected at the week 8 visit. Subjects will be inquired about compliance at every study visit and all self-reported deviations or lack of compliance will be recorded. Subjects will be asked to return diary as well as study tablets at the end of the study. If we haven't received the diary or returned tablets at the end of study, any recorded notes on self-reported compliance will be used as a basis of poor or satisfactory compliance instead.

We have chosen a cut-off of compliance to the intervention study product at 80%, in accordance with a frequently used definition for poor vs. satisfactory compliance[4].

This cutoff is also in line with other probiotic intervention studies [4-13]. Subjects with monitored poor compliance (<80%) will be excluded from the PP analyses.

8. References

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