Statistical Analysis Plan - Functional coagulation measures among healthy males randomized to either prednisolone or placebo for ten days

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A randomized, placebo-controlled, double-blinded originating from *"The effect of curcumin on the development of Prednisolone-induced hepatic insulin resistance in overweight and obese participants" (CURPRED)*-study

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Introduction

Corticosteroids have many beneficial anti-inflammatory effects, and are used to treat e.g. chronic obstructive pulmonary disease, asthma, inflammatory bowel disease, rheumatoid diseases among other inflammatory diseases [1]. Short-term treatments with corticosteroids are commonly used in acute exacerbations of COPD, which is recommended by the Global Initiative for Chronic Obstructive Lung Disease [2].

Hyperglycemia, infections, and a variety of cardiovascular and clotting disorders are all well-known side-effects of treatment with corticosteroids[3-6]. Observational studies have linked the use of glucocorticoids with venous thromboembolism including pulmonary embolism[6, 7] as well as cardio- and cerebrovascular events [8, 9].

The effect on functional coagulation tests in the use of corticosteroids is largely unknown. To our knowledge, only one study has been performed. This study was on a population of patients with Cushing's syndrome, where rotation thromboelastometry was used comparing patients with Cushing's syndrome and matched controls, clotting time in the INTEM assay was shorter in patients with Cushing's syndrome compared to controls, where the rest of parameters in all assays were similar [10]. Otherwise, data on the topic are mainly from animal studies[11-15].

We are conducting a randomized, placebo-controlled, double-blinded trial investigating the effect of prednisolone on the functional coagulation in healthy individuals in addition to the effect of prednisolone on select hemostatic parameters.

Intervention

Patients will be randomized to one of the two treatment arms:

i) Intervention group: Prednisolone 50mg capsules x 1 for 10 days

ii) Control group: Placebo capsules x for 10 days

As the study originated from the CURPRED-study, 14 of the 34 participants were treated with either curcumin or placebo in addition to the prednisolone treatment.

The analyses in described in the following will be performed by Peter Kamstrup, MD, PhD-student and Ema Rastoder, MD, PhD-student under the supervision of Professor Jens-Ulrik Jensen, Respiratory Medicine Section, Department of Medicine, Copenhagen University Hospital – Herlev and Gentofte.

This document describes in detail the analyses that will be performed on the data originating from the study, on both primary and secondary outcomes.

The analyses will be performed in accordance with the CONSORT guidelines.

Data from randomized controlled trials should be analyzed according to predefined outcomes. To comply with this, the current document is written and published online while data collection is still ongoing from participants.

Analysis population

Data will be analyzed using the intention-to-treat principle. If relevant, per-protocol analyses will also be supplied.

Any participants who withdrew consent for participating in the trial will not be included for any analyses.

To facilitate recruitment, inclusion and exclusion criteria was changed following the inclusion of the first 14 participants. Most notably, the BMI inclusion criteria was changed from ≥25 kg/m^2 to >20kg/m^2.

Sample size

Based on the Maximal Amplitude in Thromboelastography (TEG), using a paired T-test. Assumptions for the power calculation: Level of significance 5%. Power 80%. Two-sided statistics. Standard deviation 4.0 mm, detectable difference 4.0 mm. A 1:1 distribution between active treatment and placebo. This results in a sample size of 32.

Analysis software

All analyses will be performed using the Statistical Analysis Software (SAS) Enterprise 7.1 Guide. Figures will be created in R 4.1.2 with RStudio 2022.07.01+554 using the ggplot2 package.

Data analysis

Descriptive analyses – baseline characteristics at day of randomization. The following characteristics will be summarised for each study group at baseline:

Demographic characteristics

Age, years, median (IQR)

Sex, n (%)

Ethnicity (Caucasian, Asian, African, Native Hawaiian / other pacific islander, American Indian or Alaska Native, Other/mix)

Clinical characteristics

BMI

Active smoker

Previous smoker

Never smoker

History of pack years

Systolic blood pressure

Diastolic blood pressure

Heart rate

Comorbidities, n (%)

Diabetes

Respiratory disease

Heart disease

Thyroid disease

Inflammatory disease

Any bowel disease

Biochemistry

HbA1c

Follow-up data

Missing data on baseline and any of the two follow-up visits will be reported (n (%)). In case of missing data on the outcome variables, complete case analysis will be applied if data is missing completely at random, which is expected to be the case. If this should not be the case, missing data will be handled using multiple imputation.

Adherence data

For both the intervention and control group the number of participants with 100% adherence will be reported. Participants with less than 70% adherence are excluded from the study and another participant is recruited as replacement.

Primary outcomes

Change in:

1. Maximal Amplitude in TEG
2. von Willebrand factor ristocetin cofactor activation
3. Antithrombin

Secondary outcomes

Change in:

4. Reaction time (R) in TEG
5. LY30 in TEG,
6. Clot strengthening (Angle) in TEG
7. Clot formation (K) in TEG
8. D-dimer-level
9. von Willebrand factor antigen-level
10. Protein C-level
11. Prothrombin-level
12. Fibrinogen-level
13. INR

Analyses of outcomes

Primary and secondary outcomes will be analyzed using a constrained Linear Mixed Model, allowing for management of any missing data as well as the possibility of adjustments. As a necessary consequence of the changed inclusion criteria during the study, skewness in baseline values between the intervention and control groups will be evaluated. If a skewness of BMI (difference > 3 kg/m^2) exists, the primary analysis will be adjusted for the study period as well as interaction between study period and intervention, to assure there is not a difference in treatment effect difference between the two inclusion periods.

If necessary, log-transformation is used to normalize data.

A p-value of 0.05 is considered statistically significant.

Sensitivity analyses

Sensitivity analyses will be performed for each outcome, investigating the effect of curcumin-intervention as an interaction term added to the previously described analyses.

Figures and tables

Table 1 will describe baseline characteristics on the intervention and placebo group.
Table 2 will show the results of analyses on primary and secondary outcomes.

Figure 1 will be a Consolidated Standards of Reporting of Randomized Trials (CONSORT) diagram. Figure 2 will illustrate spaghetti plots for the primary outcomes. Figure 3 will illustrate spaghetti plots for the secondary outcomes.

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Conflicts of interest

None of the contributors declare any conflicts of interest.

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