Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Pharmacological Sciences 150 (2022) 49–55

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full Paper

Characterization of serotonin as a candidate biomarker of severity and prognosis of COVID-19 using LC/MS analysis

Kosuke Saito [a, 1], Rika Ishikawa [a, 1], Isao Kitamura [b], Kumiko Ogawa [c], Noriaki Arakawa [a], Yuchen Sun [a], Kazuo Imai [d, e], Takuya Maeda [e], Yoshiro Saito [a, *], Chihiro Hasegawa [f]

[a] Division of Medical Safety Science, National Institute of Health Sciences, Kanagawa, Japan
[b] Department of Clinical Laboratory, Nagoya City University East Medical Centre, Nagoya, Japan
[c] Division of Pathology, National Institute of Health Sciences, Kanagawa, Japan
[d] Department of Internal Medicine, Self-Defense Forces Central Hospital, Tokyo, Japan
[e] Department of Infectious Disease and Infection Control, Saitama Medical University, Saitama, Japan
[f] Department of Infectious Diseases, Nagoya City University East Medical Centre, Nagoya, Japan

ARTICLE INFO

Article history:
Received 16 May 2022
Received in revised form
21 June 2022
Accepted 29 June 2022
Available online 31 July 2022

Keywords:
Biomarker
COVID-19
Disease severity
Prognosis
Serotonin

ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic has been associated with high mortality worldwide. Owing to its complicated pathophysiology, diagnostic and prognostic biomarkers for effective patient management remain scarce. We analyzed kynurenine, tryptophan, and serotonin levels in the serum of patients with COVID-19 via liquid chromatography/mass spectrometry analysis. Serum serotonin levels were decreased in patients with more severe COVID-19, along with increased kynurenine and decreased tryptophan concentrations. Patients with moderate disease who subsequently worsened showed significantly lower serotonin concentrations compared with those who did not experience severe disease. Serum serotonin levels may represent a valuable biomarker for COVID-19 severity and prognosis.

1. Introduction

The coronavirus disease 2019 (COVID-19), which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in China[1] and spread rapidly worldwide causing high mortality; it was declared a global pandemic in March 2020.[2] Although some treatments, including antiviral agents and corticosteroids, and several vaccines have been recently developed and employed against the disease,[3] the number of infected people continues to rise globally with several regional pandemic waves caused by newly-emerged viral variants.[2] As of April 1, 2022, a total of 486,761,597 confirmed cases and 6,142,735 deaths associated with COVID-19 were reported by the World Health Organization.[2] The severity of COVID-19 pneumonia varies widely among patients; more than 80% of patients experience mild stage disease without requiring treatment, whereas 15% develop severe respiratory distress, of which approximately 5% rapidly progress to critical pneumonia and multiorgan injury requiring intensive care with a high risk of fatal outcomes due to cytokine storm.[1,4–7] Therefore, predicting disease progression based on the symptoms remains challenging. Although several comorbidity factors, including obesity, diabetes, hypertension, cardiomyopathy, liver dysfunction, kidney disease, heart disease, and general frailty, influence disease severity and mortality rate, they cannot definitively explain all critical cases[3,4,6,7] due to the lack of a complete understanding of the pathophysiology of COVID-19. Therefore, diagnostic and prognostic COVID-19 biomarkers that can be used at an early disease stage are still required to assess the risk of disease severity and effective clinical management.

Several mass spectrometry-based studies, including some metabolomic approaches,[8–17] have investigated alterations in the serum/plasma levels of biomolecules that could be associated with the COVID-19 pathophysiology. In particular, tryptophan metabolism was described as the top pathway affected by COVID-19 based on targeted and untargeted metabolomics studies, with significant decreases in plasma tryptophan and serotonin levels,

* Corresponding author. Division of Medical Safety Science, National Institute of Health Sciences Kanagawa 210-9501, Japan. fax: +81 44 270 6624.
E-mail address: yoshiro@nihs.go.jp (Y. Saito).
Peer review under responsibility of Japanese Pharmacological Society.
[1] Equal contributors.

https://doi.org/10.1016/j.jphs.2022.06.005

and increases in plasma kynurenine levels being described in SARS-CoV2-positive patients.[13] The major metabolic pathway of tryptophan, which converts tryptophan into kynurenine, is regulated by the rate-limiting enzyme indole 2,3-dioxygenase (IDO), which plays an immune-regulatory role and is induced by pro-inflammatory cytokines, such as interferon (IFN)-γ, in response to viral infection.[18,19] Serotonin is another metabolite of tryptophan that is altered in the serum/plasma of patients with COVID-19. Proteomic and metabolomic characterization studies showed that serum serotonin levels decrease with the increasing COVID-19 severity.[9] Other metabolomic studies have also shown a decrease in serotonin levels in the serum/plasma of patients with COVID-19.[13,17] Recently, peripheral serotonin was reported as an immune modulator[20,21]; however, changes in serotonin metabolism during COVID-19 progression remain unclear.

