Inflammation, SARS-CoV-2 infection and mental health disorders impact on systemic serotonin and dopamine metabolism

Rebuttal Letter

PSY Team

2023-05-23

# Editor

Thank you for the timely and well written piece. I have received three reviews from exceptional scholars in the field. As you can see, there are many strengths to the paper that they note and the publication is timely. However, there are also several concerns raised and I am not entirely sure they can all be sufficiently addressed to warrant publication. However, I think it is worth a shot. Please be attentive to reviewer concerns for a resubmission.

Thank you for appreciation of our manuscript and giving us a chance to address the reviewers’ issues. First we would like to thank the reviewers for their very careful, thoughtful and helpful assessment and comments. We acknowledge that such detailed reviews take a lot of time and demand a lot of commitment from the reviewers and hope that we have done them justice in the current version.

Overall, we agree that possibly due to the involvement of a large team of researchers in the study, the first version submitted was not as coherent and clear as it should have been. We have taken your advice and the suggestions of the reviewers very seriously and reworked the analysis and the text. We would like to point out that this is one of only a small number of available manuscripts addressing the role of the kynurenine and catecholamine pathway in COVID-19 and associated mental health impairment.

We have re-analysed the data to address the reviewers´ concerns and have rewritten many parts of the manuscript, thereby incorporating their suggestions in the text. In particular, we now clearly and explicitly state the gap in the literature in the Introduction and the analysis endpoints in the methods and results section. We now present our results in a ‘top - bottom’ approach. In brief, we employed the most robust and comprehensive analytic technique, multi-parameter modeling, to identify the most relevant explanatory factors affecting the kynurenine pathway affecting also tryptophan and serotonin availability and the catecholamine pathway. Subsequently, we explore these factors in more detail by correlation and network analysis, and canonical statistical tests. Concerning the manuscript text we also improved the preciseness of the language and adapted the Discussion. Please see below the point by point response for details. We hope that, our revised manuscript is now suitable for publication in *Psychoneuroendocrinology*.

# Reviewer 1

## Issue 1

The study assesses the relationship between psychological variables, inflammatory markers, and concentrations of aminoacids in individuals after SARS-CoV-2 infection compared to uninfected controls in two distinct cohorts (SIMMUN; INCOV). Moreover, the difference between the control group and different states after SARS-CoV-2 infection (acute, sub-acute, recovery) are compared to the control group within the longitudinal INCOV study cohort. In addition, the study aims to test the hypothesis that Phenylalanine, Tyrosine, Tryptophan, Kynurenine, and their respective ratios, are predicted by psychological variables inflammation, and SARS-CoV-2 infection-related variables (infection yes vs no, Anti-Antibody titers). First, it should be mentioned that the graphs are of very high quality and aesthetics and summarize the results very well. In addition, the use of a very sophisticated analysis (multiparametric linear modeling) and the detailed presentation of this analysis, and the R packages used are highly creditable. However, several weaknesses, including, biochemically wrong assumptions regarding the assessed parameters, lack of transparency regarding the pre-processing of the data, and measurement comparability between the assessed cohorts limit its significance and impact. The quality of the paper also suffers from a lack of references, scientifically inadequate language, and multiple spelling and grammatical errors. Because of the many issues that arise, I would not recommend the manuscript for publication in *Psychoneuroendocrinology*.

We thank the Reviewer for the careful assessment of our manuscript and valuable feedback which were very helpful in revising our work. First we would like to apologize for imprecise wording, not including enough references and any language errors. We have reworked large parts of the manuscript in order to better and more clearly present our data. In order to improve the clarity of our research aims and to facilitate interpretation of the results, we re-formatted the analysis pipeline and the manuscript in the following way:

* we reorganized and rewrote many parts of the **Introduction** section.
* In **Introduction**, we also more explicitly and clearly state the ‘ gap in the literature’ and our research aims in the last paragraph of the section.
* In **Methods we added the section ”Analysis endpoints” to improve clarity**
* We have included a new figure XX in which we clearly show the analysis workflow for the SIMMUN and INCOV cohort. We hope that this will make it easier and clearer to follow our analysis. Additionally we restrict the analyzed SIMMUN and INCOV cohorts to participants with the complete set of outcome and explanatory variables (**Figure 1**, **Supplementary Table S1** and **S3**)This makes the results of particular analyses more easily comparable to one another. Additionally, to investigate if and how exclusion of participant affected the SIMMUN cohort characteristic and if it could be regarded as a source of bias, we compared the excluded and analyzed individuals as presented in **Supplementary Table S2** and **Results/Characteristic of the study cohorts**.
* We excluded CRP and IL6 from analyses presented in the revised manuscript. Both markers were found to be slightly above their reference ranges (0.5 mg/dL and 7 pg/mL) solely in 10% and 4.8% of SIMMUN individuals, respectively. They are hence unlikely to play a clinically relevant role in regulation of TRP, KYN, KYN/TRP, PHE, TYR and PHE/TYR, in spite of statistically significant correlations presented in the initial manuscript. Details on this are now described in the **Supplementary Methods** of the revised manuscript.
* We provided information on all analyzed variables of the SIMMUN (**Supplementary Table S1**) and INCOV cohort (**Supplementary Table S3**) with their format, units and, if applicable, normality-stabilizing transformations.
* In order to improve clarity of the results section, we have now reordered and reformatted it. We now present the results in a ‘top - bottom’ apporach. In brief, both in the SIMMUN and the INCOV cohort, we employ multi-parameter modeling (linear regression or robust linear regression), to identify key explanatory factors significantly affecting the outcome. Subsequently, we investigate those significant predictions in more detail by ‘univariable’ correlation analysis and statistical hypothesis testing. This analysis strategy has recently been used in two COVID-related publications from our laboratory (1,2).

