We would like to thank the reviewers for their thoughtful comments on our proposal entitled: " Getting insight into effective interventions and biomarkers for post-COVID symptoms".

We are happy to see that we received one overall quality assessment "very good" (R 225697), two overall quality assessments "good " (R 13738794, R 2190922) and one "sufficient" (R 13738774).

We will respond to the reviewer comments per topic.

Choice of medication

We are pleased to learn that the reviewers are positive about our plan to evaluate selective serotonin reuptake inhibitors (SSRIs) as a potential treatment option for the post-COVID symptoms fatigue, concentration problems and post-exertional malaise (PEM).

Comment: Although the reviewers are positive towards the choice of SSRIs as a class of medicines, they do not concur with our choice of the specific SSRI to be investigated in the study namely escitalopram. The reviewers mention that fluvoxamine or fluoxetine would be a better choice, and one reviewer mentions stronger sigma 1 receptor agonist activity for fluvoxamine or fluoxetine compared to escitalopram (R 13738774, 2190922 and 225697).

Reply: We had chosen escitalopram because escitalopram is generally better tolerated than fluvoxamine or fluoxetine and has more convenient dosing, i.e., once daily versus twice daily. Escitalopram is also a sigma 1 receptor agonist (Hashimoto, Discov Ment Health 2023), albeit less strong than fluvoxamine or fluoxetine. Given that three reviewers unanimously recommended fluvoxamine or fluoxetine, we decided to choose for fluvoxamine instead of escitalopram as the SSRI to be investigated in an RCT.

Dosing level

Comment: R 13738794 had comments about the dosing level, wondered what basis would be used for titrating the dose beyond 10 mg, wondered about consequences for the blind and felt that variable dose levels may confound analysis.

Reply: We intended to titrate based upon patient experience of effects/ side effects, as is commonly done in recent antidepressant RCTS. This would have had no consequences for the blinding as placebo could also be dosed up. However, we agree with the reviewer that there are advantages to using one stable active dose. Now that we decided to choose fluvoxamine we will be dosing up to 200 mg daily and use this as the only active dose throughout the study.

Study population, inclusion and exclusion criteria

Comment: R 13738794, 13738774 and 2190922 have questions about the study population and inclusion and exclusion criteria.

Reply: we regret word count restrictions prevented us for describing them in detail. Eligible participants will be 1) previously diagnosed with a laboratory-confirmed SARS-CoV-2 infection; 2) currently severely fatigued, operationalized as a score ≥ 35 on the fatigue severity subscale of the CIS with fatigue starting or increasing substantially directly after the onset of symptoms of COVID-19 and continuing for more than 12 weeks; 3) between 18 and 70 years of age; and 4) having sufficient command of the Dutch language. Exclusion criteria are 1) contra-indications towards use of SSRIs, particularly fluvoxamine, 2) known psychiatric or somatic conditions that can explain the presence of severe fatigue; 3) current use of psychotropic drugs with the exception of low dose benzodiazepine, quetiapine (≤50 mg), trazodone and mirtazapine (≤15 mg), 4) bipolar disorder or schizophrenia, 5) currently participating in a multi-disciplinary rehabilitation program 6) current use of medicines intended to reduce post COVID symptoms.

Comment R 13738794: will patients who are availing themselves of behavioral interventions such as “pacing”, or those taking metformin off-label which may reduce incidence of post COVID be eligible?

Reply: Patients are eligible if behavioral interventions are not part in a multi-disciplinary rehabilitation program (exclusion criterion 5). Patients taking off-label metformin for post COVID are not eligible (exclusion criterion 6).

Comment R 2190922: it is unclear how the applicant will distribute into the two treatment arms ensuring equal age, gender, Pre-existing illness etc. distribution. A simple random distribution with 160 patients might lead to non-significant results if these factors are not considered. The applicants need to consider, if patients with certain pre-existing illness and treatments are excluded from the study (treatment with monoamine oxidase inhibitors etc.).

Reply: we will stratify for sex and number of medical co-morbidities I(<3 or ≥3) as these factors were previously shown to be independent predictors of persistent fatigue. Pre-existing illnesses that are excluded are listed at exclusion criteria 2 and 4. Patients on monoamine oxidase inhibitors are excluded according to exclusion criterion 1. We will include age as a covariate.

