Statistical analysis plan

MINIRICO - Mental Intervention and NIcotinamide RIboside supplementation in long COvid

January 24th, 2023

Amendment #1, January 23rd, 2025
In January 2025, prior to analyses of any results from the present trial, a detailed statistical analysis plan for the first scientific paper (reporting efficacy analyses pertaining to the primary outcome measure) have been included as an appendix to the present document (Appendix 1). In addition, slight modifications of the remaining text have been carried out to harmonize it with similar adjustments of the study protocol:

- Slight adjustment of aim, design and general procedures (Chapter 1) due to recent empirical and theoretical development in neurobiological models of symptom persistence in general. Also, errors pertaining to details of the blinding procedures have been corrected.
- Adjustments of secondary and exploratory endpoints (Chapter 3) bringing them up-to-date with recent scientific literature on the post-COVID-19 condition. In particular, executive functioning is seen as a more relevant neurocognitive marker than working memory in the population under study; the Trail Making Test, part B, is therefore included as a secondary outcome measure, substituting Digit Span Total Score which is now included as an exploratory outcome measure. Furthermore, the number of exploratory endpoints is moderately expanded to encompass more facets of potential intervention effects (on inflammation, symptoms, and physical/social functioning). The explorative endpoint related to attention bias has been removed as we were unable to establish the necessary experimental set-up.
- Addition/adjustment of other variables related to important RCT issues, such as background description of the population, compliance, therapist fidelity, and the effect of prior confidence in the interventions under study (Chapter 3).
- Addition of details to the imputation strategy and the definition of protocol deviation (Chapter 4).
- Addition of details pertaining to efficacy analyses, subgroup analyses and analyses of dose-response-relationship (Chapter 5).

Amendment #2, February 2nd, 2025

- Addition of one criterium for protocol deviation (Chapter 4)

1. Aim, study design and general procedures

Aims and design overview

Long COVID, also referred to as post-acute sequela of COVID-19 (PASC) or post-COVID-19 condition, is present in a substantial number of individuals. Two different hypothetical models of Long COVID suggest attenuated mitochondrial energy production and functional brain alterations partly caused by psychosocial load, respectively, to be key mechanisms in the underlying pathophysiology. Given the potential importance of metabolic disturbances, dietary supplement by Nicotinamide Riboside (NR, sales name Niagen®) may be beneficial. Given the potential importance of functional brain alterations and associated psychosocial factors, a tailored and personalized Mind-Body Reprocessing Therapy (MBRT) may be beneficial. The MBRT consists of 4 to 6 face-to-face therapist encounters in combination with digital resources available through the DIGNIO® interface.

The primary objective is to determine whether NR 1000 mg twice daily and/or MBRT increase health-related quality of life in individuals with Long COVID compared with care as usual and/or placebo. The Medical Outcome Study 36-item short form (SF-36) general health subscore is the primary endpoint. Secondary objectives are to determine intervention effects on six secondary endpoints: markers of inflammation (hsCRP) and executive function (trail making test), cost-effectiveness, and the patient-reported symptoms fatigue, dyspnoea, and global impression of change in symptoms, function and quality of life. Explorative objectives encompass intervention effects on additional cognitive function markers, biological markers (indices of inflammation and autonomic nervous activity), disability markers (work attendance) and patient symptoms, as well as the exploration of long-term effects, differential subgroup effects, intervention effect mediators and intervention effect predictors.

The study is a randomized controlled trial featuring a 2 x 2 factorial design where MBRT is compared with usual care and NR is compared with placebo (Figure 1). The latter comparison is double blinded. Eligible participants are individuals (18-70 years) with confirmed Long COVID interfering negatively with daily activities (such as work, socially, normal leisure activities, etc.). Participants will be recruited directly through self-referrals and referrals from general practitioners and hospital services, as well as from previous COVID-19 studies at our institution. A total of 310 participants will be enrolled. After baseline assessment (T1), the participants will be randomized 1:1 for both treatment comparisons, resulting in four treatment groups: a) MBRT and NR; b) usual care and NR; c) MBRT and placebo; d) usual care and placebo. All treatments last for three months, followed by primary endpoint assessment (T2) immediately prior to end of treatment. Total follow-up time is 12 months (T3). A comprehensive investigational program at all time points includes clinical examination, functional testing (spirometry, autonomic cardiovascular control, neurocognitive functions), sampling of biological specimens (blood) and questionnaire charting (background/demographics, clinical symptoms, psychosocial factors, study events).

