

Psychoneuroendocrinology

Status post SARS-CoV-2 infection, persistent low-grade inflammation, and mental stress impact on systemic levels of neurotransmitter precursor amino acids – a psychoneuroimmunological cohort study

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Abstract:	<p>Background: Systemic serotonin availability and the catecholamine neurotransmitter synthesis pathway have been proposed as the biological link between mental health, inflammation and physical symptoms and disorders. Here we assess serotonin and noradrenaline precursors following SARS-CoV-2 infection in individuals with and without mental health impairment.</p> <p>Methods: The cross-sectional SIMMUN cohort study included 67 SARS-CoV-2 infection convalescents and 110 uninfected controls recruited in Tyrol, Austria during the initial phase of the pandemic. Explanatory variables encompassed clinical parameters, inflammatory markers, anti-SARS-CoV-2 antibodies, and standardized scorings of anxiety, depression and mental stress. Their effects on serum markers of serotonin availability (tryptophan [TRP], kynurenone [KYN], KYN/TRP ratio) and catecholamine neurotransmitter synthesis (phenylalanine [PHE], tyrosine [TYR], PHE/TYR ratio) were assessed by correlation analysis, two-tailed T tests and multi-parameter linear modeling. Association of neurotransmitter availability markers with inflammation was additionally investigated in the published longitudinal INCOV cohort (SARS-CoV-2: n = 205, uninfected: n = 440, Su et al. 2022).</p> <p>Results: Both in the SIMMUN and INCOV collectives, serum levels of the serotonin</p>

	<p>precursor TRP and its breakdown product KYN were affected strongly by systemic inflammation. TRP concentrations were substantially lower in participants with impaired mental health. In multi-parameter modelling, systemic availability of serotonin measured by TRP, KYN and KYN/TRP, was regulated independently by inflammation, mental stress and status post SARS-CoV-2 infection. The influences of the assessed parameters on the catecholamine neurotransmitter precursor availability were heterogeneous.</p> <p>Conclusion: Inflammation, SARS-CoV-2 infection and mental stress can both independently and additively influence serum levels of neurotransmitter precursor amino acids. These pathways could thus be a biological link between COVID-19 infection and mental health via psychoneuroimmunological mechanisms.</p>
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Dear Editor,

We are herewith submitting our manuscript entitled “Status post SARS-CoV-2 infection, persistent low-grade inflammation, and mental stress impact on systemic levels of neurotransmitter precursor amino acids– a psychoneuroimmunological cohort study” for consideration as an original research article in Psychoneuroendocrinology. With this study we shed light on a possible pathophysiological link between status post SARS-CoV-2 infection and mental health. Systemic low grade inflammation and the availability of neurotransmitter precursor amino acids seem to play a mediating role and might influence each other in a bidirectional relationship. Importantly these study findings are not only relevant for SARS-CoV-2 convalescents but shed light on more generalized mechanisms linking mental and physical health.

We are looking forward to the reviews and your response.

With best regards,

Katharina Hüfner

Highlights

- Mental health impairment is common in COVID-19 convalescents
- Changes in neurotransmitter precursor amino acids might be the pathophysiological link
- Neurotransmitter precursor amino acids, clinical parameters, inflammatory markers, anti-SARS-CoV-2 antibody titers, and standardized scorings of anxiety, depression and mental stress were collected
- Data were analyzed using multi-parameter linear modeling
- Kynurenone/tryptophan was affected independently by inflammation, mental stress and status post SARS-CoV-2 infection

Abstract

Background: Systemic serotonin availability and the catecholamine neurotransmitter synthesis pathway have been proposed as the biological link between mental health, inflammation and physical symptoms and disorders. Here we assess serotonin and noradrenaline precursors following SARS-CoV-2 infection in individuals with and without mental health impairment.

Methods: The cross-sectional SIMMUN cohort study included 67 SARS-CoV-2 infection convalescents and 110 uninfected controls recruited in Tyrol, Austria during the initial phase of the pandemic. Explanatory variables encompassed clinical parameters, inflammatory markers, anti-SARS-CoV-2 antibodies, and standardized scorings of anxiety, depression and mental stress. Their effects on serum markers of serotonin availability (tryptophan [TRP], kynurenine [KYN], KYN/TRP ratio) and catecholamine neurotransmitter synthesis (phenylalanine [PHE], tyrosine [TYR], PHE/TYR ratio) were assessed by correlation analysis, two-tailed T tests and multi-parameter linear modeling. Association of neurotransmitter availability markers with inflammation was additionally investigated in the published longitudinal INCOV cohort (SARS-CoV-2: n = 205, uninfected: n = 440, Su et al. 2022).

Results: Both in the SIMMUN and INCOV collectives, serum levels of the serotonin precursor TRP and its breakdown product KYN were affected strongly by systemic inflammation. TRP concentrations were substantially lower in participants with impaired mental health. In multi-parameter modelling, systemic availability of serotonin measured by TRP, KYN and KYN/TRP, was regulated independently by inflammation, mental stress and status post SARS-CoV-2 infection. The influences of the assessed parameters on the catecholamine neurotransmitter precursor availability were heterogeneous.

Conclusion: Inflammation, SARS-CoV-2 infection and mental stress can both independently and additively influence serum levels of neurotransmitter precursor amino acids. These pathways could thus be a biological link between COVID-19 infection and mental health via psychoneuroimmunological mechanisms.

Introduction

The immune system and the brain interact at multiple levels and in a bidirectional manner with influences on one system having consequences on the other. Acute SARS-CoV-2 infection has been shown to trigger a strong systemic inflammatory response (Song et al., 2020) causing an increased permeability of the blood brain barrier (Najjar et al., 2020), so that consequences on cognition and mental health are conceivable (Bower et al., 2022). On the other hand, psychological wellbeing and mental health can influence the immune system via psychoneuroimmunological mechanisms, explaining for example the higher susceptibility and risk for a more severe disease course in individuals with mental disorders (Wang et al., 2021).

Protracted inflammation can lead to mental health impairment, namely depressive symptoms via psychoneuroimmunological mechanisms (Dantzer et al., 2008). Individual health in COVID-19 survivors shows only weak association with routine inflammatory markers, antibody titers, impaired lung function, lung lesions or cardiac deficits, but is rather influenced by measurements of health concern and mental stress (Hüfner et al., 2022a; Hüfner et al., 2022b; Matta et al., 2022; Staudt et al., 2022). Anxiety and depressive symptoms are prevalent in the general population and are increased in survivors of COVID-19 (Al-Aly et al., 2021; Huang et al., 2021; Nasserie et al., 2021; Taquet et al., 2021) and related to mental stress in individuals following COVID-19 (Hüfner et al., 2022a).

Inflammation caused by factors such as stress or infection impact the synthesis of neurotransmitters, which are in turn also modulated by mental health (Bower et al., 2022). One such mechanism involves the breakdown of tryptophan (TRP) to kynurenine (KYN) mediated by indoleamine 2,3-dioxygenase (IDO-1) (**figure 1A**). IDO-1 activity depletes TRP, which serves as a serotonin precursor. In addition, kynurenine (KYN) and further downstream catabolites such as quinolinic acid (QUIN) on their own have anxiogenic and depressogenic effects. The ratio of KYN to TRP (KYN/TRP ratio) is an

1 IDO-1 activation marker associated with anxiety and depression (Fellendorf et al., 2022; Hüfner et al.,
2 2015). QUIN acts as a N-methyl-D-aspartate (NMDA) receptor agonist and therefore amplifies the
3 excitatory and neurotoxic effect of glutamate. Inflammation therefore augments depression and
4 anxiety by shifting the metabolic pathway away from a balanced neurotransmitter homeostasis seen
5 in healthy subjects towards the anxiety- and depression-amplifying kynurenone and catabolites.
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7 Increased KYN suggestive of lowered systemic serotonin availability has recently been identified in
8 acute COVID-19 patients in comparison with uninfected controls, as well as in individuals previously
9 tested positive for SARS-CoV-2 (Bizjak et al., 2022).**Error! Bookmark not defined.**
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11 **Error! Bookmark not defined.** Reactive oxygen species (ROS) pose another link between
12 inflammation and neurotransmitter precursors. Interferon gamma (IFN- γ) was shown to trigger ROS
13 production among others by microglia (Rahimian et al., 2022). ROS in turn mediated depletion of
14 5,6,7,8-tetrahydrobiopterin (BH4), a critical co-factor for synthesis of serotonin and catecholamine
15 neurotransmitters (Neurauter et al., 2008). Furthermore, IFN- γ -stimulated macrophages and
16 dendritic cells form neopterin, a cellular marker of inflammation, instead of BH4 (Werner et al.,
17 1989). Hence, reduced BH4 availability can be reliably assessed via an increased ratio of
18 phenylalanine to tyrosine (PHE/TYR ratio) as depletion of BH4 leads to an inhibition of PHE – TYR
19 conversion by phenylalanine-hydroxylase (Capuron et al., 2011).
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21 The association of persisting low-grade inflammation in individuals with status post SARS-CoV-2
22 infection and its possible association with mental health variables is still incompletely resolved to
23 date. Herein, we explored effects of inflammation, SARS-CoV-2 infection, anti-SARS-CoV-2 humoral
24 response strength and mental disorder scoring on neurotransmitter precursor amino acid levels in
25 individuals recovering from SARS-CoV-2 infection and uninfected controls (SIMMUN cohort).
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27 Furthermore, association of inflammation with systemic availability of serotonin and catecholamine
28 neurotransmitter precursors was explored in a published longitudinal collective (INCOV, Su et al.
29 2022).
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Materials and Methods

Ethics statement

The SIMMUN study was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent prior to enrollment. This study was approved by the ethics committee of the Medical University Innsbruck, Austria (approval number: 1132/2020). No approval by the ethics committee was required for analysis of the published anonymized INCOV data set (Su et al., 2022).

Study design INCOV

The INCOV data set was previously published (Su et al., 2022) and consisted of a longitudinal assessment (diagnosis, acute disease phase, 2-3 month follow up) of individuals with COVID-19 infection as well as matched controls. Metabolomic and proteomic data were collected.

Study design SIMMUN

Results of observational single cohort SIMMUN study are presented here along the STROBE guidelines. The study consisted of an online survey (not reported here) and an in person study visit, whose results are reported here. Study enrollment started on 10. June 2020, the study visits were conducted between 17. June 2020 and 27. May 2021.

Individuals tested for SARS-CoV-2 via PCR at the University Hospital of Innsbruck Medical University (Innsbruck, Austria) were invited to participate. Additionally, inpatients and outpatients of the University Clinic for Psychiatry I and II (Innsbruck, Austria) undergoing routine SARS-CoV-2 PCR screening were invited to participate. Inclusion criteria were a SARS-CoV-2 test performed at the study site, residence in Tyrol, age 18-70 years and proficiency in German language. Exclusion criteria were active SARS-CoV-2 infection (< 14 days following a positive test), pregnancy, active malignancies, organ transplantation, prior surgery in the past 3 months, or acute or chronic

inflammatory illness and treatment with oral corticosteroids. The analysis inclusion criterion was the complete study variable data set consisting of neurotransmitter precursor availability and inflammatory markers, anti-SARS-CoV-2 antibody levels and psychometric survey (**Figure 2**).

Procedures SIMMUN

The study visit included a physician assessment, supervised completion of self-rating questionnaire and a blood sample collection.

Psychometric assessment of anxiety, depression and mental stress

To assess anxious and depressive symptoms, the Hospital Anxiety and Depression Scale (HADS) was used. The HADS comprises 14 items: a 7-item subscale on anxiety and a 7-item subscale on depression. The total possible score range for each subscale is 0 to 21, with higher scores indicating higher levels of anxiety/depression. In accordance with existing literature, a cutoff of ≥ 8 for each subscale was used to identify individuals with clinically relevant symptom load (Bjelland et al., 2002). Stress was rated with the 4-item Perceived Stress Scale and expressed as the sum of all items as described (Cohen et al., 1983).