Collectively, tryptophan, kynurenine, and serotonin are potential diagnostic biomarkers of COVID-19 progression. However, in most previous studies characterizing alterations in tryptophan and its metabolites, patients with COVID-19 (mostly severe cases) were compared with healthy controls, but few studies have evaluated such alterations during disease development or among mild, moderate, and severe disease stages.[12,13] Moreover, a comprehensive understanding of the expression profile of these metabolites during disease progression is lacking. Notably, most of these metabolomic studies used untargeted metabolomic analysis approaches, which only provide qualitative or semi-quantitative results; thus, it may be difficult to compare absolute biomarker concentrations for clinical diagnostic and/or prognostic purposes. To date, one recent study has determined the serum serotonin concentrations in patients with mild/moderate and severe COVID-19[22]; however, this previous study employed enzyme-linked immunosorbent assay (ELISA), which is associated with concerns of selectivity and specificity,[23] especially in the quantification of low molecular weight metabolites in biological fluid. Moreover, the validity of the analytical parameters of this ELISA was not described. Therefore, in this study, we investigated patients with COVID-19 at various disease stages and determined the kynurenine, tryptophan, and serotonin serum concentrations using validated liquid chromatography/mass spectrometry (LC/MS) analysis. Serum samples from moderate-stage patients were further compared based on their longitudinal progression (that is, whether or not they subsequently developed severe disease) to better characterize the clinical applicability of kynurenine, tryptophan, and serotonin levels as biomarkers of COVID-19 severity and prognosis.

2. Materials and methods

2.1. Materials

L-kynurenine and serotonin were purchased from Sigma–Aldrich (St. Louis, MO, USA). L-tryptophan was purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Serotonin-d4 and L-tryptophan-d5 were purchased from Toronto Research Chemicals (Toronto, Canada), and L-kynurenine sulfate (ring-D4, 3, 3-D2) was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). All other solvents and reagents used were of commercially available LC/MS or high-performance LC grade.

2.2. Sample collection

A total of 153 serum samples from 27 patients with SARS-CoV-2 infection confirmed via polymerase chain reaction were collected from May 15 to November 25, 2020, at a single clinical center. A total of 59 serum samples from age matched healthy subjects were collected at another clinical center. The severity of the COVID-19 pneumonia was determined at the time of blood sampling and was defined as mild, moderate, severe, and critical based on the Japanese Medical Guidance for COVID-19 applied at the hospital, which replaced the corresponding categorization protocol of the National Institutes of Health COVID-19 Treatment Guidelines.[24] General and clinical data of all samples are summarized in Table 1, and Supplemental Table 1 (patients) and **2** (healthy subjects). This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the National Institute of Health Science (No. 322, 333 and 337) and Nagoya City (University) East Medical Center (No. 20-04-314). Written informed consent was obtained from all participants.

2.3. Sample preparation

For LC/MS analysis, 30 μL of patient serum was mixed with 470 μL of 85% methanol in water containing the internal standards (12.5 ng/mL kynurenine-d6, 100 ng/mL tryptophan-d5, and 12.5 ng/mL serotonin-d4). After removing proteins via centrifugation (18,440 or 21,210 ×*g*, 10 min, 4 °C), the solution was filtered with FastRemoverC18 (GL Science, Tokyo, Japan) using an automated system (Microlab NIMBUS with an MPE2 unit; Hamilton, Reno, NV, USA). The flow-through fraction was diluted 1:3 (v/v) with water and subjected to LC/MS analysis. The preparation

Table 1
Sample characteristics in the groups of healthy subjects and COVID-19 patients.

Group	Healthy subjects (h)	Covid-19 mild (a)	Covid-19 moderate (b)	Covid-19 severe (c)	Covid-19 critical (d)	*p-value*
Number of Subjects	59	13	18	13	4	
Number of samples	59	25	62	47	19	
General data						
Age (years)	52.5 ± 7.93	55.2 ± 21.1	57.4 ± 9.89	56.0 ± 9.50	52.8 ± 4.87	*:b-d, b-h
Gender (M/F)	29/30	14/11	41/21	42/5	19/0	*:a-c, b-c, b-d,**:a-d,***:c-h, d-h
Body mass index	21.3 ± 1.56	27.0 ± 4.68	27.6 ± 4.69	26.3 ± 4.14	26.6 ± 0.77	****:a-h, b-h, c-h, d-h
Clinical data						
CRP (mg/dL)	NA	0.42 ± 0.47	3.75 ± 3.89	5.88 ± 5.39	7.89 ± 5.90	**:a-b, b-d,****:a-c, a-d
D-dimer (μg/mL)	NA	1.49 ± 2.04	1.11 ± 1.20	2.29 ± 2.16	3.37 ± 3.68	*: a-d, b-c,**:b-d
Platelet ($x10^4$/uL)	NA	30.99 ± 12.74	27.33 ± 10.70	27.20 ± 13.04	25.02 ± 6.12	

Alphabets in brackets followed by group used to present statistical differences between each groups in the column of p-value. Mean ± standard deviation. The statistical significance was shown as p-values in ANOVA followed by Tukey's test or chi-square test with Bonferroni correction: ns, not significant.
M/F; male/female, CRP; C-reactive protein. N/A; not available.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
**** $p < 0.0001$.

method used for the serum samples of the healthy subjects was slightly different because of the low volumes of patient serum samples available for analysis. Proteins were removed using an automated system by mixing the serum (50 μL) with 450 μL of 90% methanol in water containing the internal standard and filtering with FastRemover for Protein (GL Science); the following procedure was the same as described above.

2.4. LC/MS analysis

Chromatographic separation was performed using the Ultimate 3000 system (Thermo Fisher Scientific, Waltham, MA, USA) with a Triart PFP column (1.9 μm, 2.1 × 100 mm; YMC, Kyoto, Japan) at 45 °C. Mobile phase A consisted of water with 0.3% formic acid and mobile phase B consisted of methanol with 0.3% formic acid. A sample volume of 5 μL was injected. After holding 20% B for 0.5 min at a flow rate of 0.3 mL/min, a ramp gradient was used as follows: 20% B at 0.5 min, increased to 100% B at 2.5 min, held for 1.5 min before returning to 20% B at 4.1 min and holding for 0.9 min.