Comment R 13738774: age should have an upper limit as frailty and other age-related symptoms will confound results.

Reply: we agree with the reviewer and we have defined 70 years of age as an upper limit. We will stratify for medical co-morbidities I(<3 or ≥3).

fMRI

Comment R 13738794: the team need to supply details in the rationale for selection of 80% in the submaximal exercise challenge (presumably 80 percent of age-predicted maximal heart rate). Although 85% is often chosen as a target threshold in submaximal exercise testing, it is of some note that a substantial proportion of patients with Long COVID may not reach this target. The clinical protocol should also include details on the challenge, and criteria for early stopping.

Reply: While submaximal physical exertion is safe, we have chosen to 80% of the age-predicted maximal heart rate so that this procedure requires less motivation and better tolerance to exhaustive exercise, with lowered risk of invalid tests, adverse events, especially in this patient population with severe fatigue and PEM. A recent study has shown that VO2max can be predicted from 80% submaximal tests with a comparable time course to exhaustion (Petelczyc, Sci Rep 2023). Challenge will be done using a standard cycle ergometer test, which will be *terminated if participants reached 80% of their peak HR (6 minutes); or when the participant requests to stop, or when the exercise specialist/researcher feel the participants could not safely continue (Reed, Front Physiol 2020)..*

Comment R 13738794: mention is made of evaluating differences in "slope" for fMRI findings, but this is unclear.

Reply: with evaluating differences in slopes for fMRI findings we mean to say that we are looking if there is a different pattern of change over time in fMRI findings between treatment responders and non-responders (statistical intervention effect; group by time interaction).

Comment R 13738794: the MRI protocols are not described and CVs for personnel or relevant citations were not included, but it is assumed that standard protocols previously published by the lab(s) would be used to collect and analyze task/resting fMRI, arterial spin labelling and diffusion imaging measures (reviewer 13738794).

Reply: Standardized (f)MRI protocols based on protocols from large scale consortia (UK Biobank, ABCD Study) will be used that we have implemented to investigate chronic fatigue syndrome. This approach will enable later comparison between Covid induced fatigue and non-viral fatigue. We will

use in-house analyses pipelines at the Amsterdam UMC using standardized software (SPM, FSL) to investigate fMRI and diffusion [redacted] *and ASL imaging* [redacted]*. The co-investigators* [redacted] *have an extensive track record and experience with respect to fMRI, ASL, diffusion, structural brain imaging, partly in ongoing long COVID research (https://bmjopen.bmj.com/content/13/6/e072611)*

Statistical analysis and methodology

Comment: R 13738794 wondered how alpha spend was apportioned for the interim and final primary analysis.

Reply: *At the interim analysis the trial can only be stopped early for futility and not for efficacy. The null hypothesis can only be rejected at the end of the trial. Therefore, no alpha is spend at the interim analyses. The planned interim analyses does not lead to an inflation of the type I error and no adjustment is needed. In case of early stopping for futility, all secondary analyses will be considered exploratory.*

Comment: R 13738794 expressed further concern about the power of the study to evaluate a large array of secondary endpoints (e.g., proteomic biomarkers). R 13738794 didn't necessarily consider that a "deal-killer, as long as the study is powered for the primary analysis. Some of the secondary analyses might be better construed as exploratory analyses.

Reply: the study is adequately powered for the primary outcome/ primary analysis. All biomarker endpoint are indeed considered explanatory.

Comment: R 13738794 was concerned about plans to publicize results of the futility analysis. The reviewer felt that a simple statement such as that the criteria for futility were not achieved and the trial should proceed would be more appropriate.

Reply: we regret that it was unclear that by reporting the interim analysis we indeed meant that we would only report whether or not the trial would be continued.

Comment: much of the criticism of R 13738774 (2.1, 2.2, 2.6, 2.7) focuses on the fact that the reviewer feels that we propose to use a non-specific intervention that could affect multiple neurobiological pathways. R 13738774 mentions that a common phased strategy for mechanistic trials is to conduct a small study using defined targets/goals (based on effect size) for multiple variables that establish mechanism (i.e., inflammatory and/or brain response) as well as potential for clinical response (while determining the best primary outcome). If successful, a larger trial is continued using a refined set of mechanistic and clinical outcomes.