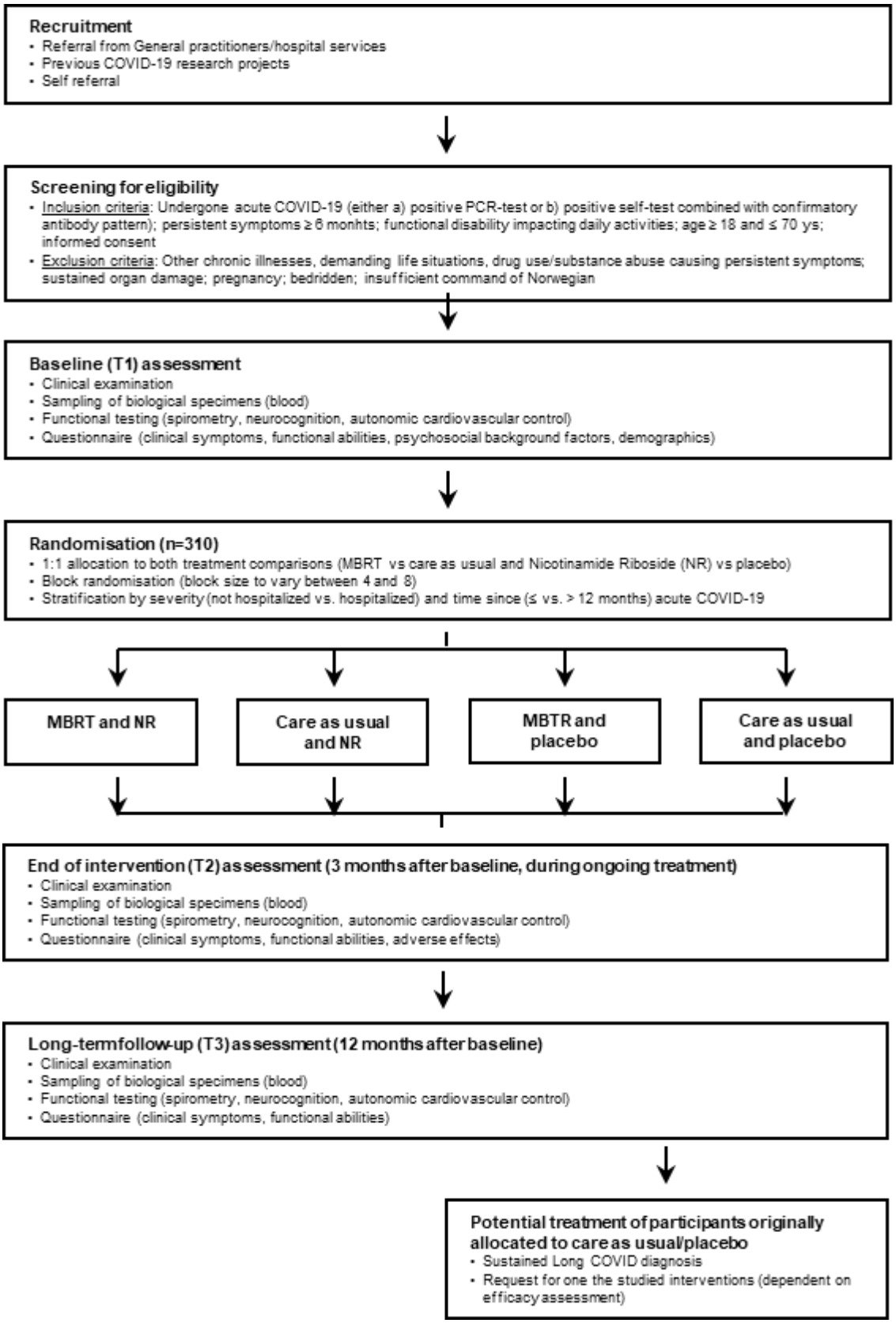

Figure 1. MINIRICO design overview

Recruitment, enrollment, randomization

Patients are recruited nation-wide in Norway. They are consecutively screened for eligibility by a telephone interview conducted by a research coordinator assessing Long COVID symptoms; functional disability; other acute COVID-19 sequels; other co-morbidities; hospitalization during acute COVID-19; and pregnancy. Patients assumed to adhere to inclusion and exclusion criteria (Table 1) are invited to the MINIRICO study center for baseline (T1) assessment.

Clinical examinations at TI are carried out by medical doctors. Long COVID patients adhering to inclusion and exclusion criteria and providing written informed consent will be formally enrolled in the study.

Enrolled patients will be block randomized to one of the four treatment combinations (MBRT and NR; care as usual and NR; MBRT and placebo; care as usual and placebo); block size will vary randomly between 4 and 8. Two stratification variables will be applied: a) Illness severity during acute COVID-19 operationalized as (1) no admission to hospital vs. (2) admission to hospital; b) Time since acute COVID-19 operationalized as (1) shorter than or equal to 12 months vs. (2) longer than 12 months. Randomization will be performed after all baseline assessments have been completed.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	*Exclusion criteria*
Fulfils diagnostic criteria for Long COVID: • Previous acute SARS-CoV-2 infection, confirmed by either a) laboratory-based PCR-test or b) self-test combined with confirmative antibody-pattern in blood. • Persistent symptoms (such as fatigue, dyspnoea, «brain fog», etc.) following acute COVID-19 for at least 6 months, and with no symptom-free interval. • Functional disability to an extent that impacts negatively on normal activities (such as work attendance, physical exercise, social activities, etc.)	Other chronic illnesses, demanding life situations or concomitant drug use/substance abuse that is considered a plausible cause of persistent symptoms and associated disability. Sustained organ damage (lung, heart, brain) following acute, serious Covid-19.
Age between 18 and 70 years	Bedridden
Signed informed consent	Pregnancy
	Insufficient command of Norwegian language

Blinding

For the NR vs. placebo comparison, the manufacturer (Chromadex Inc., Los Angeles, CA) will provide NR capsula as well as identically looking placebo capsula. These will be packed in identically looking pill boxes and given a neutral label (such as A and B). The encoding will be known by the Independent Data Monitoring Committee (IDMC) for safety reasons. Patients as well as all research personnel involved in the study will be blinded for group allocation during the stages of inclusion, intervention and end-point evaluation. In addition, they are shielded from variables that might indirectly indicate treatment allocation, such as blood NAD+ levels. The IDMC may unblind single patients in case of a Serious Adverse Event (SAE) or other medical emergencies; the result of the unblinding should not be communicated to the study personnel. Otherwise, no unblinding will take place until all participants have attended the follow-up assessment at T2 and all endpoint-evaluations (including all laboratory analyses) have been completed. The effectiveness of blinding will be assessed by asking all participants as well as the study physicians to guess group allocation at the time or primary endpoint assessment (T2).