Laboratory blood analysis

C-reactive protein (CRP), interleukin-6 (IL-6), and full blood count were determined in the University Hospital of Innsbruck's certified clinical routine laboratory. CRP was measured using a Cobas8000 C702 and IL6 using Cobas8000 E602 (Roche, Vienna, Austria). The full blood count was performed using Sysmex XN (Sysmex, Vienna, Austria). NLR (neutrophil lymphocyte ratio) was calculated. Neopterin concentrations were measured by enzyme-linked immunosorbent assay (BRAHMS Diagnostics, Berlin, Germany). TRP, KYN, PHE and TYR, were determined by high-performance liquid chromatography, as described elsewhere (Neurauter et al., 2008; Widner et al., 1997). The ratios of

KYN/TRP and PHE/TYR were calculated as indices of IDO-1 and PHA activity, respectively (Capuron et al., 2011; Fuchs et al., 1991). SARS-CoV-2 antibodies were determined by ELISA as described previously (Deisenhammer et al., 2021) .

Study endpoints

The primary endpoint was identification of inflammatory, SARS-CoV2- and mental health-related factors impacting on activity of serotonin and catecholamine metabolic pathways as assessed by levels of precursor amino acids and their breakdown products (KYN, TRP, KYN/TRP and PHE, TYR, PHE/TYR).

Bioinformatic and statistical analysis of SIMMUN and INCOV cohorts

R version 4.2.0 was employed for the data analysis.

Normalized, age- and sex-adjusted, \log_2 -transformed serum protein and metabolite levels and clinical data for the INCOV cohort were extracted from the report by Su et al. (Su et al., 2022).

Normality and homogeneity of variances was investigated by Shapiro-Wilk and Levene test, respectively. Non-parametric tests or normality/homogeneity-improving variable transformations (logarithm and square root) were used in cases of non-normal distribution. Comparison of serum metabolite and cytokine levels between uninfected controls and COVID-19 individuals at consecutive timepoints after infection was done by Kruskal-Wallis test with Benjamini-Hochberg-corrected Mann-Whitney post-hoc test (Benjamini and Hochberg, 1995). Correlations of metabolite and cytokine serum levels and of mental disorder scoring with metabolite and cytokine levels was investigated by Spearman test. Correlation of SIMMUN dataset metabolite levels with age was accomplished by Pearson test. Significance of differences in SIMMUN cohort metabolite and inflammatory marker serum concentrations between participants stratified by gender or SARS-CoV-2 was assessed by two-tailed T test.

In multi-parameter linear modeling of amino acid neurotransmitter precursors and their breakdown products, neopterin (cellular inflammation marker), SARS-CoV-2 infection status, scores of anxiety (HADS), depression (HADS) and stress (PSS-4), age and gender served as candidate explanatory variables. Modeling responses and numeric explanatory variables were normalized. The models with the complete explanatory variable set were optimized by Bayesian information criterion (BIC) driven backwards elimination of non-significant terms. The normality and homogeneity model residual assumptions were evaluated by Shapiro-Wilk and Levene test, respectively, and additionally visually inspected in standard diagnostic plots (residuals versus fitted, quantile-quantile plots).

Reproducibility of the optimized multi-parameter models was investigated by repeated cross-validation (50 repeats, 10 folds) and by comparison of the RMSE and R^2 statistics obtained with the training dataset and in cross-validation.

Details of bioinformatic and statistical analysis are provided in **Supplementary Methods**.

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Results

Characteristic of the study cohorts

Herein, two independent collectives of uninfected controls and COVID-19 convalescents were analyzed. The SIMMUN cohort included uninfected participants ($n = 110$) and individuals recovering from a PCR-confirmed SARS-CoV-2 infection ($n = 67$) recruited at the Medical University of Innsbruck, Austria. None of the participants had received anti-SARS-CoV-2 vaccination prior to enrollment.

Males represented 40% of the cohort and the median age at enrollment was 49 years. The gender and age structure of the uninfected and SARS-CoV-2 subsets was comparable. Roughly half of the SIMMUN cohort individuals was overweight or obese and suffered from at least one somatic comorbidity; these figures were similar for SARS-CoV-2-negative and -positive individuals. The rate of diagnosed psychiatric conditions in the entire SIMMUN collective was 43% and tended to be higher in the SARS-CoV-2-negative participants. HADS scores and percentage of individuals with symptoms of depression or anxiety (HADS ≥ 8) were significantly higher in the SARS-CoV-2-negative strata, there was also a tendency towards higher rating of mental stress (PSS-4) in the uninfected subset. As expected, levels of antibodies against receptor binding domain (RBD) of the S1 SARS-CoV-2 protein was dramatically higher in the SARS-CoV-2 recovering subset. Approximately three-quarters of the SIMMUN SARS-CoV-2-positive study participants experienced mild, ambulatory COVID-19 (**Table 1**).

Besides clinical, demographic and psychometric variables, the SIMMUN dataset included serum concentrations of precursors of indolamine (tryptophan [TRP]) and catecholamine neurotransmitters (phenylalanine [PHE], tyrosine [TYR]), their breakdown catabolites and breakdown ratios (kynurenine [KYN], KYN/TRP ratio and PHE/TYR ratio) along with blood markers of inflammation (interleukin 6 [IL-6], C-reactive protein [CRP], neopterin [NEO] and neutrophil/lymphocyte ratio [NLR]). In the participants who tested SARS-CoV-2 positive, these blood parameters were measured at one fixed timepoint in course of COVID-19 convalescence at median 138.5 days (IQR: 119 - 157.25) after the positive SARS-CoV-2 test .

The INCOV cohort described by Su and colleagues (Su et al., 2022) included uninfected controls ($n = 440$) and SARS-CoV-2 positive individuals ($n = 205$). Slightly more than half of the INCOV cohort were female and the percentage of females was significantly higher in the SARS-CoV-2 negative strata. The median age at enrollment in the entire INCOV cohort was 51 years. The age of the SARS-CoV-2-positive participants was significantly higher than SARS-CoV-2-negative controls' age. Approximately two-third of the INCOV cohort participants were overweight or obese and this fraction was significantly higher in the SARS-CoV-positive subset. The largest fraction of the SARS-CoV-2 INCOV subset had moderate COVID-19 (WHO ordinal outcome scale for clinical improvement: 3 - 4). In 29% of the SARS-CoV-2-positive INCOV participants the course of COVID-19 was mild (WHO: 1 - 2, ambulatory treatment) (**Table 1**).

For the INCOV collective, a wide range of serum proteins and metabolites was recorded by high throughput multiplex assays. Age- and sex-normalized serum concentrations of the indolamine neurotransmitter precursor KYN, TRP, quinolinic acid (QUIN), PHE and TYR along with the inflammatory cytokines IL-6, interleukin 10 (IL-10), tumor necrosis factor-alpha (TNF- α) and interferon-gamma (IFN- γ) were investigated. These parameters were measured at one fixed timepoint for uninfected controls and at three consecutive timepoints after COVID-19 symptoms onset: acute (median 11 days), sub-acute (17 days) disease and during recovery (64 days) (**Supplementary Table S1**).

Effects of systemic inflammation on amino acid neurotransmitter precursors

In the INCOV cohort, the maximum concentration of serum IL-6, IL-10, TNF- α and IFN- γ were detected during acute COVID-19 and returned gradually to near-uninfected levels during convalescence (**Supplementary Figure S1**). The nadir TRP concentrations were observed during acute COVID-19 and went back to levels comparable with uninfected controls during convalescence in the INCOV collective. Concomitantly, courses of TRP breakdown products, KYN and QUIN, and of

catecholamine neurotransmitter precursors PHE and TYR paralleled the time courses of inflammatory cytokines with peaking concentrations in acute COVID-19 (**Figure 3**).

Both in the SARS-CoV-2-negative and -positive SIMMUN study participants significant moderate-to-strong positive correlations between KYN and KYN/TRP ratio and inflammatory markers CRP and NEO were observed. CRP correlated significantly with PHE in both SARS-CoV-2 strata (**Figure 4A**). These findings were corroborated by a correlation analysis in the INCOV collective. KYN and QUIN concentrations were significantly positively associated with all investigated inflammatory cytokines in the SARS-CoV-2-positive INCOV strata at each timepoint after COVID-19 onset. TRP correlated significantly and negatively with each of IL-6, IL-10, TNF- α and IFN- γ in acute and sub-acute COVID-19. PHE levels were correlated positively with all analyzed cytokines in acute COVID, and with IL-6 and IL-10 in sub-acute disease. Of note, significant positive association of KYN and QUIN with IL-6 and IL-10 could be detected also in SARS-CoV-2-negative INCOV study participants, comparable to the SIMMUN cohort (**Figure 4B**).

Effects of SARS-CoV-2 infection and mental health on amino acid neurotransmitter precursors

In the SIMMUN collective we could not observe any significant differences in markers of systemic inflammation (IL6, CRP, NEO and NLR) between the SARS-CoV-2-negative individuals and individuals with status post SARS-CoV-2 infection when investigated during late recovery at median 138.5 days (IQR:119 - 157.25) after the positive SARS-CoV-2 test (**Supplementary Figure S2**). This finding is in line with the INCOV cohort data indicating near-uninfected concentrations of inflammatory cytokines at median 64 days after COVID-19 onset (**Supplementary Figure S1**). However, despite the comparable levels of systemic inflammation measured by standard laboratory values between the SARS-CoV-2 cohort strata, significantly increased KYN and tendency towards increased KYN/TRP and decreased PHE/TYR ratios were detected in COVID-19 convalescents of the SIMMUN cohort (**Figure 5A**). In line with these findings, in the INCOV cohort levels of KYN, PHE and TYR were still significantly

elevated in recovering COVID-19 participants (median 64 days after infection) as compared with
1 uninfected controls (**Figure 3**). There were no significant correlation of the investigated metabolites
2 and the strength of anti-SARS-CoV-2 immune response (Supplementary **Figure S2B**). As a readout of
3 mental health symptoms of anxiety were found significantly associated with reduced serum TRP
4 concentrations (**Figure 5B**).
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Effects of age and sex on amino acid neurotransmitter precursors

In the entire SIMMUN cohort, age significantly affected serum levels of neurotransmitter-related
17 amino acid metabolites. KYN and KYN/TRP ratio correlated positively with age with moderate
18 strength. TYR was also found to be positively associated with age, whereas PHE/TYR ratio decreased
19 with participant's age. Serum TRP concentrations were virtually age-independent (**Supplementary**
20 **Figure S3**). Blood concentrations of TRP and TYR tended to be higher in males than females. PHE/TYR
21 was found to be significantly lower in the male participants (**Supplementary Figure S4**).
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Multi-parameter modeling of amino acid neurotransmitter

To get a more thorough insight at the interplay of demographic background, inflammation, status
35 post SARS-CoV-2 infection, anti-SARS-CoV-2 antibody response, and symptoms of anxiety, depression
36 and mental stress on levels of neurotransmitter-related amino acids, we resorted to multi-parameter
37 linear regression in the SIMMUN collective. The initial models included age, sex, NEO as a
38 representative inflammation marker, status post SAR-CoV-2 infection, anti-RBD antibody titres, and
39 scores of anxiety, depression and mental stress as explanatory variables. The full linear models were
40 constructed and subsequently optimized by backwards elimination of non-significant terms. For six
41 dependent variables analyzed (TRP, KYN, KYN/TRP ratio, PHE, TYR and PHE/TYR ratio), five valid
42 multi-parameter models could be established. The PHE model had a negligible explanatory
43 performance, proved non-significant as compared with the intercept-only null model and was hence
44 not further analyzed. The remaining multi-parameter models were characterized by good
45 reproducibility and proper parameterization as indicated by comparable fit errors and R² statistic
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values in the genuine modeling dataset and cross-validation. The KYN and KYN/TRP ratio models had
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the best explanatory performance measured by R^2 of 0.22 and 0.45. The TRP, TYR and PHE/TYR ratio
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models could explain between 9% and 16% of their response variable variances (**Supplementary**
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Figure S5).
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Concentrations of the inflammatory marker NEO were identified as the sole independent factor
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associated with reduced TRP serum concentrations. Increased KYN concentration and KYN/TRP ratio
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were associated with NEO and status post SARS-CoV-2 infection. In addition, mental stress levels and
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age were found to be positively associated with higher KYN/TRP ratios in multi-parameter modeling
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(**Figure 6A**). Serum levels of TYR were found negatively regulated by inflammation gauged by blood
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NEO and the anti-RBD antibody titer. Post SARS-CoV-2 infection status and age were in turn positive
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covariates of TYR. PHE/TYR was reduced by status post SARS-CoV-2 infection and age and on the
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other hand increased by inflammation and anti-SARS-CoV-2 antibody levels (**Figure 6B**).
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Discussion

Here we investigated the bidirectional relationship between the mental and somatic disease i.e. the brain and the immune system in individuals infected with SARS-CoV-2 and uninfected controls in two separate cohorts. In both cohorts there was an association of neurotransmitter precursor amino acids, especially those related to the serotonin pathway, with markers of inflammation in uninfected individuals as well as such with acute SARS-CoV-2 infection and status post SARS-CoV-2 infection. As shown by multi -parameter modelling, parameters of TRP breakdown are influenced by mental stress, age, inflammation, and, independently of inflammation, by status post SARS-CoV-2, all of them leading to lower availability of serotonin. On the other hand parameters of the catecholamine neurotransmitter synthesis pathway are affected by age, anti-SARS-CoV-2 antibody levels, inflammation, and, independently of inflammation, by the status-post SARS-CoV-2.

