SEMALCO- Statistical Analysis Plan

Does the Glucagon-like Peptide 1 (GLP-1) receptor agonist semaglutide reduce alcohol intake in patients with alcohol use disorder and comorbid obesity?

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Abbreviations

AUD	Alcohol Use Disorder
AUDIT	Alcohol use disorder identification test
BOLD-response	Blood-oxygen-level-dependent response
CBT	Cognitive behavioral therapy
DSM-5	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion tension imaging
fMRI	Functional magnetic resonance imaging (brain-scan)
GABA	gamma-aminobutyric acid
GLP-1RA	Glucagon-like peptide-1 receptor agonist
Heavy drinking day	Alcohol consumption over 60/48 g (men/women) of alcohol per day
ICD-10	International Classification of Diseases
MRS	Magnetic Resonance Spectroscopy
PEth	Phosphatidylethanol - Biomarker for alcohol consumption
RCT	Randomised clinical trial
REDCap	Web application for building and managing online surveys and databases
TLFB	Time-Line-Follow-Back Method (recording of heavy drinking days)
WHO	World Health Organization

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Introduction

Alcohol use disorder (AUD) is a chronic brain disorder characterised by loss of control of alcohol intake, a negative state when not consuming alcohol, and compulsive alcohol behaviour, leading to relapse.[1] Globally, alcohol use is a huge burden, and an estimated 280 million people globally suffer from AUD.[2] In 2016, three million deaths were caused by the harmful use of alcohol. In Denmark, 14% of the Danish population has a harmful consumption of alcohol, and 3% fulfils the criteria for AUD.[3] Despite this, the treatment gap is wide compared to other mental health disorders[4] - a Danish study has reported an all-cause cumulative 15-year mortality rate of 29% after a first-time hospital contact due to alcohol.[5] This high mortality rate might be due to medical conditions and complications such as alcoholic liver disease, injuries,[1] fracture,[5] heart disease, stroke, cancer,[6] or suicide.[7] In this perspective, AUD has serious consequences for the individual, families and friends[6] and the society due to higher health care[5] and socio-economical costs.[2,8] This is also why alcohol is reported as the most harmful drug of addiction; the harm to both users and others taken into consideration.[9]

One of the best-documented modalities in the treatment of AUD is cognitive-behavioural therapy (CBT).[10–12] The underlying neuroanatomical basis of alcohol addiction and treatment effects of CBT are not yet established, though functional Magnetic Resonance Imaging (fMRI) studies have begun to elucidate the neuroanatomical basis. The pharmacological treatment of AUD is considered an important supplement to psychological therapy. Disulfiram,[13] a substance that blocks alcohol-metabolising enzymes resulting in increased acetaldehyde concentrations, was introduced in Denmark many years ago and is still the most used drug against AUD in Denmark. Newer pharmacological agents such as acamprosate[13] and naltrexone[13] have been introduced to the clinic. However, despite numerous randomised clinical trials (RCT), these compounds have not gained widespread dissemination, probably because the effect of these substances has been modest, with a less than 10% increase in abstinent rate compared to placebo.

As the success rates of CBT are moderate and the synergistic effects of adding pharmacological treatment – as described above – are quite limited, strong needs still exist for efficient, molecular targets in the medical treatment of alcohol use disorder.[14]

GLP-1-based therapy was introduced to the market in 2006, and since then, several GLP-1 (GLP-1RAs) have been approved for treating type 2 diabetes and/or obesity. The proposed GLP-1RA semaglutide is reported to be superior to previous GLP-1 RAs approved for weight loss,[15] and was approved by the FDA to treat obesity in June 2021 (Wegovy®). Several pre-clinical studies report of GLP-1RAs crossing the blood-brain

barrier to some extent,[16–19] and semaglutide is reported to access the brainstem, septal nucleus, and hypothalamus.[16]

Several GLP-1RAs have been evaluated in pre-clinical addiction models regarding their effects on alcohol consumption in mice, rats, and nonhuman primates. Also, register-based cohort studies, brain imaging, and preliminary clinical data indicate that GLP-1RAs could be a potential new treatment for AUD.[20] However, larger RCTs are needed to investigate whether these beneficial findings on alcohol consumption can be extended to encompass patients with known alcohol use disorder.[21]

