The effect of inflammation, SARS-CoV-2 infection, age and mental health on serotonin, and kynurenine and catecholamine pathway metabolites

Rebuttal Letter

PSY Team

2023-06-11

# 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 reviewers’ concerns. 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 and hope that we have done them justice in the revised manuscript.

Overall, we agree that the initial manuscript version was not sufficiently coherent and clear likely because of the involvement of a large team of researchers in the study. Following your advice and the reviewers’ suggestions, we reworked thoroughly the analysis and the text. We would like to point out that this is one of very few manuscripts characterizing activity of the kynurenine and catecholamine pathways in COVID-19 (Bizjak et al., 2022; Ceballos et al., 2022; Dewulf et al., 2022; Lionetto et al., 2021; Saito et al., 2022; Santiago-Mujika et al., 2022). Importantly, their role in symptoms of mental health disorders in COVID-19 patients has been proposed (Bower et al., 2022; Kucukkarapinar et al., 2022). By analyzing two cohorts, we were able to identify key factors affecting the activity of those metabolic pathways and investigate their temporal activity profile in acute and sub-acute SARS-CoV-infection and recovery, which adds to novelty of our work.

We have re-analysed the data to address the reviewers´ concerns and have rewritten many parts of the manuscript following the reviewers’ suggestions while keeping within the word limits of the journal. Despite extensive changes on the manuscript text, the main results remain unaltered. In particular, we now clearly and explicitly state the gap in the literature in **Introduction** and the analysis endpoints in the **Methods** and **Results** sections. To facilitate interpretation of our results, we now follow a top - bottom analysis approach. In brief, we employed the most robust and comprehensive analytic technique, multi-parameter modeling, to identify the most relevant explanatory factors affecting the key metabolites and activity markers of the kynurenine pathway, serotonin availability and the catecholamine pathway. Subsequently, we explore these factors in more detail by correlation and network analysis, and canonical statistical tests. Following the reviewer’s suggestion we also keep analysis results in the SIMMUN and INCOV cohort separately for the sake of clarity. We have re-arrange and rewritten the Discussion section taking into account the reviewer´s suggestions.

Please see below the point by point response for details. Please notice that due to the scale of changes in the text, it was not feasible to mark the changes introduced during revision. To meet the journals’ requirements concerning the maximal number of tables and figures, we had to eliminate the original figure 1 (model of investigated pathways) and move the scheme of analysis inclusion and strategy flowchart to **Supplementary Material** (**Supplementary Figure S1**). Since this improved flowchart is very helpful for the understanding of our analysis, we would move it back to the main text if we are granted to exceed the maximum figure number upon acceptance. 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 convey our research aims, results and their interpretation in a more clear way. For these reasons, we re-formatted the analysis pipeline and the manuscript in the following way:

* We unified nomenclature of the metabolic pathways investigated in our manuscript and term them now ‘kynurenine pathway’ and ‘catecholamine pathway’ in line with prominent review articles (Badawy, 2017; Lukić et al., 2022; Meiser et al., 2013)
* We reorganized and rewrote many parts of **Introduction**.
* We also more explicitly and clearly state the ‘gap in the literature’ and our research aims in the last paragraph of **Introduction**.
* We included an **Analysis endpoints** section in **Methods**. Here we describe our analytic approach, define outcome variables and candidate explanatory factors.
* We show the analysis strategy for the SIMMUN and INCOV cohort in **Supplementary Figure S1**. We hope that this makes it easier to follow our analysis. Additionally we restrict the analyzed SIMMUN and INCOV cohorts to participants with the complete set of outcome and explanatory variables (**Supplementary Figure S1**, **Supplementary Table S1** and **S3**). This makes the results of particular analyses more easily comparable to one another. Additionally, we compared the excluded and analyzed individuals as presented in **Supplementary Table S2** and **Results/Characteristic of the 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 be biologically and clinically relevant predictors of the kynurenine and catecholamine pathway activity. Details on this are now described in **Supplementary Methods**.
* 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 **Results**, we have now reordered and reformatted this section. We now present the results in a ‘top - bottom’ approach. 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 predictors in more detail by ‘univariable’ correlation analysis and statistical hypothesis testing. This analysis strategy has recently been used in two COVID-19-related publications of our group (Hüfner et al., 2023, 2022).

