University of Zurich[UZH]

Epidemiology, Biostatistics
and Prevention Institute,
Biostatistics Department
+41 44 634 46 41
www.biostat.uzh.ch

Omega-3 Fatty Acids as First-Line Treatment in Pediatric Depression

Statistical Analysis Plan for Gregor Berger, Isabelle Häberling

Kelly Reeve (*kelly.reeve@uzh.ch*)
Ulrike Held

Version 1.0 of May 23, 2022

List of abbreviations

AD	antidepressant
AE	adverse event
CDRS-R	Children's Depression Rating Scale - Revised
DIKJ	Children's Depression Inventory (Depressionsinventar für Kinder und Jugendliche)
EPA	eicosapentaenoic acid
KIDscreen-CAT-27	quality of life questionnaire
pMDD	pediatric major depressive disorder
SIQ-Jr	Suicide Ideation Questionnaire-Junior
SUSAR	Suspected Unexpected Serious Adverse Reaction

Introduction

1 Type of trial

This is a 36-week multi-center 1:1 randomized, double-blind, placebo-controlled, two-armed, parallel group study investigating the superiority of EPA-rich Omega-3 to placebo in the treatment of children and adolescents with pediatric major depressive disorder. The randomization included a minimization algorithm based on the following factors: site, age (8-12 vs. 13-18), gender (male vs. female), and hsCRP (<1 mg/l; 1-3 mg/l; >3mg/l). For sites offering both in- and out-patient care, in-/out-patient type was used as an additional minimization factor. The trial aimed to recruit children/teenagers between 8 and 18 years old with a primary diagnosis of major depressive disorder (DSM-IV, K-SADS-PL) and at least moderate depressive symptoms (CDRS-R $\geq$ 40).

In order to include a representative sample of moderately and severely ill children with major depressive disorders, the use of clinically required SSRIs is permitted. The anticipated proportion of patients receiving additional antidepressants at some point during follow-up is estimated to be around 30%. The use of additional antidepressants is expected to influence primary outcome measurements and be influenced by treatment group, with greater antidepressant use hypothesized in the control arm. Antidepressant use may therefore bias the estimation of the omega-3 treatment effect.

2 Scope of the statistical analysis plan

This statistical analysis plan (SAP) is based on the protocol for the trial SNF 33IC30 166826 "Omega-3 Fatty Acids as First-Line Treatment in Paediatric Depression" version 6 dated 09.03.2021. Its scope is limited to the primary trial objectives, namely efficacy and safety. This SAP includes further details regarding the statistical analysis of the primary and secondary efficacy and safety endpoints to be conducted after closure of the database. Specifically, the following changes were introduced:

- A single primary outcome, longitudinal CDRS-R total score, was selected;
- Remission and response were changed from additional primary outcomes to confirmatory secondary outcomes, while recovery was removed as redundant;
- The scope of the primary trial analysis was reduced, reserving exploration of the relationships between inflammatory and lipid markers and symptoms and treatment response for follow-up analyses described in future analysis plans and manuscripts;
- Further details for statistical analyses per outcome were specified;
- A method assuming MNAR was explicitly specified for the primary analysis of the primary outcome and sensitivity analyses exploring robustness to the selected assumptions were specified.

Changes to the statistical analyses made across protocol versions are documented in the final section of this document.

3 Objectives

The study protocol defines the following study objectives:

1. To investigate the therapeutic efficacy and safety of omega-3 fatty acids rich in EPA in pMDD;
2. to demonstrate clinically meaningful effects of omega-3 fatty acid treatment;
3. to investigate inflammatory and bioactive lipid markers as response predictors;
4. to investigate the relationship between psychopathology (in particular suicidal ideation), illness course and cognition in relation to inflammatory and bioactive lipid markers.

For the purpose of this statistical analysis plan, we focus on the primary trial objectives, i.e., efficacy and safety objectives, as described below.

3.1 Primary objective

- To demonstrate the superiority of daily EPA-rich Omega-3 over placebo as an adjunct to the standard of care (German S3 guidelines) in children and adolescents with pediatric major depressive disorder.

3.2 Secondary objectives

- To demonstrate the clinically meaningful efficacy of daily EPA-rich Omega-3 relative to placebo as an adjunct to the standard of care (German S3 guidelines) in children and adolescents with pediatric major depressive disorder.
- To compare the safety and tolerability of daily EPA-rich Omega-3 versus placebo as an adjunct to the standard of care (German S3 guidelines) in children and adolescents with pediatric major depressive disorder.