The mass spectrometer used was TSQ-Quantiva (Thermo Fisher Scientific), which was operated in the multiple-reaction monitoring mode. The samples separated via chromatography were ionized via heated electrospray ionization in positive mode. Ion source parameters were as follows: Ion source voltage, 3.5 kV; ion-transfer tube temperature, 350 °C; vaporizer temperature, 250 °C. The gas settings were as follows: Sheath gas, 40 arbitrary units (Arbs); auxiliary gas, 10 Arbs; sweep gas, 1 Arb. The following multiple-reaction monitoring transitions (m/z) were monitored at collision energies of 10 V with Ar 1.5 Torr: Tryptophan 205.1/188.1, tryptophan-d5 210.1/192.0, kynurenine 209.1/192.1, kynurenine-d6 215.1/198.1, serotonin 177.2/160.1, and serotonin-d4 181.2/164.1. Obtained raw data were processed using TraceFinder 4.1 (Thermo Fisher Scientific) and Prism 9 (GraphPad Software, San Diego, CA, USA). Determined concentrations of kynurenine, tryptophan, and serotonin in individual serum samples are shown in Supplemental Table 1 (patients) and **2** (healthy subjects).

2.5. Validation of LC/MS analysis

The following parameters of LC/MS analysis were assessed for quantitative analysis: Calibration curve, carryover, parallelism, precision, and relative accuracy. Calibration standards were prepared in water at for kynurenine and serotonin (20, 40, 100, 200, 400, 1,000, 2,000, and 4000 ng/mL), and for tryptophan (150, 300, 750, 1,500, 3,000, 7,500, 15,000, and 30,000 ng/mL). The dilution of serotonin calibration standards must be conducted with polypropylene tubes but not glass vialsA. Two quality control (QC) samples (QCa and QCb) were prepared by pooling serum samples from patients with various lung diseases. In addition, the lowest QC sample (QC diluted) was prepared by diluting other samples of pooled sera with 10% bovine serum album in phosphate-buffered saline, and the spiked QC sample (QC spiked) was prepared by spiking another sample of pooled sera with the highest calibration standard concentration.

The calibration curves were established using the normalized peak area (analyte/IS) of the calibration standards in duplicate using Prism 9 (GraphPad Software). A linear regression model with $1/X^2$ weighting was used to obtain the calibration curve equation. Calibration curves were assessed using the correlation coefficient (r) and relative error (RE, %) of the back-calculated concentration against the nominal concentration of each calibration standard. Carryover was assessed by comparing the peak area of the blank sample injected immediately after the highest calibration standard concentration with that of the lowest calibration standard concentration. Parallelism was assessed by calculating the slope ratio between the calibration curve calculated using the calibration standards and the response curve calculated using four QC samples. Within-run precision and relative accuracy were determined by measuring QC samples in six replicates; three replicates of three individual batches were used to determine between-run precision and relative accuracy. Reinjection reproducibility was assessed by the relative value (%) of the second injection in comparison with the first injection; the second injection was conducted after sample vial storage at 4 °C for over 12 h.

2.6. Statistical analysis

All statistical analyses were performed using Prism 9 (GraphPad Software). Significant differences in numerical characteristics and metabolite concentrations of patients with COVID-19 and healthy subjects were assessed using analysis of variance (ANOVA) followed by post-hoc Tukey's test for more than two groups and an unpaired t-test for two groups. Significant differences in categorical characteristics were assessed using the Chi-square test with Bonferroni correction. Receiver operating characteristic analysis was performed to assess the potential of the metabolites as prognostic/diagnostic biomarkers.

3. Results

3.1. Analytical method validation

A summary of the LC/MS validation assay for kynurenine, tryptophan, and serotonin analysis is shown in Supplemental Table 3. Overall, the RE of the back-calculated concentrations of kynurenine, tryptophan, and serotonin were within ±3.59, 1.81, and 4.95%, respectively, and the correlation coefficient for all analytes was greater than 0.999. Carryover of kynurenine, tryptophan, and serotonin was not detected. In parallelism evaluation, the slope ratio of the calibration standards and QC samples was 96.60, 97.71, and 98.76% for kynurenine, tryptophan, and serotonin, respectively. The within-run precision of the four QC samples was within 9.75% for kynurenine, 9.27% for tryptophan, and 9.36% for serotonin, whereas the between-run precision was within 5.05% for kynurenine, 3.20% for tryptophan, and 6.32% for serotonin. The within-run relative accuracy of the four QC samples was 94.47–98.33% for kynurenine, 96.69–101.03% for tryptophan, and 98.45–104.68% for serotonin, whereas the between-run relative accuracy was 94.47–104.37% for kynurenine, 96.69–103.08% for tryptophan, and 92.81–104.68% for serotonin. The reinjection reproducibility was within ±1% of the original values for all three analytes. As these results were compatible with the acceptance criteria for drugs outlined in the Guidance/guidelines of bioanalytical method validation using LC/MS,[25] the herein used LC/MS method was validated for the quantitative analysis of kynurenine, tryptophan, and serotonin.