Hypotheses

Treatment with semaglutide will:

- Cause a greater decrease in alcohol consumption compared with placebo, measured as the total number of heavy drinking days, in patients with AUD and comorbid obesity
- Cause a reduced fMRI BOLD response compared with placebo in reward processing regions (ventral and dorsal striatum, putamen, nucleus accumbens, and caudate), including the septal area
- Cause a greater increase in brain GABA levels compared with placebo, measured with an MRS scan

Study Methods

Trial design

The SEMALCO trial is a 26-week, randomised, double-blinded, placebo-controlled clinical trial.[22] Comparative treatment regimens were once-weekly injections with semaglutide 2.4 mg (Wegovy®) or placebo (saline) as a supplement to cognitive behavioural therapy. Baseline data were collected at the screening session. Follow-up meetings were scheduled at weeks 6, 8, 12, 16, 20, and a final evaluation at week 26. A subgroup of patients had a combined baseline fMRI- and MRS scan performed before receiving their first injection and again when finalising the study.

Randomisation

The participants were randomised into two groups: semaglutide or placebo. The randomisation was stratified in terms of age, sex, and baseline alcohol consumption, i.e., the number of heavy drinking days in the recorded 30 days before inclusion measured by the TLFB method (6-11, 12-17, 18-23, 24-30 days). The randomisation tool in REDCap was used for this stratified randomisation. Patients, investigators, other

caregivers performing assessments, and persons performing data analysis were blinded from the time of randomisation until the time of database unlock. To maintain the blinding, the participants were blindfolded, and as the active pen made a small sound when released, the patients were also provided with music in their ears. An un-blinded nurse prepared the injection in a separate room and gave the injection immediately after.

Sample size

The sample size calculation was based on the primary endpoint, i.e., the change in heavy drinking days from baseline to end of follow-up (week 26). No other studies have investigated the effects of semaglutide in treatment-seeking patients with alcohol use disorder. The sample size calculation is thus based on the alcohol study by Bogenschutz et al.,[23] investigating psilocybin-assisted psychotherapy as a novel treatment of AUD. They reported a reduction in the total number of heavy drinking days of 46.77% in the intervention group and 25% in the placebo group. With a power of 90%, an alpha of 5%, and an estimated standard deviation (SD) of 26.44, a total of 64 patients, 32 patients in each treatment group, is needed. Large drop-out rates are often observed in AUD intervention trials, with attrition rates between 10% and 35%.[24] Assuming a 40% drop-out rate, the total required sample size is 108, with 54 patients in each treatment group. All patients will be included at one research site (Psychiatric Centre Copenhagen, Frederiksberg Hospital).

Framework, Statistical interim analysis and stopping guidance

No superiority, equivalence, or noninferiority hypothesis testing framework was performed.

No interim analysis was performed.

Timing of final analysis

All analyses will be carried out with the treatment groups still blinded and labelled as “Treatment Group ~~A~~1” and “Treatment Group ~~B~~2”. Before dividing participants into “group ~~A~~ 1” and “group ~~B~~2”, the statistical analysis plan will be completed, signed, and uploaded at ClinicalTrials.gov, and the data set locked. The final unblinding of treatment groups (semaglutide or placebo), will not be carried out until all statistical analyses are performed.

Post-hoc analyses, if performed, will be conducted unblinded.

Timing of outcome assessments

All visits are planned from the date of the first injection and are performed at week 7, 9, 13, 17, 21, and 27.

All irregularities are recorded as protocol deviations, and allowed visit windows are within ± 2 weeks.

Statistical Principles

Confidence intervals and P-values

Statistical significance will be assessed using an alpha level of 0.05, with two-sided testing and a 95% confidence interval. There will be no adjustment for multiplicity, and no additional adjustments for covariates, except baseline values, will be performed.

Adherence and protocol deviations

Adherence to the intervention was defined by no more than three missed consecutive injections or six missed injections in total during the 26-week period. The un-blinded project nurses kept an electronic individual injection schedule per patient, tracking the extent of exposure to the assigned treatment. Adherence to the intervention will be presented in a table showing the distribution of injection percentages and the number of therapy sessions. Protocol deviations will be presented in a table divided into the categories: study procedure, eligibility, and randomisation.