For the MBRT vs. care as usual comparison, blinding of participants and study personnel is not possible due to the nature of the intervention. However, endpoint evaluation will be carried out by personnel blinded for group allocation.

Efficacy assessment and multiplicity adjustments

The primary efficacy endpoint of both intervention is the Medical Outcome Study 36-item short form (SF-36) general health (GH) subscore.[2] This subscore is based upon 5 single items, and has a range from 0 – 100 according to the standard scoring algorithm. The GH subscore is a generic measure of health-related quality of life that has been extensively used in previous intervention trials; also, the reliability and validity in the Norwegian population is well established, and norm data exists.

The 2 x 2 factorial design of the present study implies that two primary hypotheses are tested simultaneously (the NR vs. placebo comparison and the MBRT vs. usual care comparison, respectively). For both treatment comparisons, the level of significance for the primary end-point analyses is set at $\alpha = 0.05$.

A priori, we assume no interactions between these treatments; still, an interaction effect cannot be ruled out. For analysis of a potential interaction effect, as well as for the secondary efficacy endpoints, a testing procedure that controls the family wise error rate (FWER) at the overall 5% level will be applied. However, as previous research indicates significant correlation between several PROMs in Long COVID patients,[5] the Bonferroni correction method is not considered to be the best solution for FWER correction; rather, a resampling procedure such as the one suggested by Romano and Wolf will be applied.[4]

As for the exploratory endpoints, no multiplicity adjustments will be carried out.

2. Power calculation

The power calculation is based on the primary endpoint. A difference of 10 points of the GH subscore is considered clinically significant.[6] The distribution of SF-36 subscores among Long COVID sufferers are unknown, but a large Norwegian survey of the general population reported mean score of 73 and standard deviations (SDs) between 20 and 23 across both sexes and all age groups.[2] If SD is set to be 25 in the population under study, the study should aim to include a total of 310 participants. This yields a power of at least 90 % (α=0.05) to detect a small to medium effect size. If as many as 20 % of the participants (n=62) are lost to follow-up or subjected to another protocol violation at T2, the study still has a power of at least 85 % to detect the same effect sizes in per-protocol analyses.

3. Variables

Variable group	Variable subgroup/explanations
BACKGROUND AND PREDICTOR VARIABLES	
Background, demographics, etc	Sex Age Body Mass Index (BMI) Ethnicity Chronic diseases/comorbidities Medicines Vaccines Diagnosis of acute COVID-19 Severity of acute COVID-19 (**stratification variable**) Time since acute COVID-19 (**stratification variable**)

	Adherence to post-infective fatigue syndrome case definition (**subgrouping variable)**
Social and behavioural markers	Household members
	Socioeconomic level/Level of education
	Chronic disease, family member
	Smoking
	Alcoholic beverages, illicit drugs
	Average level of physical activity prior to acute infection
	UCLA loneliness questionnaire, total sum score
Psychological traits	NEO-FFI-30, subscore neuroticism
	Penn State Worry Questionnaire, total score
Symptoms, function and quality of life	SF-36 subscores, not used elsewhere
	Post-infective fatigue syndrome accompanying symptoms (fatigue; PEM; general infectious symptoms; cognitive, respiratory, digestive, cardiac, ENT, autonomic symptoms)
Clinical findings	General clinical examination, pathological findings
	Neurological examination, pathological findings
	Pregnancy tests (only women 18-50 years of age)
Blood analyses	Haemoglobin
	Leucocyte count with differential count
	Platelet count
	C-reactive protein (CRP)
	Sodium
	Potassium
	Calcium
	Creatinine
	Carbamide
	ALT
	Albumin
	Bilirumin
	CK
	LDH
	HbA1c
	Vitamin B_{12}
	D-dimer
	INR
	Ferritin
	NT-proBNP
	Troponin T
	TSH
	Folic acid
	SARS-CoV-2-Antibodies (nucleocapsid and RBD)
Organ function tests	Blood pressure
	Heart rate
	Respiratory rate
	Tympanic temperature
	SpO_2
	Spirometry indices
	ECG indices (including indices of Heart Rate Variability)
Cognitive function tests	Trail making test, A and B
	Digit span test
	HVLT-R (Hopkins Verbal Learning Test-Revised)
EFFICACY VARIABLES	
Primary outcome measure	The Medical Outcome Study 36-item short form (SF-36), general health subscore at T2
Secondary outcome measures	hsCRP (high-sensitive assay)
	Trail Making Test, part B
	Chalder Fatigue Questionnaire, total sum score
	Medical Research Council dyspnoea scale
	Patient Global Impression of Change (PGIC) inventory
	Incremental cost-effectiveness ratio, using the 36-item short form (SF-36) general health subscore to determine quality-adjusted life years.
Exploratory outcome measures	Interleukin (IL)-6
	Digit Span Test, total score
	Penn State Worry Questionnaire (PSWQ), total sum score
	Heart rate variability (HRV) indices in the time and frequency domain using a 5-minute ECG recording obtained during supine rest
	PEM items from the DePaul Symptom Questionnaire, total average score across five items
	Brief Pain Inventory (BPI), average score
	Cognitive difficulties, average score across four items
	Karolinska sleep questionnaire (KSQ), total sum score
	Hospital Anxiety and Depression Symptoms (HADS), anxiety score
	Hospital Anxiety and Depression Symptoms (HADS), depression score
	Smell and/or taste abnormalities, average score across two items
	SF-36 physical function subscore
	SF-36 social function subscore