3.2. Sample characteristics of patients with COVID-19 and healthy subjects

The characteristics of the patients with COVID-19 and healthy subjects enrolled in the study are shown in Table 1. Although the healthy subjects were age-matched to those included in the COVID-19 group, a slight difference in age between the control group and patients with moderate symptoms was observed. In addition, age differences were detected between the moderate and critical COVID-19 groups. Regarding gender ratio, several differences among the different-stage patients and control groups were observed. In contrast, the body mass index (BMI) of patients with

COVID-19 was significantly higher than that of healthy subjects but was similar among those in different disease stage groups. In agreement with previous studies, the C-reactive protein (CRP) and D-dimer values, which are potential biomarkers for COVID-19 severity, also increased with disease severity, whereas platelet numbers were not significantly changed.

3.3. Changes in kynurenine, tryptophan, and serotonin concentrations, and their relationship with COVID-19 severity

An overall increase in kynurenine and a decrease in tryptophan and serotonin serum concentrations were observed in patients with COVID-19 as the disease severity increased (Fig. 1). However, only serotonin concentration significantly changed in all disease severity stages compared with the control group, whereas kynurenine and tryptophan levels only significantly changed in the moderate, severe, and critical stages. In addition, among the COVID-19 groups, serotonin concentrations were significantly different in four comparisons, whereas kynurenine and tryptophan concentrations were significantly different in one and three comparisons, respectively. The average concentrations of serotonin were 160.70 ng/mL in healthy subjects, 111.94 ng/mL in patients with mild-stage disease, 75.61 ng/mL in patients with moderate-stage disease, 58.86 ng/mL in patients with severe-stage disease, and 36.98 ng/mL in patients with critical-stage disease. Therefore, these results suggested that, among tryptophan and its metabolites, serotonin was the most promising biomarker associated with COVID-19 severity.

Next, we examined the impact of the general background of the patients (age, sex, and BMI) on serum serotonin concentration as some significant differences were detected among the patients and healthy subjects groups (Table 1). Nevertheless, further analysis of the data in relation to the median age and BMI of the healthy subjects showed no significant differences in serum serotonin concentrations between young and old patients, males and females, and patients with low and high BMI (Supplemental Table 4). These results suggested that the effect of the patient background was negligible on the serum serotonin concentrations, at least in healthy subjects.

3.4. Prognostic performance of serum serotonin levels in moderate-stage COVID-19

Further analysis was performed to investigate the prognostic value of serotonin, kynurenine, and tryptophan to differentiate patients who had stable moderate-stage disease from those who had moderate-stage disease and subsequently developed severe and/or critical-stage disease (Table 2). Analysis of the clinical data showed that the CRP values were significantly higher in patients who subsequently developed severe and/or critical-stage disease than those in patients who did not. Similarly, serum serotonin levels were significantly lower in patients who subsequently developed severe and/or critical-stage disease compared with

Table 2
Sample characteristics in the groups of moderate stage COVID-19 patients before and without worsening.

Group	Before worsening (A)	Without worsening (B)	*p-value*
Number of subjects	6	7	
Number of samples	9	25	
General data			
Age (years)	54.1 ± 4.81	60.6 ± 13.5	
Gender (M/F)	8/1	15/10	
Body mass index	30.5 ± 3.63	26.7 ± 5.45	
Clinical data			
CRP (mg/dL)	8.57 ± 5.16	4.40 ± 3.67	*: A-B
D-dimer (μg/mL)	0.61 ± 0.32	0.68 ± 0.48	
Platelet (x10^4/uL)	18.2 ± 6.92	24.1 ± 9.34	

Alphabets in brackets followed by group used to present statistical differences between each groups in the column of p-value. Mean ± standard deviation. The statistical significance was shown as p-values in student t-test or chi-square test: ns, not significant.
M/F; male/female, CRP; C-reactive protein.
* $p < 0.05$.