## Issue 2

**Introduction:** In the introduction the term “psychoneuroimmunological mechanisms” is mentioned twice. I would appreciate it if at least some of the mechanisms which are relevant to the paper are specified.

As outlined in **Issue 1**, we re-ordered and re-wrote the entire **Introduction** section. Following discussions in the core author team, we decided to replace the general term ‘psychoneuroimmunological’ with more direct descriptions of the mechanisms we are referring to i.e. the role of inflammation on IDO activity are the effect of glucocorticoids on TDO activity. We have also changed the title of the manuscript.

## Issue 3

**Introduction:** Some statements made in the introduction are without reference, very general, and the conclusions weakly justified. For instance, TRP, KYN, and KYN/TRP are listed as markers of serotonin availability. However, the available evidence for the relationship between increased degradation of TRP to KYN and its effects on serotonin availability is controversial as only 1-2% of tryptophan metabolism goes into the serotonin pathway (<https://doi.org/10.3390/metabo12060514>). In addition, line 60 mentions a depressiogenic and an anxiogenic effect of QUIN, and an anxiety- and depression-amplifying effect of kynurenine and catabolites is stated in line 9 (page 3). Should this statement refer to existing cross-sectional findings (e.g., higher QUIN in patients with MDD compared to healthy controls) it is very generalized and not sufficiently justified. If the statement refers to evidence that suggests a causal relationship, a reference is urgently needed.

We agree that the role of the kynurenine pathway metabolites as well as the associated availability of tryptophan and serotonin in mental health are controversial and definitely need a very differential view on the topic. We find that one of the many challenges is that many mechanistic studies are done in the animal model, making the transfer to the human situation questionable. On the other hand human studies are quite divergent and there are quite some influencial theoretical manuscripts on the issue, but, in relation, surprisingly few original research manuscripts. We have taken very seriously the comment by the reviewer and decided to give a more balanced view on the topic of kynurenine and catecholamine pathways and their role in mental health while still keeping the text relatively compact. We now give a more clear, but short introduction to the two biochemical pathways in the introduction section. We provide an ample discussion of the possible role of these pathways in linking mental and physical health. In the Discussion we discuss the potential sources of the very heterogenous findings in the literature (such as e.g. subtypes of depressive disorders ) and also the question of central and peripheral measurements in more detail (a short statement was included in the original manuscript). Also in the Limitation section we have added a comment that some researchers are not convinced on the role of serotonine as well as the KYN or catecholamine pathway in depression and other psychiatric disease. Additionally, we addressed the question how TRP levels may affect systemic serotonin availability in the original data by, modelling circulating serotonin levels in the INCOV cohort (10) as a function of its precursors and competitor metabolites (TRP, KYN, QUIN), timepoint of SARS-CoV-2 infection, inflammatory cytokines and age. As presented in **Figure 3**, we could indeed find a significant positive association of plasma TRP and serotonin in multi-parameter modeling, which is expected to be independent of age, inflammation and infection time course. In a more detailed correlation and network analysis of the INCOV dataset (**Figure 5A**), we could observe a moderate-strength correlation of circulating serotonin and TRP levels in acute and sub-acute infection. This suggests that systemic serotonin synthesis may be limited by blood TRP availability to some extent during SARS-CoV-2-dependent inflammation, which was characterized by lower TRP and elevated KYN levels (**Figure 4**).

## Issue 4

**Introduction:** The introduction sentence of the last paragraph in the introduction seems not adequate for the following hypotheses. It highlights that low-grade inflammation (as I understand it, in the case of this study IL-6 and CRP concentrations) is associated with mental health. However, the presented hypotheses use IL-6 and CRP and mental stress, anxiety, and depressive symptoms as predictors in their analysis (multi-parameter linear modeling) and TRP, KYN, KYN/TRP, PHE, TRY, PHE/TRY as dependent variables.

We are sorry for this unclarity. In the revised manuscript this has been clarified. Please refer to the last paragraph of the **Introduction**, the subsection “analysis endpoints” in the **Methods/**,as well as **Supplementary Tables S1** and **S3**As described in the **Supplementary Methods**, only a minute subset of the SIMMUN study participants had CRP or IL-6 concentrations above the reference levels. Thus, biological effect of those two inflammatory markers is doubtful and we decided to exclude them from the revised analysis pipeline.

## Issue 5

**Methods:** while the SIMMUN cohort is described in detail, basic information about the INCOV cohort is missing and should be provided.

We agree with the Reviewer that the INCOV cohort deserves a more thorough description, we left this out of the original version of the manuscript because the data are already published, but it made it difficult to understand the current manuscript, so we now this information in the e **Methods/Procedures** section of the revised manuscript and in **Supplementary Methods** (10,11).

## Issue 6

**Methods:** Before the statistical analysis, several transformations (log2, ln, and square root) were applied. However, it is not transparent how each parameter is transformed. I would appreciate it if this could be further specified.

We provide now these information in **Methods** and **Supplementary Methods**. Variable format, transformations and, if appropriate, stratification schemes are presented in **Supplementary Tables S1** and **S3**.