4 Estimands

4.1 Primary estimand

Effectiveness (i.e., 'de facto', 'treatment policy') estimand The effectiveness estimand quantifies the average treatment effect of EPA-rich Omega-3 relative to placebo over 36 weeks, as an adjunct to the German S3 guideline standard of care, in all randomized subjects regardless of adherence to treatment or initiation of antidepressants.

4.2 Secondary estimand

Efficacy (i.e., 'du jure', 'hypoethetical') estimand The efficacy estimand quantifies the average treatment effect of EPA-rich Omega-3 relative to placebo over 36 weeks, as an adjunct to the German S3 guideline standard of care, if all randomized subjects had adhered to the randomly assigned treatment for the entire trial duration.

5 Endpoints

5.1 Primary endpoints

- CDRS-R total score trajectories from baseline to week 36 (measured at baseline, 6, 12, 24, and 36 weeks)

5.2 Secondary endpoints

5.2.1 Confirmatory secondary endpoints

- Remission (CDRS-R total score less than or equal to 28) at any point during the 36 weeks of trial observation
- Response (30% reduction in CDRS-R total score) at any point within 12 weeks from baseline
- Participants newly initiating antidepressant medication (or newly increasing dose if on antidepressant at baseline) at any point during the 36 weeks of trial observation
- Number of days on trial before new antidepressant initiation (or new antidepressant dose increase if on antidepressant at baseline)
- KIDscreen-CAT-27 overall quality of life score at 36 weeks
- DIKJ depression score at 36 weeks

5.2.2 Supportive secondary endpoints

Effect endpoints

- Number of days in hospital
- Lower outpatient service use

Safety endpoints

- Suicidal ideation (SIQ-Jr) from baseline to week 36 (measured at baseline, 6, 12, 24, and 36 weeks)
- Number of SUSARs from baseline to week 36

Statistical considerations

6 Sample size determination

The sample size calculations were performed for the primary outcome, as well as closely related secondary outcomes in order to ensure all questions of high interest are sufficiently powered. All calculations assume a parallel two-arm design with a 1:1 randomization ratio.

6.1 CDRS-R Difference

Meta-analyses selecting studies of omega-3 fatty acids with high proportion of EPA in aMDD as primary diagnosis found an SMD between 0.28-0.56 (see Protocol references 160-166). However, no studies were performed in pMDD, except for one very small (n=20) pilot study in childhood depression with a large effect size (SMD=1.2) (see Protocol reference 167).

A two-sample t-test (equal variances) would require 74 participants per treatment arm to have 90% power to detect an optimistic standardized mean difference of 0.54 on the CDRS-R scale at the 0.05 two-sided significance level. The trial would then require a total of 148 participants.

As the placebo response rate in minors is probably higher compared to that reported in the single center RCTs in adults summarized in the meta-analyses referenced above, we calculated our sample size estimation under more conservative assumptions as well.

The sample size calculation above for testing a difference in CDRS-R scores considered a relatively optimistic effect size and did not account for loss to follow-up. A two-sample t-test (equal variances) would require 111 participants per treatment arm to have 80% power to detect a smaller standardized mean difference of 0.4 on the CDRS-R scale at the 0.05 two-sided significance level. The total sample size needed would then be 222 participants.

6.2 Difference in Proportion of Responders

A two-proportion z-test would require 97 participants per treatment arm to have 80% power to detect a difference in response of 20% (with 60% response in the treatment group and 40% response in the control group) at the 0.05 two-sided significance level. Accounting for a 10% loss to follow-up results in a total sample size of 216 participants.

7 Statistical analyses

Analysis sets The following analysis sets are defined in accordance with the ICH-E9 guidelines:

- The full analysis set (FAS) includes all randomized subjects. The FAS will be used for 'as randomized' evaluation. Only in exceptional cases may subjects be excluded from the FAS. In such cases the reason for exclusion will be justified and documented.
- The safety analysis set includes all randomized subjects receiving at least one dose of randomized treatment. The safety analysis set will be used for 'as treated' evaluation.

Baseline comparability of randomized groups Baseline descriptive variables of participants (including antidepressant use) will be summarized overall and by treatment arm using suitable measures of central tendencies and variability for continuous data (means or medians, SD or IQR) and frequencies and proportions for categorical data. No hypotheses are tested.

The number of participants lost to follow-up, initiating antidepressants, and adhering to the protocol will each be tabulated by treatment arm and visit. Baseline characteristics will also be summarized across the groups of participants completing the trial, lost to follow-up, and initiating antidepressant use.