Inflammatory markers correlate with neurotransmitter precursor amino acids

Collectively, the temporal relationships and correlations between cytokines and neurotransmitter-related amino acid metabolites suggest reduced systemic availability of serotonin and elevated availability substrates for dopamine/adrenaline/noradrenaline synthesis mediated by systemic inflammatory reaction during COVID-19. Additionally, activity of TRP/KYN/QUIN degradation pathway was positively associated with COVID-19-independent systemic inflammation as indicated by the correlation analysis results in SARS-CoV-2-negative individuals.

Interaction of mental and somatic symptoms on neurotransmitter precursor amino acids of the serotonin pathway

The results of multi-parameter modeling suggest SARS-CoV-2-dependent and -independent inflammation as well as SARS-CoV-2 related factors dependent and independent of inflammation on

outcome parameters. The additive effects of inflammation, status post SARS-CoV-2 infection, mental stress and age may hence lower systemic availability of the serotonin precursor TRP and predispose to depressive or anxious disorders (Dantzer et al., 2008) (**Figure 1B**). This finding i.e. the interaction of mental and physical health with an additive effect on neurotransmitter precursor amino acid levels has been observed by our group independently of COVID-19 (Hüfner et al., 2015). We have also shown previously that not only acute but also chronic somatic diseases can interact with mental health presumably via their bi-directional influence on neurotransmitter precursor amino acid levels (Hüfner et al., 2019). We have also demonstrated previously the influence of acute mental stress on KYN/TRP values (Hüfner et al., 2020).

Sickness behaviour in humans comprises among others symptoms of depressed and anxious mood, social disconnection, fatigue, cognitive disturbance, and psychomotor slowing (Bower et al., 2022). This is a clear indication that inflammatory changes are associated also with alterations of mental status (Dantzer et al., 2008) and especially for the KYN and TRP pathway data are now also available in a larger metaanalyses (Hunt et al., 2020). Alterations of KYN levels and the serotonin pathway activity were described in acute COVID-19 using a metabolomics approach, with this pathway being the most prominently affected of all of the investigated compounds (Thomas et al., 2020). Elevated KYN levels were also found in the urine of COVID-19 patients and associated with disease severity (Dewulf et al., 2022; Robertson et al., 2020). These findings were summarized in a recent metaanalysis confirming the alterations of KYN/TRP ratio in COVID-19 and especially in its severe manifestations (Almulla et al., 2022). Furthermore, profound alterations of amino acid turnover and KYN metabolism were identified as a unique phenotype of COVID-19 (Lawler et al., 2021).

Low grade inflammation is a transdiagnostic feature of many psychiatric disorders and psychopathological symptoms (Miller and Raison, 2016). Recently, elevated levels of pre-pandemic inflammation makers could be associated with a 40% greater risk of developing depressive

symptoms in the early months of the pandemic, and mental stress was proposed as the mediating factor (Hamilton et al., 2021). Protracted systemic inflammation beyond acute phase of COVID-19 was linked to reduced antioxidative glutathion in the brain as well as with depressive symptoms (Poletti et al., 2022). Elevated inflammatory markers during acute COVID-19 were shown to predict psychopathology at three months follow up, underlining the role of inflammation. This finding was supported by observation of protective effects of cytokine-blocking agents in acute COVID-19 against development of depressive symptoms during recovery (Benedetti et al., 2021). In our local cohort the increases in KYN/TRP are reflective of higher IDO-1 activity and related to inflammation as well as SARS-CoV-2 status. Serum metabolomics and proteomics data from the INCOV cohort (Su et al., 2022) indicate strongly, that systemic availability of the serotonin precursor TRP and circulating amounts of KYN and QUIN, the products of IDO1-mediated TRP breakdown stays under control of systemic inflammation during acute COVID-19 and recovery. Interestingly, in both analyzed cohorts, serum levels of standard inflammatory cytokines were comparable in fully recovered COVID-19 patients and uninfected controls. The potential role of KYN/TRP and IDO-1 activation has been summarized in a recent hypothesis paper (Eroğlu et al., 2021) and KYN has been suggested as a potential marker of in individuals with status post COVID-19 (Bizjak et al., 2022). Markers of serotonin availability, especially KYN and TRP have been proposed as predictors of increased mental stress, anxiety and depression following COVID-19 infection (Kucukkarapinar et al., 2022).

43 **Tetrahydrobiopterin and neurotransmitter precursor amino acids of the 44 noradrenaline pathway**

49 Mental stress has been proposed to play an important role in recovery from COVID-19: both factors
50 could act as a “double hit” with synergistic effects of psychological stress and infection on
51 inflammation (Bower et al., 2022; Hüfner et al., 2022a). Changes in the availability of BH4 can lead to
52 alterations in PHE/TYR also independently of inflammation as a direct biochemical mechanism
53 (Hüfner et al., 2020). PHE/TYR was reduced with age and in individuals and status post SARS-CoV-2
54 infection. A similar pattern was found in individuals with no or mild depression were a negative
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correlation with PHE/TYR was found indicating a direct biochemical mechanism related to BH4 availability (Hüfner et al., 2021). Reduced BH4 availability can be the result of impaired synthesis, low recycling from BH₂ or oxidation by ROS (Thony et al., 2000; Werner-Felmayer et al., 2002). Changes in BH4 availability can also influence the production of serotonin (Haroon et al., 2012). BH4 is also important for nitric oxide synthesis and thus involved in oxidative stress; an interaction between nitric oxide and the HPA- axis is increasingly recognized (Yilmaz et al., 2007). It could be a sign of a salutogenetic feedback mechanism that inflammation as assessed by neopterin and anti SARS-CoV-2 antibodies regulate PHE/TYR levels in an opposite direction as the status post SARS-CoV-2 infection.

Limitations

The major limitation of the SIMMUN study is the limited sample size, however, the recruitment during a defined and early stage of the pandemic is also an advantage since influences due to vaccinations or multiple COVID-19 viral variants were eliminated. The cohorts consisted of hospital patients along with patients of psychiatric facilities, which resulted in a selection bias toward subjects with high rate of somatic and psychiatric comorbidities. In the INCOV cohort ratios of KYN/TRP and PHE/TYR could not be analyzed due to the fact that only transformed data were available. For the INCOV cohort, psychometric measures were unavailable and data of somatic symptoms were recorded only for the SARS-CoV-2-infected participants making validation of multi-parameter modeling results in the SIMMUN collective impossible. Furthermore, the time interval between SARS-CoV-2 infection and the study visit varied substantially. Finally, in both cohorts, alterations of neurotransmitter precursors were analyzed cross-sectionally and at the systemic level, which does not have to reflect metabolic changes of the central nervous system. Many of the neurotransmitter precursor amino acids readily cross the blood-brain barrier so it is possible that fluctuations in the blood levels of these metabolites directly affect their concentration and metabolism in the brain (Schwarcz et al., 2012). More preclinical studies are urgently needed to elucidate these findings.

Conclusions

Here we show that status post COVID-19 infection as well as mental health and inflammation are associated with changes in KYN/TRP and PHE/TYR levels as a surrogate marker of the serotonin and noradrenaline transmitter pathway jointly but yet independently. This indicates that there are effects of SARS-CoV-2 infection, which go beyond those of inflammation, while there are also inflammatory effects not related to SARS-CoV-2. These findings could help to further explore the biological mechanisms linking SARS-CoV-2 infection, inflammation and mental health parameters in a bi-directional way.

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Bernhard Holzner has intellectual property rights to the CHES software tool used for questionnaire data collection.

Jens Lehmann reports consultation for Evaluation Software Development GmbH, the software company developing CHES.

All other authors report no conflicts of interest related to the current manuscript.

Data and code availability

Anonymized patient data will be made available upon request to the corresponding author. The INCOV cohort data are publicly available. The study pipeline code is available at
https://github.com/PiotrTymoszuk/SIMMUN_validation.

Supplementary Materials

Tables and Figures

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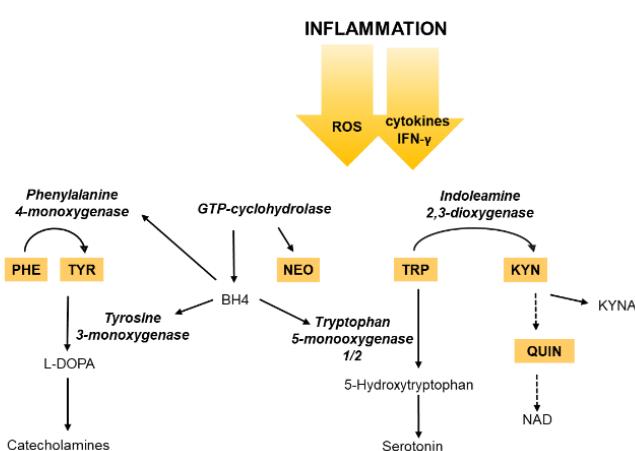
Variable	Uninfected	SARS-CoV-2	Test	Significance
Participants, n	110	67		
Sex	female: 64% (70) male: 36% (40) complete: n = 110	female: 54% (36) male: 46% (31) complete: n = 67	χ^2	ns (p = 0.25)
Age, years	48 [IQR: 35 - 57] range: 18 - 69 complete: n = 110	50 [IQR: 32 - 56] range: 20 - 68 complete: n = 67	Mann-Whitney	ns (p = 0.69)
Body mass class ^a	normal: 53% (54) overweight: 34% (34) obesity: 13% (13) complete: n = 101	normal: 52% (34) overweight: 28% (18) obesity: 20% (13) complete: n = 65	χ^2	ns (p = 0.42)
Somatic comorbidity	56% (59) complete: n = 106	45% (29) complete: n = 64	χ^2	ns (p = 0.25)
Psychiatric comorbidity	54% (59) complete: n = 110	34% (23) complete: n = 67	χ^2	p = 0.019
HADS anxiety score ^b	7 [IQR: 3 - 12] range: 0 - 20 complete: n = 110	3 [IQR: 2 - 7] range: 0 - 19 complete: n = 67	Mann-Whitney	p = 0.0027
HADS depression score ^b	5 [IQR: 1 - 10] range: 0 - 20 complete: n = 110	3 [IQR: 1 - 6] range: 0 - 17 complete: n = 67	Mann-Whitney	p = 0.028
Depression or anxiety screening positive, HADS $\geq 8^b$	48% (53) complete: n = 110	24% (16) complete: n = 67	χ^2	p = 0.0022
PSS-4 stress score ^c	6.5 [IQR: 3 - 10] range: 0 - 16 complete: n = 110	5 [IQR: 3 - 8] range: 1 - 13 complete: n = 67	Mann-Whitney	ns (p = 0.082)
anti-RBD SARS-CoV-2, IgG, AU ^d	0.31 [IQR: 0.28 - 0.34] range: 0.22 - 0.99 complete: n = 110	16 [IQR: 14 - 17] range: 0.34 - 25 complete: n = 67	Mann-Whitney	p < 0.001
COVID-19 severity ^e		mild: 75% (50) moderate: 19% (13) severe-critical: 6% (4) complete: n = 67		
Sex	female: 59% (261) male: 41% (179) complete: n = 440	female: 50% (102) male: 50% (103) complete: n = 205	χ^2	p = 0.028
Age, years	50 [IQR: 41 - 58] range: 19 - 80 complete: n = 440	57 [IQR: 42 - 69] range: 18 - 89 complete: n = 205	Mann-Whitney	p < 0.001
Body mass class ^a	normal: 38% (167) overweight: 30% (134) obesity: 32% (139) complete: n = 440	normal: 24% (36) overweight: 36% (53) obesity: 39% (58) complete: n = 147	χ^2	p = 0.012

Variable	Uninfected	SARS-CoV-2	Test	Significance
Ethnicity	Asian: 11% (44) Black or African-American: 6.2% (26) White: 81% (337) Other: 2.6% (11) complete: n = 418	Asian: 14% (28) Black or African-American: 9.3% (19) White: 51% (104) Other: 26% (54) complete: n = 205	χ^2	p < 0.001
COVID-19 severity ^b		mild: 29% (60) moderate: 43% (88) severe: 18% (37) critical: 9.8% (20) complete: n = 205		

Table 1. Characteristic of the local SIMMUN cohort and INCOV cohort (shaded in grey).