Analysis populations

All analyses will be performed using the intention-to-treat principle on subjects who received at least one dose of the trial compound (Wegovy® or placebo).

Trial Population

Screening data

Screening data will be presented in the CONSORT flowchart.

Eligibility

Individuals aged 18-70, BMI ≥30, diagnosed with AUD according to ICD 10 and DSM-5, with an AUDIT score >15 and at least six heavy drinking days in the past 30 days.* Furthermore, no severe psychiatric-, neurological- or somatic disease, history of alcohol withdrawal seizures, diabetes, other substance use disorder, or concomitant pharmacotherapy against alcohol use disorder. Please see the protocol paper for the full list of in- and exclusion criteria.[22]

The 30-day period will be the 30 consecutive days with the biggest alcohol intake (most heavy drinking days **and the largest amount of total alcohol intake) out of the 40 days prior to the evaluation, measured by the TLFB method*

Recruitment

Recruitment began on the 13th of June, 2023 and ended on the 14th of February, 2025. The last patient's last visit is in medio August, 2025. All participants were recruited from the capital region of Copenhagen and the region of Zealand, Denmark.

Withdrawal/Follow up

All participants who have withdrawn from the intervention and have been included for at least 12 weeks were encouraged to participate in the final follow-up at week 26, including brain scans at the time of withdrawal. Due to safety concerns, all participants were contacted by telephone during week 31 to evaluate any side effects. Drop-out data will be presented in a Kaplan-Meier survival curve as a function of the number of injections received (the week they dropped out).

Baseline Patient characteristics

Baseline characteristics will be descriptively summarised in a table assessed by the intervention.
List of baseline characteristics to be summarised:

- Sex, No. (%)
- Age, mean (SD)
- Age group (+/- 40 years)
- Education (lower secondary school, upper secondary school, vocational education + short-cycle higher education, medium cycle higher education + higher education), No. (%)
- Marital status (Cohabitation + married), No. (%)
- Job status (yes, no), No. (%)
- Previous treatment with a GLP-1 receptor agonist (yes, no), No. (%)
- Previous pharmacological treatment for AUD (disulfiram, acamprosate, naltrexone, nalmefene), No. (%)
- AUDIT score, mean (SD)
- AUDIT-C score, mean (SD)
- ICD-10 number of diagnostic items (4,5,6), No. (%)

- DSM-5 group (mild (2-3), moderate (4-5), severe (>5)), No. (%)
- Heavy drinking days*, mean (SD)
- Heavy drinking days group/randomisation strata* (6-11, 12-17, 18-23, 24-30), No. (%)
- Days without alcohol consumption (0-days)*, mean (SD)
- Total alcohol consumption (grams)*, mean (SD)
- Plasma PEth, mean (SD)
- WHO risk level (low, medium, High, very high), No. (%)
- Weight (kilogram), mean (SD)
- Body mass index (BMI), mean (SD)
- Plasma HbA1c, mean (SD)

  The 30-day period will be the 30 consecutive days with the biggest alcohol intake (most heavy drinking days **and the largest amount of total alcohol intake) out of the 40 days prior to the evaluation, measured by the TLFB method.*

Analysis

Primary endpoint

The primary endpoint is the change in alcohol consumption, defined as the change in the percentage of heavy drinking days during a period of 30 consecutive days*, from baseline to end of follow-up after 26 weeks, adjusted for baseline (expressed in percentage points (pp)). A heavy drinking day is defined as more than 60/48 grams (men/women) of alcohol in one day, measured with the validated timeline followback (TLFB) method.[25]

The 30-day period will be the 30 consecutive days with the biggest alcohol intake (most heavy drinking days **and the largest amount of total alcohol intake) out of the 40 days prior to the evaluation, measured by the TLFB method.*

Secondary endpoints

Changes from baseline to last assessment after 26 weeks of treatment with semaglutide vs placebo in:

1. Total alcohol consumption (gram ethanol/last 30 consecutive days*)
2. Number of days without alcohol consumption (last 30 consecutive days*)
3. Change in drinks per day (last 30 consecutive days*)
4. Time to relapse, defined as the time to first alcohol intake
5. Time to first heavy drinking day