## 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 systemic, i.e. peripheral availability of serotonin as well as its association with kynurenine pathway activity in mental health are controversial, and definitely warrants a critical discussion (Brown et al., 2021; Goldstein et al., 1999; Marx et al., 2020; Moncrieff et al., 2022). We have tried to give a more balanced view on the topic in the revised Introduction and Discussion sections, taking the reviewer´s concerns and suggestions into account. On one hand, we find that one of the many challenges is that many mechanistic studies are done in animal models and their significance for humans is not well documented (Brown et al., 2021). On the other hand, human studies are quite equivocal and, while there are influential theoretical manuscripts on the issue, surprisingly few original research has been published so far (Brown et al., 2021).

We now give a more clear, but short introduction to the biochemical pathways in **Introduction**. We provide critical discussion of the possible role of these pathways in linking mental and physical health. In **Discussion** and **Limitations** we comment on the controversy over systemic serotonin availability as well as kynurenine and catecholamine pathway in depression and other mental disorders (Brown et al., 2021; Goldstein et al., 1999; Marx et al., 2020; Moncrieff et al., 2022; Schwarcz et al., 2012).

Additionally, to address the question whether TRP levels are associated with systemic serotonin availability in SARS-CoV-2 infection, we modeled circulating serotonin levels in the INCOV cohort (Su et al., 2022) as a function of its precursor and competitor metabolites (TRP, KYN, QUIN), timepoint of SARS-CoV-2 infection, inflammatory cytokines and age. As presented in Figure 2, we found a significant positive association of plasma TRP and serotonin in multi-parameter modeling, which is expected to be independent of age, inflammation and infection timepoints. In a more detailed correlation and network analysis of the INCOV dataset (Figure 4A, Supplementary Figure S6), we observed significant, weak-to-moderate strength correlation of circulating serotonin and TRP levels in acute and sub-acute infection.

## 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, we rephrased the aims, and define outcomes and explanatory factors. Please refer to the last paragraph of **Introduction** and the subsection **Analysis endpoints** of **Methods**.

## Issue 5

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

We included short methodological information of the INCOV cohort in **Methods** (subsection **Procedures**) and in **Supplementary Methods** (Su et al., 2022, 2020).

## 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 this 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** (subsection **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 included them in our analyses. In the revised manuscript, we applied robust linear regression with the MM algorithm and Huber’s psi function (Huber, 2011; Ripley, 2022) for multi-parameter modeling (**Figure 2**) and time-course modeling of the INCOV dataset (**Figure 3**). Additionally, we specify in legends of figures with INCOV cohort data 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’ (Benjamini and Hochberg, 1995).

## 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 (Bjelland et al., 2002; Zigmond and Snaith, 1983) 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 S2A**), we decided use McDonald’s as a consistency measure instead of the more popular Cronbach’s (McDonald, 1999; Revelle, 2015). The PSS-4 tool demonstrated a good and the HADS scales an excellent consistency, as presented in **Supplementary Figure S2B**.

## 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 S5** 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**. 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 Cohen (Cohen, 2013) (**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, 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. Instead we present p values and effect size statistics in plots and tables.

## 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 by ‘subgroup’ or ‘subset’.

## Issue 14

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

Since we re-wrote and shortened the **Introduction** section, we did not included subheadings. To facilitate interpretation, each topic of **Introduction** is described in a separate paragraph. We now included subheadings in **Discussion**.

## 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 **Methods** instead of **Results**. Furthermore, we describe the rationale for using the INCOV dataset in the last paragraph of **Introduction** and the **Analysis endpoints** subsection of **Methods**. Information on measurement of proteins and metabolites is provided in **Methods** (subsection Procedures) and **Supplementary Methods**. Unfortunately, data incompatibilities of the two cohorts hindered us from analyzing them in one comprehensive model with direct validation in the other (canonical training - test 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**).

## 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** section. 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 (SIMMUN: NEO, NLR, INCOV: IL6, IL10, TNF and IFNG) 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).

We restructured and improved **Discussion**. Large parts of **Discussion** were reorganized and rewritten as suggested by reviewer 2 (**Issue 40**). We hope that you agree with the structure proposed by the other reviewer which we have now followed. We have also included new references to back and critically interpret our results. 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 as the co-factor for synthesis of indolamine and catecholamine neurotransmitters (Capuron et al., 2011; Geisler et al., 2013; Neurauter et al., 2008; Vancassel et al., 2018).