Numeric variables are presented as medians with interquartile ranges. Categorical variables are presented as percentages and counts within complete observations.

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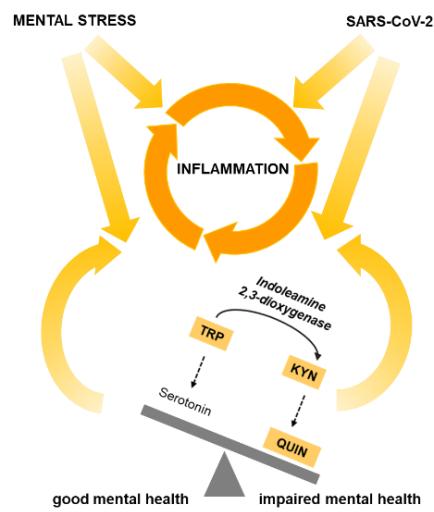


Figure 1. Effect of inflammation on neurotransmitter precursor synthesis pathways.

A) Description of established pathways investigated in the current study. Pro-inflammatory cytokines, most importantly interferon (IFN)- γ , and reactive oxygen species (ROS) are released during the cellular immune response. Tryptophan (TRP) conversion to kynurenine (KYN) is increased due to the activation of indoleamine 2,3-dioxygenase, and also downstream metabolism is altered, affecting the formation of e.g. quinolinic acid (QUIN). In macrophages and dendritic cells, pteridine synthesis is shifted towards formation of the immune activation marker neopterin. Inflammatory conditions may reduce the oxidation-labile tetrahydriobiopterin (BH4), which is cofactor for several monooxygenases, including phenylalanine 4-monooxygenase and tyrosine 3-monooxygenase. (KYNA= kynurenic acid, NAD = nicotinamide adenine dinucleotide, L-DOPA = 3,4-dihydroxyphenylalanine). Figure adapted from (Gietl et al., 2023).

B) Summary of findings from the current study. A proinflammatory milieu stimulates IDO1-mediated conversion of tryptophan (TRP) to kynurenine (KYN) and further promotes the catabolic route towards the formation of neurotoxic quinolinic acid (QUIN). Reduced TRP availability may affect the synthesis of the anti-depressive and anxiolytic neurotransmitter serotonin. SARS-CoV-2 dependent inflammation but also mental stress may sustain a chronic inflammatory state, thus keeping a vicious cycle going. SARS-CoV-2 and mental stress also exert inflammation-independent effects on serotonin availability. During recovery from SARS-CoV-2 infection, TRP to KYN conversion and further formation of QUIN is stimulated. Although the direct relevance of the observed peripheral immunometabolic

events on the central nervous system needs to be investigated in more detail, we hypothesize that this ongoing dysregulated processes predispose to depression or anxiety. Depression and anxiety symptoms are on the other hand known to be paralleled by decreased TRP, thus a bidirectional relationship is established acting again on the same circuits.

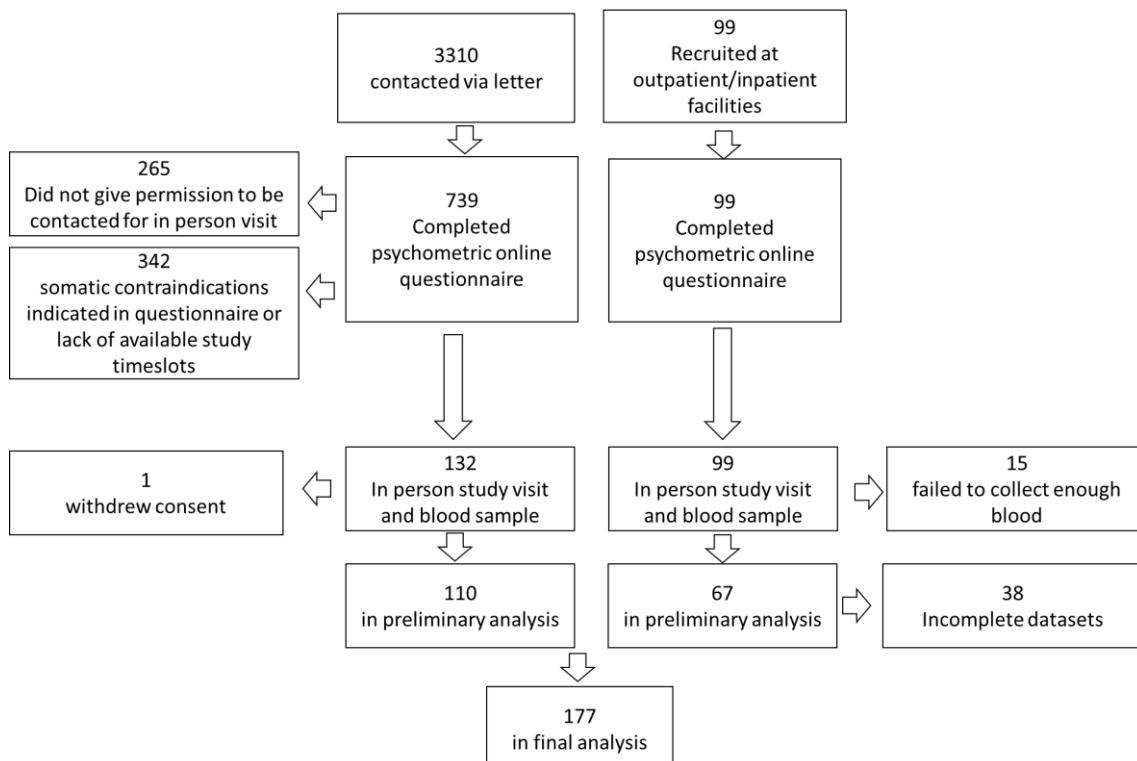


Figure 2 Flowchart of participant recruitment and dropout in the SIMMUN cohort

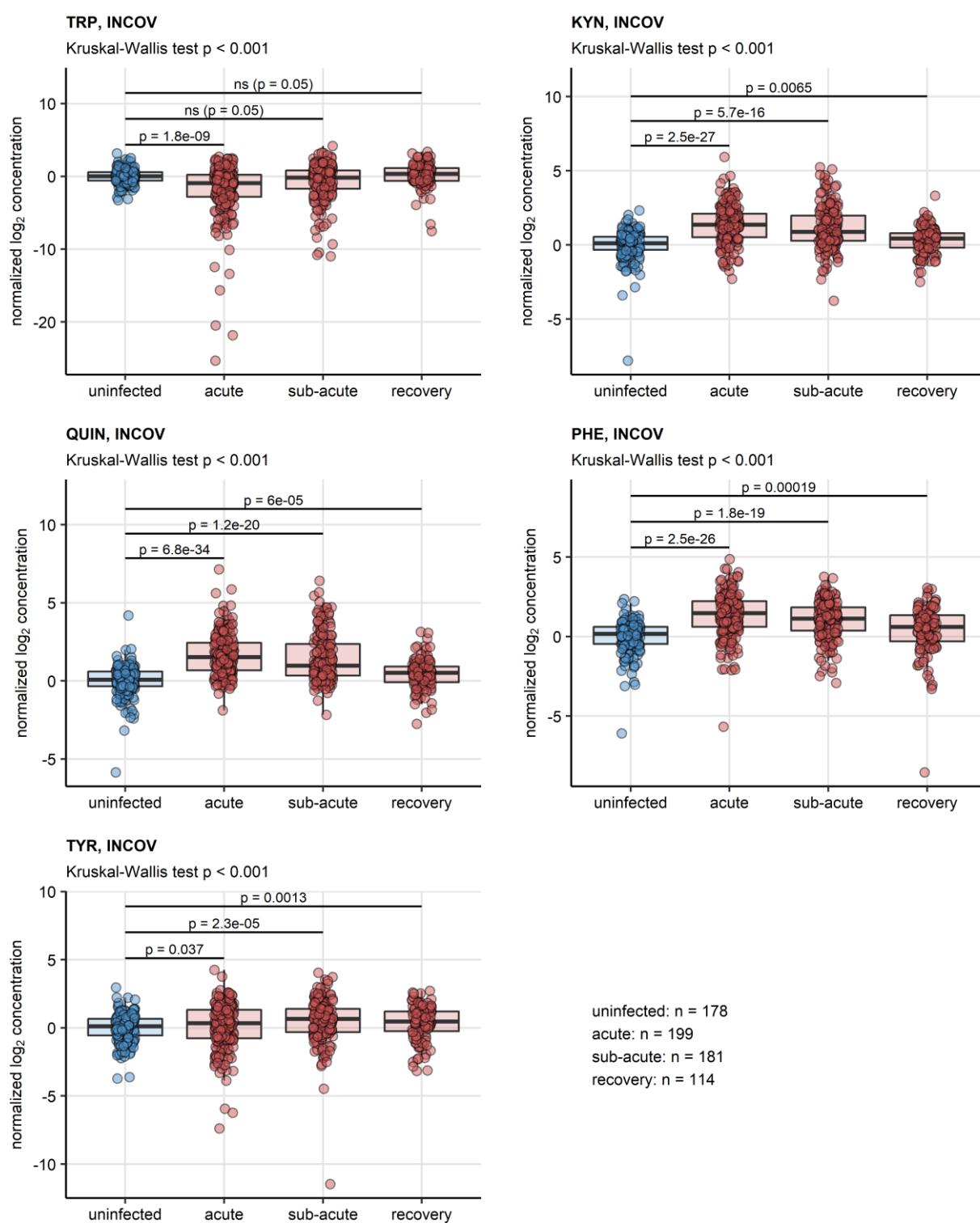


Figure 3. Serum levels of neurotransmitter precursor and their decay products in course of COVID-19 and recovery, INCOV cohort.

Serum levels of tryptophan (TRP), kynurenine (KYN), quinolinic acid (QUIN), phenylalanine (PHE) and tyrosine (TYR) in serum of uninfected controls and COVID-19 individuals during acute, sub-acute and

recovery phase of the disease in the INCOV cohort. Statistical significance was determined by Kruskal-Wallis test with Benjamini-Hochberg-corrected Mann-Whitney U test. Normalized serum level concentrations are presented in box plots. Points represent single samples. The Kruskal-Wallis test results are indicated in the plot captions. Results of the post-hoc tests are indicated in the plots. Numbers of complete observations are displayed under the plots.

