The Statistical Analysis Plan of the HI-LOOP Study

This Statistical Analysis Plan (SAP) was written according to the guidelines proposed by Yuan et al. [1] and by Gamble et al. [2] for randomized clinical trials.

1. SAP versions, dates and reasons for revision

Version #1: February 24th, 2026

2. Trial registration number

NCT Number: NCT07409649

3. Study title

Influence of intermittent hypoxemia on Loop Gain in healthy subjects: HI-LOOP

4. SAP contributors with roles and responsibilities

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5. Background and rationale of study

Sleep apnoea hypopnoea syndrome (SAHOS) is responsible for a number of co-morbidities, notably cardiovascular, and is currently widespread throughout the world, with significant healthcare costs.

Current research in this field focuses on exploring different phenotypes in patients with the disease, in order to be able to offer increasingly personalised care.

Several mechanisms can contribute to the development of obstructive sleep apnoea hypopnoea syndrome (OSAH), including ventilatory control, which can be assessed using Loop Gain. In addition, the various mechanisms contributing to the occurrence of different phenotypes in the context of OSAH depends on an endothelial response mediated by extracellular vesicles, which play a role in intercellular communication and which, in the context of OSAH, are responsible for vascular hyperreactivity in response to intermittent hypoxia.

As part of our protocol, we would like to study ventilatory control via Loop Gain in intermittent hypoxia with study of the phenotype of microvesicles in healthy subjects in order to better elucidate the mechanisms contributing to the onset of sleep apnoea in the absence of confounding factors (obesity, cardiovascular pathologies, etc.).

6. Study type

This is a prospective, interventional, comparative, randomized, single-blind, 2-groups with cross-over and a wash-out period of 7 days, single-centre pathophysiological study in healthy volunteers.

7. Objectives and related endpoints (units and calculations used to derive outcomes)

7.1. Primary objective

The primary objective is to evaluate the effect of hypoxemia conditions during 6 hours on the Loop Gain in healthy patients.

The primary outcome is the difference between the average value (over 5 minutes) of the Loop Gain before and after 6 hours experimental condition. The loop gain is defined by the product of the plant and controller gains.

7.2. Secondary objectives

The secondary objectives are:

- To compare the evolution of the controller gain and plant gain between hypoxia condition and ambient air condition. The evolution of the controller and the plant gains will be defined by the difference between, respectively, the average value (over 5 minutes) of the controller gain and the average value of the plant gain before and after experimental condition.
- To compare the change in the quantity of extracellular vesicles between hypoxia condition and ambient air condition. The change in the quantity of extracellular vesicles will be defined by the difference between before and after experimentation for each of the following extracellular vesicles: CD41, CD235a, CD45, CD146, CD66b, CD11b, CD62L CD62E, CD62P, CD62P/platelets ratio, CD162, annexin V
- To evaluate the correlation between the change in the quantity of extracellular vesicles and the evolution of the Loop Gain, by distinguishing between subjects exposed to intermittent hypoxia and ambient air.
- To compare the evolution of the Loop Gain between hypoxia condition and ambient air condition, by distinguishing patients with or without hyperventilation syndrome. The hyperventilation syndrome will be determined by a Nijmegen score upper or equal to 23 calculated at the inclusion visit.

8. Inclusion criteria

- Healthy volunteer aged [18 – 45] years
- IMC between [18 – 25] kg/m^2
- No known sleep disorders
- Free subject, without guardianship or curatorship or subordination
- Patients benefiting from a Social Security system
- Having given their informed consent.

9. Non-inclusion criteria

- Active smokers or quit smoking less than 3 months ago and total consumption of more than 10 packs-years
- Alcohol or drug addiction
- Heavy coffee drinker (>3 espressos per day)
- History of acute mountain sickness (presence of symptoms such as vertigo, headache, nausea/vomiting, and incapacitating fatigue during or after a stay at high altitude)
- High-altitude living (above 3,000 meters, continuously for more than 6 months during the last 10 years)
- History of respiratory disease and/or cardiovascular and/or renal and/or neurological disease (migraines, epilepsy)
- Diabetes
- Anemia, sickle cell disease
- Any medication associated with oxygen metabolism and any psychotropic medication (anxiolytics, sedatives, antidepressants, neuroleptics, muscle relaxants, etc.) that may interfere with motor and respiratory control, muscle strength or sleep quality
- Women of childbearing age without effective contraception
- Concurrent participation in another clinical research study affecting respiratory control or respiratory muscles

- Persons benefiting from enhanced protection, namely minors, persons deprived of their liberty by a judicial or administrative decision, persons staying in a health or social car institution, adults under legal protection and patients in emergency situations.

