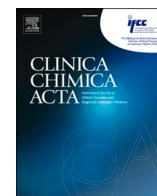




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Alteration of the kynurenine pathway is inversely associated with the humoral immune response in patients with SARS-CoV-2

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ABSTRACT

Background: The scale and the course of antibody production in patients with SARS-CoV-2 is highly variable. Factors involved in the immune regulation during the infection may play a major role in the antibody response. We investigated the relationship between the inflammatory markers of the kynurenine pathway and the concentration of antibodies against SARS-CoV-2 in infected patients 8–11 days after admission.

Methods: The study included 72 SARS-CoV-2 - positive inpatients hospitalized between August 2020 and April 2021. The plasma concentrations of tryptophan, kynurenine, anti-SARS-CoV-2 antibodies and the leucocyte count were measured 8–11 days after admission. The kynurenine/tryptophan ratio (KYN/TRP ratio) was calculated. Tertiles based on the values for tryptophan, kynurenine, KYN/TRP ratio and the leucocytes were generated.

Results: Statistically significant correlations were observed between anti-SARS-CoV-2 antibodies and tryptophan, kynurenine, KYN/TRP ratio and the leucocytes (p-values < 0.001–0.007). The high kynurenine and KYN/TRP ratio tertiles showed significantly lower antibody titers compared to the low tertiles (p-values 0.017 and < 0.001). The low tryptophan and leucocytes tertiles showed significantly lower antibody titers compared to the high tertiles (p-values 0.001 and 0.008).

Conclusion: Patients with higher activation levels of the kynurenine pathway tended to develop lower anti-SARS-CoV-2 antibody titers.

The markers of the kynurenine pathway, tryptophan and kynurenine, have been highlighted recently as modulators of the immune response to SARS-CoV-2 with a potential predictive value for the disease outcome [1,2]. Inflammatory stimuli are the main triggers for the activation of the indolamine-pyrrole 2,3-dioxygenase (IDO) [3]. This enzyme catalyzes the oxidation of tryptophan to its metabolite kynurenine. Two isoforms of IDO have been described. IDO1 is broadly expressed in various tissues, while IDO2 has been found in liver, kidney and antigen presenting cells [4]. However, data on the relationship to the B-cell immunity with antibody production, particularly in the context to SARS-CoV-2 infections, are scarce.

Therefore, we aimed to evaluate possible associations between the magnitude of the antibody response in patients with SARS-CoV-2 and

markers of the kynurenine pathway. Data of 72 inpatients with confirmed SARS-CoV-2 infection, which were hospitalized during the period of August 2020 to April 2021, were evaluated. All patients were not vaccinated against SARS-CoV-2. A panel of laboratory analyses comprising the tryptophan metabolism (e.g. tryptophan (TRP), kynurenine (KYN), KYN/TRP ratio), anti-SARS-CoV-2 antibodies against the spike (s) -protein, C-reactive protein (CRP) and hematological parameters (e.g. leukocytes, lymphocytes, hemoglobin, platelets), performed from blood samples collected 8–11 days after admission, was investigated. Tryptophan and kynurenine were measured by high-performance liquid chromatography (HPLC) with a simultaneous ultraviolet and fluorimetric detection system [5]. Anti-SARS-CoV-2 antibodies were measured on a cobas e801 (Roche Diagnostics, Rotkreuz, Switzerland)