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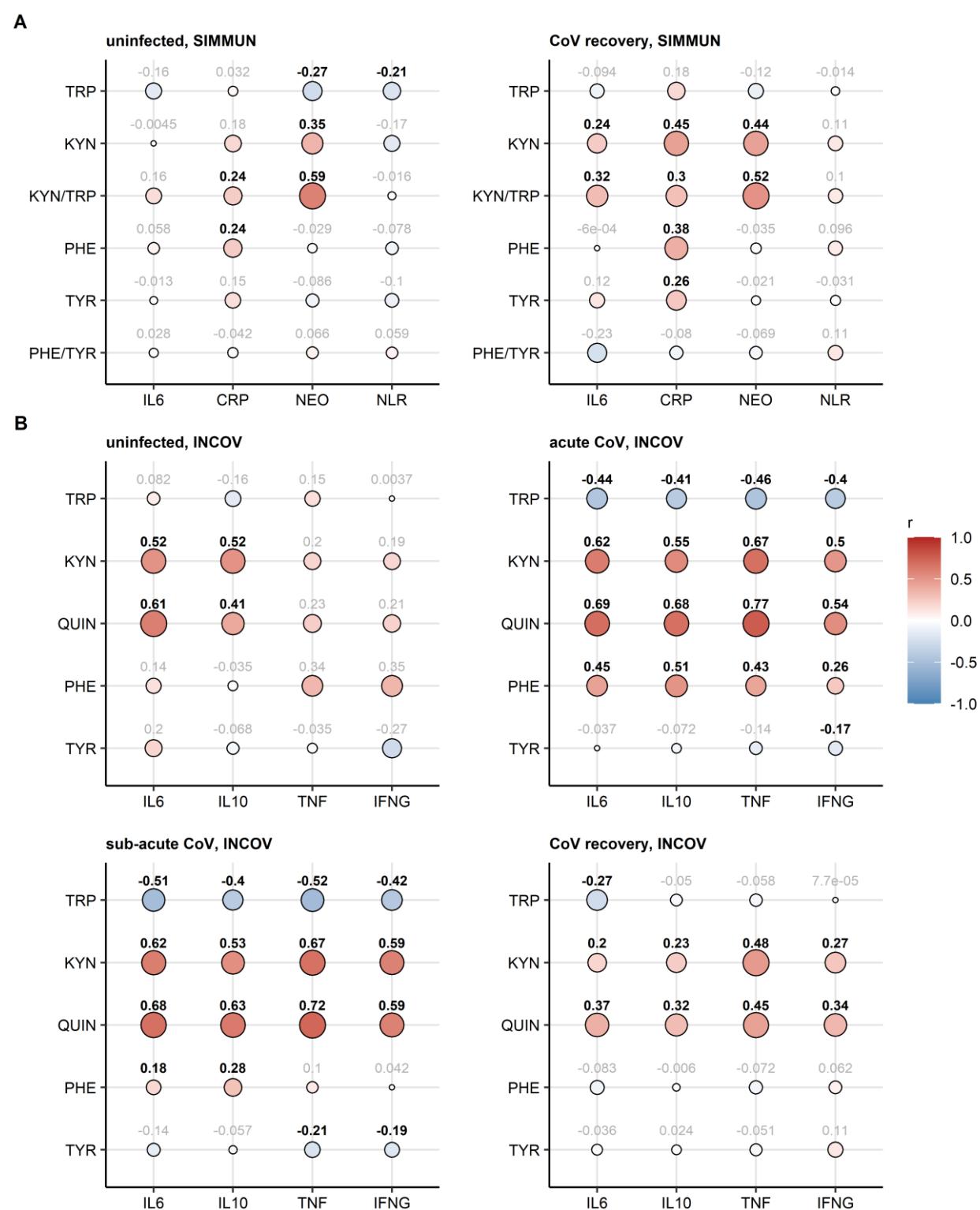


Figure 4. Correlation of serum levels of aminoacid precursors of neurotransmitters and their decay products with markers of inflammation.

Serum levels of tryptophan (TRP), kynurenine (KYN), quinolinic acid (QUIN), kynurenine/triptophan ratio (KYN/TRP), phenylalanine (PHE), tyrosine (TYR) and phenylalanine/tyrosine ratio (PHE/TYR) were correlated with serum levels of inflammatory markers interleukin 6 (IL6), interleukin 10 (IL10), tumor necrosis factor-alpha (TNF), interferon-gamma (INF γ), C-reactive protein (CRP), neopterin (NEO) and neutrophil/lymphocyte ratio (NLR) in uninfected and SARS-CoV-2-infected individuals from the SIMMUN (A) and INCOV cohort (B). Statistical significance was assessed by Spearman test.

Correlation coefficients are presented in bubble plots. Point sizes correspond to absolute values of correlation coefficient. Point color corresponds to the correlation coefficient value. Correlation coefficients for significant effects are highlighted in bold.

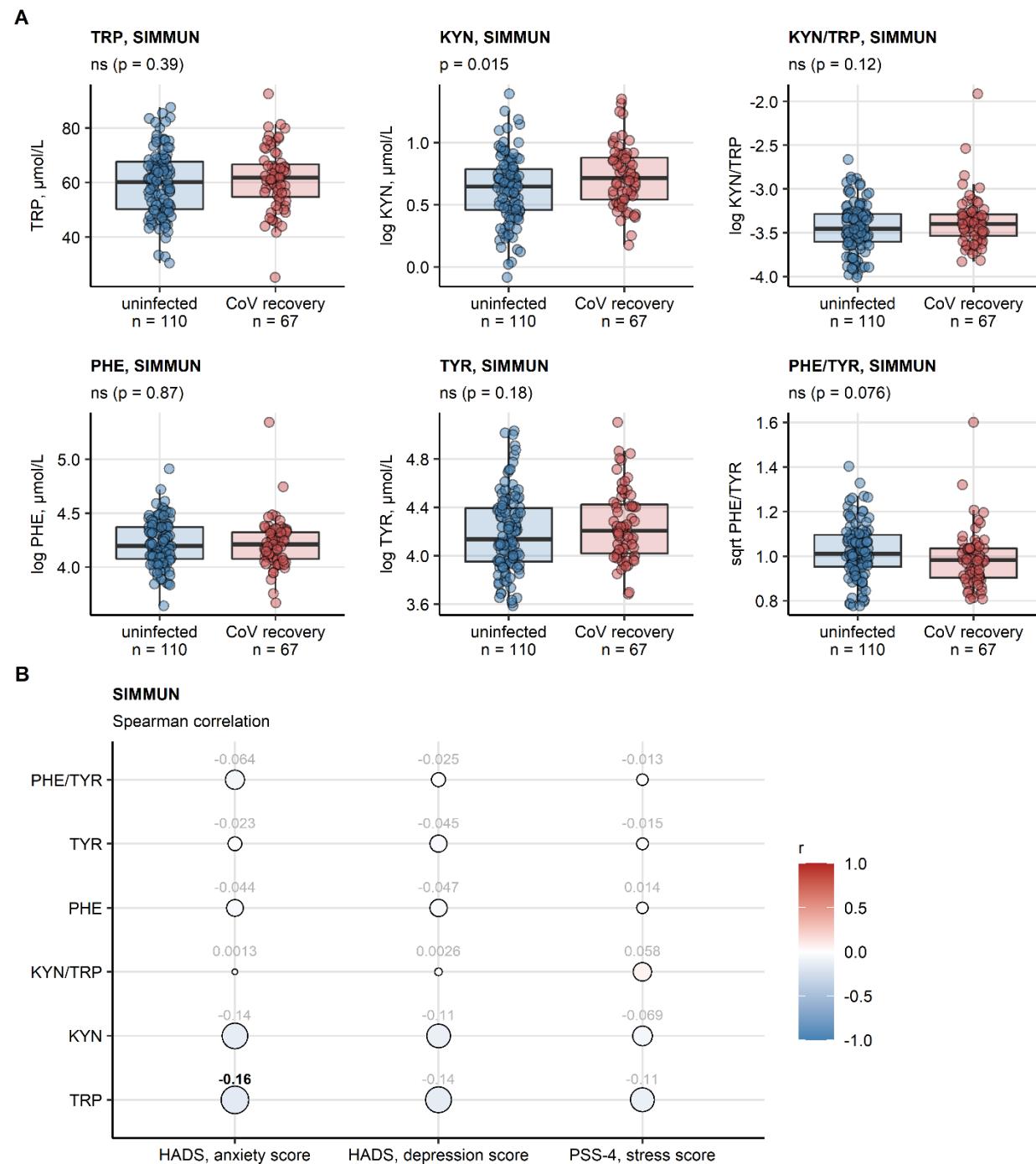


Figure 5. Association of neurotransmitter precursor amino acids and their breakdown products with SARS-CoV-2 infection and anxiety, depression and mental stress in the SIMMUN cohort.

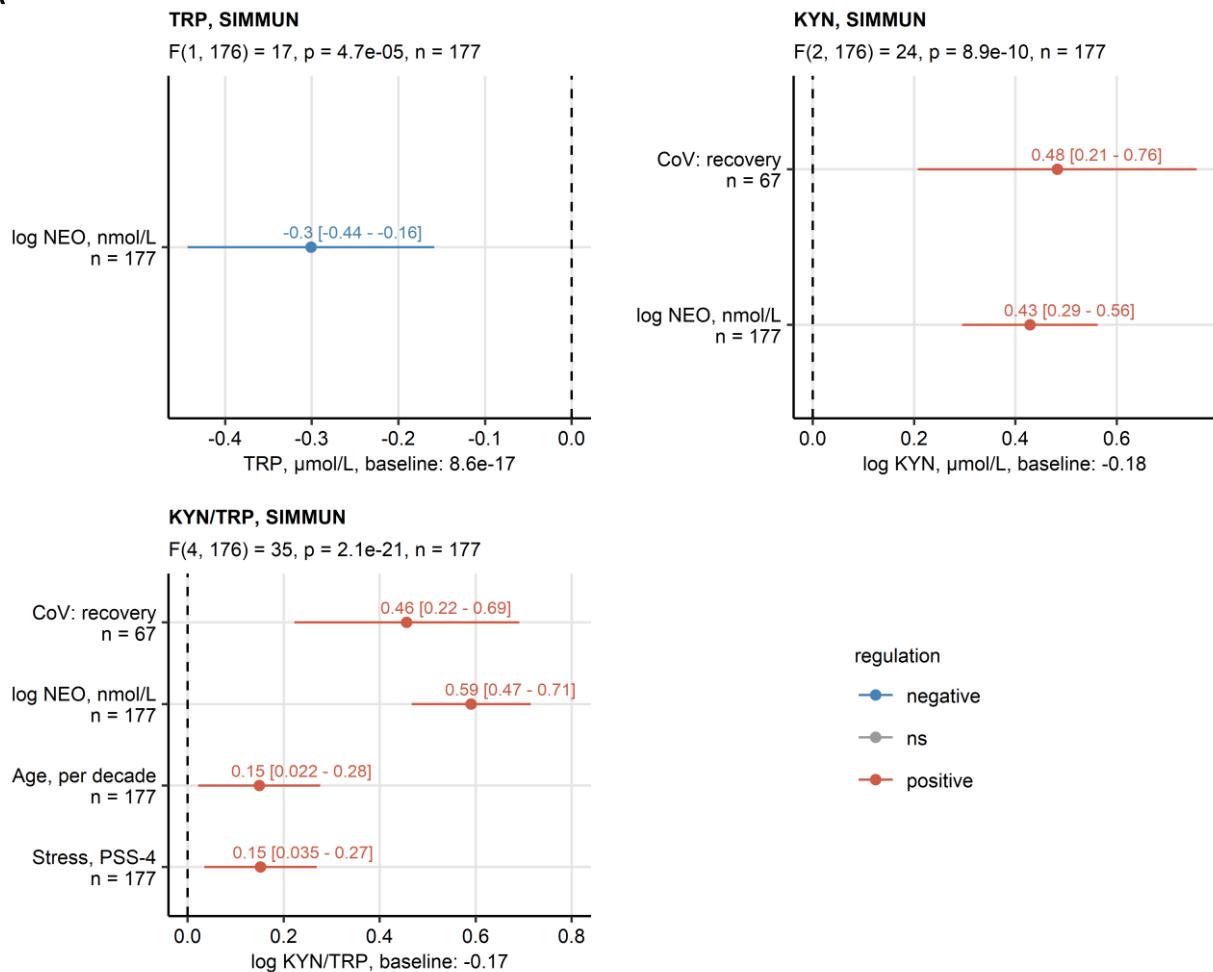
(A) Serum levels of tryptophan (TRP), kynurenine (KYN), kynurenine/tryptophan ratio (KYN/TRP), phenylalanine (PHE), tyrosine (TYR) and phenylalanine/tyrosine ratio (PHE/TYR) in the SIMMUN study participants stratified by SARS-CoV-2 infections status. Statistical significance was determined by two-tailed T test. Serum levels are shown in box plots. Points represent single observations. Significance p

values are displayed in the plot captions, numbers of complete observations are indicated in the plot

axes.

