2023

Neurofilament light chain, inflammatory markers, calcitonin gene-related peptide, and kynurenine metabolites in patients with severe post-concussive symptoms

STATISTICAL ANALYSIS PLAN

NCT ID NOT YET ASSIGNED, FEBRUARY 28TH, 2023

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Statistical analyses plan

At baseline (before randomization):

1) **Concentration differences between groups**
Concentration differences of biomarkers between patients and controls were assessed using two-way ANOVAs with gender and group (patient/control) as factors. This approach was used since gender differences are known for some biomarkers.
Before the test was conducted, normality was assessed using QQ-plots after log transformation and Barlett's test was used to test for equal variances. Interaction between group and gender was tested, and removed from the model if it was non-significant.
If statistical significantly differences were found, a post-hoc-analyses using Fisher's protected least-significant difference were conducted.
If Barlett's test showed unequal variances, a mixed model allowing for variance heterogeneity was used.
If data was not normally distributed, Kruskal Wallis test was used.

Neurofilament light chain was included as the primary outcome in this study since it is highly specific for brain tissue, and multiple studies have shown concentration differences between healthy controls and concussion patients.
Calcitonin-gene related peptide was included as a primary outcome as well (separate study) since it is related to headache.

2) **Correlation between symptom score and biomarkers**
The correlation between biomarkers and symptom score measured by the Rivermead Post Concussion Symptoms Questionnaire was tested by Spearman's Rank Correlation or Pearson correlation coefficient (depending on whether the data were normally distributed or not).

Neurofilament light chain and TNF-alfa were included as the primary outcomes since multiple previous studies have found an association.

At follow-up (after randomization):
1) To test whether the concentrations changed at follow-up, a paired t-test was used on all concussion patients regardless of the intervention (n=54). Before conducting the test, normality of the differences was assessed using QQ-plots, and variances were assessed using Bland Altman plots. If the data were not normally distributed or had unequal variance, a Wilcoxon signed-rank test was used.

2) To test whether the non-pharmacological intervention had an effect on the biomarkers, a two-way ANOVA on concentration differences (baseline – follow-up) was performed with gender and intervention arm as the two factors. This was included as a secondary outcome in the study, since we

did not believe that the biomarker change would be sensitive enough to detect changes in RPQ/psychology due to the non-pharmacological intervention.