SAFETY VARIABLES	
Efficacy variables, cf. above	Analyses of deterioration
Clinical findings, organ function test and blood analyses	Analyses of pathological findings; suicidal intent
Questionnaire	Health care contacts and treatment initiations Occurrence of novel diseases/illnesses Depression subscore from the Hospital Anxiety and Depression Scale (HADS) inventory
Spontaneous reports of adverse events/serious adverse events	
COMPLIANCE VARIABLES	
Compliance with the NR vs. placebo intervention	Ratio between actual and expected number of capsula NAD+ levels in whole blood
Compliance with the MBRT vs. usual care intervention	Number of self-directed activities per week, charted at day 25 and T2
STUDY DESIGN CHARACTERISTICS	
Screening results	Number of screened, number/characteristics of excluded/declined, reasons for exclusion
Lost to follow up	Total number, reasons for being lost to follow-up, number of incomplete cases
Time span from T1 to T2	
Number of outpatient appointments MBRT	Charted at T2
Protocol deviations	Primary outcome missing; lost to follow-up; low compliance; therapy discontinuation; other chronic disorders; severe illness/trauma; other treatment for post-COVID-19 condition
Prior confidence in treatment	Prior confidence in MBRT and NR, respectively, charted at T1
Prior preference of treatment, NR over MBRT	Charted at T1
Guess on treatment allocation	Study physicians' and participants' guess on NR/placebo allocation at T2
OTHER VARIABLES	
Effect of brief intervention	The Medical Outcome Study 36-item short form (SF-36), general health subscore, administered to participants in the MBRT arm immediately after the first medical appointment
Effect on long-term work attendance	Linkage with the Norwegian Labour and Welfare Administration registry on sick leave
Therapist fidelity to MBRT	Audio recording and subsequent content analyses of a random selection of therapist-patient-encounters

4. Analysis sets

Full analysis set

The 'full analysis set' is defined as all patients who were included and randomised (n = 310). This 'full analysis set' will be used for intention-to-treat analyses of efficacy, as described below. Generally, missing values will replaced by multiple imputation using the mi chained procedure in STATA. The number of data sets will be guided by the proportion of cases that are incomplete. All available data from background, study design and efficacy variables will be used to generate imputed data sets (for details, cf. appendix below). Rubin's rule will be used to combine estimates and standard errors. As for missing values in single items belonging to the SF-36 inventory: if less than 50 % the values are missing in a composite subscore, these missing data points will be ignored in the computation (i.e., averages will be calculated across remaining values), in accordance with current recommendations. If more than 50 % of the single item values are missing in a composite subscore, the subscore will be regarded as missing as well.

Per protocol analysis set

The 'per protocol analysis set' is defined as all patients in the 'full analysis set' that completed the treatment period (12 weeks) without any of the following protocol deviations:

- Interruption of therapy
- Lost to follow-up (including participant withdrawal)
- Primary outcome measure missing

- Low compliance with the NR vs. placebo intervention, defined as a ratio between actual and expected number of capsula lower than 3 SD from the mean value.
- Low compliance with the MBRT intervention, defined as less than four therapy sessions OR a total number of self-directed activities (charted at T2) lower than 3 SD from the mean value.
- Diagnosed with another chronic disorder during the study period.
- Experiencing a severe illness or trauma during the study period.
- Commencing other treatment for long COVID during the study period.
- T2 assessment performed too late in relation to the NR vs placebo intervention; i.e., after ingestion of all available NR/placebo capsula.

Missing data will not be imputed in the per protocol analysis set. The 'per protocol analysis set' will be used for per protocol assessment of efficacy and reported as sensitivity analyses in scientific publications (cf. below). The fraction of this set that was allocated to NR and MBRT interventions, respectively, will be used for analyses of dose-response relationship.

Safety analysis set
The 'safety analysis set' is defined as all participants that actually received an intervention (or part thereof). Missing values will not be imputed in the safety analysis set.

5. Statistical methods

The main results of the trial will be presented following the CONSORT recommendations for reporting of factorial randomized trials.[3]

General considerations
Continuous variables will be reported with parametric (mean/standard deviation) or non-parametric (median, quartiles) descriptive statistics, depending on the distribution. Ordinal/nominal variables will be reported as frequency tabulation. All statistical tests will be carried out two-sided. A p-value ≤ 0.05 is considered statistically significant. Test multiplicity adjustments will be carried out as described above. For statistical tests of intervention outcome (cf below), variables having a skewed distribution will be considered transformed in order to achieve an approximate normal distribution.