(B) Serum levels of TRP, KYN, KYN/TRP, PHE, TYR and PHE/TYR were correlated with anxiety, depression and stress scores in the SIMMUN study participants ($n = 177$). Statistical significance was assessed by Spearman test. Correlation coefficients are presented in bubble plots. Point sizes correspond to absolute values of correlation coefficient. Point color corresponds to the correlation coefficient value. Correlation coefficients for significant effects are highlighted in bold.

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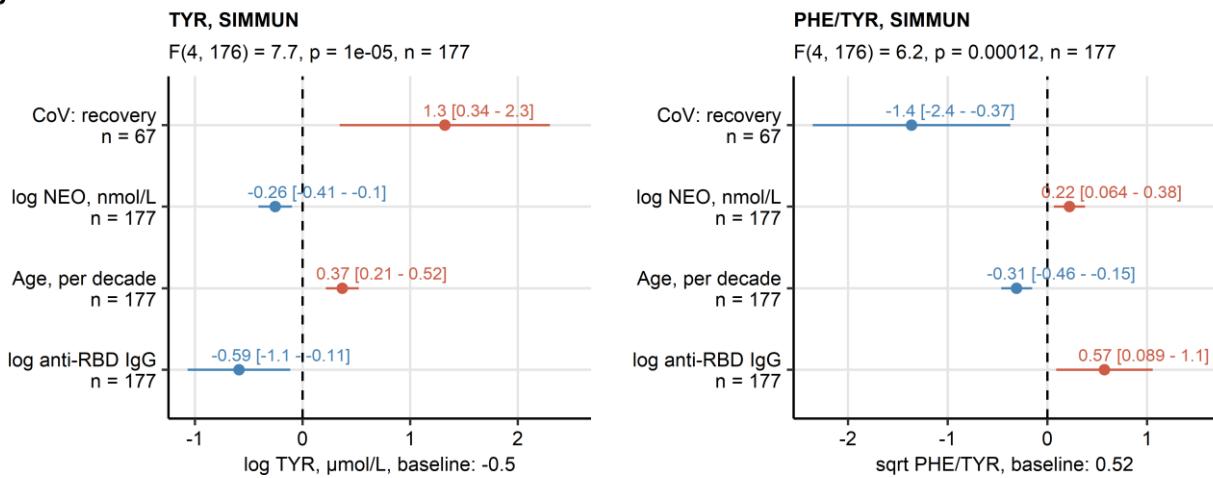


Figure 6. Results of multi-parameter modeling of amino acid neurotransmitter precursor and their breakdown products.

Effects of inflammation (neopterin, NEO), SARS-CoV-2 infection status, titer of immunoglobulin gamma against the receptor binding domain of the S1 SARS-CoV-2 protein (anti-RBD IgG), scores of anxiety, depression and mental stress, age and sex was investigated by multi-parameter linear

regression with backward elimination of non-significant terms. Overall model validity was assessed by likelihood-ratio test (LRT). Significant model coefficient estimates with 95% confidence intervals are presented in Forest plots. LRT results (F values, degrees of freedom and p values) and total numbers of complete observations are indicated in the plot captions. Model intercepts (baseline) are indicated in the X axis titles.

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Conflicts of interest

The study was supported by GZ 71134 from Land Tirol to Katharina Hüfner.

Piotr Tymoszuk owns the Data Science as a Service Tirol enterprise and works a free-lance data scientist and biostatistician. He has received an honorarium for statistical data analysis and minor manuscript work.

Bernhard Holzner has intellectual property rights to the CHES software tool used for questionnaire data collection.

Jens Lehmann reports consultation for Evaluation Software Development GmbH, the software company developing CHES.

All other authors report no conflicts of interest related to the current manuscript.

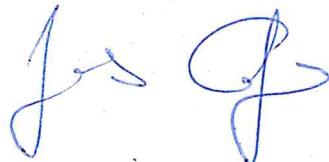
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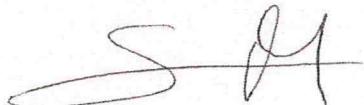
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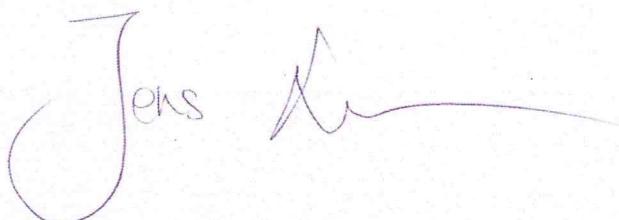
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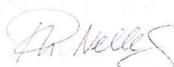
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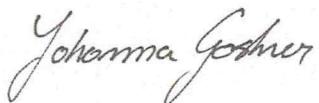
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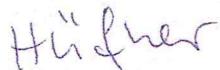
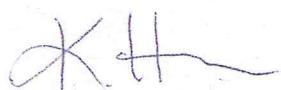
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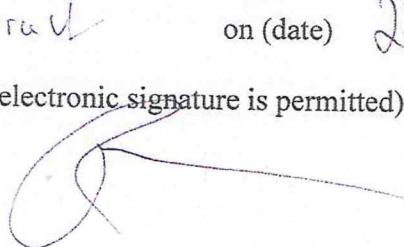
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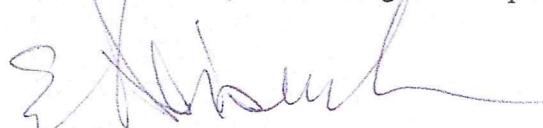
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Status post SARS-CoV-2 infection, persistent low-grade inflammation, and mental stress impact on systemic levels of neurotransmitter precursor amino acids – a psychoneuroimmunological cohort study

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Running title: Neurotransmitter precursor amino acid levels and COVID-19

Status post SARS-CoV-2 infection, persistent low-grade inflammation, and mental stress impact on systemic levels of neurotransmitter precursor amino acids

– a psychoneuroimmunological cohort study

Supplementary Material

Supplementary Methods

Software

Proteome and metabolome data were analyzed with R version 4.2.0. General data transformation tasks were accomplished with the *tidyverse* package bundle (1), *rlang* (2) and the development package *trafo* (<https://github.com/PiotrTymoszuk/trafo>). Statistical data testing was done with the packages *rstatix* (3), *ggpubr* (4) along with the development package *ExDA* (<https://github.com/PiotrTymoszuk/ExDA>). In linear modeling, base R functions, the development packages *lmqc* (<https://github.com/PiotrTymoszuk/lmqc>) and *caretExtra* (<https://github.com/PiotrTymoszuk/caretExtra>), and the package *caret* (5) were utilized.

Results were visualized with tools provided by the packages *ggplot2* (6), *cowplot* (7) and *ExDA*. Manuscript and supplementary tables were created with *flextable* (8). Supplementary Material file was written in the *rmarkdown* environment (9) and rendered with the *knitr* (10) and *bookdown* (11) packages.

INCOV cohort data import and transformation

Proteome and metabolome data in form of normalized, age- and sex-adjusted, log₂-transformed serum levels as well as clinical information (sex, SARS-CoV-2 infection status, COVID-19 severity, timepoint, post-COVID-19 syndrome status and particular symptoms) for the INCOV cohort were extracted from supplementary tables accompanying the report by Su and colleagues (12).

Characteristic of the INCOV cohorts is presented in **Table 2**. Numbers of available INCOV cohort samples and the sampling timepoints are shown in **Supplementary Table S1**.

Variable distribution and transformation

Distribution normality and variance homogeneity of normalized cytokine and metabolite serum levels was assessed by Shapiro-Wilk and Levene test, respectively. The distribution testing revealed substantial deviations from normality for multiple study parameters. For this reason statistical hypothesis testing in the INCOV dataset was done with non-parametric tests. For SIMMUN cohort metabolite and inflammation marker levels (KYN, KYN/TRP ratio, PHE, TYR and PHE/TYR ratio, NEO, CRP, IL-6, anti-RBD IgG) used in analysis of correlation with age and mental disorder scoring, comparison between participants stratified by SARS-CoV-2 infection status or gender as well as for linear modeling, logarithm and square root transformations was used, which improved both normality and variance homogeneity.

Statistical hypothesis testing

Comparison of normalized serum cytokine and metabolite levels between uninfected controls and COVID-19 individuals from the INCOV cohort at consecutive timepoints after symptom onset was done with Kruskal-Wallis test. Differences between SARS-CoV-2-negative controls and consecutive timepoints of COVID-19 were investigated with Mann-Whitney post-hoc U test corrected for multiple testing with Benjamini-Hochberg method (13).

Correlation of serum cytokine and metabolite levels in uninfected controls and COVID-19 individuals at consecutive timepoints in the INCOV and SIMMUN datasets was analyzed by Spearman test. Correlation of serum cytokine and metabolite levels with scores of anxiety, depression and stress was assessed by Spearman test. Correlation of SIMMUN dataset metabolite levels with age was assessed by Pearson test.

Comparison of SIMMUN dataset metabolite and inflammatory marker levels between participants stratified by SARS-CoV-2 infection status or gender was done with two-tailed T test.

Multi-parameter linear regression with backwards elimination

Effects of the inflammation marker (NEO), SARS-CoV-2 infection status, titer of immunoglobulin gamma against the receptor binding domain of the S1 SARS-CoV-2 protein (anti-RBD IgG), anxiety (HADS), depression (HADS) and stress scoring (PSS-4), age and gender on systemic levels of aminoacid neurotransmitter precursor and their decay products (TRP, KYN, KYN/TRP ratio, PHE, TYR and PHE/TYR ratio) was assessed by multi-parameter linear regression with backwards elimination. Modeling responses and numeric explanatory variables were transformed with log or squared root functions to improve normality and normalized.

Full models including the complete set of explanatory variables listed above were constructed (function `make_lm()`, package `lmc`) and optimized by Bayesian information criterion (AIC) driven backwards elimination of non-significant terms (method `step()`, package `lmc`). Normality and homogeneity of distribution of the model residuals was checked by Shapiro-Wilk and Levene test, respectively (method `summary(type = 'assumptions')`, package `lmc`) and visually inspected in standard diagnostic plots of model residuals (residuals vs fitted, quantile-quantile plots, method `plot()` called for the model objects). Fit stats (R^2 and root mean squared error [RMSE]) were retrieved from the model objects by calling `summary(type = 'fit')` (package `lmc`). Validity of the optimized models was determined by likelihood-ratio test (LRT) versus the respective null models (method `anova()`). Reproducibility and proper parameterization of the optimized multi-parameter models was investigated by repeated cross-validation (50 repeats, 10 folds, function `train(method = 'lm')`, package `caret`) and by comparison of the RMSE and R^2 .

statistics obtained with in the training dataset and in cross-validation (method `summary()`, package *caretExtra*). As presented in **Supplementary Figure S5A**, similar values of error fit in the training and cross-validation data suggest good reproducibility of the optimized models and lack of over-parameterization.

Data and code availability

Anonymized local cohort data will be made available upon request to the corresponding author. The INCOV cohort data are publicly available. The study pipeline code is available at https://github.com/PiotrTymoszuk/SIMMUN_validation.

