Amendment to the statistical Analysis Plan

Title

Hypoxic burden, ventilatory burden and OSA endotype analysis

Additional analysis planned in the study: A randomized cross over trial exploring the Sleep Revolution diagnostic and therapeutic pathway in patients with obstructive sleep apnea

- **Referred to the original Study Protocol Number:**
  SR 001.3
- **Amendment number**: 1.0
- **Date for amendment:** 3rd of November 2025

The following amendment has been formulated after a new software package for data analysis has become available for future analysis of the sleep studies within the study.

Background:

New data analysis tools will be utilized on the data in the study. Automated analysis software developed by Nox Medical; Reykjavik, Iceland, and the Star Sleep Research Group from the University of Eastern Finland in Kupio will extract additional respiratory parameters from the polysomnography/sleep analysis system (PSG/SAS, Nox A1) recordings used in the study referred to above. In the NOX software package, respiratory events are detected through a deep learning model based primarily on RIP signals, supplemented by nasal cannula input when available. The postprocessing stage computes both classical sleep parameters, such as the Apnea–Hypopnea Index (AHI) and Respiratory Disturbance Index (RDI), and a number of additional research metrics that characterize the physiological burden and mechanisms of obstructive sleep apnea (OSA). The ABOSA software by the STAR team will analyse the hypoxic load during sleep. In detail, these novel metrics include measures such as:

A) **Ventilatory burden (VB),** which quantifies the cumulative deviation of ventilation from eupneic levels during respiratory events. It represents the **event-specific area under the normalized ventilation signal**—capturing both the depth and duration of hypoventilation—summed per hour of sleep (Labarca et al., 2023, PMID: 37418748). VB characterizes the total ventilatory reduction during respiratory events rather than simply counting events or measuring downstream effects such as desaturation or arousal. In large cohorts, higher VB has been associated with incident cardiovascular disease and

mortality, with a magnitude similar to hypoxic burden (Labarca et al., 2023). Because VB explained most of the variation in hypoxic burden, it likely represents the underlying ventilatory disturbance that gives rise to hypoxemia. In this study, VB will be calculated as average event depth × average event duration × AHI, expressed in [% eupnea × min / h], reflecting the same principle described by Labarca et al. (2023).

B) Hypoxic Burden (cumulative desaturation load, also described as the desaturation severity parameter), a physiologically informed metric that quantifies the cumulative impact of oxygen desaturation events during sleep in patients with obstructive sleep apnea (OSA). It is calculated as the total area under the oxygen saturation curve during respiratory events, integrating the **frequency, depth, and duration of desaturations**. Several methods to calculate hypoxic burden are described and an internationally accepted standard for calculating and reporting hypoxic burden is not defined yet. In the study we have at least two software solutions for advanced oximetry analysis available: ABOSA and NOX analysis software for overnight hypoxic load. Elevated hypoxic load during sleep has been associated with increased risk of cardiovascular and neurocognitive outcomes, making it an important research topic to analyse the different parameters as diagnostic and prognostic markers in sleep medicine.

C) Pathophysiological endotyping
In addition, the NOX software package derives sleep-apnea endotypes based on established physiological trait modelling, providing estimates of **loop gain, arousal threshold, upper-airway collapsibility and compensation, circulatory delay, and ventilatory response to arousal**. These traits can be quantified using advanced analysis of polysomnography data, enabling a more personalized approach to diagnosis and treatment. Unlike traditional metrics like the apnea-hypopnea index (AHI), endotyping provides insight into the mechanisms driving sleep apnea in each individual. This approach aims to support precision medicine by guiding targeted sleep apnea therapies such as oral appliances, upper airway surgery, hypoglossal/phrenic nerve stimulation or pharmacotherapy. However, endotyping is not yet part of clinical routine procedures and several scientific questions have been recently published in a consensus statement (Tolbert et al. 2025. PMID 40737283). The study data set fits very well to answer the current questions regarding the clinical validity of endotyping in OSA.

Outcome parameters within the three outlined thematic areas will be calculated by the novel algorithms on the data cluster at Reykjavik University by experienced researchers within the Sleep Revolution network. This provides new opportunities for addressing the following and additional future scientific questions posed to the dataset.

New analysis proposals to be listed in an amendment of the statistical analysis plan:

<u>Methodological questions (reliability, precision, noise etc.), for example:</u>

A) What are normal and patient population means of
   a. Hypoxic burden
   b. Ventilatory burden
   c. OSA pathophysiological endotypes

B) Night to night variability: The setting of the study with three nights of self-applied polysomnography in more than 700 patients allows the calculation of night-to-night variability of
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
C) Within night variability: The night will be subdivided in tertiles of quartiles and within and between night variability of the following metrics will be characterized:
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
D) Sleep stage dependent variability: The night will be subdivided in the EEG-based sleep stages Ni-N3 and REM sleep and the within and between night variability of the following metrics will be characterized:
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
E) What amount of data and which quality of data is necessary to get a reliable assessment of
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
F) Compare SAS derived (manual scoring) to HSAT + AI (automatic scoring) in terms of
    a. AHI and other traditional PSG derived parameters
    b. Hypoxic Burden, Ventilatory Burden and sleep apnea endotypes

<u>Association between the new metrics and sleep apnea severity and comorbidities</u>

A) What is the association between established **sleep apnea severity** measures (frequency, duration, hypoxia) and
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
B) What is the association between **sleep apnea symptoms** and
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
C) What is the association between objective and subjective **cognitive function** and
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
D) What is the association between different **measures of body composition and obesity** and
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes

E) What is the association between **breathing routes during sleep** (predominately oral/nasal or combined) and
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
F) What is the association between **cardiovascular comorbidity** (e.g. hypertension, ischemic heart disease) and
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
G) What is the association between **respiratory disease** (e.g. COPD, asthma, respiratory failure) and
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes
H) What is the association between **metabolic comorbidity** (e.g. diabetes, hyperlipidemia, hypothyreosis) and
    a. Hypoxic burden
    b. Ventilatory burden
    c. OSA pathophysiological endotypes

<u>Do these new metrics influence the final diagnosis of sleep apnea and the decision for treatment?</u>

The study enables to analyse the predictors for the final diagnosis of sleep apnoea at the end of the diagnostic process. Thereby the following analysis will be performed:

- Predictive capacity of the three parameters for the final diagnosis and severity classification of OSA
- Predictive capacity of the three parameters for the sleep doctor's Clinical Global Impression Scale rating at the end of the diagnostic process (AHI, ODI, HB, VB, endotypes, comorbidities)
- Predictive capacity of the three parameters for the final decision on treatment (yes/no) and type of treatment (CPAP versus oral device, other treatment combinations)

<u>Do these new metrics have predictive capacity for treatment outcome?</u>

To be analysed in the following treatment related questions: Association between the three novel diagnostic parameters and

A) Adherence with and acceptance of Positive Airway Pressure (PAP) Treatment
B) Reduction of daytime sleepiness with OSA treatment?
C) Efficacy of non-PAP treatments?
D) Frequency and severity of side effects with PAP- or other treatments.
E) Changes of blood pressure, heart rate, and vascular stiffness by CPAP treatment
F) Other treatment aspects.