Study Protocol Title: Impact of Platelet-Rich Fibrin on Donor Site Regeneration and Functional Recovery after ACL Reconstruction with BPTB Autograft

Version 01, 21 September 2020

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Version/Date: Version 01, 21 September 2020

NCT number: Not Available

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Statistical Analysis Plan (SAP)

Study title: Sensory deficit following anterior cruciate ligament reconstruction with bone patellar-tendon bone autograft: platelet-rich fibrin (PRF) could provide a solution
Design: Single-center prospective cohort pilot study with two parallel groups (PRF / Vivostat group vs. Standard group) and repeated follow-up measurements
Sample size: 53 male patients (Vivostat n = 24; Standard n = 29)
Primary endpoint: Sensory deficit at the donor site over time (4, 8 and 12 months)
Secondary endpoints: Subjective knee function scores (Modified Cincinnati, IKDC, Tegner Lysholm, Tegner Activity Score); demographic and perioperative characteristics; safety

1. Analysis Populations

- **Full Analysis Set (FAS):** All patients who met inclusion criteria, underwent ACL reconstruction with BPTB autograft, were assigned to one of the two cohorts (Vivostat vs. Standard) and had at least one postoperative follow-up assessment. All efficacy analyses (sensory deficit and subjective scores) will be based on the FAS.
- **Safety Set:** All operated patients for whom group assignment (Vivostat vs. Standard) is known. Safety (complications, adverse events) will be summarized descriptively in this set.

No separate per-protocol analysis is planned because all 53 included patients completed follow-up according to the study results.

2. Endpoints

2.1 Primary endpoint

The **primary endpoint** is the **presence of sensory deficit at the donor site** at postoperative follow-ups:

- Sensory deficit is assessed in a predefined skin area lateral to the incision and coded as a binary variable:
    - **0 = no sensory deficit** (normal perception in all points);
    - **1 = sensory deficit present** (numbness or altered sensation in more than three tested points).

Measurements are performed at:

- 4 months after surgery,
- 8 months after surgery,
- 12 months after surgery.

The primary focus of the analysis is:

1. **Between-group comparison** (Vivostat vs. Standard) of the proportion of patients with sensory deficit (0/1) at each time point, with special emphasis on the 12-month result.
2. **Within-group change over time** (4 vs. 8 vs. 12 months) in the proportion of patients with sensory deficit.

2.2 Secondary endpoints

Secondary endpoints include:

- **Subjective functional scores:**
  - Modified Cincinnati score (continuous),
  - IKDC score (continuous),
  - Tegner Lysholm score (continuous),
  - Tegner Activity Score (ordinal/continuous).
    Each is measured preoperatively (baseline) and at 12 months postoperatively.
- **Demographic and perioperative characteristics:**
  - Age (years),
  - Body mass index (BMI, kg/m²),
  - Time from injury to surgery (months),
  - Length of hospitalization (days).

These are used to describe and compare the two groups at baseline.

- **Safety endpoints:**
  Clinical complications (infection, wound problems, thromboembolic events, range of motion restriction, graft rupture) observed during follow-up. These are summarized descriptively (frequency and percentage in each group).

3. General Statistical Principles

- All statistical tests will be **two-sided**.
- The nominal significance level is **$p < 0.05$**.
- For multiple pairwise comparisons of the same endpoint (e.g., sensory deficit across the three follow-up time points), **Bonferroni correction** will be applied ($\alpha\_corrected = 0.05$ / number of comparisons).

- Continuous variables will be summarized as **mean, standard deviation, median and range**.
- Categorical variables will be summarized as **absolute frequencies and percentages**.
- The distribution of continuous variables will be assessed using the **Kolmogorov–Smirnov** and **Shapiro–Wilk** tests. Because most continuous variables deviate from normality or sample sizes are modest, **non-parametric tests** will be used for between- and within-group comparisons.

All analyses will be performed using **R software**, version 4.3.1 (2023-06-16).

4. Baseline Comparisons

Baseline comparability of the Vivostat and Standard groups will be assessed for:

- age, BMI, time from injury to surgery, length of hospitalization.

Since these are continuous variables, they will be compared between groups using the:

- **Wilcoxon rank-sum test** (Mann–Whitney U test).