Reply: the primary aim of the proposed study is to investigate if SSRI treatment, and specifically fluvoxamine, is effective in reducing post COVID symptoms of fatigue, concentration problems and PEM. Thus, the primary aim is to investigate if it works and not how it works. This primary aim corresponds more to a pragmatic trial than to a mechanistic one. Consequently, the primary study design is more pragmatic than mechanistic. As the reviewer points out, there are several potential pathways through which SSRIs could reduce post COVID symptoms. Therefore, our additional aims are directed at identifying differences between treatment responders and non-responders in biomarkers as this could shed light on underlying pathophysiology of post COVID.

Response rate

Comment R 13738774: a 50% response rate is expected after the SSRI, which is similar to responses rates to placebo in many antidepressant trials (2.3). This is also lower than the expected response rate for CBT from the prior study of the investigators showing 60% response 9 (2.7).

Reply: we believe an expected response rate of 50% is realistic based on available evidence. The only study so far, reporting on response to SSRI treatment initiated among patients with already existing post COVID symptoms found a response rate of 63%. However, this was not a placebo-controlled

study so that a possible placebo effect cannot be excluded. The CBT trial was conducted among patients with severe post COVID fatigue who had a relatively short duration of post COVID symptoms. The likelihood of treatment response usually decreases with duration of symptoms. Therefore, we believe a response rate below 60% would be more likely to be achieved among a group of patients with a prolonged duration of symptoms.

Feasibility

Comment R 13738774: 16 patients per month in year 1 are proposed to meet the criteria for the interim analysis. This seems extreme considering lower levels of COVID infection over the past two years. Existing COVID cohorts established during the pandemic will be a recruitment source, but whether many will still have significant fatigue (due to COVID) or meet the unclear inclusion criteria (e.g., is there a cut point for time from infection?) is not discussed. The staff proposed are also adequate for a normal study, but it seems impossible for them to handle 16 patients enrolled per month for a study that would last a minimum of 12 weeks per patient, and in year 2 include scans and other assessments.

Reply: Indeed, the number of new Covid-19 infections has been low over the past 2 years. Yet, the number of patients with post COVID in the Netherlands is estimated to be 90.000 (estimate Maatschappelijk Impact Team, juni 2023). The number of patients enrolled in the cohorts from which we aim to recruit is ≈ 25.000. With 8% to 10% of previous SARS-CoV-2 infected persons developing post COVID this would represent 2000 to 2500 persons. If needed, we will recruit outside of the cohorts. With the personnel requested, i.e., post-docs for study coordination and neuro-imaging, a research assistant and a research physician, we will be able to include 16 patients per month and conduct scans and assessments. The capacity for fMRI scanning is sufficient for these numbers of patients.

Effect of CBT

Comment R 13738794:claims are made about the study results providing further insights into the value of CBT in patients with PASC, but the analyses planned after the trial's primary endpoint read out at 12 weeks are not described. Nor is the duration of follow-up on CBT. CBT will be offered to non-responders after 12 weeks, presumably as a stand-alone intervention? There is a reference in a late section of the proposal to gaining insight into the combined use of SSRI therapy and CBT, but I assume this is meant to imply sequential use? Note, also, that this will be a heterogeneous population of patients with ongoing fatigue, who may or may not have been treated with an SSRI, presumably treated in an uncontrolled fashion with CBT, all of which represent challenges in secondary analyses of the value of CBT.

Reply: CBT would indeed be offered as a stand-alone intervention to non-responders at week 12. The reviewer correctly assumes that there would be sequential use of SSRIs and CBT. The duration of CBT would be 20 weeks (this information was presented in the Figure study design). The reviewer correctly states that this would be a heterogeneous group of patients with ongoing fatigue. We would like to clarify that the rationale of offering CBT to non-responders was to be able to look at differences in biomarkers between treatment responders versus non-responders because we know from our previous RCT that about 60% of patients can be expected to respond to CBT. We aim to get insight in the relation between treatment response and changes in biomarkers irrespective of the way the treatment response has been achieved (CBT of SSRI).

Budged

Comment R 13738774: it is not clear why the pharmacy fees are higher than the neuroimaging fees.

Reply: pharmacy fees are high due to the high costs of manufacturing study medication (both fluvoxamine and placebo) in accordance with GMP legislation.