## Issue 25

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

We apologize, this phrase was removed.

## 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.

## 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 structure of the revised manuscript, we do not use scatter plots to illustrate monotone correlations between non-normally distributed variables any more. Correlation analysis results in the INCOV cohort are now presented as simple bubble correlograms or visualized as force-directed graphs (Briatte et al., 2021; Csardi and Nepusz, 2006).

# 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 by 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 those relevant predictors. To make the analysis of the two investigated cohorts easier to follow, we separated the results obtained with the SIMMUN and INCOV cohort. In more detail, we employ the SIMMUN cohort as an ‘exploratory’ cohort 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 **Discussion** 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. We are assuming that some inconsistencies in the first version of the manuscript arose from working on the manuscript in a large study 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.

## 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 and use consistently ‘cohort’ instead. 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 initial manuscript version. In the final paragraph of **Introduction**, we now present the ‘gap in the literature’ and the specific aim of our analysis. Our study did not have a fully exploratory character, since the candidate explanatory factors: age, sex, body mass index, mental and physical disorders, smoking and alcohol consumption, depression, anxiety, mental stress and inflammation have already been described to be associated with serotonin availability, and kynurenine and catecholamine pathway activity in previous research, also from our own research consortium (Hüfner et al., 2021, 2020, 2019; Hüfner et al., 2015).

## 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 manuscript.

## 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 rearranged the complete **Results** section. 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 manuscript. We removed the sentence from **Discussion** referring to somatic symptoms, since those were not systematically surveyed in the SIMMUN cohort. Following your suggestion, we use the terms ‘mental disorders’ and ‘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 -> HADS 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 **Discussion** according to the following scheme:

* A brief generalizing summary of the results obtained with both cohorts and discussion of discrepant findings
* Effects of inflammation and infection, including SARS-CoV-2, on the kynurenine pathway as well as systemic levels of serotonin
* Effects of inflammation and infection on the catecholamine pathway and systemic availability of dopamine
* Mutual effects of mental health-related factors (e.g. depression, anxiety and mental stress) on activity of the kynurenine and catecholamine pathways, and systemic serotonin and dopamine availability. We provided a balanced and critical discussion of the evidence here.

## Issue 41

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

We removed this reference from **Discussion** of the revised manuscript.

# 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. We now consistently use the terms ‘serotonin’, ‘kynurenine pathway’ (TRP, KYN, QUIN) and ‘catecholamine pathway’ (PHE, TYR, dopamine sulfate) in the revised manuscript.

## 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. As described in **Issues 1** and **38**, we present the results in cohort-wise manner, for the sake of clarity. We also included the analysis strategy scheme as **Supplementary Figure S1**. Unfortunately we had already reached the maximum figure number allowed so that this could not be included into the main text.

## 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 **Discussion** and now report citations (Brown et al., 2021; Fellendorf et al., 2022; Hunt et al., 2020; Maes et al., 2012; Marx et al., 2020; Moncrieff et al., 2022)] on the pros and cons of KYN pathway involvement in depression in the text and the **Limitations** section.

## Issue 47

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

This was re-written as suggested.

## Issue 48

p. 15, line 62 - where ???

The ‘???’ was likely a typo in a figure reference in the manuscript markdown document. We have paid special attention to the proper syntax in the revised text.

## Issue 49

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

This was re-written as suggested.

## 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 **Supplementary Figure S1**.

## 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

Badawy, A.A.B., 2017. Kynurenine pathway of tryptophan metabolism: Regulatory and functional aspects. International Journal of Tryptophan Research 10. <https://doi.org/10.1177/1178646917691938/ASSET/IMAGES/LARGE/10.1177_1178646917691938-FIG2.JPEG>

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

Bizjak, D.A., Stangl, M., Börner, N., Bösch, F., Durner, J., Drunin, G., Buhl, J.L., Abendroth, D., 2022. Kynurenine serves as useful biomarker in acute, Long- and Post-COVID-19 diagnostics. Frontiers in immunology 13. <https://doi.org/10.3389/FIMMU.2022.1004545>

