STATISTICAL ANALISIS PLAN

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Physical Activity and Fitness in Pediatric Inflammatory Bowel Disease (PAFIT–IBD)

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Study Description

This document contains the **Statistical Analysis Plan** for the study titled *"Physical Activity and Fitness in Pediatric Inflammatory Bowel Disease (PAFIT–IBD)"*, a prospective single-arm interventional study evaluating the effects of a structured physical activity intervention on physical activity, physical fitness, inflammation, and clinical outcomes in children and adolescents with inflammatory bowel disease.

Important Note

This document was prepared for submission to **ClinicalTrials.gov** in accordance with the requirements of the **Protocol Registration and Results System (PRS)**. Uploaded documents may be posted publicly on the ClinicalTrials.gov website after PRS review.

This document does **not contain any personally identifiable information about study participants**.

1. Introduction

This Statistical Analysis Plan (SAP) describes the statistical methodology that will be used for the analysis of data collected in the PAFIT-IBD study.

The SAP specifies:

- analysis populations
- definitions of outcomes
- statistical methods
- handling of missing data
- sensitivity analyses
- software used for statistical analyses.

This document has been prepared prior to database lock and statistical analysis in order to ensure transparency, reproducibility, and methodological rigor.

2. Study Objectives

2.1 Primary Objective

To evaluate the effect of a **12-week structured physical activity intervention** on **cardiorespiratory fitness** in children and adolescents with inflammatory bowel disease.

2.2 Secondary Objectives

1. To describe physical activity and physical fitness levels in pediatric IBD.
2. To evaluate associations between physical activity and inflammatory markers.
3. To assess relationships between physical fitness and clinical outcomes.
4. To evaluate changes in inflammatory markers after the intervention.
5. To assess changes in quality of life, fatigue, and mental health.
6. To evaluate whether improvements in physical fitness mediate clinical outcomes.
7. To evaluate the sustainability of intervention effects at 6–12 month follow-up.

3. Study Design and Statistical Framework

This study follows a **prospective longitudinal single-arm intervention design**.

Participants are assessed at:

1. **Baseline (T0)**
2. **Post-intervention (T1, after 12 weeks)**
3. **Follow-up (T2, 6–12 months)**

The intervention effect will be evaluated using **within-participant comparisons**.

Each participant serves as their own control.

The primary statistical framework will include:

- paired analyses
- longitudinal mixed-effects models
- regression models
- mediation analysis.

4. Analysis Populations

4.1 Intention-to-Treat Population (ITT)

The ITT population will include **all participants enrolled in the intervention** with at least one baseline assessment.

Participants will be analyzed according to the intervention assigned regardless of adherence.

The ITT population will be the **primary analysis population**.

4.2 Per-Protocol Population (PP)

The per-protocol population will include participants who complete **at least 70% of the scheduled intervention sessions**.

This population will be used for **sensitivity analyses**.

4.3 Safety Population

The safety population will include **all participants who attend at least one intervention session**.

Adverse events occurring during the intervention will be summarized descriptively.

5. Sample Size Justification

The sample size was calculated based on the expected change in **cardiorespiratory fitness**, measured by the **20-meter shuttle run test (20mSRT)**.

Assumptions:

- Expected effect size: Cohen's $d = 0.5$
- Significance level: $\alpha = 0.05$
- Statistical power: 80%
- Paired pre–post design

The minimum required sample size is **34 participants**.

Considering expected drop-out rates between **15–30%**, the target recruitment is **approximately 100 participants**.

This sample size allows sufficient power for:

- primary outcome analysis
- secondary outcomes
- exploratory longitudinal models.

6. Study Variables

6.1 Primary Outcome

Cardiorespiratory fitness

Measured using the **20-meter shuttle run test (20mSRT)**.

Outcome metrics:

- number of completed stages
- estimated VO_2max (if calculated).

Primary endpoint:

Change between **baseline (T0)** and **post-intervention (T1)**.

6.2 Secondary Outcomes

Physical Activity

Measured using **accelerometry over 7 days**.