Population characteristics
The four treatment allocation groups will be compared using descriptive statistics only (ie., no statistical tests will be applied)

Outcome of intervention
No interaction between the two interventions is hypothesized; potential interaction effects will be formally assessed by generalized linear models (cf. appendix for details). The included and randomised participants (ie. the full analysis set) will be subjected to intention-to-treat analyses comparing the group allocated to NR with the group allocated to placebo *and* the group allocated to MBRT with the group allocated to treatment as usual. Separate general linear models (ANCOVA) will be applied for both comparisons. Also, separate analyses of all efficacy variables at both time points (T2 and T3) will be carried out. For investigation of long-term effects (T3), analyses based on mixed models for repeated measurements will be reported as sensitivity analyses The baseline (T1) values of each efficacy outcome measure as well as the two stratification variables (time since acute COVID-19 *and* severity of acute COVID-19) will be included as covariates in each ANCOVA model. The null hypothesis is

no differences in efficacy variables between the treatment allocation groups. Primary endpoint is the Medical Outcome Study 36-item short form (SF-36) general health (GH) subscore. Secondary and exploratory outcome measures are defined in paragraph 3 above. For each statistical analysis of efficacy, the net intervention effect (the mean difference between groups at T2) will be calculated from the parameters of the fitted general linear model and reported with 95 % confidence intervals. In addition, effect size (Cohen's *d*) will be reported.

An identical methodological approach will be applied for per protocol analyses of intervention effects, based upon the per protocol analysis set.

Subgroup analyses

The outcome of both interventions will be explored in the subgroup of participants adhering to the modified Fukuda-criteria for post-infective fatigue syndrome.[1] A formal caseness assessment of all included participants will be performed at baseline (T1), following an algorithm as described elsewhere.[5] In addition, outcome of both interventions will be explored in the sub-group of participants with PEM (post-exertional malaise) score in the upper quartile at baseline (T1).

The full analysis set will be applied for subgroup analyses. A differential outcome will be tested for all efficacy variables at both time points, applying a general linear model including relevant interaction terms.

Dose-response relationship

From the per protocol analysis set, the patients who were allocated to NR and MBRT interventions, respectively, will be subjected to analyses of dose-response relationships. For NR allocation, the NAD+ concentration in whole blood at T2 (cf. above) will serve as the independent variable. For MBRT allocation, the total number of self-directed activities will serve as the independent variable.

The association between dose and response will be explored separately for all efficacy variables at T2, applying general linear models.

Safety endpoints

Safety data will be summarized descriptively through appropriate data tabulations and descriptive statistics, based upon the safety analysis set. No statistical tests will be carried out.

Interim analysis

No interim analysis of efficacy variables will be carried out. Safety data will be monitored by the independent monitoring committee during the treatment period.

Predictors of treatment effects

A prediction analysis of treatment effects will feature a methodological set-up similar to a recent observational cohort study of COVID-19 patients,[5] exploring associations between a wide range of background and T1-variables (independent variables) and T2-effector variables (dependent variables) by regression analyses. The PPAS will be applied in these analyses. The independent variables include:

- Previous infectious diseases: Time since acute COVID-19, genetic variant of SARS-CoV-2, reinfection with SARS-CoV-2, other infectious events in the aftermath of acute COVID-19
- Previous immunizations: Vaccination against COVID-19 (date(s), type(s)), other vaccinations in the aftermath of acute COVID-19.
- Previous and current medical history: Diagnoses of other chronic diseases, current medication

- Severity of acute COVID-19: Hospitalization (days), intensive care unit admission (days), respiratory support, cardiovascular support, neurological sequels, thromboembolic events, immunological and infectious markers during hospital stay (CRP, viral replication numbers).
- Current clinical symptoms and functional disability
- Psychological traits (neuroticism, worrying tendencies) and social features (socioeconomic level, loneliness, substance abuse)

6. References

1. Hickie I, Davenport T, Wakefield D, Vollmer-Conna U, Cameron B, Vernon SD, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ. 2006;333(7568):575.
2. Jacobsen EL, et al. Norwegian reference values for the Short-Form Health Survey 36: development over time. Qual Life Res 2018; 27: 1201-12.
3. Kahan BC, et al. Reporting of factorial randomized trials. Extension of the CONSORT 2010 statement. JAMA 2023; 330: 2106-14.
4. Romano JP, Wolf M. Exact and approximate stepdown methods for multiple hypothesis testing. Journal of the American Statistical Association. 200^5; 100 (469): 94–108.
5. Selvakumar J, Havdal LB, Brodwall E, et al. Prevalence and predictors of post-COVID-19 condition among non-hospitalised adolescents and young adults: a controlled prospective observational study. *Submitted*
6. Wyrwich KW, Thierney WM, Babu AN, et al. A comparison of clinically important differences in health-related quality of life for patients with chronic lung disease, asthma, or heart disease. Health Serv Res 2005; 40: 577–92

7. Signatures

We hereby vouch for the fidelity of the study to this statistical analysis plan.