## Issue 7

Methods: Furthermore, when measured within clinical routine laboratory values of CRP and IL-6 are in many cases lower the limits of detection (e.g., 0.3 mg/ml). Was this the case in this study? If so, it would be useful for the replicability to know how the authors dealt with this issue.

As specified now in **Methods/Procedures**, values beyond or at their detection limits were substituted with the lower or upper detection limit. CRP and IL6 were removed from the revised analysis pipeline for the reasons specified in **Issue 1**.

## Issue 8

**Methods:** Kruskal-Wallis test with Man-Whitney U tests are applied to assess the differences be-tween uninfected individuals and infected individuals at different time points. While the pairwise comparisons are adequate for this research question, the overall statistic of the Kruskal-Wallis test is not useful in this context, as it tests whether there is an over-all difference between independent groups. I would therefore suggest using a linear model with predefined contrasts (uninfected vs. acute; uninfected vs. sub-acute; uninfected vs. recovery) inclusive control for multiple tests. If the assumptions of linear regression are not fulfilled, robust methods can be used (e.g., R-package: MASS; <https://www.rdocumentation.org/packages/MASS/versions/7.3-58.2/topics/rlm>). Additionally, the correlations between the assessed dependent variables seem rather high, why controlling for multiple comparisons using Benjamini & Yekutieli procedure (2001) or even robust MANOVAS (see: <https://cran.r-project.org/web/packages/MANOVA.RM/vignettes/Introduction_to_MANOVA.RM.html>) could be also necessary. Additionally, the terms “acute”, “sub-acute and”recovery” should be described in the notes of **Figure 3**.

We are grateful for these suggestions and have taken them up In the revised manuscript, we applied robust linear regression with the MM algorithm and Huber’s psi function (12,13) for multi-parameter modeling (**Figure 3**) and time-course modeling of the INCOV dataset (**Figure 4**). Additionally, we specify in the figure legends of the INCOV cohort results the median time interval between diagnosis and sampling for the acute, sub-acute and recovery timepoints. As suggested, we applied multiple testing adjustment throughout the analysis pipeline. Except for multi-parameter modeling, p values were adjusted separately for each analysis step (e.g. correlation analysis, time-course modeling) with the false discovery rate method known also as ‘Benjamini-Hochberg’ (14).

## Issue 9

**Psychometric assessment:** In the description of the psychometric assessment, the source of the HADS is missing as well as a calculation of the reliability of the used questionnaires for the SIMMUN study cohort.

We apologize for the missing references and cite the seminal HADS paper and a report dealing with the clinically relevant HADS cutoffs (15,16) in the revised text. We also checked consistency of the PSS-4 and HADS psychometric tools employed in our report. Since the HADS scales, and in particular, the HADS depression tool were found to be non-tau-equivalent by factors analysis (**Supplementary Figure S1A**), we decided use McDonald’s as a consistency measure instead of the more popular Cronbach’s (17,18). The PSS-4 tool demonstrated a good and the HADS scales an excellent consistency, as presented in **Supplementary Figure S1B**.

## Issue 10

**Results:** First, it would be useful to distinguish between cohorts in the descriptive characterization of cohorts. Furthermore, concrete numbers should be given instead of rough descriptions (e.g., “about half” (page 8, line 19)). Second, assessing the effect as “dramatic” is not appropriate in a scientific context. If at all, existing assessments based on effect sizes can be made using generally accepted definitions (e.g., according to Cohen).

We concur with the Reviewer, that a comparison between the cohorts is useful for interpretation of the results. We present significant results of the comparison between the SIMMUN and INCOV cohort in **Supplementary Table S4** and elaborate on them in **Results/Characteristic of the study cohorts**. The cohorts differed in age and sex distribution, rates of SARS-CoV-2 infection and infection severity. In particular, most INCOV study participants experienced moderate-to-critical COVID-19 and required hospitalization. This may explain the stronger effects of inflammation and infection timepoint on inflammatory cytokines and the metabolites of interest, which we discuss in **Discussion** and **Limitations**. In the revised text, Figures and Tables we also present effect size metrics for statistical hypothesis tests, correlation and modeling. We interpret them as proposed in the handbook by Jacob Cohen (19) (**Supplementary Methods**).

## Issue 11

**Results:** In general, numbers (test statistics, p-values) are missing in the results part and should be added. Additionally, effect sizes should be presented.

We present now p values and effect sizes or model with 95% confidence intervals (19), as appropriate, for the most important findings in Abstract and Results. Yet, for the sake of readability, we were not able to provide significance and effect size information for all effects. In particular, we do not specify p values and effect sizes for non-significant effects and state instead clearly that the effect missed statistical significance. We abstained from referencing test statistics such as or , since they depend on the number of complete observations and hence do not deliver reliable information on the effect size.

## Issue 12

**Results:** “Tends to be higher”/ “Slightly more” is scientifically incorrect. Please replace the terms and add the test statistic and p-value

Following your suggestion, we avoid this wording in the revised manuscript. Please refer to **Issue 11** for details.

## Issue 13

**Results:** The terminology of strata (page 10, line 10) can be misleading as no stratification was performed. Probably it would be better to use the term group or subgroup.

Following your suggestion, we have replaced the term ‘strata’ in the revised text.

## Issue 14

**Introduction:** The use of subheadings would be helpful to facilitate the understanding of the first part of the introduction.

