STATISTICAL ANALYSIS PLAN

Effect of L-menthol on breathlessness and exercise capacity in COPD: a randomized crossover trial

Study code:	Ment-COPD
Date:	2025-11-17
NCT number:	NCT05888597
Sponsor:	Region Skåne, Represented by Pia Malmqvist Lung- och allergikliniken, SUS Lund
Principal Investigator	Zainab Ahmadi Pulmonary physician

Statistical Analysis Plan

The **primary outcome** is the post-dose difference in breathlessness intensity ratings during exercise at isotime, defined as the highest equivalent 1-minute interval of exercise completed by a given participant during each constant-load CPET. The **co-primary outcome** is the post-dose difference in exercise endurance time (EET), defined as the duration of loaded pedaling during constant-load CPET. Secondary outcomes include additional physiological and perceptual responses assessed at isotime and at peak exercise (end of test).

Data Presentation:

- Normally distributed continuous variables will be expressed as mean ± standard deviation (SD).
- Skewed continuous variables will be expressed as median with range or interquartile range (IQR).
- Categorical variables will be presented as frequencies and percentages.

Comparisons:

- Baseline characteristics will be compared using independent t-tests (continuous normal), Wilcoxon rank-sum tests (continuous non-normal), and chi-square tests (categorical).
- For CPET outcomes, within-subject comparisons between menthol and placebo will be performed using paired t-tests for normally distributed variables and Wilcoxon signed-rank tests for non-normal variables. Categorical outcomes will be analyzed using McNemar's test.
- CPET variables will be compared between conditions at isotime and peak exertion.

Modeling:
Changes during CPET may be further analyzed using mixed-effects regression models to account for repeated measures.
Reporting:
Estimates will be presented as mean differences (menthol minus placebo) with 95% confidence intervals (CIs). Statistical significance will be defined as two-sided $p < 0.05$.
Software:
Analyses will be conducted using Stata (StataCorp LP; College Station, TX) [or specify alternative if applicable].

Figures

Figures will be adapted from Abdallah et al. (2017) to illustrate perceptual and physiological responses during constant-load exercise testing. These will include:

- **Panels a–c:** Symptom ratings (breathlessness intensity, breathlessness unpleasantness, and leg discomfort) plotted against time during exercise for menthol and placebo conditions.
- **Panels d–f:** Post-dose differences (menthol minus placebo) at isotime and peak exercise for breathlessness intensity, unpleasantness, and exercise endurance time (EET), including individual participant data and group means with 95% CIs.
- **Panels g–i:** Distribution of individual responses and indication of clinically meaningful thresholds (e.g., minimally clinically important difference).

These figures will visualize symptom trajectories, treatment effects, and variability across participants, highlighting both statistical and clinical significance.

Figure 1: CONSORT diagram showing the flow of participants through each stage of the study