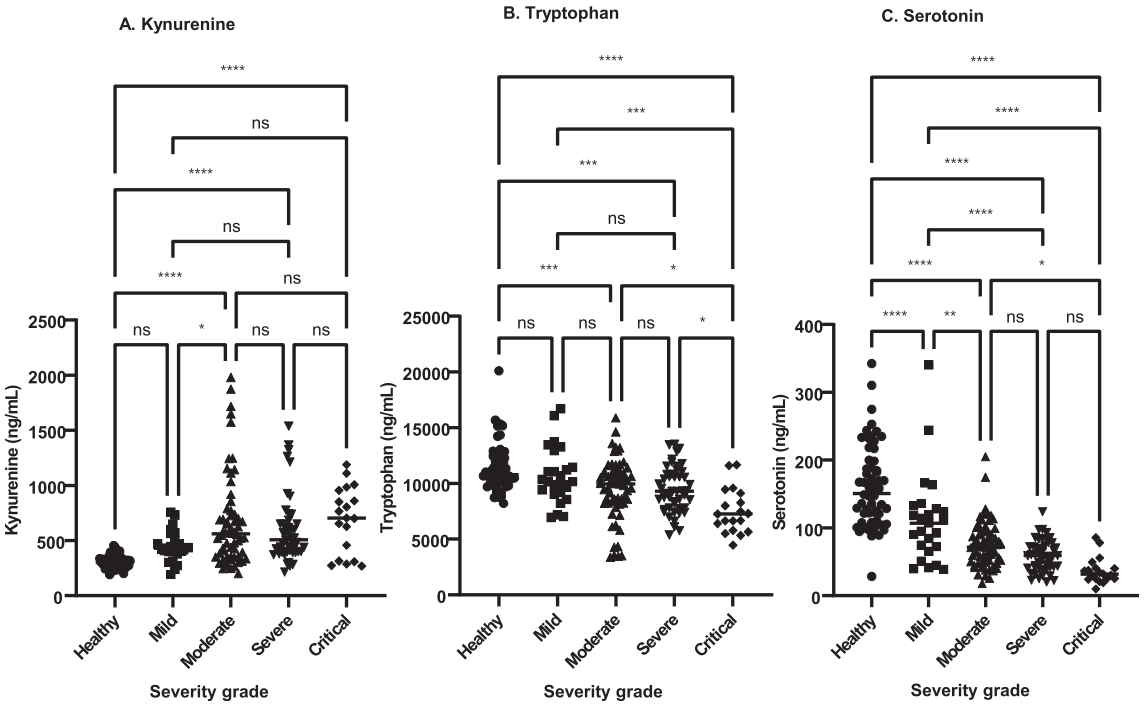

Fig. 1. Kynurenine (A), tryptophan (B), and serotonin (C) concentrations in the serum of healthy subjects and patients with COVID-19. Each dot represents individual samples. ns, not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ by ANOVA followed by Tukey's test.

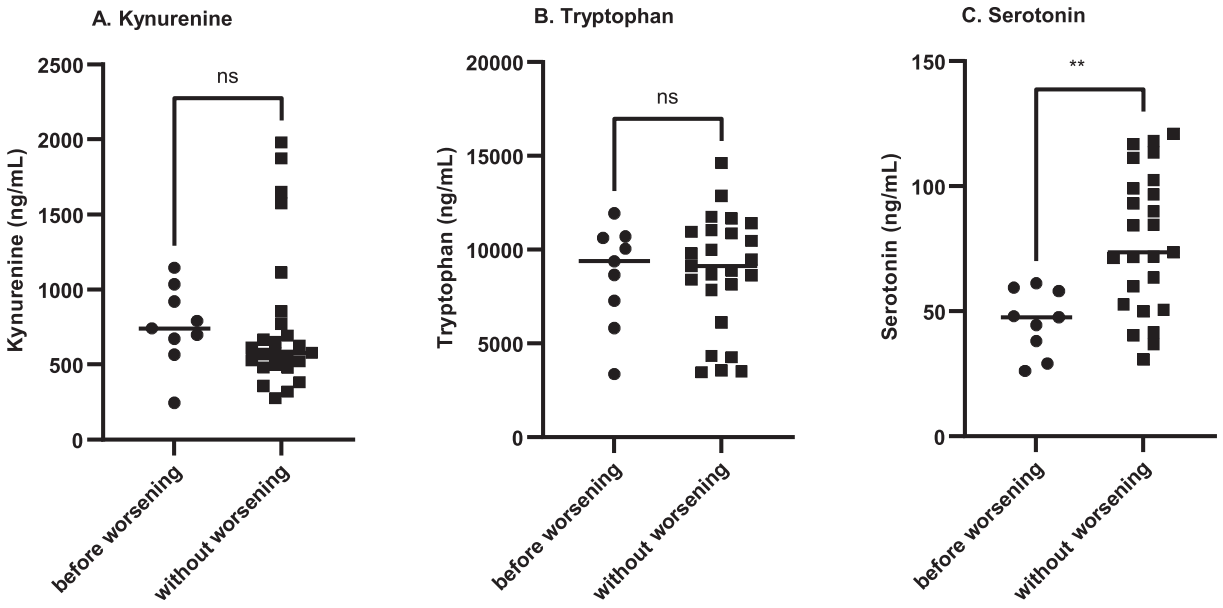

Fig. 2. Kynurenine (A), tryptophan (B), and serotonin (C) concentrations in the serum of moderate-stage patients with COVID-19. Comparison between the samples of patients with moderate-stage COVID-19 who subsequently developed severe disease (before worsening) and those who did not develop severe disease (without worsening). ns, not significant; $^{**}p < 0.01$ by ANOVA followed by Tukey's test.

those in patients who did not (Fig. 2). Neither kynurenine nor tryptophan levels showed such a trend. Thus, as well as CRP values, serotonin concentration was also associated with the prognosis of patients with moderate-stage COVID-19.

Supported by the above-described results, the potential clinical utility of serotonin levels for COVID-19 prognosis in the moderate disease stage, where further transition to more severe stage would require critical care, was evaluated by establishing receiver operating characteristic curves. As shown in Fig. 3, the area under the curve (AUC) to differentiate between patients who did or did not subsequently develop severe and/or critical-stage disease from moderate-stage disease was 0.840 for serotonin, which was even better than that obtained for CRP (0.747). We then determined the correlation between serotonin and CRP levels in the serum of these patients with moderate-stage COVID-19 but found no significant correlation (Supplemental Fig. 1). The lack of correlation between the CRP and serum serotonin concentrations was consistent with that reported previously.[22] Thus, we also constructed a prognostic model using logistic regression analysis with serotonin concentrations and CRP values. The following equation was used: score $= 2.42 + 0.369 \times$ CRP $- 0.101 \times$ serotonin. As shown in Fig. 3B, the AUC of the prognostic model was 0.88. Taken together, these results indicated that serotonin had greater potential as a prognostic biomarker than CRP and that its differentiating ability can be further improved by combining it with CRP.