We re-wrote large parts of the **Introduction** and Discussion of the revised manuscript. The Introduction is separated by paragraphs, the discussion has subheadings. If desired sub-headings can also be introduced to the Introduction, we have avoided it at this point because we are not sure if this is common practice.

## Issue 15

**Results:** The statement that TRP is a precursor of IDO is not correct!

Thanks for the attentive assessment, this misleading wording was removed.

## Issue 16

**Results:** The description of the time between the infection and the assessment of 138.5 days leads to the question of how the time between the infection and the assessment was assessed. Maybe it would be better to use complete instead of half and quarter days as units.

This time interval refereed to the SARS-CoV-2 infection diagnosis via PCR test, which we specify in the revised manuscript. We now also round them to complete days, as suggested.

## Issue 17

**Results:** h. Table 1: I would suggest adding further lines in the table as “heading” lines specifying the cohorts described in the following parts.

For the sake of clarity, we present characteristic of the SIMMUN and INCOV cohorts in separate tables (**Table 1** and **Table 2**).

## Issue 18

**Results:** Page 9 lines 24-41; This paragraph should be in the Methods instead of the Results. Furthermore, the rationale is missing: Why was INCOV used as a comparison or additional study to your own data? In addition, information on the measurement /measurement system (for INCOV) should be provided. Were the measuring probes used in SIMMUN and INCOV comparable?

As suggested we now list the variables of interest in the **Methods** section instead of **Results**. Furthermore, we describe the rationale for using the data of the INCOV cohort in our analyses in the last paragraph of **Introduction** and the section “analysis endpoints” of the **Methods/Analysis endpoints**. Unfortunately the incompatibility of designs and datasets of these two cohorts hindered us from developing one comprehensive model in one cohort and direct validation in the outer (canonical training - test or external validation setup). We list this issue in **Limitations**.

## Issue 19

**Results**: The term acute and sub-acute should be defined.

We provide the time intervals for the acute, sub-acute and recovery timepoints in the INCOV cohort in the revised manuscript (**Methods**, **Results**, **Figures**). Of note, those time intervals fit well with the definition of acute, sub-acute and long-COVID/recovery timepoints in our recent population study (24).

## Issue 20

**Results:** What does the term “virtually age-independent” mean? I would appreciate it if concrete numbers are stated here instead.

We have removed all imprecise wording while rewriting the Results sections. Thank you for pointing this out.

## Issue 21

**Results:** Page 11 lines 35 to 54 should be part of the statistical description in the Method section.

This part of the manuscript was removed during revision. Following your suggestion, we keep details on procedures and statistical analysis in the **Methods** section.

## Issue 22

**Results:** Page 15 line 28: standard inflammatory cytokines should be named more specifically.

We are sorry for this unclarity. In the revised text, we name inflammatory markers (NEO, NLR, cytokines) more explicitly.

## Issue 23

**Discussion:** The discussion of the study results is rather shallow: Results should be better integrated into existing evidence. Furthermore, there is no discussion of the results of depressive symptoms (HADS).

In the revised manuscript, we restructured and improved the **Discussion**. Large parts of the discussion were reorganizied and rewritten in the order suggested by reviewer 2 (issue 40). We hope that you agree with the structuring proposed by the other reviewer which we have now followed. We have also included new references to back up our discussion points. The limitations section was also expanded and improved.

## Issue 24

**Discussion:** The statement that “BH4 is also important for nitric oxide synthesis and therefore involved in oxidative stress” is not correct. (Page 16) BH4 is an essential Co-factor of NO-synthases that produce NO with its function as a vasodilator and additional anti-pathogenic effects. Only if BH4 is missing, there is an uncoupling of NOS with subsequently increased reactive oxygen/nitrogen species. Thus, your “therefore involved in oxidative stress” is misleading in normal physiological states.

We have removed most of the discussion on oxidative stress from the revised text since it is likely not so important for interpretation of our data. Instead we focus more on BH4

## Issue 25

**Discussion:** Page 14 line 60: “pre-pandemic inflammation markers” is probably not the right term. Maybe rewrite the sentence.

We apologize, this is really misleading and was reworded.

## Issue 26

**Highlights:** In the Highlights, the results and perhaps the conclusion should be emphasized more than the theoretical background (Highlights 1 and 2) or the methodology (Highlights 3 and 4).

*The highlights were adapted according to your suggestions.Katharina: please respond, I don’t have this manuscript part at all in pre-revision manuscript files.*

## Issue 28

**General:** Furthermore, there are several grammatical flaws and spelling mistakes. Some of these mistakes are listed here. However, the manuscript would benefit from professional language correction.

Thank you for your careful assessment. . We have paid particular attention to the style, grammar and proper scientific language in the revised manuscript. For particular language issues:

* *Page 3 line 24: mediate instead of mediated.* - corrected as suggested
* *Page 3 line 29: sentence incomplete* - rewritten
* *Page 4 l. 35. Results of the SIMMUN study* - does not apply, **Results** were rewritten
* *Page 4: line 37: in-person study visit sounds not familiar: better use on-site investigation* - we use the wording ‘on-site study visit’ in the revised manuscript
* *Page 4 line 38 is grammatically not correct: probably better […] the results of which are re-ported here* - rewritten
* *Page 5 line 14: The term physician assessment should be replaced by a better term* - we use ‘general medical assessment’ instead.
* *Page 6 line 11: The hyphen is missing at “inflammatory”* - does not apply, the section was rewritten
* *Page 8: lines 55-56: In the participants who were tested…* - rewritten
* *Page 8 line 58: timepoint in [the?] the course sounds strange (and the article is missing), may-be better time between infection and assessment.* - changed to ‘timepoints of SARS-CoV-2 infection’ and ‘days after diagnosis via PCR’
* *Page 9 line 52: What are nadir TRP concentrations?* - This was changed to ‘minimum’
* *Page 11 line 7: a comma is missing.* - does not apply, the section was rewritten.
* *Page 12 line 24: “on the other hand” without “on the one hand”* - we removed this colloquial phrase and other similar wording from the revised text
* *Page 13: line 4: a comma is missing* - rewritten
* *Page 14 line 14: […] via their bidirectional influence* - rewritten
* *Page 14 line 34: please delete the term “larger”* - done as suggested.
* *Page 15, line 35: of in individuals* - rewritten
* *Page 16, line 16: incomplete* - rewritten

## Issue 29

**Supplementary Material:** As a criterion to compare models, the Bayesian information criterion is stated with AIC as an abbreviation.

We apologize for the typo, it was corrected to ‘BIC’.

## Issue 30

**Supplementary Figure S2:** It is not clear why fitted generalized adaptive models are presented as graphs, as the associated statistic (Spearman’s rho) assesses monotone relationships.

We concur with the Reviewer’s view, that visualization of trends for monotone associations is problematic. Due to profound changes in the manuscript structure, we do not show scatter plots to illustrate monotone correlations between non-normally distributed variables in the revised version. Correlation analysis results in the INCOV cohort are now presented as simple bubble correlograms or visualized as force-directed graphs (34,35).

# Reviewer 2

## Issue 31

In this paper, the authors show the relationship between alterations in markers of inflammation, aminoacid precursors, and symptoms of depression, anxiety, and stress in individuals COVID-19-positive or not. They relate the results of these observations to the results obtained in another immune and metabolic study carried out at different time points of the COVID-19 trajectory and already published by a group of different investigators. The methodology is excellent as can be expected from a research team specialized in the study of inflammation and its impact on the kynurenine metabolism pathway. The results are original and sufficiently important to be published. The main problem with the current manuscript is the confusion coming from the mode of presentation that mixes up in the results section data from the two studies. The discussion is circumvoluted and difficult to follow.

We thank the Reviewer for appreciation of out text and the constructive feedback. Weaknesses in structure of the analyses were already pointed out be the Reviewer 1. We addressed them by employing the multi-parameter modeling results for identification of the most vital explanatory factors that affect neurotransmitter metabolism and subsequent more specific correlation analyses and statistical testing with the identified most relevant predictors. To make the analysis of the two investigated cohorts easier to follow, we separated the results obtained with the SIMMUN and INCOV collective. In more detail, we employ the SIMMUN cohort as an ‘exploratory’ collective to identify factors affecting TRP, KYN, KYN/TRP, PHE and PHE/TYR from a wider palette of demographic, clinical, psychometric, inflammatory and SARS-CoV-2 associated variables. Subsequently, we attempted to validate the most important ones: age, inflammation and SARS-CoV-2 infection in the INCOV cohort. Finally, we re-wrote the **Discussion** section with a special focus on the crosstalk between inflammation and neurotransmitter biosynthesis and, in part controversial, relevance of systemic neurotransmitter availability for the central nervous system and mental health.

## Issue 32

There are a few typos in the text, e.g., othe (line 6 instead of other).

Thanks for the careful assessment. We paid special attention to the grammar and spelling of the revised text.

## Issue 33

The text would benefit from further editing by a specialized scientific English editing service

*The first author of the manuscript has extensive experience in scientific English writing, we are assuming that some inconsistencies in the first version of the manuscript arose from working on the manuscript in quite a large team. The first author has now carefully reworked the text and we hope that this has now lead to a more consistent style and better quality. There were indeed a lot of issues to be corrected.*

## Issue 34

Line 7: by collectives you probably mean cohorts or population samples.

Yes, in the manuscript text the term ‘collective’ had the same meaning as ‘cohort’. We removed the word collective since this obviously was not easy to understand. For practical reasons, we avoid the wording ‘population sample’, which may suggest a random and hence representative draw from the general population. In fact neither the SIMMUN cohort enriched in psychiatric patients nor the INCOV cohort enriched in hospitalized COVID-19 patients pose a representative sample of the general population during the pandemic (see **Limitations**).

## Issue 35

The introduction ends with the statement that the study was conducted in an exploratory manner. Was that the objective of the present study in the absence of any specific hypothesis?

As also pointed out by Reviewer 1, the last paragraph of **Introduction** was not written clearly enough in the first version . In the final part of **Introduction**, we present now the ‘gap in the literature’, which is the still incompletely characterized effect of SARS-CoV-2 infection on neurotransmitter precursor amino acid availability, as well as the aim of our analysis, which was characterization of factors affecting the neurotransmitter turnover in a cohort of SARS-CoV-2-infected individuals with and without mental health impairment. Our study did not have a fully exploratory character, since the candidate explanatory factors: age, sex, body mass class, mental and somatic conditions, smoking and alcohol, depression, anxiety, stress and inflammation have already been described to affect serotonin and dopamine metabolism in previous research, also from our own research consortium (e.g. Hüfner et al .2015, 2019, 2020,2021)

## Issue 36

Page 9, line 34 and elsewhere in the text when referring to immune factors that are measured, use the term variables instead of parameters.