Variables:

- Moderate-to-vigorous physical activity (MVPA, min/day)
- Total sedentary time
- Activity bouts
- Total activity counts

Self-reported physical activity:

- PAQ-C
- PAQ-A

Physical Fitness

Measured using the **ALPHA fitness battery**:

- Handgrip strength
- Standing long jump

- 4 × 10 m agility test
- Cardiorespiratory fitness

Clinical Variables

Disease activity:

- PCDAI (Crohn's disease)
- PUCAI (ulcerative colitis)

Inflammatory Biomarkers

- Fecal calprotectin
- C-reactive protein (CRP)

Patient-Reported Outcomes

- IMPACT-III (quality of life)
- PedsQL Fatigue Module
- RCADS (anxiety and depression)
- ADHD Rating Scale

Cognitive Function

- BENCI neuropsychological battery

Nutritional Status

- BMI z-score
- Lean mass
- Body composition

7. General Statistical Principles

7.1 Significance Level

All statistical tests will be two-sided.

Statistical significance will be set at:

$p < 0.05$

For multiple comparisons in secondary analyses, false discovery rate (FDR) correction may be applied.

7.2 Confidence Intervals

All estimates will be reported with **95% confidence intervals**.

7.3 Data Distribution

Continuous variables will be evaluated for normality using:

- Shapiro–Wilk test
- visual inspection of histograms
- Q-Q plots.

Non-normally distributed variables may be:

- log-transformed
- analyzed using non-parametric tests.

8. Descriptive Analysis

Baseline characteristics will be summarized using:

Continuous variables

- mean ± standard deviation (normal distribution)
- median and interquartile range (non-normal distribution)

Categorical variables

- frequencies
- percentages.

Baseline characteristics may include:

- age
- sex
- disease type
- disease duration
- medication
- baseline physical fitness.

9. Primary Outcome Analysis

The primary analyses will evaluate **changes in objectively measured physical activity and physical fitness following the structured physical activity intervention**.

Primary outcomes will include both **physical activity variables (accelerometry-based)** and **physical fitness variables (ALPHA fitness battery)**.

All primary outcomes will be evaluated as **within-participant changes between baseline (T0) and post-intervention (T1)**.

9.1 Physical Activity Outcomes

Physical activity will be objectively measured using **accelerometry over 7 consecutive days** at each assessment point.

The following accelerometer-derived variables will be considered **primary physical activity outcomes**:

- **Moderate-to-vigorous physical activity (MVPA)** expressed as minutes per day.
- **Total sedentary time**, expressed as minutes per day.
- **Physical activity patterns**, including:
    - number and duration of MVPA bouts
    - fragmentation of physical activity and sedentary time.

Accelerometer data will be processed using standardized cut-points for pediatric populations.

Valid data will require:

- a minimum of **4 valid days**
- including **at least one weekend day**
- with **≥10 hours of wear time per day**.

Mean daily values will be calculated across valid days.

Changes in these variables between baseline and post-intervention will be analyzed using:

- **paired t-tests** for normally distributed variables
- **Wilcoxon signed-rank tests** for non-normal distributions.

Effect sizes will be calculated using **Cohen's d**.

9.2 Self-Reported Physical Activity

Subjective physical activity will be assessed using validated questionnaires:

- **PAQ-C** for children
- **PAQ-A** for adolescents.

These questionnaires will provide a summary physical activity score ranging from 1 to 5.

Changes in questionnaire scores between baseline and post-intervention will be analyzed using:

- paired t-tests or Wilcoxon signed-rank tests.

Correlations between **self-reported and accelerometer-derived physical activity variables** will be evaluated using Pearson or Spearman correlation coefficients.

9.3 Physical Fitness Outcomes

Physical fitness will be assessed using the **ALPHA fitness battery adapted for the hospital setting**.

The following variables will be analyzed as **primary physical fitness outcomes**:

Cardiorespiratory Fitness

Measured using the **20-meter shuttle run test (20mSRT)**.

Outcomes:

- number of completed stages
- estimated VO_2max (when applicable).

Changes between baseline and post-intervention will be analyzed using paired statistical tests.

Muscular Strength

Muscular strength will be evaluated using:

- **Handgrip strength test (handgrip dynamometry)**
- **Standing long jump test**

For handgrip strength, the **maximum value obtained across attempts** will be used for analysis.

Standing long jump distance will be recorded in centimeters.