4. Discussion

This study aimed to identify diagnostic and prognostic biomarkers of COVID-19 severity. A validated quantitative LC/MS was employed to measure the serum concentrations of kynurenine, tryptophan, and serotonin in patients with COVID-19 at various stages of severity. Notably, the alteration in serotonin concentrations was associated with disease severity even in mild stage disease. Furthermore, serum serotonin concentrations were found to be associated with severity and prognosis for moderate COVID-

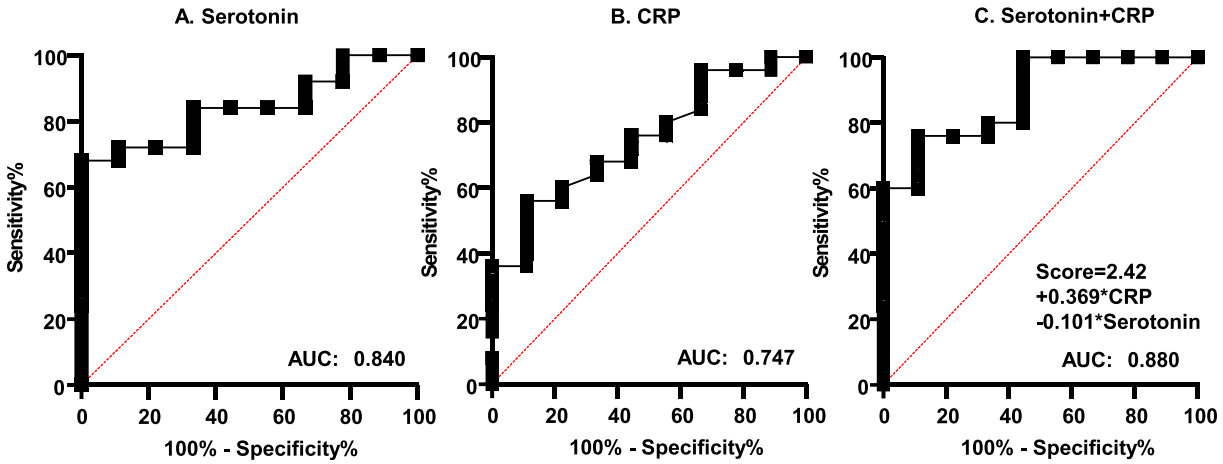

Fig. 3. Receiver operating characteristic curves of serotonin (A) and CRP (B) concentrations, and diagnostic model generated using serotonin and CRP (C) levels in patients with moderate-stage COVID-19 between those who subsequently did or did not develop severe disease. AUC, area under the curve; CRP, C-reactive protein.

19. To the best of our knowledge, this is the first study to show the potential of serotonin as a prognostic biomarker for the risk of developing severe and/or critical COVID-19 from moderate COVID-19.

A clear decrease in serum serotonin concentrations was observed in patients with COVID-19 as disease severity worsened. This observation is in agreement with that of a previous study, which demonstrated lower serum serotonin concentration in patients with severe COVID-19 than in patients with mild/moderate COVID-19; the previous study determined the serum concentration of serotonin using ELISA.[22] Important physiological roles of serotonin have been reported in the immune, vascular, and digestive systems, as well as in the central nervous system.[20,21] Therefore, the decreased serum concentrations of serotonin may be associated with the immune status of patients with COVID-19. Although the underlying mechanism is unclear, several metabolomic studies have revealed increased kynurenine and decreased tryptophan levels, along with a decrease in serotonin levels in patients with COVID-19.[8–13,15–17] In agreement with these findings, increased kynurenine and decreased tryptophan levels were also observed in the present study. Some of these previous studies proposed that the upregulation of the IDO enzyme in patients with COVID-19 may play a role in increased tryptophan conversion into kynurenine during the immune response to COVID-19. Regarding serotonin homeostasis, it has been proposed that IDO activation leads to significant consumption of tryptophan and limits its availability for serotonin production.[20] As IDO is also involved in the conversion of serotonin to formyl-5-hydroxykynurenamine,[20] its upregulation may also decrease the serum serotonin concentration by enhancing serotonin metabolism. Taken together, the decrease in serotonin levels as disease severity progressed can be attributed to the upregulation of IDO caused by SARS-CoV-2 infection.

In the present study, a decrease in serotonin concentrations was observed even between healthy subjects and patients with mild COVID-19, whereas alterations in tryptophan and kynurenine concentrations were not significant (Fig. 1). Furthermore, the prognostic ability of serotonin (but not of kynurenine and tryptophan) for further progression to more severe stages was confirmed in patients with moderate-stage COVID-19 (Figs. 2 and 3). Therefore, there may be another mechanism other than the upregulation of IDO underlying the decrease in serotonin concentrations. To date, there is no established mechanism associated with COVID-19; however, recent reports have suggested several possibilities regarding the decreased serum serotonin levels. Serotonin is metabolized by IDO but also by monoamine oxidases (MAOs) and is synthesized from 5-hydroxy-L-tryptophan by L-dopa decarboxylase (DDC).[20] In 2021, Cuperlovic-Culf et al.[26] suggested an increase in MAO activity in patients with COVID-19 evidenced by alterations in several metabolite ratios upon reanalysis of previously published metabolomics data.[9] Alternatively, *in vitro* experiments on epithelial cell lines (VeroE6 and A549) infected with SARS-CoV-2 revealed a strong negative correlation between viral RNA and *DDC* mRNA levels,[27,28] which implied that patients with COVID-19 may have decreased *DDC* levels in their epithelial cells. Thus, IDO-independent alterations in serotonin homeostasis in patients with COVID-19 may also contribute to the decreased serum serotonin levels.