We agree that the term ‘parameter’ may be misleading, especially in context of modeling and its results. We changed it to ‘variable’ throughout the revised text.

## Issue 37

Legend of Figures: the use of the term decay is inappropriate, you probably mean metabolites

This was reworded in the revised versiond.

## Issue 38

The results are difficult to read as you mix up the two cohorts both in the text and in the figures. It would be preferable to first present the results of your own cohort, the SIMMUN cohort, and then test the generality of the results by assessing whether the same variations are found in the INCOV cohort with the limitation that this can only be done for the relationship between immune factors and neurotransmitter precursors.

We agree and how now rearranged the complete results. Please refer to **Issues 1** and **31** for details.

## Issue 39

You utilize the terms mental disease, mental stress, mental symptoms, etc… Be more specific and replace these terms by what you measured, i.e., HADS-anxiety, HADS-depression, and PSS. You mention somatic symptoms in the discussion but what they refer to remains obscure. You also refer to somatic diseases. Pathologists would rather speak of physical disorders as opposed to psychiatric disorders.

Thank you for your suggestions. We have specified the terms according to your suggestions throughout the revised version of the text. + We removed the sentence from **Discussion** referring to somatic symptoms, since those were not systematically surveyed in the SIMMUN cohort. Following your suggestion, we term ‘somatic conditions’ ‘physical disorders’ consequently in the revised text.

## Issue 40

The discussion is convoluted and needs to be simplified. It would be easier to follow if you would first discuss the significance of the association between inflammation and neurotransmitter precursors and then the association between these two sets of factors and what you call mental health measured by HADS and PSS, first in the immune -> HDAS symptomatology or kynurenine -> HDAS symptomatology and then in the stress -> immune direction, remembering that in the case of your own cohort, the cross-sectional nature of your study does not allow you to get further than describing associations.

We thank the reviewer for this specific suggestion on the discussion section which we have gratefully taken up and rewritten large parts of the discussion section.

## Issue 41

Page 15: the study by Benedetti is at best suggestive but does not demonstrate anything.

We have remove this reference

# Reviewer 3

## Issue 42

Considering the disease SARS CoV-2 and its consequences in the form of post-covid syndrome, the presented work is highly relevant.

We thank the Reviewer for appreciation of our manuscript and the valuable feedback.

## Issue 43

In the abstract, the authors introduce …markers of serotonin availability (TRP, KYN, KYN/TRP ratio)….(line 24). This statement is not accurate, as Serotonin is formed from TRP, but there is no KYN in its catabolic pathway. Only if the catabolism of TRP moves more to the kynurenine pathway, less TRP goes to the serotonin pathway, and the availability of serotonin is reduced. This argument is similar to the line 44.

We absolutely agree with the reviewer and have now used the words “KYN pathway affecting also TRP and serotonin availability” or KYN pathway and related metabolites to account for this fact’ in the revised text.p

## Issue 44

The principle point is that it would help the reader to orient himself in such a large amount of data if the authors completed the manuscript with a design scheme of the INCOV and SIMMUN study

Thank you for the suggestions, this was added (figure XX)

## Issue 45

In line 16 on p. 6 are introducing breakdown products as KYN, TRP, KYN/TRP ratio ….. It would be more precise to introduce them in the order they are formed: TRP, KYN, KYN/TRP ratio ….pls, correct everywhere in the manuscript similar p. 9, line 29 ….. For example, correct position is given on the line 52 of p.11

Thank you, we made the changes you suggest throughout the revised manuscript.

## Issue 46

p. 14, line 34 : a large meta-analyses … include more citations

We have improved this part of the Discussion and now report citations on the pros and cons of KYN pathway involvemen in depression in the text and the limimitations section (e.g. We re-

## Issue 47

p. 15, line 38 - correct as a note in the abstract

*Katharina, could you please respond? I don’t have the submission text with the line numbering and can’t find the fragment. Thanks!*

## Issue 48

p. 15, line 62 - where ???

*Thanks you this was corrected.*

## Issue 49

put one line above the grey text stating that it is INCOR

*Same as above!*

## Issue 50

the given data do not match mathematically - check or explain why

We apologize for the typos concerning numbers in the study inclusion scheme. Please refer to the correct numbers in the revised **Figure 1**.

## Issue 51

explain used abbreviations in the text below

We are sorry for that. We explain now all abbreviations used in **Figures** in their legends.

# References

1. Hüfner K, Tymoszuk P, Ausserhofer D, Sahanic S, Pizzini A, Rass V, Galffy M, Böhm A, Kurz K, Sonnweber T, et al. Who Is at Risk of Poor Mental Health Following Coronavirus Disease-19 Outpatient Management? *Frontiers in Medicine* (2022) 9: doi: [10.3389/fmed.2022.792881](https://doi.org/10.3389/fmed.2022.792881)

2. Hüfner K, Tymoszuk P, Sahanic S, Luger A, Boehm A, Pizzini A, Schwabl C, Koppelstätter S, Kurz K, Asshoff M, et al. Persistent somatic symptoms are key to individual illness perception at one year after COVID-19 in a cross-sectional analysis of a prospective cohort study. *Journal of Psychosomatic Research* (2023) 169:111234. doi: [10.1016/J.JPSYCHORES.2023.111234](https://doi.org/10.1016/J.JPSYCHORES.2023.111234)