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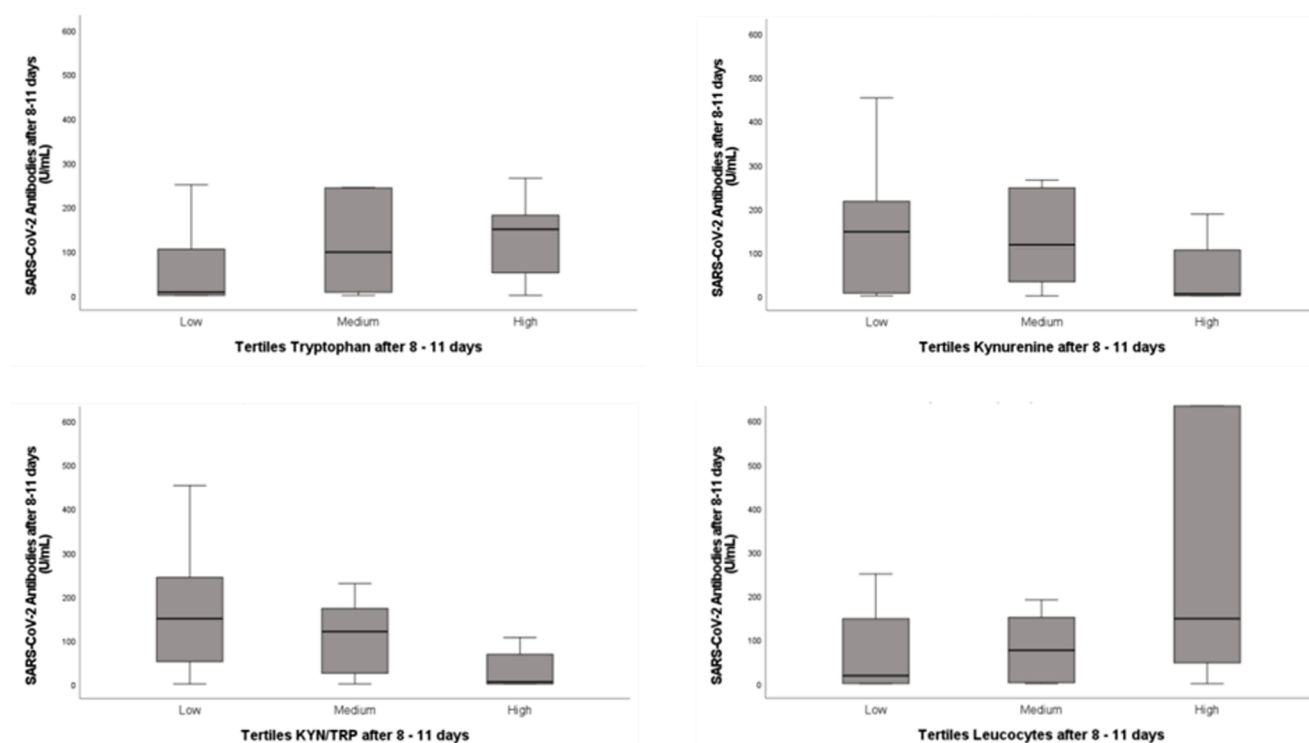


Fig. 1. Comparisons of SARS-CoV-2 antibodies between tertiles for tryptophan (TRP), kynurenine (KYN), KYN/TRP ratio and the leucocyte count 8–11 days after admission in 72 hospitalized patients. Kruskal-Wallis test was used for subgroup comparisons: TRP: $p = 0.003$; KYN: $p = 0.009$; KYN/TRP ratio: $p < 0.001$; leucocytes: $p = 0.025$.

and the CRP on a cobas c503 by standard laboratory procedures. The hematological parameters were measured on a XN-3000 (Sysmex Corporation, Kobe, Japan) analyzer. The study cohort was divided into tertiles based on the values of tryptophan, kynurenine, KYN/TRP ratio and the leucocyte count. For tryptophan, the low tertile comprised subjects with tryptophan levels $< 41.77 \mu\text{mol/L}$, the intermediate tertile $41.77\text{--}55.08 \mu\text{mol/L}$ and the high tertile $> 55.08 \mu\text{mol/L}$. The cut-off values for kynurenine tertiles were $< 3.52 \mu\text{mol/L}$ and $> 5.36 \mu\text{mol/L}$, for the KYN/TRP ratio < 8.0 and > 12.8 , and for the leucocyte count $< 7.14 \text{ G/L}$ and $> 11.23 \text{ G/L}$. The Kruskal-Wallis test was performed to determine differences of the SARS-CoV-2 antibody titers between the tertiles for each parameter. Spearman's rho (ρ) was calculated to assess correlations between parameters of tryptophan metabolism and hematological parameters with titers of anti-SARS-CoV-2 antibodies. A p -value < 0.05 was considered statistically significant. The analyses were performed using SPSS 26.0 statistical software (SPSS Inc., Chicago, IL, USA).

After 8–11 days, the majority of patients showed a humoral response with a median (Q1–Q3) concentration of SARS-CoV-2 antibodies of 97.6 (3.9 – 163.0) U/mL. Eleven patients presented antibody concentrations below the detection limit of the assay. Statistically significant correlations between the concentrations of anti-SARS-CoV-2 antibodies and the levels of kynurenine, tryptophan, KYN/TRP ratio, leucocytes and platelets were observed 8–11 days after admission (see supplementary material). Furthermore, statistically significant correlations between the markers of the kynurenine pathway and CRP after 8–11 days were observed (all p -values < 0.05). The relationship between the tertiles of tryptophan, kynurenine, KYN/TRP ratio and leucocytes 8–11 days after admission are presented in Fig. 1. The lowest tertile of the KYN/TRP ratio showed a statistically significant lower median (Q1 – Q3) age than the intermediate and high tertiles (68 (63–74) vs 76 (67–82) and 75 (73–82) years, p -values = 0.007 and 0.003). Likewise, a statistically significant difference of the median (Q1 – Q3) age was found between the high and low kynurenine tertiles (76 (74–82) vs 71 (62–77) years, p