The present data further demonstrated the potential prognostic ability of serum serotonin for assessing the risk of developing severe and/or critical COVID-19 from moderate COVID-19. To date, the role of serotonin in COVID-19 prognosis has been unknown. However, serotonin treatment was reported to suppress the release of interleukin (IL)-6 and tumor necrosis factor (TNF)-α in cultured macrophages and lymphocytes,[29] and of INF-γ in cultured whole blood.[30] Nau et al. reported that the activation of the serotonin 2A receptor inhibits the intestinal production of IL-6 and the increase in circulating IL-6 levels caused by TNF-α in mice.[31] Taken together, these results indicate that serotonin may suppress the release of pro-inflammatory cytokines, such as IL-6, which may explain why patients with COVID-19 with low serum serotonin concentrations had a higher risk of developing severe and/or critical disease from moderate COVID-19. Contrary to its anti-inflammatory effect, serotonin is known as accelerate blood coagulation,[32] which may cause further increase the severity of COVID-19. However, as shown in Fig. 1, the serum serotonin concentrations in all patients with moderate-stage COVID-19 were mostly lower than those in healthy subjects. Therefore, at least among the patients with moderate-stage COVID-19 whom we recruited, excessive blood coagulation by serotonin may not have played a role in the progression of disease.

As serotonin plays important physiological roles in the immune, vascular, digestive, and central nervous systems,[20,21] the decreased serotonin concentration with COVID-19 progression may also contribute to some of the symptoms of COVID-19. In particular, studies have demonstrated an association between COVID-19 and depression.[33–35] It has also been reported that the serum serotonin levels inversely correlated with the degree of anxiety and depression[36]; thus, decreased serotonin levels with disease progression may be one of the causes of depressive syndrome of COVID-19, although the cause and effect remain unclear. On the contrary, the role of serotonin in the gastrointestinal symptoms has also been proposed. Ha et al. demonstrated increased plasma serotonin levels with COVID-19 severity and its strong correlation with the incidence of diarrhea.[37] However, the plasma concentration of serotonin, which reflects pre-clotting state of the blood, could not be simply compared with serum serotonin concentration, which reflects post-clotting state of the blood. Thus, the association between serum serotonin levels and diarrhea remains unclear, warranting further research. In addition, an inverse correlation of plasma and serum serotonin levels against COVID-19 severity could not simplify the role of serotonin in COVID-19, but simultaneous analysis of plasma and serum serotonin levels may be capable of addressing the pathophysiological implication of serotonin on COVID-19.

This study has several limitations. First, owing to the limited number of patients in a single hospital, we used several serum samples from the same patient on different days and at different stages. This limitation also made it difficult to recruit healthy subjects with matching backgrounds to patients at all stages of COVID-19, although there was no influence of age, sex and BMI on serum serotonin concentrations in healthy subjects. However, there are still concerns of confounding influence of an unidentified patient background factor on serum serotonin concentration in patients with COVID-19. Second, detailed clinical information of the patients, especially regarding comorbidities, was not available. This may be important considering that alterations in serum serotonin concentrations are observed in several other diseases, including cancer, human immunodeficiency virus infection, and depression.[20] Therefore, caution should be exercised when considering the clinical application of serum serotonin concentrations as a predictor of COVID-19 progression in patients with other relevant diseases. Larger sample size and comprehensive consideration of clinical information are required to complement the present findings.

5. Conclusion

Using a validated method, the present study revealed dynamic changes in serotonin concentrations during severe COVID-19 progression. In particular, serotonin concentrations are significantly decreased in patients with moderate COVID-19 who subsequently

develop severe/critical conditions compared with those who experienced the stable moderate disease. Therefore, serum serotonin concentration is a possible biomarker of the severity and prognosis of COVID-19.

Funding

This work was supported by a grant awarded by the Japan Agency for Medical Research and Development (AMED) (Grant Number: 20fk0108300).

Declaration of competing interest

There are no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphs.2022.06.005.