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

Bower, J.E., Radin, A., Kuhlman, K.R., 2022. Psychoneuroimmunology in the time of COVID-19: Why neuro-immune interactions matter for mental and physical health. Behaviour Research and Therapy 154, 104104. <https://doi.org/10.1016/J.BRAT.2022.104104>

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

Brown, S.J., Huang, X.F., Newell, K.A., 2021. The kynurenine pathway in major depression: What we know and where to next. Neuroscience & Biobehavioral Reviews 127, 917–927. <https://doi.org/10.1016/J.NEUBIOREV.2021.05.018>

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

Ceballos, F.C., Virseda-Berdices, A., Resino, S., Ryan, P., Martínez-González, O., Peréz-García, F., Martin-Vicente, M., Brochado-Kith, O., Blancas, R., Bartolome-Sánchez, S., Vidal-Alcántara, E.J., Albóniga-Díez, O.E., Cuadros-González, J., Blanca-López, N., Martínez, I., Martinez-Acitores, I.R., Barbas, C., Fernández-Rodríguez, A., Jiménez-Sousa, M.Á., 2022. Metabolic Profiling at COVID-19 Onset Shows Disease Severity and Sex-Specific Dysregulation. Frontiers in Immunology 13, 3155. <https://doi.org/10.3389/FIMMU.2022.925558/BIBTEX>

Cohen, J., 2013. Statistical Power Analysis for the Behavioral Sciences. Statistical Power Analysis for the Behavioral Sciences. <https://doi.org/10.4324/9780203771587>

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

Dewulf, J.P., Martin, M., Marie, S., Oguz, F., Belkhir, L., De Greef, J., Yombi, J.C., Wittebole, X., Laterre, P.F., Jadoul, M., Gatto, L., Bommer, G.T., Morelle, J., 2022. Urine metabolomics links dysregulation of the tryptophan-kynurenine pathway to inflammation and severity of COVID-19. Scientific reports 12. <https://doi.org/10.1038/S41598-022-14292-W>

Fellendorf, F.T., Bonkat, N., Dalkner, N., Schönthaler, E.M.D., Manchia, M., Fuchs, D., Reininghaus, E.Z., 2022. Indoleamine 2,3-dioxygenase (IDO)-activity in Severe Psychiatric Disorders: A Systemic Review. Current Topics in Medicinal Chemistry 22. <https://doi.org/10.2174/1568026622666220718155616>

Geisler, S., Gostner, J.M., Becker, K., Ueberall, F., Fuchs, D., 2013. Immune activation and inflammation increase the plasma phenylalanine-to- tyrosine ratio. Pteridines 24, 27–31. <https://doi.org/10.1515/PTERID-2013-0001/MACHINEREADABLECITATION/RIS>

Goldstein, D.S., Swoboda, K.J., Miles, J.M., Coppack, S.W., Aneman, A., Holmes, C., Lamensdorf, I., Eisenhofer, G., 1999. Sources and physiological significance of plasma dopamine sulfate. The Journal of clinical endocrinology and metabolism 84, 2523–2531. <https://doi.org/10.1210/JCEM.84.7.5864>

Huber, P.J., 2011. Robust Statistics. International Encyclopedia of Statistical Science 1248–1251. <https://doi.org/10.1007/978-3-642-04898-2_594>

Hüfner, K., Fuchs, D., Blauth, M., Sperner-Unterweger, B., 2019. How acute and chronic physical disease may influence mental health – An Analysis of neurotransmitter precursor amino acid levels. Psychoneuroendocrinology 106, 95–101. <https://doi.org/10.1016/j.psyneuen.2019.03.028>

Hüfner, K., Galffy, M., Egeter, J., Giesinger, J.M., Arnhard, K., Oberacher, H., Gostner, J.M., Fuchs, D., Sperner-Unterweger, B., 2020. Acute and Chronic Mental Stress both Influence Levels of Neurotransmitter Precursor Amino Acids and Derived Biogenic Amines. Brain Sciences 10. <https://doi.org/10.3390/BRAINSCI10060322>

Hüfner, K., Giesinger, J.M., Gostner, J.M., Egeter, J., Koudouovoh-Tripp, P., Vill, T., Fuchs, D., Sperner-Unterweger, B., 2021. 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 14. <https://doi.org/10.1177/11786469211039220>