6. WHO alcohol risk levels (last 30 consecutive days*)
7. Alcohol craving (PACS score)
8. Alcohol Use Disorder Identification Test (AUDIT) score
9. Alcohol Use Disorder Identification Test (AUDIT-C) score
10. Blood Phosphatidylethanol (PEth)
11. Fibrosis-4 (FIB4) score
12. Blood gamma-glutamyl transferase (GGT)
13. Blood alanine transaminase (ALT)
14. Blood mean corpuscular volume (MCV)
15. Plasma amylase
16. Plasma semaglutide
17. Body weight (kg)
18. Blood pressure (mmHg)
19. Pulse (PR)
20. Waist circumference (cm)
21. BMI
22. Glycaemic control parameters (HbA1c)
23. Drug Use Disorders Identification Test (DUDIT) score
24. WHO quality of life - BREF (WHOQOL-BREF) score
25. Fagerströms Test for Nicotine Dependence score

Descriptive statistics

The number of cognitive behavioural sessions during the follow-up period will be described by treatment group in order to assess if the individuals in the two treatment groups received equal amounts of therapy, and including any self-selected post-intervention treatment undertaken by the participants, if any. Also, the change in the percentage of heavy drinking days during a period of 30 consecutive days*, from baseline to end of follow-up after 26 weeks will be plotted against (a) the maximum semaglutide dose during the 26 weeks of treatment, and (b) the weight loss at the end of follow-up.

Analysis methods

All continuous outcomes, i.e., change in metabolic parameters, weight, body composition parameters, alcohol use, etc. will be analysed using baseline adjusted ANCOVA. The two time to event outcomes (time to first alcohol intake and time to first heavy drinking day) will be analysed using Cox regression.

We will use residual plots and qq-plots in combination with Wally plots[26] to validate the normality assumption of the residuals. If the distributional assumption about normality of the residuals does not hold then we will first see if a log-transform of the outcome will result in a model fit that does not appear to violate the assumptions of normality. If the log-transform still does not hold, our next step will be to consider if the lack of fit is caused by inflation, e.g. due to too many observations being zero. Should that be the case we will replace the standard ANOVA model with a zero-inflated gaussian model for the analyses. Should none of these approaches work we will use quantile regression and model the median outcome instead.

Missing data/sensitivity analysis

Missing data will be imputed with the use of multiple imputations in the mice package[27] in R software[28], method = "pmm" (predictive mean matching), and 100 imputed datasets. The missing baseline and endpoint data will be imputed based on a linear imputation model based on all available repeated measures data for the outcome in question. As a sensitivity analysis, the complete case analysis will be performed. For the outcomes of heavy drinking days, total alcohol intake, and days without alcohol consumption two further sensitivity analyses regarding the missing data mechanism will be conducted: 1) assume that all non-observed individuals at 26 weeks have reverted to their pre-study drinking habit, and 2) assume that all non-observed individuals at 26 weeks have halved their drinking compared to the value at baseline.

Subgroup analysis

We will perform the following subgroup analysis for the primary outcome (change in alcohol consumption) and for the outcomes of total alcohol consumption and number of days without alcohol consumption:

- Baseline heavy days (6-11, 12-17, 18-23, 24-30)
- DSM-5 group (mild (2-3), moderate (4-5), severe (>5))

Tertiary analyses

Tertiary analyses will be conducted and reported in subsequent secondary publications:

- Proteomic fingerprint
- Brain imaging:
  - Brain alcohol cue-response (Blood oxygenation level-dependent signal (BOLD)) in reward-processing brain regions (ventral and dorsal striatum, putamen, nucleus accumbens, and caudate), including the septal area assessed by fMRI brain scans
  - Brain GABA levels (cortical, caudate, and putamen) assessed by MRS brain scans

- Resting state functional connectivity
- Diffusion tensor imaging (DTI)

Harms

Safety data has been collected in the 26 weeks of inclusion and up until 5 weeks after termination of the study for all individuals. The safety outcomes were classified as adverse events (AE), adverse reactions (AR), serious adverse events (SAE), serious adverse reactions (SAR), and suspected unexpected serious adverse reactions (SUSAR). All collected safety data will be summarized in a table with number of cases.

Statistical software

Statistical analyses will be performed using R.

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