3. Goldstein DS, Swoboda KJ, Miles JM, Coppack SW, Aneman A, Holmes C, Lamensdorf I, Eisenhofer G. Sources and physiological significance of plasma dopamine sulfate. *The Journal of clinical endocrinology and metabolism* (1999) 84:2523–2531. doi: [10.1210/JCEM.84.7.5864](https://doi.org/10.1210/JCEM.84.7.5864)

4. Moncrieff J, Cooper RE, Stockmann T, Amendola S, Hengartner MP, Horowitz MA. The serotonin theory of depression: a systematic umbrella review of the evidence. *Molecular Psychiatry 2022* (2022)1–14. doi: [10.1038/s41380-022-01661-0](https://doi.org/10.1038/s41380-022-01661-0)

5. Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, Gomes-da-Costa S, Lane M, Sanches M, Diaz AP, et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. *Molecular Psychiatry 2020 26:8* (2020) 26:4158–4178. doi: [10.1038/s41380-020-00951-9](https://doi.org/10.1038/s41380-020-00951-9)

6. Teunis C, Nieuwdorp M, Hanssen N. Interactions between Tryptophan Metabolism, the Gut Microbiome and the Immune System as Potential Drivers of Non-Alcoholic Fatty Liver Disease (NAFLD) and Metabolic Diseases. *Metabolites 2022, Vol 12, Page 514* (2022) 12:514. doi: [10.3390/METABO12060514](https://doi.org/10.3390/METABO12060514)

7. Lukić I, Ivković S, Mitić M, Adžić M. Tryptophan metabolites in depression: Modulation by gut microbiota. *Frontiers in Behavioral Neuroscience* (2022) 16:367. doi: [10.3389/FNBEH.2022.987697/BIBTEX](https://doi.org/10.3389/FNBEH.2022.987697/BIBTEX)

8. Brown SJ, Huang XF, Newell KA. The kynurenine pathway in major depression: What we know and where to next. *Neuroscience & Biobehavioral Reviews* (2021) 127:917–927. doi: [10.1016/J.NEUBIOREV.2021.05.018](https://doi.org/10.1016/J.NEUBIOREV.2021.05.018)

9. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: When physiology meets pathology. (2012) 13: doi: [10.1038/nrn3257](https://doi.org/10.1038/nrn3257)

10. Su Y, Yuan D, Chen DG, Ng RH, Wang K, Choi J, Li S, Hong S, Zhang R, Xie J, et al. Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell* (2022) 185:881–895.e20. doi: [10.1016/J.CELL.2022.01.014](https://doi.org/10.1016/J.CELL.2022.01.014)

11. Su Y, Chen D, Yuan D, Lausted C, Choi J, Dai CL, Voillet V, Duvvuri VR, Scherler K, Troisch P, et al. Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19. *Cell* (2020) 183:1479. doi: [10.1016/J.CELL.2020.10.037](https://doi.org/10.1016/J.CELL.2020.10.037)

12. Huber PJ. Robust Statistics. *International Encyclopedia of Statistical Science* (2011)1248–1251. doi: [10.1007/978-3-642-04898-2\_594](https://doi.org/10.1007/978-3-642-04898-2_594)

13. Ripley B. MASS: Support Functions and Datasets for Venables and Ripley’s MASS. (2022) <https://cran.r-project.org/package=MASS>

14. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)* (1995) 57:289–300. doi: [10.1111/j.2517-6161.1995.tb02031.x](https://doi.org/10.1111/j.2517-6161.1995.tb02031.x)

15. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale: An updated literature review. *Journal of Psychosomatic Research* (2002) 52:69–77. doi: [10.1016/S0022-3999(01)00296-3](https://doi.org/10.1016/S0022-3999(01)00296-3)

16. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta psychiatrica Scandinavica* (1983) 67:361–370. doi: [10.1111/J.1600-0447.1983.TB09716.X](https://doi.org/10.1111/J.1600-0447.1983.TB09716.X)

17. Revelle W. Package ’psych’ - Procedures for Psychological, Psychometric and Personality Research. *R Package* (2015)1–358. <https://cran.r-project.org/web/packages/psych/index.html http://personality-project.org/r/psych-manual.pdf>

18. McDonald RP. *Test theory: A unified treatment*. 1st Editio. New Yor: Psychology Press (1999). doi: [10.4324/9781410601087](https://doi.org/10.4324/9781410601087)

19. Cohen J. Statistical Power Analysis for the Behavioral Sciences. *Statistical Power Analysis for the Behavioral Sciences* (2013) doi: [10.4324/9780203771587](https://doi.org/10.4324/9780203771587)

20. Capuron L, Schroecksnadel S, Féart C, Aubert A, Higueret D, Barberger-Gateau P, Layé S, Fuchs D. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms. *Biological psychiatry* (2011) 70:175–182. doi: [10.1016/J.BIOPSYCH.2010.12.006](https://doi.org/10.1016/J.BIOPSYCH.2010.12.006)

21. Widner B, Werner ER, Schennach H, Wachter H, Fuchs D. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. *Clinical Chemistry* (1997) 43:2424–2426. doi: [10.1093/CLINCHEM/43.12.2424](https://doi.org/10.1093/CLINCHEM/43.12.2424)

22. Neurauter G, Schrocksnadel K, Scholl-Burgi S, Sperner-Unterweger B, Schubert C, Ledochowski M, Fuchs D. Chronic immune stimulation correlates with reduced phenylalanine turnover. *Current drug metabolism* (2008) 9:622–627. doi: [10.2174/138920008785821738](https://doi.org/10.2174/138920008785821738)