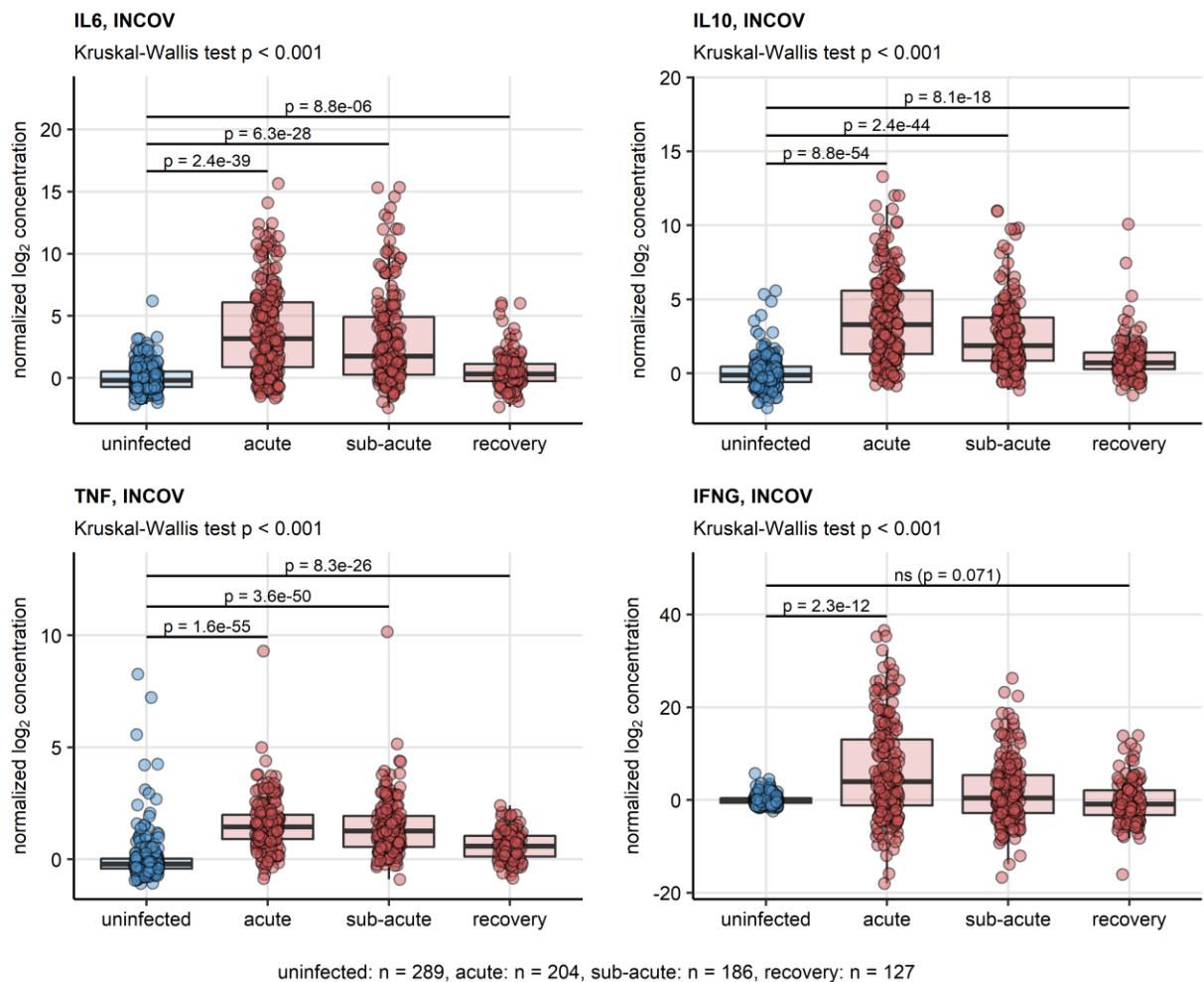
Supplementary Tables

Supplementary Table S1: Number of available samples and sampling timepoints in the INCOV cohort.

Time point	Days post infection ^a	Sample number
healthy		440
acute	11 [7.9 - 16]	205
sub-acute	17 [12 - 23]	187
recovery	64 [53 - 90]	127

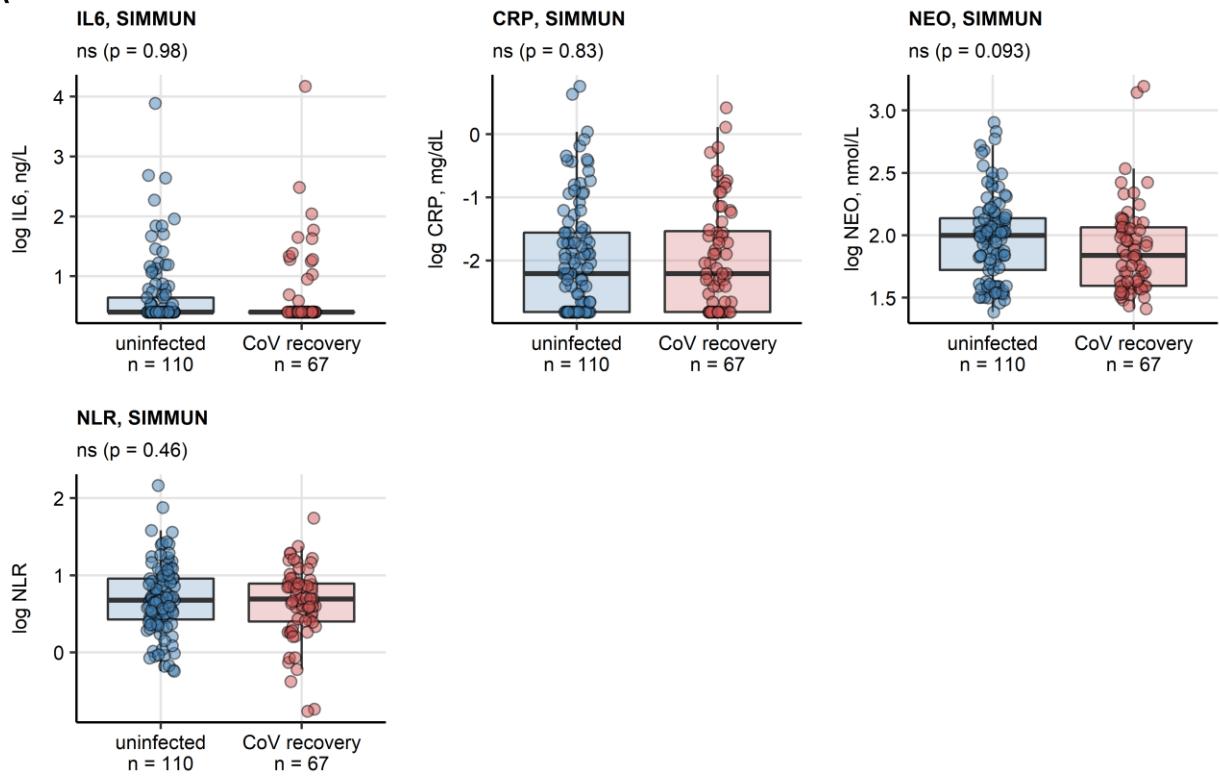
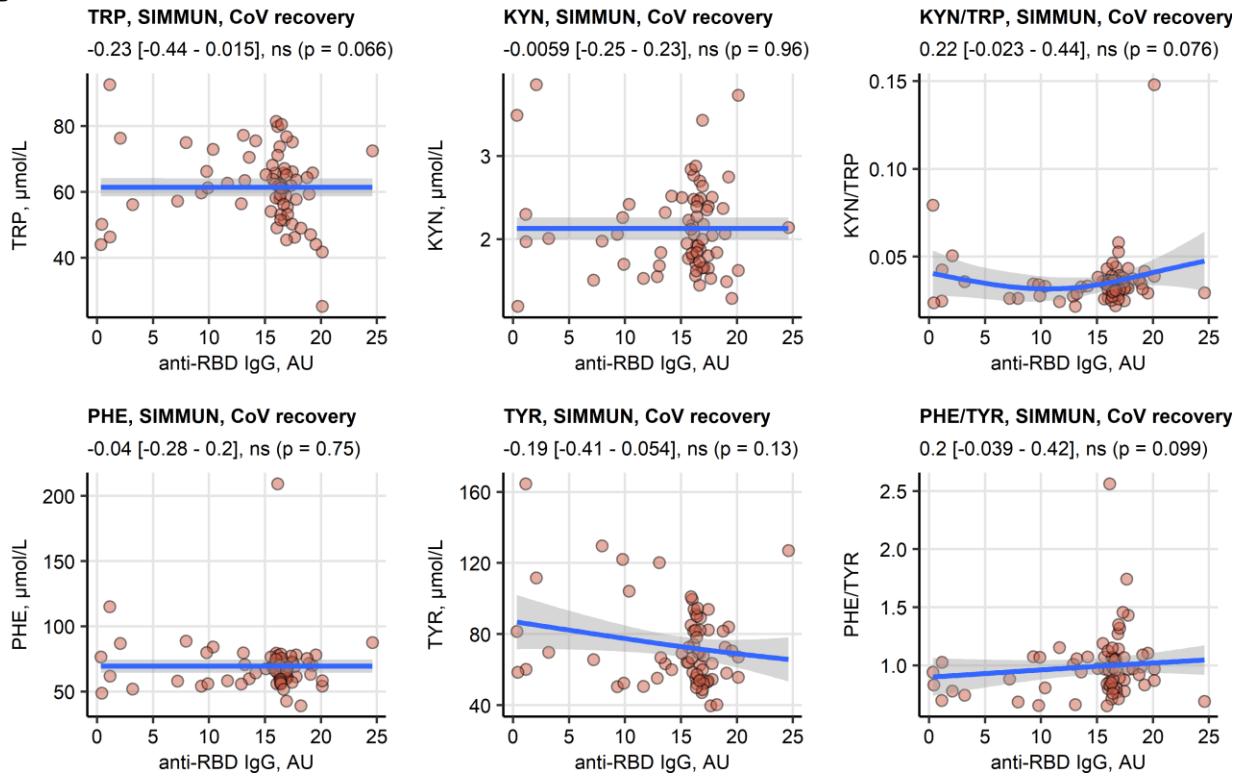
^aMedian with interquartile range, days after symptom onset.

Supplementary Figures



Supplementary Figure S1. Serum levels of inflammatory cytokines in course of COVID-19 and recovery, INCOV cohort.