= 0.012). The Kruskal-Wallis test revealed statistically significant differences between the categories for each parameter (p -values $< 0.001\text{--}0.025$). The median (Q1 – Q3) titers of anti-SARS-CoV-2 antibodies were lower in the high kynurenine (4.7 (0.0 – 10.5) vs 146.5 (6.2 – 216.5) U/mL, $p = 0.017$) and KYN/TRP ratio (4.7 (0.0 – 67.6) vs 149.0 (50.9 – 243.0) U/mL, $p < 0.001$) tertiles compared to the low tertiles. The high tryptophan (149.5 (50.9 – 181.0) vs 7.2 (0.0 – 104.5) $\mu\text{mol/L}$, $p < 0.001$) and leucocytes (148.0 (47.4 – 721.5) vs 18.1 (0.3 – 148.0) U/mL, $p = 0.008$) tertiles showed higher median antibody titers compared to the low tertiles. Accordingly, the highest mean antibody titer was measured in the low KYN/TRP ratio and high leucocytes subgroup of patients, while there was only a low antibody response in the high KYN/TRP and low leucocytes tertiles.

Our results indicate a possible suppressive effect of an activated kynurenine pathway on the anti-SARS-CoV-2 antibody response after 8–11 days. There are publications indicating an immunosuppressive effect of the activated kynurenine pathway in the B-cell immunity [4,6]. It was shown that hemodialysis patients with an insufficient antibody response to hepatitis B vaccination had significantly higher IDO-activities than those with sufficient antibody titers [4]. Soundaki et al. reported an increased humoral alloimmunity in lymphocytes from four subjects after addition of an IDO-inhibitor [6]. Thus, IDO might play a role as an inhibitor of humoral alloimmunity [6]. In contrast, there is evidence that an activated kynurenine pathway is associated with increased B-cell responses. Bonezi et al. reported a correlation between the number of antibody-secreting B cells and IgG-titers with the concentrations of tryptophan and kynurenine in cells infected with the Dengue virus [7]. However, no correlation was seen in cells infected with the Zika virus or the Yellow fever virus [7]. Pigott et al. showed that the activation of IDO is an important step in the differentiation of autoreactive B cells to antibody-secreting cells [8]. These proinflammatory properties of an activated kynurenine pathway may be due to differences in the regulatory pathways between IDO1 and IDO2 [9]. In mouse models, IDO2 was identified as a mediator of autoreactive B-

cell development [9]. IDO2 also influenced the antibody production in models of influenza [9]. Hence, a proinflammatory effect of IDO2 in B cells was hypothesized [9]. Furthermore, we found a statistically significant positive correlation between the leucocyte count and anti-SARS-CoV-2 antibodies. We observed the lowest antibody titers in patients ranging in the high kynurenine and low leucocyte tertile. Thus, we hypothesize that immunoregulatory dysregulations might lead to a diminished or at least delayed humoral response.

We evaluated the markers of the kynurenine pathway and the antibody response about 10 days after admission, thus in an early stage of humoral response. Neutralizing antibodies against SARS-CoV-2 tend to appear seven to 15 days after onset of the disease with a peak after 14 to 22 days [10]. IgG-antibodies usually reach their peak between three to seven weeks [10]. Hence, an investigation of the course of antibody titers over a wider period to assess long term effects on antibody production would be of interest. The high KYN/TRP ratio tertile showed a significantly higher median age than the medium and low tertiles, while the high kynurenine tertile showed a significantly higher age compared to the low tertile. Therefore, age might be a confounding factor in this study. However, published data have not yet revealed a clear association between age and SARS-CoV-2 antibody response [10]. Due to the limited sample size and lacking clinical data, we could not relate our findings to the disease outcome. Larger studies with comprehensive clinical follow-up procedures are needed. Finally, an inference of mechanisms in the development of the disease from our data is not possible. Particularly the influences of activated IDO1 and IDO2 were not investigated. Hence, the pathophysiological mechanisms of an activated kynurenine pathway on the B-cell function in SARS-CoV-2 infected patients remain to be elucidated.

In conclusion, this study highlights the potential interaction between the kynurenine pathway and the immune response in SARS-CoV-2 infected patients. Patients with high kynurenine, high KYN/TRP ratios and low leucocytes tended to develop lower anti-SARS-CoV-2 antibody titers after 8–11 days. For the investigation of the possible underlying pathomechanisms, further studies are needed.

CRedit authorship contribution statement

Simon Michaelis: Conceptualization, Formal analysis, Writing – original draft. **Sieglinde Zelzer:** Conceptualization, Methodology. **Christopher Schneider:** Formal analysis, Investigation. **Wolfgang J. Schnedl:** Supervision. **Andreas Baranyi:** Methodology. **Andreas Meinitzer:** Conceptualization, Methodology. **Markus Herrmann:** Supervision. **Dietmar Enko:** Writing – review & editing, Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cca.2022.10.005>.

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