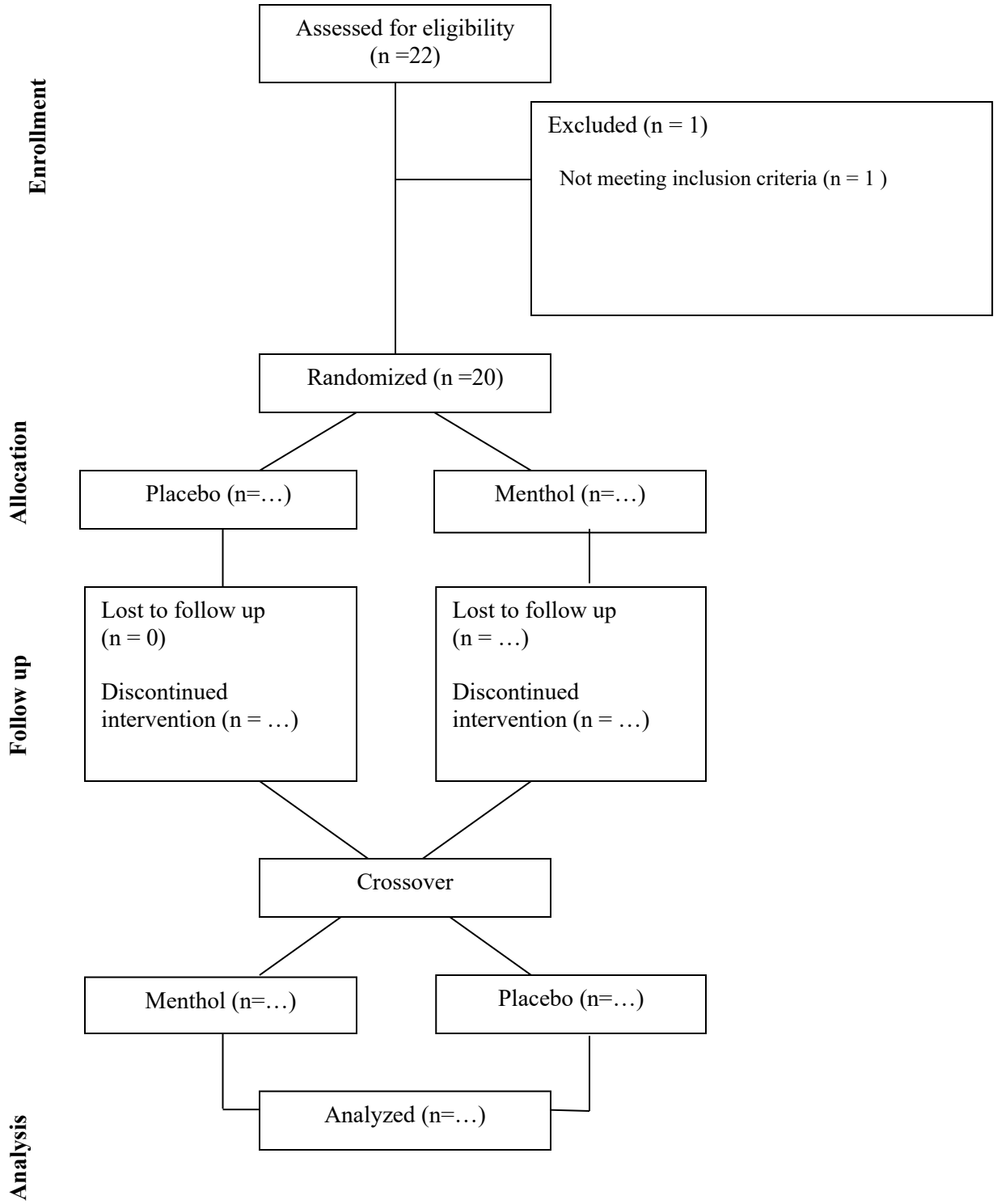

Table 1: Baseline participant characteristics (t-test for continuous variables with normal distribution, ranksum test for continuous non-normal variables and chi-square for categorical variables).

Parameter	Menthol first	Placebo first	All participants
N (%)			
Male:female (n)			
Age, mean years (SD)			
Height (cm)			
Weight (kg)			
BMI kg/m^2 , mean (SD)			
Smoking history, pack-years (SD)			
Physical activity during normal week			
Post-bronchodialator pulmonary function (spirometry)			
FEV1, L (SD)			
FEV_1, % predicted (SD)			
FVC, L (SD)			
FVC, % predicted (SD)			
FEV_1/FVC, L (SD)			
FEV_1/FVC, % of predicted (SD)			
Breahlessness and health status			
mMRC 0-4			
CAT total Score			
CAT breathlessness			
CAT activity limitation			
MDP A1 total			
Dyspnea 12			
Dyspnea severity likert			
SF12 q1 – q12			
Change between visit 1 and 2 (GIC-scale)			
Change between visit 2 and 3 (GIC-scale)			
Underlying conditions			
Ischaemic heart disease			
Angina Pectoris			
AF			
Heart failure			
Hypertension			
Hyperlipidemia			
Diabetes			

Obstructive sleep apnea			
Asthma			
Other lung disease			
Tuberculosis			
Rheumatic disease			
Stroke			
Cancer			
Depression or anxiety			
None			
Other			
Medications			
Diuretics			
Beta blocker			
Calcium antagonist			
Renin-angiotensin system blocker			
Insulin			
Other diabetic medication			
Anti hyperlipidemic drug			
Inhalators for COPD/asthma			
Anticoagulants			
Antidepressants/anxiolytics			
Sleep medication or other benzodiazepines			
Other			
COPD medication			
LABA+LAMA			
LABA+LAMA+ICS			
LABA			
LAMA			
LABA+LAMA+Daxas			
LABA+LAMA+ICS+Daxas			
Physiological responses at incremental cycle test (visit 1)			
Cycle exercise time (min)			
Workload, W			
Workload, % of predicted			
Heart rate, beats/min			
Breathing frequency (breaths/min)			
SpO_2 (%)			
$V_{O2,}$ mL/kg/min,			
V_{O2} (% of predicted),			
V'_{CO2} (ml/kg/min)			

RER			
$V'_{E,}$ L/min			
$V_{T,}$ L			
V'_E/V'_{CO2}			
Perceptual responses during test (visit 1)			
Breathlessness intensity, Borg units			
Breathlessness discomfort, Borg units			
Leg discomfort, Borg units			
Reasons for stopping exercise (visit 1)			
Breathlessness (n)			
Leg discomfort (n)			
Breathlessness and Leg discomfort (n)			
Breathlessness ratings after test (visit 1)			
MDP (A1 + dimensions)			
D12			
Self-reported motivation for completing test (visit 1)			

Table 2: Effect of menthol versus placebo on physiological and perceptual responses at a standardized submaximal time during constant-load exercise testing (isotime) and at the symptom limited peak of constant-load exercise testing among individuals with Chronic obstructive pulmonary disease. (t-test for continuous variables with normal distribution, ranksum test for continuous non-normal variables and chi-square for categorical variables). **(Visit 2 and 3)**