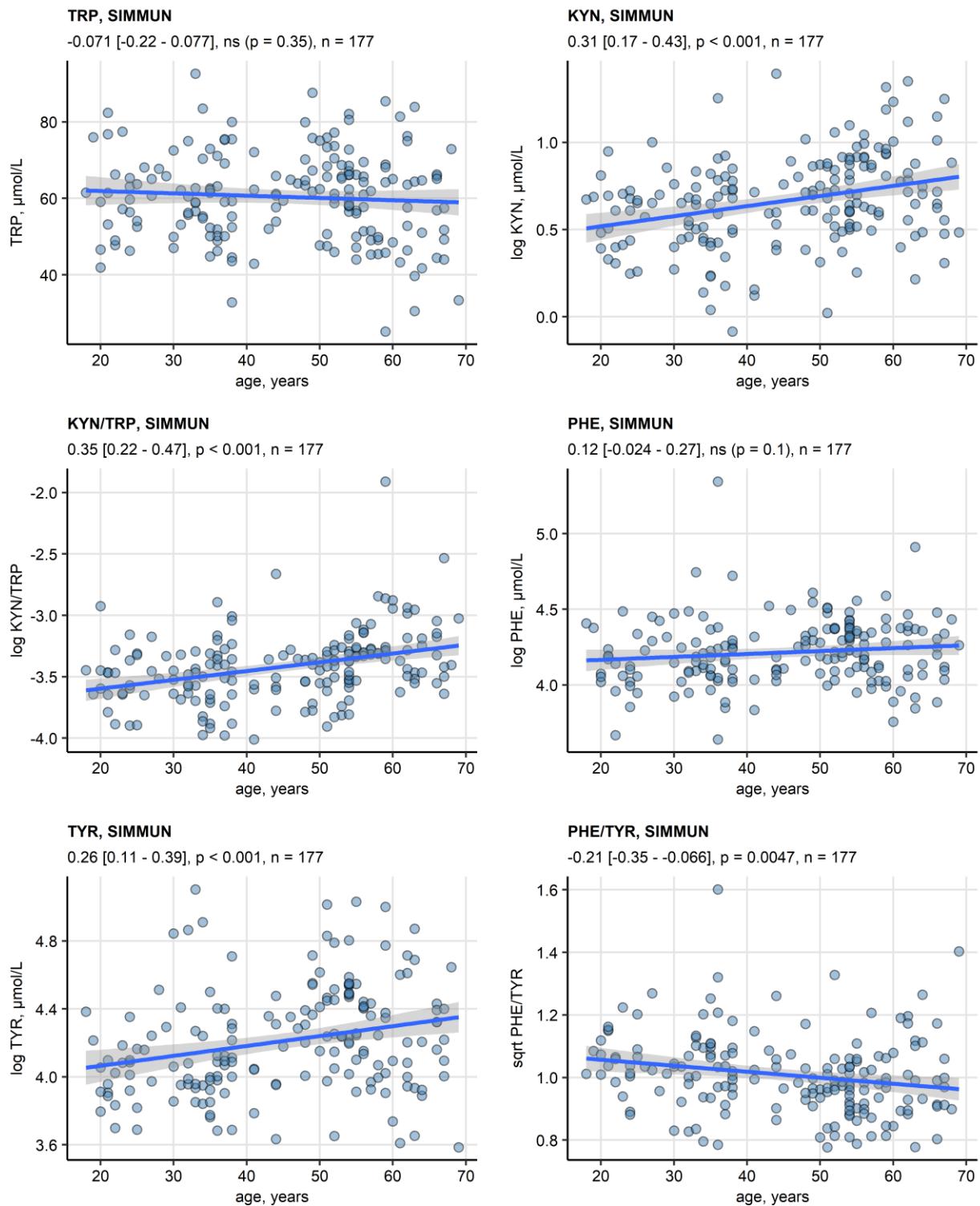
Serum levels of interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor alpha (TNF- α) and interferon-gamma (IFN- γ) in serum of uninfected controls and COVID-19 individuals during acute, sub-acute and recovery phase of the disease in the INCOV cohort. Statistical significance was determined by Kruskal-Wallis test with Benjamini-Hochberg-corrected Mann-Whitney U test. Normalized serum level concentrations are presented in box plots. Points represent single samples. The Kruskal-Wallis test results are indicated in the plot captions. Results of the post-hoc tests are indicated in the plots. Numbers of complete observations are displayed under the plots.

A**B**

Supplementary Figure S2. Association of inflammatory markers with SARS-CoV-2 convalescence in the SIMMUN cohort. Association of neurotransmitter precursor amino acids and their breakdown products with anti-SARS-CoV-2 antibody response in the SIMMUN cohort.

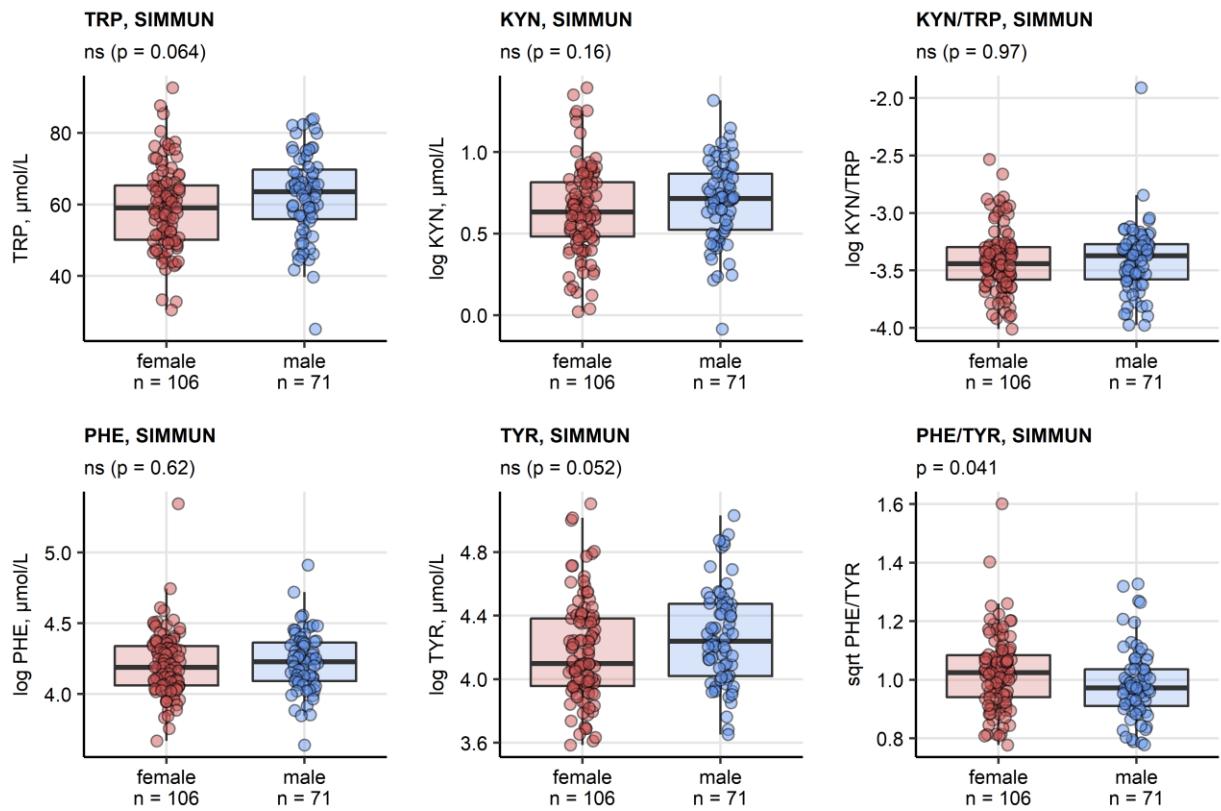
(A) Serum levels of interleukin 6 (IL-6), C-reactive protein (CRP), neopterin (NEO), and neutrophil-leukocyte ratio (NLR) in the SIMMUN study participants stratified by SARS-CoV-2 infections status. Statistical significance was determined by two-tailed T test. Serum levels are shown in box plots. Points represent single observations. Significance p values are displayed in the plot captions, numbers of complete observations are indicated in the plot X axes.

(B) Correlation of TRP, KYN, KYN/TRP, PHE, TYR and PHE/TRP with the titer of immunoglobulin gamma to receptor binding domain of the SARS-CoV-2 S1 protein (anti-RBD IgG, arbitrary units [AU]) in the SIMMUN cohort ($n = 177$) was investigated by Spearman test. Points represent single observations, blue lines with gray ribbons depict fitted generalized additive model (GAM) trends with 95% confidence intervals. Values of correlation coefficients with 95% confidence intervals and significance are indicated in the plot captions.



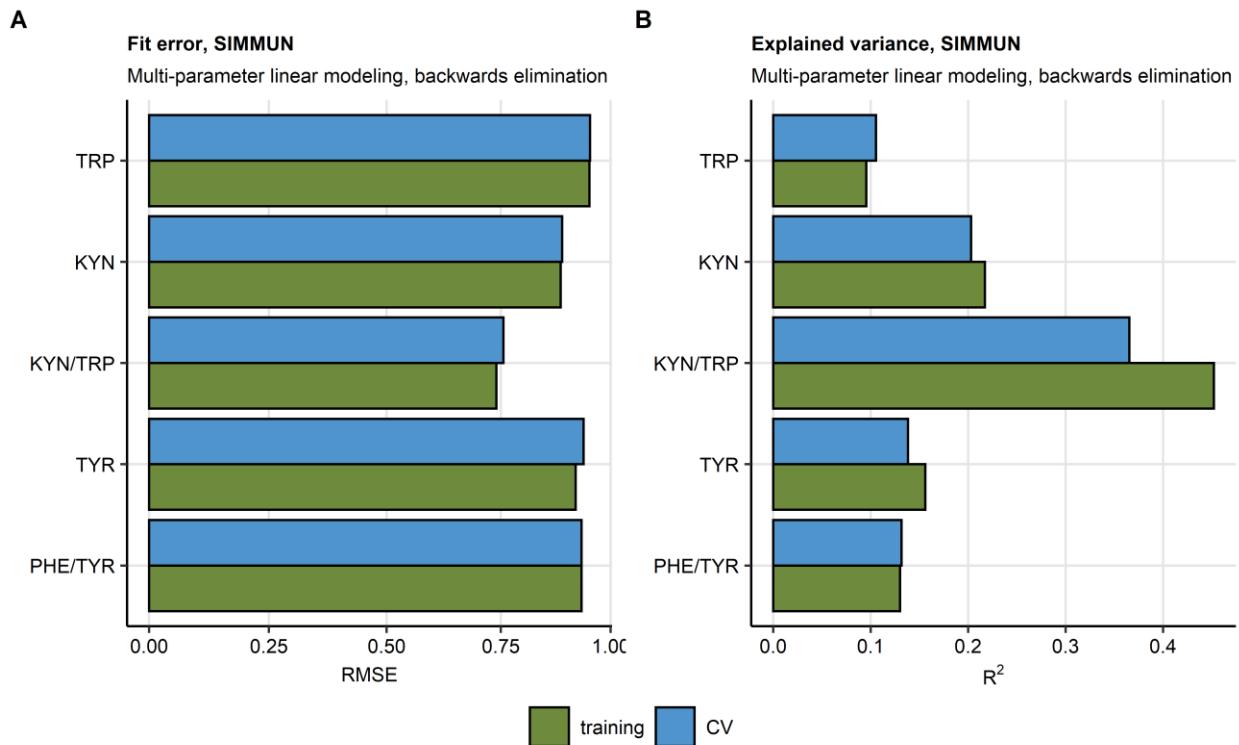
Supplementary Figure S3. Correlation of neurotransmitter precursor amino acids and their breakdown products with age.

Correlation of tryptophan (TRP), kynurenine (KYN), kynurenine/tryptophan ratio (KYN/TRP), phenylalanine (PHE), tyrosine (TYR) and phenylalanine/tyrosine ratio (PHE/TRP) with age in the SIMMUN cohort was investigated by Pearson test. Points represent single observations, blue lines with gray ribbons depict fitted linear trends with 95% confidence intervals. Values of correlation coefficients with 95% confidence intervals, significance and numbers of complete observations are indicated in the plot captions.



Supplementary Figure S4. Levels of neurotransmitter precursor amino acids and their breakdown products in females and males.

Serum concentrations of tryptophan (TRP), kynurenine (KYN), kynurenine/tryptophan ratio (KYN/TRP), phenylalanine (PHE), tyrosine (TYR) and phenylalanine/tyrosine ratio (PHE/TRP) in female and male participants of the SIMMUN study. Statistical significance was assessed by two-tailed T test. Serum levels are shown in box plots. Points represent single observations. Significance p values are displayed in the plot captions. Numbers of complete observation are indicated in the plot X axes.



Supplementary Figure S5. Root mean square error and R-squared statistics for multi-parameter linear models of neurotransmitter precursor amino acids and their breakdown products in the SIMMUN cohort.

Multi-parameter linear regression models of serum levels of tryptophan (TRP), kynurenine (KYN), kynurene/tryptophan ratio (KYN/TRP), phenylalanine (PHE), tyrosine (TYR) and phenylalanine/tyrosine ratio (PHE/TRY) in the SIMMUN models were optimized by backwards elimination and their reproducibility was tested by repeated cross-validation (CV, 50 repeats, 10 folds). Values of root mean square error (RMSE, A) and R^2 (B) in the training data set and CV are plotted.

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