Speed and Agility

Speed and agility will be assessed using the **4 × 10 meter shuttle run test**.

The outcome will be expressed as **time in seconds**, with lower values indicating better performance.

Body Composition

Body composition will be evaluated using:

- **Bioelectrical impedance analysis**
- **Body mass index (BMI) z-score**

BMI z-scores will be calculated according to **age- and sex-specific pediatric reference values**.

9.4 Statistical Analysis of Physical Fitness Outcomes

Changes in physical fitness variables between baseline and post-intervention will be analyzed using:

- **paired t-tests** for normally distributed variables
- **Wilcoxon signed-rank tests** for non-normally distributed variables.

In addition, **linear mixed-effects models** will be applied to evaluate longitudinal changes across:

- baseline (T0)
- post-intervention (T1)
- follow-up (T2).

Model structure:

Outcome ~ Time + Age + Sex + Disease Type + (1 | Participant)

This model will account for **within-participant repeated measures**.

Possible covariates:

- age
- sex
- baseline disease activity
- BMI.

9.5 Effect Size Estimation

Effect sizes will be calculated for all primary outcomes.

For continuous variables:

Cohen's d will be calculated as:

$$d = \frac{Mean_{post} - Mean_{baseline}}{SD_{baseline}}$$

Effect size interpretation:

- 0.2 → small effect
- 0.5 → moderate effect
- 0.8 → large effect.

9.6 Multiple Comparison Considerations

Because multiple primary variables are analyzed within the domains of physical activity and physical fitness, p-values may be adjusted using **False Discovery Rate (FDR) correction** in sensitivity analyses.

However, the primary interpretation will focus on:

- effect sizes
- consistency across related outcomes
- clinical relevance.

10. Secondary Outcome Analyses

10.1 Changes Over Time

Changes across the three timepoints (baseline, post-intervention, follow-up) will be analyzed using:

Linear mixed-effects models.

Model structure:

Outcome ~ Time + (1 | Participant)

This approach accounts for repeated measures within individuals.

10.2 Association Analyses

Associations between variables will be assessed using:

Pearson correlation (normal distribution)

or

Spearman correlation (non-normal distribution).

10.3 Multivariable Regression Models

Regression models will evaluate associations between:

- physical activity
- physical fitness
- inflammatory markers
- patient-reported outcomes.

General model:

$$\text{Outcome} = \beta 0 + \beta 1\ \text{Fitness} + \beta 2\ \text{Age} + \beta 3\ \text{Sex} + \beta 4\ \text{Disease Activity} + \varepsilon$$

11. Mediation Analysis

To evaluate whether **physical fitness mediates the relationship between physical activity and clinical outcomes**, mediation models will be conducted.

Approach:

Baron & Kenny framework or structural equation modeling.

Indirect effects will be estimated using:

bootstrapping (5,000 resamples).

12. Subgroup Analyses

Exploratory subgroup analyses may be performed according to:

- age group (children vs adolescents)
- disease type (Crohn's vs ulcerative colitis)
- baseline disease activity.

These analyses will be considered **exploratory**.

13. Missing Data

Missing data will be evaluated to determine the pattern:

- Missing completely at random (MCAR)
- Missing at random (MAR)
- Missing not at random (MNAR)

If appropriate:

Multiple imputation will be applied.

Sensitivity analyses will compare:

- complete-case analysis
- imputed datasets.

14. Sensitivity Analyses

Sensitivity analyses will include:

1. Per-protocol population analysis
2. Models adjusted for baseline covariates
3. Alternative model specifications.

15. Adverse Events Analysis

Adverse events will be summarized descriptively.

Reported variables:

- type of event
- severity
- relationship to intervention.

Incidence rates will be reported as:

number of events / number of participants.

16. Software

All statistical analyses will be conducted using:

R (version ≥ 4.0)

or

SPSS (version ≥ 27)

Additional packages for advanced modeling may include:

- lme4
- mediation
- lavaan.

17. Data Reporting

Results will be reported following:

- CONSORT recommendations for intervention studies
- STROBE recommendations for observational analyses.

Tables and figures will include:

- baseline characteristics table
- outcome change plots
- regression model summaries
- mediation diagrams.