General statistical considerations Statistical analysis results will generally be presented as point estimates with two-sided 95% confidence intervals. A limited number of analysis results will additionally be reported with p-values, which will be interpreted as indicative of superiority when the value is less than 5% and the estimated treatment contrast favors the omega-3 treatment arm.

Descriptive statistics for all efficacy and safety endpoints will be presented according to treatment arm.

The baseline values are collected at visit 1 (0 weeks), which is the first week after the lead-in phase. Further measurements are taken at visits 2 through 5 at 6, 12, 24, and 36 weeks.

The clinical trials registry number (03167307) will be used as a seed number, if needed.

Missing data By design, several baseline model covariates cannot be missing, as they are required for minimization during randomization. We distinguish between study dropouts and intervention dropouts. Study dropouts are participants whom withdraw consent, are monotone not available for follow-up visits, or are removed from the trial according to the protocol due to non-compliance (< 60% pills taken). Intervention dropouts are participants whom newly initiate an off-trial antidepressant or are newly prescribed an increased dose of an off-trial antidepressant prescribed before baseline.

Methods to handle missing data are outlined per analysis type below. Where multiple is used, 50 imputed datasets will computed. Results across datasets will be combined using Rubin's rule.

Modeling In case of covariate imbalance on baseline covariates other than antidepressant use, omega-3 index, age, gender, and hsCRP, these covariates will also be included in the models described below. In case of computational convergence issues, simpler models will be estimated.

7.1 Primary endpoint

- CDRS-R total score from baseline (post-placebo-lead-in) to week 36

The primary analysis consists of a joint model linking (1) a linear mixed effects model for CDRS-R total score over time with (2) a Cox proportional hazards model for the risk of the event new initiation of or increase in antidepressant use (intervention dropout) and (3) a Cox proportional hazards model for the risk of study dropout as a competing event. This assumes that whether a participant has missing data and at what time this occurs contains additional information that should be taken into account and corresponds to an MNAR missing data mechanism.

Participants are censored at whichever occurs first of: antidepressant initiation/increase, loss to follow up, consent withdrawal, and study exit due to non-adherence. Participants lost to follow up, withdrawing consent, and exiting the study (as per protocol) due to non-adherence are considered alike and these events considered one event type: study dropout.

The linear mixed effects model for the longitudinal CDRS-R total score outcome $Y_i(t)$ measured at time t for participant i takes the following form:

$$
\begin{aligned}
Y_i(t) &= m_i(t) + \varepsilon_i(t), \\
m_i(t) &= \beta_0 + \beta_1 \text{intervention}_i + \beta_2 t_{base} + \beta_3 t_{6weeks} + \beta_4 t_{12weeks} + \beta_5 t_{24weeks} + \beta_6 t_{36weeks} \\
&\quad + \beta_7 t_{base} * \text{intervention}_i + \beta_8 t_{6weeks} * \text{intervention}_i + \beta_9 t_{12weeks} * \text{intervention}_i \\
&\quad + \beta_{10} t_{24weeks} * \text{intervention}_i + \beta_{11} t_{36weeks} * \text{intervention}_i \\
&\quad + \beta_{12} \text{age}_i + \beta_{13} \text{gender}_i + \beta_{14} \text{hsCRP}_i \\
&\quad + \beta_{15} \text{antidepressant} + \beta_{16} \text{omega-3 index} \\
&\quad + b_{i0} + b_{i1} t + b_{s0},
\end{aligned}
$$

where time t is considered a factor (while also investigating possibility of linearizing time effect) and randomized treatment group (intervention) and the interaction between randomized treatment group and time are included as fixed effects. The baseline values of the minimization factors age, gender, and hsCRP, as well as baseline antidepressant use and omega-3 index are included as covariates. The baseline outcome measure is modeled in the response, not as a covariate. The terms b_{i0}, b_{i1}, and b_{s0} represent participant-specific random intercepts and slopes and site-specific random intercepts. The random effects are assumed to be normally distributed and an unstructured covariance matrix will be assumed. The error term $\varepsilon_i(t)$ is assumed to be normally distributed with mean zero and variance σ^2, independent of the random effects and is independent over time. An overall treatment effect for CDRS-R score at 36 weeks ($\beta_1 + \beta_{11}$) will be reported with a 95% confidence interval and corresponding p-value.