Oslo, Norway and Sydney, Australia; January 2023

Vegard Bruun Bratholm Wyller
Principal investigator MINIRICO

Erin Cvejic
Biostatistician MINIRICO

Appendix 1: Detailed Statistical Analysis Plan for the primary scientific publication from the MINIRICO trial

January 23rd, 2025

This appendix outlines in detail the statistical analyses of the main results from the MINIRICO trial at the T2 follow-up timepoint (i.e., immediately after completion of the interventions). The plan has been developed prior to database lock, and prior to any statistical analyses of the material. *Analyses of missingness and construction of the Full Analysis Set (para. 1.a.), as well as the main statistical analyses of efficacy (para. 2.a.-d.) will be performed by biostatisticians not otherwise affiliated with any part of the study and blinded for both treatment allocations.*

1. Data sets

a. Analysis of missingness, imputation strategy, and construction of the Full Analysis Set (FAS)

The Full Analysis Set (FAS) is defined as all patients who were included and randomised (n = 310) and all variables specified in paragraphs 2, 3 and 4 below, cf. chapter 4 above. Missing datapoint/cases will be evaluated with the Little's Missing Completely at Random (MCAR) test. If $p > 0.05$, missing data will be replaced exploiting the Multiple Imputation by Chained Equations (MICE) technique. The number of imputed datasets will be guided by the proportion of missingness in the dataset in such a way that the number of datasets equals the percentage of subjects with any missing data. If $p \leq 0.05$, a series of sensitivity analyses examining different imputation mechanisms will be carried out to examine the consistency of conclusions drawn in regard to treatment efficacy.

b. Protocol deviations and construction of the Per-Protocol Analysis Set (PPAS)

The following variables will be used to assess protocol deviations, cf. chapter 4 above:

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION
Protocol deviations	Primary outcome missing; lost to follow-up; low compliance; therapy discontinuation; other chronic disorders; severe illness/trauma; other treatment for post-COVID-19 condition

The number of each protocol deviation across the four intervention groups will be reported. Low compliance with the NR intervention is defined as a ratio of taken vs. prescribed capsules of < 3 standard deviations from the mean value (cf. paragraph 6, below). Low compliance with the MBRT intervention is defined as < 4 therapy session OR a total number of self-directed activities < 3 standard deviations from the mean value (cf. paragraph 6, below). One participant may have more than one protocol deviation. Total number of protocol deviations and total number of cases in the PPAS will be reported.

c. Construction of the Safety Analyses Set (SAS)

The SAS consists of all participants that actually received an intervention (or part thereof), independent of any protocol deviation. Missing values will not be imputed in the SAS.

d. Transformation of variables

Transformation of variables will only be performed if necessary for complying with formal requirements of planned statistical analyses. Information on variable transformation will be reported.

2. Study participant characteristics

a. Background data

The following variables will be reported as background data for the study population (n=310):

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION
Background, demographics, etc Social and behavioural markers Symptoms, function and quality of life Clinical findings Blood analyses Organ function tests Cognitive function tests Primary, secondary and exploratory outcome measures (cf. below) Time span from T1 to T2 Number of outpatient appointments MBRT Prior preference of treatment, NR over MBRT	Cf. table in chapter 3, above. Mix of categorical, ordinal and continuous variables

Variables will be reported across all four intervention groups as well as totals for the entire study population, using descriptive statistics as appropriate (mean/standard deviation; median/interquartile range; numbers/proportions). No statistical tests will be carried out. Baseline (T1) values will be used unless otherwise indicated.

b. PIFS caseness subgrouping

Each participant will be assessed according to the modified Fukuda-criteria of post-infective fatigue syndrome (PIFS), cf. chapter 5 above. Classification in three groups (certain PIFS case; uncertain PIFS case; no-PIFS case) will be performed by two researchers independently; if disagreement, the classification will be discussed with a third researcher until consensus is reached. The classification results as well as the applied algorithm will be reported.

3. Study design characteristics

a. Screening and attritional analyses

The following variables will be used:

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION
Screening results	Number of screened, number/characteristics of excluded/declined, reasons for exclusion
Background, demographics, etc.	Sex Age Diagnosis of acute COVID-19 Severity of acute COVID-19 Time since acute COVID-19

Number and proportions will be reported. For participants who fulfilled inclusion criteria, an attritional analysis featuring logistic regression will be carried out assessing the associations between characteristics of the invited individuals and their decision to decline/accept the invitation to participate in the study. Odds ratios will be reported with 95 % confidence intervals. P-values will not be adjusted for multiplicity.

b. Lost to follow-up and data missingness; number and analyses
The following variables will be used:

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION
Lost to follow up at T2	Total number, reasons for being lost to follow-up, number of incomplete cases
Background, demographics, etc	Cf. table in chapter 3, above.
Social and behavioural markers	
Primary, secondary and exploratory outcome measures (cf. below)	

Number and proportions of losses to follow-up will be reported, using the PPAS. In addition, descriptive statistics for key background variables and all outcome measures at T1 will be reported across the group lost to follow-up and the group remaining in the study, respectively. Also, for all these variables, associations to being lost to follow-up will be formally assessed by logistic regression; odds ratios will be reported with 95 % confidence intervals, p-values will not be adjusted for multiplicity.

c. Quality check of blinding for the NR/placebo allocation:
The following variable will be used to assess whether the NR/placebo-allocation was indeed double blinded, using the PPAS:

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION
Guess on treatment allocation	Study physicians' and participants' guess on NR/placebo allocation at T2

Numbers and proportions will be reported. Chi-square test will be carried out separately for physicians' guess vs. actual allocation and participants' guess vs. actual allocation; p-values will not be adjusted.