Results will be presented as in Table 1 (mean, SD, median, range, and p-values). These analyses are descriptive and serve to document the similarity of groups at baseline in this non-randomized cohort.

5. Analysis of Subjective Functional Scores

For each subjective functional score (Modified Cincinnati, IKDC, Tegner Lysholm, Tegner Activity), the following analyses will be performed:

1. **Between-group comparison at baseline:**
    - Vivostat vs. Standard, using **Wilcoxon rank-sum test**.
2. **Between-group comparison at 12 months:**
    - Vivostat vs. Standard, using **Wilcoxon rank-sum test**.
3. **Within-group change from baseline to 12 months:**
    - For each group separately, the change over time will be tested using the **Wilcoxon signed-rank test** (paired, non-parametric).

P-values will be reported for:

- Baseline vs. 12-month comparison within each group;

- Between-group comparisons at baseline and at 12 months (as in Table 2).

The Tegner Activity Score will be analyzed in the same way; in the current dataset, no statistically significant change over time was observed.

6. Analysis of Sensory Deficit (Primary Endpoint)

6.1 Between-group comparisons at each follow-up

At 4, 8 and 12 months, the proportions of patients with and without sensory deficit (0 vs. 1) will be summarized for each group.

- For **between-group comparisons** (Vivostat vs. Standard) at each time point, a **Chi-squared test** will be performed when expected cell counts are adequate. If assumptions are not met, **Fisher's exact test** may be used.
- In the current dataset, statistical significance is expected at 8 months ($p < 0.05$) and 12 months ($p < 0.01$), but not at 4 months.

Results will be reported as in Table 3, with counts, percentages, and p-values.

6.2 Within-group change over time

To assess changes in sensory deficit over time (4 vs. 8 vs. 12 months):

- For **overall comparison across the three time points** within each group and in the total sample, **Cochran's Q test**will be used.
- If Cochran's Q test is significant, **pairwise McNemar's χ^2 tests** will be used to compare:
  - 4 vs. 8 months,
  - 4 vs. 12 months,
  - 8 vs. 12 months.

Bonferroni correction will be applied for these three pairwise tests ($\alpha = 0.05/3 \approx 0.0167$). The corrected p-values and interpretation will be presented as in Table 4.

These analyses identify the postoperative interval with the most pronounced sensory recovery (in the current study: mainly between 4 and 8 months in the Vivostat group, with stabilization thereafter).

7. Power Calculation

After completing data collection, a **post-hoc power calculation** for the primary endpoint at 12 months will be performed:

- Observed proportions without sensory deficit at 12 months:
  - Vivostat group: 79% (0.79),
  - Standard group: 34% (0.34).
- Group sizes: $n_1 = 24$ (Vivostat), $n_2 = 29$ (Standard).
- Test: two-sided pooled z-test for difference in proportions, $\alpha = 0.05$.

The achieved power for detecting this difference is **0.93**, indicating that, despite being a pilot study, the sample size was sufficient to detect the observed effect size at 12 months with high probability.

This power calculation is descriptive and does not change the interpretation of the primary analysis.

8. Handling of Missing Data

In the conducted study all 53 patients completed follow-up; therefore, **no imputation of missing data** was necessary.

For the purposes of the statistical plan:

- In the event of missing follow-up data for future or extended analyses, only **complete-case analyses** will be performed (patients with available data for a given endpoint).
- The number and proportion of patients with missing data for each time point and endpoint would be reported descriptively.
- No multiple imputation or advanced methods are planned, given the pilot nature and small sample size.

9. Sensitivity and Exploratory Analyses

Given the modest sample size, no predefined subgroup analyses are planned. However, exploratory analyses could include:

- graphical presentation of sensory recovery over time in both groups (e.g., line plot of proportions without deficit, as in Figure 6),
- exploratory correlations between sensory deficit and subjective scores at 12 months (using Spearman correlation).

All such analyses will be clearly labeled as exploratory.

10. Deviations from the SAP

Any deviations from the planned statistical methods (e.g., use of alternative tests due to distributional assumptions) will be documented, justified and reported in the final manuscript or registry entry.