10. Exclusion criteria

- SpO_2 < 97% in ambient air at rest
- Detection of pregnancy in women of childbearing age
- Detection of acute mountain sickness (Lake Louise score > 0)
- Abnormalities in spirometry, ventilatory polygraphy, electrocardiogram, and/or hemoglobinometry prior to randomization
- Ventilation minute signal, PETCO2 or PETO2 unusable
- Irreversible deterioration in the volunteer's health during the study period, likely to affect the experimental conditions.

11. Definition of the two compared experimental conditions

- The test experimental condition is defined by a day with intermittent hypoxia application via hypoxia chamber.
- The control experimental condition is defined by a day under ambient air.

12. Randomization details

Randomization will be used to determine the order in which each subject will participate over the two days:

- Arm A: control experimental condition, then test experimental condition after a washout period of between 7 and 28 days.
- Arm B: test experimental condition, then control experimental condition after a washout period of between 7 and 28 days.

Subjects will be randomized with a 1:1 allocation using a secure web-based random-number generator.

13. Sample size calculation

The expected difference before/after experiment is 0.07 under test experimental condition [3]. We expect no loop gain variation (difference = 0) under control experimental condition. Considering an absolute difference of 0.07, with a standard deviation of 0.09, with a two-sided alpha risk of 5% and a power of 90%, we need to include 20 subjects in this cross-over study. In order to comply with the normality conditions used for calculating the sample size, the needed sample size is 30 subjects. With a non-usable data rate of 15% and a rate of ineligible subjects after review at the inclusion visit of 10%, we plan to include 40 subjects.

14. Interim analysis (timing, person, adjustment of the significance level)

No interim analysis was planned.

15. Guidelines for an early stop of the study

The study may be prematurely suspended in the event of unexpected adverse events. On the same token, in the event of unexpected developments or new information and showing that the objectives of the study or the clinical program are unlikely to be reached, the sponsor may be persuaded to prematurely suspend the study.

No statistical discontinuation criteria have been drawn up.

16. Reporting the representativity of the studied population

A flowchart (**Deliverable #1**) will be decomposed according to the following sets. The effectives and the related differences in-between two consecutive sets will be reported. The causes of the related differences will also be listed.

- **The screened set**. The patients having signed the informed consent.
- **The intention-to-treat (ITT) analysis set.** The patients who were randomized according to their allocated group, regardless of their adherence to the entry criteria, regardless of the intervention they received, and regardless of subsequent withdrawal from intervention or deviation from the protocol.
- **The full analysis set (FAS)**. The previous ITT population, excluding participants who failed to meet major entry criteria [4] and participants with no data post-randomization or with missing primary outcome.

17. Withdrawals and follow-up data

Patients may be withdrawn early from the clinical investigation for several reasons, including but not limited to consent withdrawal, lost to follow-up, death, non-operated patients. The investigator will document the reasons for premature study discontinuation. The withdrawals will be exhaustively described in the flowchart.

18. Definition of the studied population

All the analyses will be performed based on the full analysis set.

Patients who have withdrawn their consent prior to randomization will be replaced. If a patient withdraws consent, he/she will be asked for permission to use data obtained prior to withdrawal and to collect the primary outcome. If this is achieved, the patient will be included in the final analyses. If the patient declines, all data from that patient will be destroyed (except for safety data) and a new patient will be randomized.

19. Level of statistical significance (p-values, confidence intervals, one- or two-sided)

For the primary and secondary objectives, statistical significance will be achieved for p-value < 5% by using two-sided alternative hypothesis and two-sided 95% confidence interval.