Hüfner, K., Oberguggenberger, A., Kohl, C., Geisler, S., Gamper, E., Meraner, V., Egeter, J., Hubalek, M., Beer, B., Fuchs, D., Sperner-Unterweger, B., 2015. Levels in neurotransmitter precursor amino acids correlate with mental health in patients with breast cancer. Psychoneuroendocrinology 60, 28–38. <https://doi.org/10.1016/J.PSYNEUEN.2015.06.001>

Hüfner, K., Tymoszuk, P., Ausserhofer, D., Sahanic, S., Pizzini, A., Rass, V., Galffy, M., Böhm, A., Kurz, K., Sonnweber, T., Tancevski, I., Kiechl, S., Huber, A., Plagg, B., Wiedermann, C.J., Bellmann-Weiler, R., Bachler, H., Weiss, G., Piccoliori, G., Helbok, R., Loeffler-Ragg, J., Sperner-Unterweger, B., 2022. Who Is at Risk of Poor Mental Health Following Coronavirus Disease-19 Outpatient Management? Frontiers in Medicine 9. <https://doi.org/10.3389/fmed.2022.792881>

Hüfner, K., Tymoszuk, P., Sahanic, S., Luger, A., Boehm, A., Pizzini, A., Schwabl, C., Koppelstätter, S., Kurz, K., Asshoff, M., Mosheimer-Feistritzer, B., Pfeifer, B., Rass, V., Schroll, A., Iglseder, S., Egger, A., Wöll, E., Weiss, G., Helbok, R., Widmann, G., Sonnweber, T., Tancevski, I., Sperner-Unterweger, B., Löffler-Ragg, J., 2023. 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 169, 111234. <https://doi.org/10.1016/J.JPSYCHORES.2023.111234>

Hunt, C., Macedo e Cordeiro, T., Suchting, R., Dios, C. de, Cuellar Leal, V.A., Soares, J.C., Dantzer, R., Teixeira, A.L., Selvaraj, S., 2020. Effect of immune activation on the kynurenine pathway and depression symptoms – A systematic review and meta-analysis. <https://doi.org/10.1016/j.neubiorev.2020.08.010>

Kucukkarapinar, M., Yay-Pence, A., Yildiz, Y., Buyukkoruk, M., Yaz-Aydin, G., Deveci-Bulut, T.S., Gulbahar, O., Senol, E., Candansayar, S., 2022. Psychological outcomes of COVID-19 survivors at sixth months after diagnose: the role of kynurenine pathway metabolites in depression, anxiety, and stress. Journal of Neural Transmission 129. <https://doi.org/10.1007/s00702-022-02525-1>

Lionetto, L., Ulivieri, M., Capi, M., De Bernardini, D., Fazio, F., Petrucca, A., Pomes, L.M., De Luca, O., Gentile, G., Casolla, B., Curto, M., Salerno, G., Schillizzi, S., Torre, M.S., Santino, I., Rocco, M., Marchetti, P., Aceti, A., Ricci, A., Bonfini, R., Nicoletti, F., Simmaco, M., Borro, M., 2021. Increased kynurenine-to-tryptophan ratio in the serum of patients infected with SARS-CoV2: An observational cohort study. Biochimica et biophysica acta. Molecular basis of disease 1867. <https://doi.org/10.1016/J.BBADIS.2020.166042>

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

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

Marx, W., McGuinness, A.J., Rocks, T., Ruusunen, A., Cleminson, J., Walker, A.J., Gomes-da-Costa, S., Lane, M., Sanches, M., Diaz, A.P., Tseng, P.T., Lin, P.Y., Berk, M., Clarke, G., O’Neil, A., Jacka, F., Stubbs, B., Carvalho, A.F., Quevedo, J., Soares, J.C., Fernandes, B.S., 2020. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. Molecular Psychiatry 2020 26:8 26, 4158–4178. <https://doi.org/10.1038/s41380-020-00951-9>

McDonald, R.P., 1999. Test theory: A unified treatment, 1st Editio. ed. Psychology Press, New Yor. <https://doi.org/10.4324/9781410601087>

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

Moncrieff, J., Cooper, R.E., Stockmann, T., Amendola, S., Hengartner, M.P., Horowitz, M.A., 2022. The serotonin theory of depression: a systematic umbrella review of the evidence. Molecular Psychiatry 2022 1–14. <https://doi.org/10.1038/s41380-022-01661-0>