The competing survival models for time to event r (intervention dropout or study dropout) take the form:

$$
\lambda_{ir}(t) = \lambda_{0r} \exp\{\gamma_1 \text{intervention} + \alpha_r m_i(t)\}
$$

where the cause-specific Cox models include intervention as a time-independent effect and the estimated true trajectory of CDRS-R as a time-varying effect. The parameters α_r measure the strength of association between the longitudinal outcome and risk of the corresponding dropout event.

7.1.1 Subgroup analysis for the primary endpoint

The consistency of the treatment effect will be evaluated by adding an interaction term between treatment effect, time, and omega-3 index to the model described above. The p-value for the comparison between the interaction model and the simpler model will be used to determine consistency.

7.1.2 Sensitivity analyses for the primary endpoint

1. **Typical ITT analysis**: In order to compare results with other trials, the linear mixed model part of the analysis model above will be applied to the full analysis set, with all participants analyzed as randomized. The data from intervention dropout participants will be used as observed, i.e, the outcomes observed while on non-randomized antidepressants will be used. The mixed model assumes that missing data is missing at random. This analysis is expected to be biased toward the placebo arm, as the number of participants requiring antidepressants is expected to be higher in the placebo arm and the additional antidepressant will improve outcomes.

2. **Modified ITT analysis**: The first sensitivity analysis will be repeated, but the data from intervention dropout participants will be considered missing in the mixed model upon non-randomized antidepressant initiation/dose increase. The responses of participants with missing data will be assumed similar to those with complete data and similar covariate characteristics. The bias resulting from imbalance in the use of non-randomized antidepressants across treatment groups is curtailed, but the amount of data available for analysis, and therefore power, is diminished.

3. **Typical PP analysis**: In order to address the efficacy estimand, the sensitivity analysis model above will be applied to all full analysis set data during which participants were 'on protocol'. This means the participants are adhering to the treatment they were randomized to and are not using additional antidepressants. Non-compliers and those taking non-randomized antidepressants are excluded completely (i.e., complete case analysis). This yields an estimate of efficacy that is unbiased under the MAR assumption. It assumes that participants with missing data (those with additional antidepressants and those not adhering to randomized treatment) would have had similar outcomes to participants completing the study in the same treatment arm and with similar baseline characteristics and score trajectories. However, the use of additional anti-depressants is not considered to be random across participants, but rather related to intermediate outcomes and possibly treatment assignment. Selection bias is then expected to affect this estimate. Additionally, the reduction in participants decreases the power to detect a treatment effect.

4. **Delta-based multiple imputation for MNAR analysis**: The data after intervention dropout or study dropout is considered to be missing. Multiple imputation under MAR will be performed separately within each treatment arm (White et al 2011) using imputation models containing the minimization factors, baseline antidepressant use and omega-3 index. It will be assumed that participants initiating/increasing non-randomized antidepressant use would have had worse outcomes than observed had they not used the non-randomized antidepressant. A range of mean differences δ from a 25% increase in CDRS-R score (25% worse outcome) to a 100% increase (by 25% increments) in the CDRS-R score in the intervention dropouts as compared to the observed cases will be considered. Delta will then be added to the imputed

CDRS-R scores for the participants with intervention dropout. Results will be combined using Rubin's rule. This is repeated for each delta.

7.2 Secondary endpoints

7.2.1 Confirmatory secondary endpoints

The binary confirmatory secondary outcomes include:

- **Remission**, defined as a CDRS-R total score < 28 at any time up until 36 weeks
- **Response**, defined as a 30% drop in CDRS-R total score at any time up until **12 weeks**
- **Intervention dropout**, defined as initiation of non-randomized antidepressant use while on trial for participants not on a non-randomized antidepressant at baseline or an increase in antidepressant dose while on trial for participants on a non-randomized antidepressant at baseline

Unadjusted proportions per treatment arm will be presented with 95% confidence intervals. To adjust for baseline antidepressant use, omega-3 index, and CDRS score, as well as the minimization factors age, gender, and hsCRP, these binary outcomes will each be assessed using log-binomial models with treatment arm as the effect of interest. Each model will be interpreted in terms of the the adjusted risk ratio (RR) comparing the outcome probability in the omega-3 group to that in the placebo group ($RR_{\text{omega-3/placebo}}$). The null hypothesis that the risk ratio is equal to 1 (no difference in risk across treatment groups) will be rejected if the 95% confidence interval for the risk ratio does not include 1. If the 95% confidence interval for the risk ratio is above 1, omega-3 treatment will be considered superior.