20. Plan and rationale for controlling the type I error (multiplicity of tests)

We will not consider the inflation of the type I error. The level of the proof will be the higher for the primary outcome. The statistical analyses related the secondary outcomes will be considered as exploratory results with a risk of false positive.

21. Sub-group analyses and/or tests of interactions

No sub-group analyses or tests of interactions are planned.

22. Description of baseline patient characteristics

Demographic variables and other baseline characteristics will be summarized in a descriptive table (**Deliverable #2**) for the full analysis set.

The means, the standard deviations and the number of missing values will be presented for each arm and marginally for the following continuous variables: age (in years), body mass index (in kg/m^2), Nijmegen's score (0-64), Lake Louise score (0-12), heart rate (in bpm), systolic blood pressure (in mmHg), diastolic blood pressure (in mmHg), SpO2 (in %), hemoglobin (in g/dL), apnea-hypopnea index (per hour), Forced Expiratory Volume in 1 second / Forced Vital Capacity (in %).

The effectives, the percentages of patients in each category and the number of missing values will be presented for each arm and marginally for the following categorical variables: gender (male/female).

No p-value will be reported. The balance diagnostics of baseline covariates between the two randomized arms will be achieved by estimating standardized differences [5]. Unbalances will be considered for values higher than 10%.

23. Analysis of the outcomes

The outcomes of the patients will be described and compared for the full analysis set.

The Loop Gain, the controller gain, the plant gain and each extracellular vesicles will be describe before and after experimentation according to the experimental condition (Deliverable #3).

We expect very low percentage of missing data on the primary and secondary outcomes.

The interaction between the experimental condition and the period will be tested using a mixed model for each outcome to verify that there is no effect related to the order of experimental conditions. If we find a significant interaction, the analysis will be performed using only the first period.

For the main analysis, a linear mixed model approach will be used to estimate the difference between the primary outcome measured in the two compared groups (test and control experimental conditions). The experimental condition and the period will be included as fixed effects and the subject will be included as random effect to account for within-subject correlations arising from the cross-over design. The difference between the two experimental conditions and its 95% confidence interval will be presented (Deliverable #4).

The same strategy will be used to estimate the effect of hypoxia on the evolution of the controller gain, plant gain and the quantity of extracellular vesicles. The differences between the two experimental conditions and their 95% confidence interval will be presented in the same table (Deliverable #4).

To evaluate the change in the quantity of extracellular vesicles according to the change in Loop Gain, linear models will be constructed for each extracellular vesicle with the Loop Gain difference before/after the experimentation. We will look for the best Loop Gain transformation (logarithm, loess, B-spline function…). The best transformation will be determined by selecting the model that minimizes the Akaike information criterion (AIC). The likelihood-ratio test will be performed between the model chosen with the Loop Gain as adjustment factor and the model without Loop Gain. This strategy will be performed by separating data relating to test experimental condition and relating to control experimental condition.

Loop Gain evolution will be modeled with a linear mixed model. The experimental condition and the period will be included as fixed effects and the subject will be considered as random effect. This strategy will be performed by separating patients with hyperventilation syndrome and patients without hyperventilation syndrome.

24. Graphical presentation of treatment effects

We will present, for subjects exposed to test experimental condition and for those exposed to control experimental condition, the evolution of each extracellular vesicles quantity according to the change in Loop Gain. The p-value of the likelihood-ratio test will be presented (Deliverable #5).

25. References

[1] Yuan I, Topjian AA, Kurth CD, Kirschen MP, Ward CG, Zhang B, et al. Guide to the statistical analysis plan. Paediatr Anaesth. mars 2019;29(3):237‑42

[2] Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA. 19 déc 2017;318(23):2337‑43.

[3] Gederi E, Nemati S, Edwards BA, Clifford GD, Malhotra A, Wellman A. Model-based estimation of loop gain using spontaneous breathing: A validation study. Respir Physiol Neurobiol. 15 sept 2014;201:84-92.

[4] Fergusson D, Aaron SD, Guyatt G, Hébert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. BMJ. 2002 Sep 21;325(7365):652-4.

[5] Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med. 10 nov 2009;28(25):3083-107.