4. Efficacy analyses
The following variables are to be exploited for efficacy analyses:

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION	SCORING/UNIT AND DISTRIBUTION
Primary outcome measure	The Medical Outcome Study 36-item short form (SF-36), general health subscore	Scoring 0-100, where higher scores mean better general health. Semi-continuous, approximate normal distribution assumed
Secondary outcome measures	hsCRP (high-sensitivity assay)	Unit is mg/L. Continuous, leftward skewing assumed
	Trail Making Test, part B	Unit is sec. Continuous, approximate normal distribution assumed
	Chalder Fatigue Questionnaire, total sum score	Scoring 0-33, where higher scores mean more fatigue. Semi-continuous, approximate normal distribution assumed
	Medical Research Council dyspnoea scale	Scoring 0-4 (Likert scale), where higher scores mean more dyspnoea. Ordinal, leftward skewing assumed
	Patient Global Impression of Change (PGIC) inventory	Scoring 1-7 (Likert scale), where higher scores means stronger improvement of health. Ordinal, no prior assumption on distribution
Exploratory outcome measures	Interleukin (IL)-6	Unit is pg/mL. Continuous, leftward skewing assumed
	Digit Span Test, total score	Scoring 0-32, where higher scores means better digit span. Semi-continuous, approximate normal distribution assumed
	PEM items from the DePaul Symptom Questionnaire, total average score across five items	Scoring 0-100, where higher scores mean more PEM. Semi-continuous, approximate normal distribution assumed
	Cognitive difficulties, average score across four items	Scoring 1-5, where higher scores mean more cognitive difficulties. Semi-continuous, approximate normal distribution assumed

Karolinska sleep questionnaire (KSQ), total sum score	Scoring 12-72, where higher scores mean better sleep. Semi-continuous, approximate normal distribution assumed
Hospital Anxiety and Depression Symptoms (HADS), anxiety score	Scoring 0-21, where higher scores mean more anxiety symptoms. Semi-continuous, approximate normal distribution assumed
Hospital Anxiety and Depression Symptoms (HADS), depression score	Scoring 0-21, where higher scores mean more depressive symptoms. Semi-continuous, approximate normal distribution assumed
Smell and/or taste abnormalities, average score across two items	Scoring 1-5, where higher scores mean more smell/taste abnormalities. Semi-continuous, leftward skewing assumed.
SF-36 physical function subscore	Scoring 0-100, where higher scores mean better physical function. Semi-continuous, approximate normal distribution assumed
SF-36 social function subscore	Scoring 0-100, where higher scores mean better social function. Semi-continuous, approximate normal distribution assumed

a) Simple intervention effects within four treatment groups and tests of interactions between interventions

Using the PPAS, the mean/median values at T1 and T2 as well as the difference will be computed for all outcome variables across all four treatment groups. For the variable Patient Global Impression of Change (PGIC), there is no baseline (T1) value; hence, only T2-values are reported. Interactions between the two interventions will be formally assessed by generalized linear models across all outcome variables. For the secondary outcome measures, p-values for the tests of interaction will be adjusted by a resampling procedure (cf. Chapter 1, above), keeping the family wise error rate (FWER) at 5 %. For the exploratory outcome measures, p-values for the tests of interaction will not be adjusted.

b) Main analyses of efficacy

If there is no significant interaction between the two interventions for any primary/secondary outcome measure (i.e., all adjusted p-values for tests of interactions > 0.05, cf. above), we will perform separate analyses comparing the group allocated to NR with the group allocated to placebo and the group allocated to MBRT with the group allocated to care as usual using the FAS. General linear models (ANCOVA) will be applied for both comparisons. The baseline (T1) values of each efficacy outcome measure as well as the two stratification variables (time since acute COVID-19 *and* severity of acute COVID-19) will be included as covariates in each analysis. For each statistical analysis of efficacy, the fitted general linear model will provide: i) The estimated mean value in the intervention group at T2; ii) The estimated mean value in the control group at T2; iii) The net intervention effect (the mean difference between the intervention group and the control group at T2) with 95 % confidence interval; iv) The p-value for the net intervention effect; v) The effect size (Cohen's *d*) of the net intervention effect. All values will be pooled across the imputed datasets, and the p-values of the secondary outcome measures will be adjusted by a resampling procedure (cf. Chapter 1, above), keeping the FWER at 5 %.

Alternatively, if there is a significant interaction between the two interventions for any primary/secondary outcome measure (i.e., one or more adjusted p-values for tests of interactions ≤ 0.05), combined analyses (with the interaction term included in the analysis model) will be performed, with estimates as described above accounting for the interaction effect.

c) Analyses of efficacy across subgroups

The outcome of both interventions will be explored across two subgroups (cf. above), using the FAS: i) The subgroup classified as certain PIFS cases compared with those with another PIFS classification; ii) The subgroup having PEM scores in the upper quartile at baseline (T1)

compared with those having PEM scores in the three lower quartiles. A differential outcome will be tested for all outcome variables at T2 applying general linear model (ANCOVA) that include relevant interaction terms. For each subgroup, estimated means, net intervention effect with 95 % confidence intervals, p-values and Cohen's d will be reported (cf. above). In addition, p-values for the interaction will be reported. No multiplicity adjustments will be carried out.

d) Sensitivity analyses

Two sensitivity analyses will be performed: i) Analyses of efficacy using the same approach as outlined above (paragraph 4.b.), but with PPAS replacing the FAS; ii) Analyses of efficacy using ordinal regression instead of general linear models for variables with an ordinal or close-to-ordinal distribution (assumed to encompass the Medical Research Council dyspnoea scale; Patient Global Impression of Change (PGIC) inventory; and Smell and/or taste abnormalities). For these analyses, the FAS will be exploited, and odds ratio with 95 % confidence intervals and p-values will be reported.