Parameter, mean (SD)	**Rest**			**Isotime**			**Peak**		
	Menthol	**Placebo**	**Mean difference (95% CI)**	**Menthol**	**Placebo**	**Mean difference (95% CI)**	**Menthol**	**Placebo**	**Mean difference (95% CI)**
Physiological responses									
Cycle exercise time, min									
Heart rate, beats/min									
Breathing frequency, breaths/min									
SpO2, %									
$V'_{O2,}$ ml/kg/min									
$V'_{O2,}$ % of predicted									
V'_{CO2} (ml/kg/min)									
RER									
$V'_{E,}$ L/min									
$V_{T,}$ L									
V'_E/V'_{CO2}									
PETH CO2									
Perceptual responses									
Breathlessness intensity (Borg CR10)									
Breathlessness unpleasantness (Borg CR10)									
Leg discomfort (Borg CR10)									
Significant breathlessness (>2, n (%)									
Reasons for stopping test									
Breathlessness (n)									
Leg discomfort (n)									

Breathlessness and Leg discomfort (n)									
Level of self-reported motivation									
Breathlessness ratings after tests									
MDP									
Dyspnea 12									

Mean differences are for menthol minus placebo.

Figures:

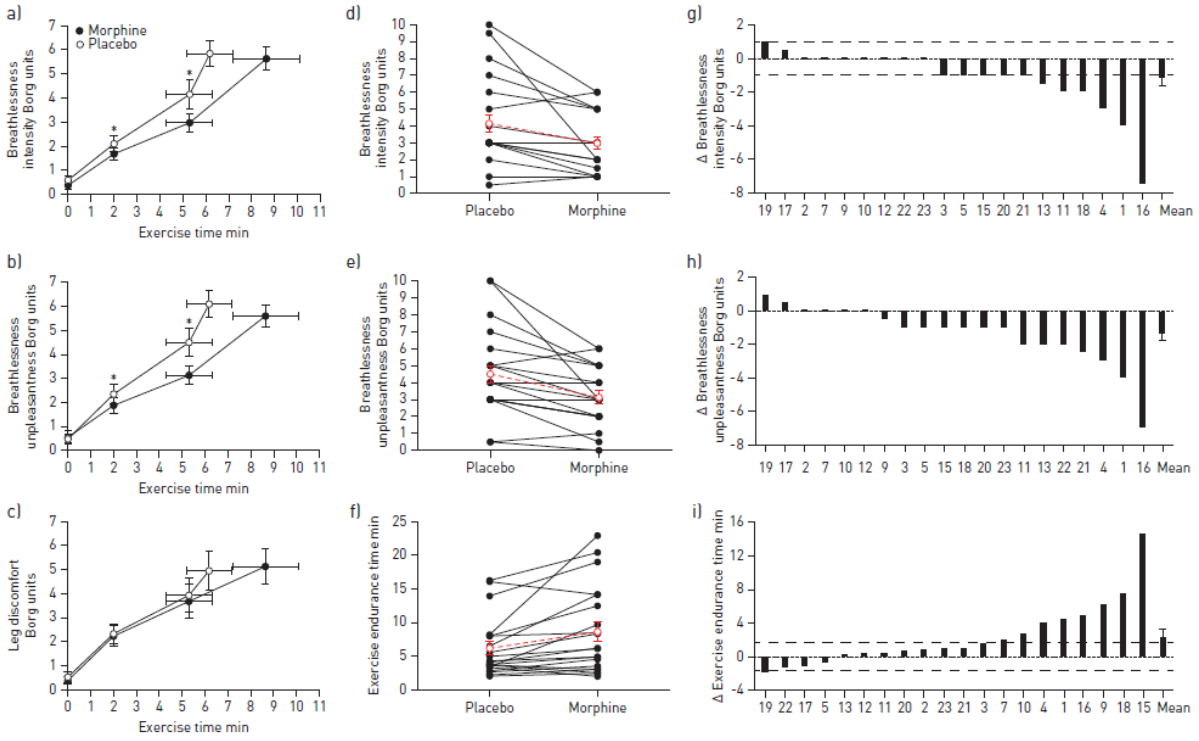

FIGURE 2 Effect of immediate-release oral morphine *versus* placebo on exertional breathlessness and exercise endurance in adults with advanced chronic obstructive pulmonary disease (COPD) and chronic breathlessness syndrome. Mean±SEM a) breathlessness intensity ratings, b) breathlessness unpleasantness ratings and c) leg discomfort ratings at rest and during constant-load cycle exercise testing at 75% of peak incremental power output. Individual participant post-dose values and post-dose differences in d and g) breathlessness intensity ratings during exercise at isotime, e and h) breathlessness unpleasantness ratings during exercise at isotime and f and i) exercise endurance time, where red symbols with dashed horizontal lines in panels d, e and f) denote mean±SEM. Dashed horizontal lines in panels g and i denote minimally clinically important difference for breathlessness intensity [36] and exercise endurance time [37]. Δ: post-dose difference (*i.e.*, morphine *minus* placebo). *: p<0.05 *versus* placebo.

Example from Abdallah 2017 - panels (a,b och c) to be reported for each of "intensity,unpleasantness and leg discomfort". To be presented as the above example but change "Morphine" to "Menthol".