References

1. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet*. 2020;395:470–473.
2. World Health Organization. Coronavirus disease (COVID-19) pandemic. www.who.int. Accessed April 1, 2022.
3. Rahman S, Montero MTV, Rowe K, Kirton R, Kunik Jr F. Epidemiology, pathogenesis, clinical presentations, diagnosis and treatment of COVID-19: a review of current evidence. *Expet Rev Clin Pharmacol*. 2021;14:601–621.
4. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382:1708–1720.
5. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020;8:475–481.
6. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395:1054–1062.
7. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med*. 2020;180:934–943.
8. Danlos FX, Grajeda-Iglesias C, Durand S, et al. Metabolomic analyses of COVID-19 patients unravel stage-dependent and prognostic biomarkers. *Cell Death Dis*. 2021;12:258.
9. Shen B, Yi X, Sun Y, et al. Proteomic and metabolomic characterization of COVID-19 patient sera. *Cell*. 2020;182:59–72. e15.
10. Fraser DD, Slessarev M, Martin CM, et al. Metabolomics profiling of critically ill coronavirus disease 2019 patients: identification of diagnostic and prognostic biomarkers. *Crit Care Explor*. 2020;2:e0272.
11. Kimhofer T, Lodge S, Whiley L, et al. Integrative modeling of quantitative plasma lipoprotein, metabolic, and amino acid data reveals a multiorgan pathological signature of SARS-CoV-2 infection. *J Proteome Res*. 2020;19:4442–4454.
12. Marín-Corral J, Rodríguez-Morató J, Gomez-Gomez A, et al. Metabolic signatures associated with severity in hospitalized COVID-19 patients. *Int J Mol Sci*. 2021;22:4794.
13. Thomas T, Stefanoni D, Reisz JA, et al. COVID-19 infection alters kynurenine and fatty acid metabolism, correlating with IL-6 levels and renal status. *JCI Insight*. 2020;5:e140327.
14. Su Y, Chen D, Yuan D, et al. Multi-omics resolves a sharp disease-state shift between mild and moderate COVID-19. *Cell*. 2020;183:1479–1495. e20.
15. Robertson J, Gostner JM, Nilsson S, et al. Serum neopterin levels in relation to mild and severe COVID-19. *BMC Infect Dis*. 2020;20:942.
16. Lionetto L, Ulivieri M, Capi M, et al. Increased kynurenine-to-tryptophan ratio in the serum of patients infected with SARS-CoV2: an observational cohort study. *Biochim Biophys Acta, Mol Basis Dis*. 2021;1867:166042.
17. Xiao N, Nie M, Pang H, et al. Integrated cytokine and metabolite analysis reveals immunometabolic reprogramming in COVID-19 patients with therapeutic implications. *Nat Commun*. 2021;12:1618.
18. Gelpi M, Hartling HJ, Ueland PM, et al. Tryptophan catabolism and immune activation in primary and chronic HIV infection. *BMC Infect Dis*. 2017;17:349.
19. Wu H, Gong J, Liu Y. Indoleamine 2, 3-dioxygenase regulation of immune response (Review). *Mol Med Rep*. 2018;17:4867–4873.
20. Arreola R, Becerril-Villanueva E, Cruz-Fuentes C, et al. Immunomodulatory effects mediated by serotonin. *J Immunol Res*. 2015;2015:354957.
21. Herr N, Bode C, Duerschmied D. The effects of serotonin in immune cells. *Front Cardiovasc Med*. 2017;4:48.
22. Soria-Castro R, Meneses-Preza YG, Rodríguez-López GM, et al. Severe COVID-19 is marked by dysregulated serum levels of carboxypeptidase A3 and serotonin. *J Leukoc Biol*. 2021;110:425–431.
23. Sakamoto S, Putalun W, Vimolmangkang S, et al. Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites [published correction appears in *J Nat Med*. 2018 Jan 5]. *J Nat Med*. 2018;72:32–42.
24. National Institutes of Health. Clinical spectrum of SARS-CoV-2 infection. www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/. Accessed April 1, 2022.
25. U.S. Food and Drug Administration. *Bioanalytical method validation guidance for industry*; 2018. www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry. Accessed April 1, 2022.
26. Cuperlovic-Culf M, Cunningham EL, Teimoorinia H, et al. Metabolomics and computational analysis of the role of monoamine oxidase activity in delirium and SARS-COV-2 infection. *Sci Rep*. 2021;11:10629.
27. Mpekoulis G, Frakolaki E, Taka S, et al. Alteration of L-Dopa decarboxylase expression in SARS-CoV-2 infection and its association with the interferon-inducible ACE2 isoform. *PLoS One*. 2021;16:e0253458.
28. Nataf S. An alteration of the dopamine synthetic pathway is possibly involved in the pathophysiology of COVID-19. *J Med Virol*. 2020;92:1743–1744.
29. Kubera M, Maes M, Kenis G, Kim YK, Lasoń W. Effects of serotonin and serotonergic agonists and antagonists on the production of tumor necrosis factor alpha and interleukin-6. *Psychiatr Res*. 2005;134:251–258.
30. Kubera M, Kenis G, Bosmans E, Scharpé S, Maes M. Effects of serotonin and serotonergic agonists and antagonists on the production of interferon-gamma and interleukin-10. *Neuropsychopharmacology*. 2000;23:89–98.
31. Nau Jr F, Yu B, Martin D, Nichols CD. Serotonin 5-HT2A receptor activation blocks TNF-α mediated inflammation *in vivo*. *PLoS One*. 2013;8:e75426.
32. De Clerck F. The role of serotonin in thrombogenesis. *Clin Physiol Biochem*. 1990;8(Suppl 3):40–49.
33. Kong X, Kong F, Zheng K, et al. Effect of psychological-behavioral intervention on the depression and anxiety of COVID-19 patients. *Front Psychiatr*. 2020;11:586355.
34. Dai LL, Wang X, Jiang TC, et al. Anxiety and depressive symptoms among COVID-19 patients in Jianghan Fangcang shelter hospital in Wuhan, China. *PLoS One*. 2020;15:e0238416.
35. Wu C, Zhou Z, Ni L, et al. Correlation between anxiety-depression symptoms and immune characteristics in inpatients with 2019 novel coronavirus in Wuhan, China. *J Psychiatr Res*. 2021;141:378–384.
36. Moroianu LA, Cecilia C, Ardeleanu V, et al. Clinical study of serum serotonin as a screening marker for anxiety and depression in patients with type 2 diabetes. *Medicina (Kaunas)*. 2022;58:652.
37. Ha S, Jin B, Clemmensen B, et al. Serotonin is elevated in COVID-19-associated diarrhoea. *Gut*. 2021;70:2015–2017.