In general, no multiplicity adjustments will be carried out for the sensitivity analyses.

e) Analyses of recovery

A recovery threshold for the primary outcome (SF-36 general health subscore) is defined as the mean value in the general population (~75). Numbers/proportions within each intervention group meeting the recovery threshold will be reported for T1 and T2, using the PPAS. Differences between groups at each time point will be analyzed by chi-square tests; p-values will not be adjusted.

f) Analyses of dose-response associations

In addition to the outcome variables listed above, the following variables will be exploited for analyses of dose-response associations, using the PPAS:

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION	SCORING/UNIT AND DISTRIBUTION
Compliance with the NR vs. placebo intervention	NAD+ levels in whole blood	Unit pmol/µL. Continuous, approximate normal distribution assumed.
Compliance with the MBRT vs. usual care intervention	Number of self-directed activities per week, charted at T2	Ordinal, no prior assumption on distribution

Dose-response-associations will be assessed by generalized linear modelling. For study participants receiving MBRT, we will sequentially assess all outcome variables at T2 as independent variables against 'Total number of self-directed activities at T2' as dependent variable; the baseline (T1) value of each independent variable will be included as covariate. Likewise, for study participants receiving NR, we will sequentially assess all outcome variables as independent variables against 'NAD^+-levels in whole blood at T2' as dependent variable. Regression coefficients with 95 % confidence intervals and p-values will be reported; no multiplicity adjustments will be performed.

5. Safety analyses

The following variables will be exploited for safety analyses, using the SAS:

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION	SCORING/UNIT AND DISTRIBUTION
Selected outcome variables charted at T2, cf. paragraph 4 above.	Analyses of deterioration in general health (primary outcome measures), physical function, social function, PEM, cognitive	Categorical variables, dichotomous distribution (deterioration vs. no deterioration)

	difficulties, and depressive symptoms (exploratory outcome measures).	
Other (selected) symptom variables charted at T2, cf. paragraph 2 above.	Analyses of deterioration in nausea, bloating, headache, and dizziness	Categorical variables, dichotomous distribution (deterioration vs. no deterioration)
Clinical findings, organ function test, and laboratory analyses charted at T2	Analyses of pathological findings in vital signs, clinical (incl. neurological) examination, blood laboratory tests, ECG-recordings, pregnancy test, suicidal intent	Categorical variables, dichotomous distribution (present vs. not present)
Questionnaire distributed two times during the intervention period (two and five weeks after T1) as well as spontaneous reports	Health care contacts and treatment initiations	Categorical variables, dichotomous distribution (present vs. not present)
	Occurrence of novel diseases/illnesses	Categorical variables, dichotomous distribution (present vs. not present)
	Depression subscore from the Hospital Anxiety and Depression Scale (HADS) inventory	Categorical variable, dichotomous distribution (above vs. below cutoff-value for potential severe depression (≥ 15))
Spontaneous reports of adverse events/serious adverse events (AE/SAE)		String variables

For the selected outcome variables and other symptom variables charted at T2, the number/proportions experiencing decreased functional capabilities/increased symptom load will be reported across all four treatment groups. Likewise, for clinical findings, organ function tests and laboratory analyses charted at T2, the number/proportions with any pathological findings will be reported across all four treatment groups. As for health care contacts, treatment initiations, novel diseases/illnesses and severe depressive symptom load, number of occurrences will be summarized within each category and reported across all four treatment groups. All AEs and SAEs will be reported one-by-one across all four treatment groups. Finally, all participants receiving either NR, MBRT or both and experiencing a deterioration of ≥ 10 points in general health score (primary outcome measure) from T1 to T2 will be subjected to a qualitative assessment whereby clinical records will be explored to identify potential causes of the deterioration. No statistical tests will be used for the safety analyses.

6. Compliance

The following variables will be exploited for compliance analyses:

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION	SCORING/UNIT AND DISTRIBUTION
Compliance with the NR vs. placebo intervention	Ratio between taken and prescribed number of capsules, calculated at T2	Ratio, no prior assumption on distribution
	NAD+ levels in whole blood at T2	Unit pmol/µL. Continuous, approximate normal distribution assumed.
Compliance with the MBRT vs. usual care intervention	Number of self-directed activities per week, charted at day 25 and T2	Ordinal, no prior assumption on distribution

The ratio between taken and prescribed capsules and the NAD+-levels in whole blood will be reported with mean and standard deviation across all four treatment groups. Likewise, the number of self-directed activities pr week at day 25 and T2 will be reported with mean and standard deviation among those who received the MBRT intervention.

7. Therapist fidelity

The following variables will be exploited for analysis of therapist fidelity to the MBRT intervention:

VARIABLE GROUP	VARIABLE SUBGROUP/EXPLANATION
Therapist fidelity to MBRT	Audio recording and subsequent content analyses of a random selection of therapist-patient-encounters

All therapist-patient encounters have been audio recorded. A random selection of 20 % will be reviewed by a psychologist not otherwise affiliated with the study, and dichotomously scored as adherent/non-adherent with the pre-specified intervention protocol. Numbers and proportions will be reported; no statistical tests will be performed.