These models will include data from all randomized participants and analyze the participant data according to the treatment arms participants were randomized to (as-randomized). Data after intervention dropout will be considered missing. Multiple imputation under MAR will be used to impute longitudinal CDRS-R scores for study and intervention dropouts before the binary outcomes are determined. MI will be performed separately within each treatment arm (White et al 2011) using imputation models containing the minimization factors, baseline antidepressant use and omega-3 index.

As for the primary outcome, a delta-based sensitivity analysis approach will be used to address MNAR. For analysis of intervention dropout, imputation is only needed for participants with study dropout before intervention dropout and no sensitivity analysis is planned.

The continuous confirmatory secondary outcomes include:

- self-reported **quality of life** measured by the KIDscreen-CAT-27 overall score at 36 weeks
- **Depression severity** measured by DIKJ at 36 weeks

These continuous outcomes will each be assessed using ANCOVA linear regression models with the 36-week measurement as the outcome, treatment arm as the effect of interest, and the baseline measurement, antidepressant use, omega-3 index, CDRS-R score, and the minimization factors age, gender, and hsCRP as covariates.

The treatment coefficient denotes the mean difference in outcome score across the treatment groups. The null hypothesis that the mean scores are equal across treatment groups will be rejected

if the 95% confidence interval for the treatment group coefficient does not include 0. If the 95% confidence interval for the treatment group coefficient is above 0 for KIDscreen-CAT-27 (below 0 for DIKJ), omega-3 treatment will be considered superior with respect to its effect on quality of life (depressive symptoms).

These models will include data from all randomized participants and analyze the participant data according to the treatment arms participants were randomized to (as-randomized). Data after intervention dropout will be considered missing. Multiple imputation under MAR will be used to impute scores at 36 weeks for study and intervention dropouts. MI will be performed separately within each treatment arm (White et al 2011) using imputation models containing the minimization factors, baseline antidepressant use, omega-3 index, and baseline score. As for the primary outcome, a delta-based sensitivity analysis approach will be used to address MNAR.

Sub-domains will be described with tables or figures.

The final confirmatory secondary outcome is:

- the time on trial before initiation of new or increased antidepressant use (intervention dropout).

A Kaplan Meier approach will be used to estimate unadjusted median time to this event overall and per treatment arm. A log rank test will be used to compare time to intervention dropout across treatment arms.

7.2.2 Supportive secondary endpoints

Effect endpoints

- **Number of days in the hospital**
- **Outpatient service use**

These endpoints will be described using summary statistics across treatment arm.

Safety endpoints

- **Suicidal ideation** from baseline to week 36 will be modeled jointly by a linear mixed effects model for SIQ-Jr score over time combined with Cox regression for time to intervention and study dropout, analogously to the CDRS-R total score primary outcome model.
- **Serious adverse events** from baseline to week 36 will be tabulated per type and treatment arm using the safety analysis set. If appropriate, the difference in proportion (with 95% confidence interval) will be estimated.

8 Changes to the statistical analyses planned in the protocol

Version	Explanation
IICT submission	First version of study plan
Frontiers protocol	Study protocol after funding review and peer review, addition of concomitant treatment description, addition minimization factor for unit type (in-/outpatient), description of sensitivity analyses to account for antidepressant use
UZH protocol	Refinement of secondary (supportive/exploratory) outcomes and their analyses
Statistical analysis plan	Focusing the analysis plan on measures of efficacy and safety for reporting of primary trial results. Updating method for dealing with informative missingness.

9 References

N. M. Laird and J. H. Ware. Random-effects models for longitudinal data. Biometrics 1982; 38:963–74

R J A Little and L Yau. Intent-to-treat analysis for longitudinal studies with drop-outs. Biometrics 19996; 52:1324–33.

S Mazumdar and K S Liu and P R Houck and C F Reynolds. Intent-to-treat analysis for longitudinal clinical trials: coping with the challenge of missing values. Journal of Pyschatric Research 1999; 33:87–95

Michele L Shaffer and Vernon M Chinchilli. Including multiple imputation in a sensitivity analysis for clinical trials with treatment failures. Contemporary Clinical Trials 2007 28:130–137

Ian R. White and C Bamias and P Hardy and S. Pocock and J Warner. Randomized clinical trials with added rescue medication: some approaches to their analysis and interpretation. Statistics in Medicine 2001; 20:2995–3008.

R version and packages used to generate this report:
R version: R version 4.0.1 (2020-06-06)
Base packages: stats, graphics, grDevices, utils, datasets, methods, base
Other packages: pwr, readxl, reporttools, xtable, knitr
This document was generated on May 23, 2022 at 12:26.