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

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

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

Saito, K., Ishikawa, R., Kitamura, I., Ogawa, K., Arakawa, N., Sun, Y., Imai, K., Maeda, T., Saito, Y., Hasegawa, C., 2022. Characterization of serotonin as a candidate biomarker of severity and prognosis of COVID-19 using LC/MS analysis. Journal of Pharmacological Sciences 150, 49–55. <https://doi.org/10.1016/J.JPHS.2022.06.005>

Santiago-Mujika, E., Heinrich, K., George, S., Forton, C., Madaj, Z., Burmeister, A.R., Sims, M., Pospisilik, A., Brundin, P., Graham, S.F., Brundin, L., Beaumont, W., 2022. Increased levels of circulating neurotoxic metabolites in patients with mild Covid19. bioRxiv 2022.06.22.497189. <https://doi.org/10.1101/2022.06.22.497189>

Schwarcz, R., Bruno, J.P., Muchowski, P.J., Wu, H.Q., 2012. Kynurenines in the mammalian brain: When physiology meets pathology. <https://doi.org/10.1038/nrn3257>

Su, Y., Chen, D., Yuan, D., Lausted, C., Choi, J., Dai, C.L., Voillet, V., Duvvuri, V.R., Scherler, K., Troisch, P., Baloni, P., Qin, G., Smith, B., Kornilov, S.A., Rostomily, C., Xu, A., Li, J., Dong, S., Rothchild, A., Zhou, J., Murray, K., Edmark, R., Hong, S., Heath, J.E., Earls, J., Zhang, R., Xie, J., Li, S., Roper, R., Jones, L., Zhou, Y., Rowen, L., Liu, R., Mackay, S., O’Mahony, D.S., Dale, C.R., Wallick, J.A., Algren, H.A., Zager, M.A., Wei, W., Price, N.D., Huang, S., Subramanian, N., Wang, K., Magis, A.T., Hadlock, J.J., Hood, L., Aderem, A., Bluestone, J.A., Lanier, L.L., Greenberg, P.D., Gottardo, R., Davis, M.M., Goldman, J.D., Heath, J.R., 2020. Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19. Cell 183, 1479. <https://doi.org/10.1016/J.CELL.2020.10.037>

Su, Y., Yuan, D., Chen, D.G., Ng, R.H., Wang, K., Choi, J., Li, S., Hong, S., Zhang, R., Xie, J., Kornilov, S.A., Scherler, K., Pavlovitch-Bedzyk, A.J., Dong, S., Lausted, C., Lee, I., Fallen, S., Dai, C.L., Baloni, P., Smith, B., Duvvuri, V.R., Anderson, K.G., Li, J., Yang, F., Duncombe, C.J., McCulloch, D.J., Rostomily, C., Troisch, P., Zhou, J., Mackay, S., DeGottardi, Q., May, D.H., Taniguchi, R., Gittelman, R.M., Klinger, M., Snyder, T.M., Roper, R., Wojciechowska, G., Murray, K., Edmark, R., Evans, S., Jones, L., Zhou, Y., Rowen, L., Liu, R., Chour, W., Algren, H.A., Berrington, W.R., Wallick, J.A., Cochran, R.A., Micikas, M.E., Wrin, T., Petropoulos, C.J., Cole, H.R., Fischer, T.D., Wei, W., Hoon, D.S.B., Price, N.D., Subramanian, N., Hill, J.A., Hadlock, J., Magis, A.T., Ribas, A., Lanier, L.L., Boyd, S.D., Bluestone, J.A., Chu, H., Hood, L., Gottardo, R., Greenberg, P.D., Davis, M.M., Goldman, J.D., Heath, J.R., 2022. Multiple early factors anticipate post-acute COVID-19 sequelae. Cell 185, 881–895.e20. <https://doi.org/10.1016/J.CELL.2022.01.014>

Vancassel, S., Capuron, L., Castanon, N., 2018. Brain Kynurenine and BH4 Pathways: Relevance to the Pathophysiology and Treatment of Inflammation-Driven Depressive Symptoms. <https://doi.org/10.3389/fnins.2018.00499>

Zigmond, A.S., Snaith, R.P., 1983. The hospital anxiety and depression scale. Acta psychiatrica Scandinavica 67, 361–370. <https://doi.org/10.1111/J.1600-0447.1983.TB09716.X>