Statistical analysis plan

MINIRICO - Mental Intervention and NIcotinamide RIboside supplementation in long COvid

January 24nd, 2023

1. Aim, study design and general procedures

Aims and design overview

Long COVID, also referred to as post-acute sequela of COVID-19 (PASC), is present in a substantial number of individuals. Two different hypothetical models of Long COVID suggest attenuated mitochondrial energy production and 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 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 cognitive function (digit span 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 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).

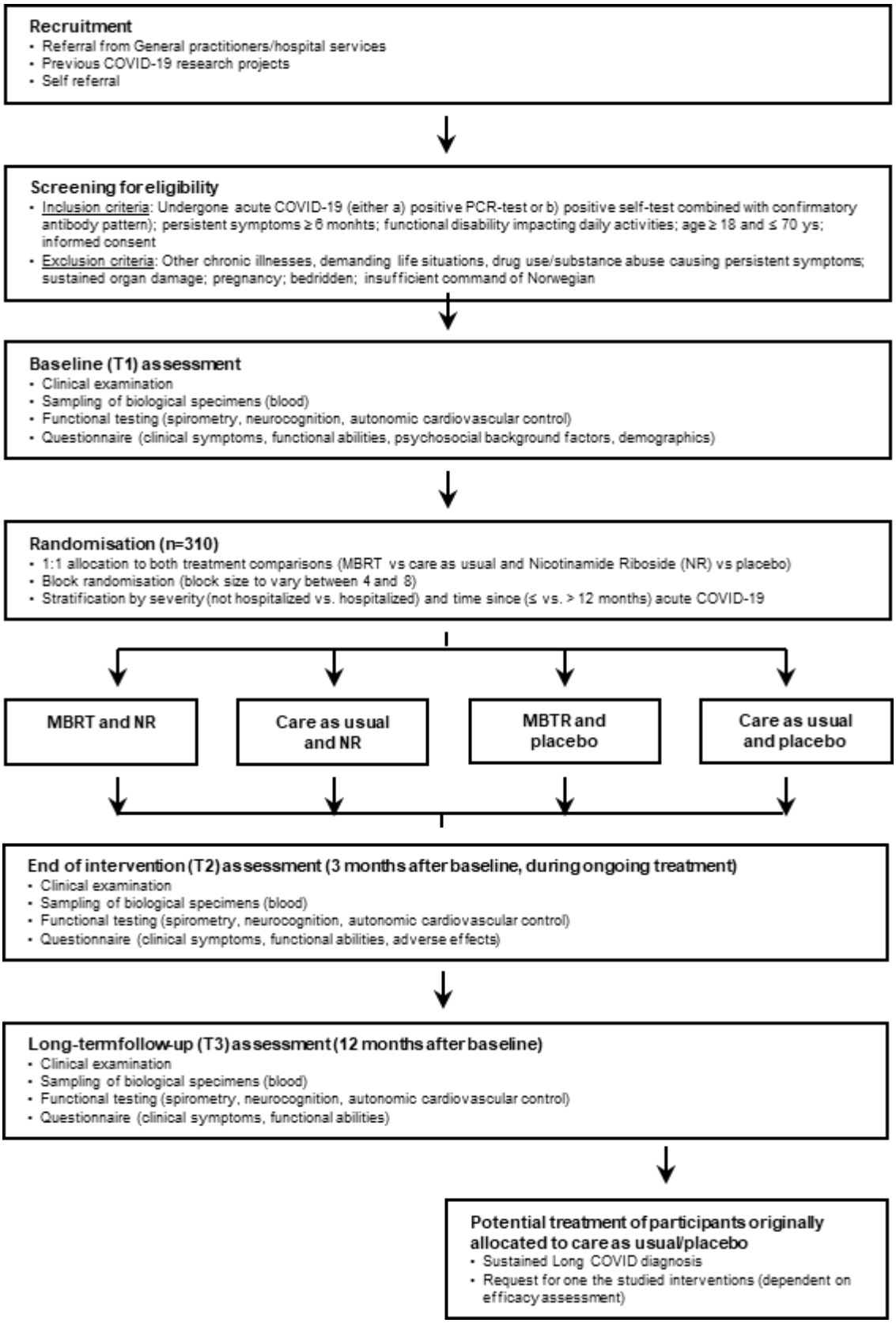

Figure 1. MINIRICO design overview

Recruitment, enrollment, randomization

Patients are recruited nation-wide. 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*
Fullfils 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 deliberately 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 final follow-up assessment (T3) 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 a 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,[4] 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.[3]

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.[5] The scatter 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
BACKGROUND AND PREDICTOR VARIABLES	
Background, demographics, etc	Sex Age Body Mass Index (BMI) Ethnicity Chronic diseases Medicines Vaccines 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
	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	Sleep disturbances
	Depression and anxiety
	Post-infective fatigue syndrome accompanying symptoms
Blood analyses	Haemoglobin
	Leucocyte count
	Platelet count
	Sodium
	Potassium
	Creatinine
	ALT
	Albumin
	Vitamin B_{12}
	D-dimer
	Ferritin
	NT-proBNP
	Troponin T
	SARS-CoV-2-Antibodies (nucleocapsid and RBD)
	EBV antibodies (VCA IgG and IgM, EBNA IgG)
Organ function tests	Blood pressure
	Respiratory rate
	Tympanic temperature
	SpO_2
	FVC (functional vital capacity)
Cognitive function tests	HVLT-R, immediate recall
	HVLT-R, delayed recall
	HVLT-R, recognition index
	Trail making test, total score
EFFICACY VARIABLES	
Primary endpoint variable	The Medical Outcome Study 36-item short form (SF-36), general health subscore
Secondary endpoint variable	hsCRP
	Digit span, total score
	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 variables	A computerised Function Acquisition Speed Test (FAST)
	A computerised test of attention bias towards illness-related words
	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
	Karolinska sleep questionnaire (KSQ), total sum score
	Hospital Anxiety and Depression Symptoms (HADS), total sum score
	Upper airways symptoms, two single items
SAFETY VARIABLES	
Questionnaire results	Pre-specified potential side-effects of NR and MBRT, charted throughout the intervention period
	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	Pre-specified questions on time allocated to recommended exercises and digital resources
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

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. Missing values will replaced by multiple imputation using chained equations (MICE). The number of imputations will be guided by the proportion of missingness in the dataset.

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
- Primary endpoint measurements missing
- Low compliance with the NR vs. placebo intervention, defined as a ratio between actual and expected number of capsula higher 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.

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 intervention 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

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 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
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. General linear models (ANCOVA) will be applied for both comparisons. Separate tests for all efficacy variables at both time points (T2 and T3) will be carried out. The baseline values of each efficacy endpoint 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. 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. For each statistical analysis of efficacy, the net intervention effect (the mean change in the intervention group minus the mean change in the control group) will be calculated from the parameters of the fitted general linear model and reported with 95 % confidence interval.

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.[4]

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. Results of subgroup analyses will be presented if the interaction $p < 0.10$.

Dose-response relationship

From the per protocol analysis set, the patients that were allocated to NR intervention will be subjected to analyses of dose-response relationships. The NAD+ concentration in whole blood at T2 (cf. above) 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,[4] 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

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4. 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*
5. 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

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