23. Meiser J, Weindl D, Hiller K. Complexity of dopamine metabolism. *Cell Communication and Signaling* (2013) 11:1–18. doi: [10.1186/1478-811X-11-34/FIGURES/5](https://doi.org/10.1186/1478-811X-11-34/FIGURES/5)

24. Sahanic S, Tymoszuk P, Ausserhofer D, Rass V, Pizzini A, Nordmeyer G, Hüfner K, Kurz K, Weber PM, Sonnweber T, et al. Phenotyping of Acute and Persistent Coronavirus Disease 2019 Features in the Outpatient Setting: Exploratory Analysis of an International Cross-sectional Online Survey. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* (2022) 75:e418–e431. doi: [10.1093/cid/ciab978](https://doi.org/10.1093/cid/ciab978)

25. Hüfner K, Giesinger JM, Gostner JM, Egeter J, Koudouovoh-Tripp P, Vill T, Fuchs D, Sperner-Unterweger B. Neurotransmitter Precursor Amino Acid Ratios Show Differential, Inverse Correlations with Depression Severity in the Low and High Depression Score Range. *International Journal of Tryptophan Research : IJTR* (2021) 14: doi: [10.1177/11786469211039220](https://doi.org/10.1177/11786469211039220)

26. Sun Y, Drevets W, Turecki G, Li QS. The relationship between plasma serotonin and kynurenine pathway metabolite levels and the treatment response to escitalopram and desvenlafaxine. *Brain, Behavior, and Immunity* (2020) 87:404–412. doi: [10.1016/J.BBI.2020.01.011](https://doi.org/10.1016/J.BBI.2020.01.011)

27. Hüfner K, Oberguggenberger A, Kohl C, Geisler S, Gamper E, Meraner V, Egeter J, Hubalek M, Beer B, Fuchs D, et al. Levels in neurotransmitter precursor amino acids correlate with mental health in patients with breast cancer. *Psychoneuroendocrinology* (2015) 60:28–38. doi: [10.1016/J.PSYNEUEN.2015.06.001](https://doi.org/10.1016/J.PSYNEUEN.2015.06.001)

28. Fellendorf FT, Bonkat N, Dalkner N, Schönthaler EMD, Manchia M, Fuchs D, Reininghaus EZ. Indoleamine 2,3-dioxygenase (IDO)-activity in Severe Psychiatric Disorders: A Systemic Review. *Current Topics in Medicinal Chemistry* (2022) 22: doi: [10.2174/1568026622666220718155616](https://doi.org/10.2174/1568026622666220718155616)

29. Paul-Savoie É, Potvin S, Daigle K, Normand E, Corbin JF, Gagnon R, Marchand S. A deficit in peripheral serotonin levels in major depressive disorder but not in chronic widespread pain. *The Clinical journal of pain* (2011) 27:529–534. doi: [10.1097/AJP.0B013E31820DFEDE](https://doi.org/10.1097/AJP.0B013E31820DFEDE)

30. Hunt C, Macedo e Cordeiro T, Suchting R, Dios C de, Cuellar Leal VA, Soares JC, Dantzer R, Teixeira AL, Selvaraj S. Effect of immune activation on the kynurenine pathway and depression symptoms – A systematic review and meta-analysis. (2020) 118: doi: [10.1016/j.neubiorev.2020.08.010](https://doi.org/10.1016/j.neubiorev.2020.08.010)

31. Geisler S, Gostner JM, Becker K, Ueberall F, Fuchs D. Immune activation and inflammation increase the plasma phenylalanine-to- tyrosine ratio. *Pteridines* (2013) 24:27–31. doi: [10.1515/PTERID-2013-0001/MACHINEREADABLECITATION/RIS](https://doi.org/10.1515/PTERID-2013-0001/MACHINEREADABLECITATION/RIS)

32. Luporini RL, Pott-Junior H, Di Medeiros Leal MCB, Castro A, Ferreira AG, Cominetti MR, de Freitas Anibal F. Phenylalanine and COVID-19: Tracking disease severity markers. *International Immunopharmacology* (2021) 101:108313. doi: [10.1016/J.INTIMP.2021.108313](https://doi.org/10.1016/J.INTIMP.2021.108313)

33. Fanet H, Capuron L, Castanon N, Calon F, Vancassel S. Tetrahydrobioterin (BH4) Pathway: From Metabolism to Neuropsychiatry. *Current neuropharmacology* (2021) 19:591–609. doi: [10.2174/1570159X18666200729103529](https://doi.org/10.2174/1570159X18666200729103529)

34. Briatte F, Bojanowski M, Canouil M, Charlop-Powers Z, Fisher JC, Johnson K, Rinker T. ggnetwork: Geometries to Plot Networks with ’ggplot2’. (2021) <https://cran.r-project.org/package=ggnetwork>

35. Csardi G, Nepusz T. The igraph software package for complex network research. *InterJournal* (2006) Complex Sy:1695. [https://igraph.org](https://igraph.org/)

36. Maes M, Berk M, Goehler L, Song C, Anderson G, Gałecki P, Leonard B. Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. *BMC Medicine* (2012) 10:1–19. doi: [10.1186/1741-7015-10-66/TABLES/1](https://doi.org/10.1186/1741-7015-10-66/TABLES/1)