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Evaluation of inflammatory biomarkers and vitamins in hospitalized patients with SARS-CoV-2 infection and post-COVID syndrome

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Abstract

Objectives: Concentrations of neopterin, kynurenine and kynurenine/tryptophan ratios predict prognosis and the need for oxygen therapy in patients hospitalized for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The aims of the present study were to evaluate the changes of these biomarkers early in the course of infection, the association with the prior coronavirus disease (COVID-19) vaccination and therapeutic administration of Anti-SARS-CoV-2 monoclonal antibodies, investigation of other potential biomarkers including neuropilin, 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine in patients hospitalized with SARS-CoV-2 infection and an assessment of these biomarkers and vitamins A, E and D in patients with post-COVID syndrome.

Methods: Urine and blood samples were obtained on the 1st to the 4th day and 4th to 7th day from 108 patients hospitalized with COVID-19. Chromatography tandem mass spectrometry methods were used to analyse neopterin, kynurenine, tryptophan, liposoluble vitamins, and DNA damage biomarkers.

Results: A statistically significant decrease of neopterin, kynurenine and kynurenine/tryptophan ratios was observed on after 4th to 7th day of hospitalization, and concentrations of these biomarkers were increased in patients with poor prognosis and subsequent post-COVID syndrome. The concentrations of remaining biomarker and vitamins were not associated with outcomes, although markedly decreased concentrations of vitamin A, E and D were noted.

Conclusions: The concentrations of neopterin, kynurenine and kynurenine/tryptophan ratios decrease during the course of infection SARS-CoV-2 and are associated with the post-COVID syndrome. No other prognostic biomarkers were identified.

Keywords: COVID-19; neopterin; kynurenine; tryptophan; vitamins; post-COVID

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can activate innate and adaptive immune responses and result in a massive inflammatory reaction later in the course of the disease. This uncontrolled inflammatory response may lead to local and systemic tissue damage [1]. The majority of individuals with coronavirus disease (COVID-19) fully recover. Based on the latest available evidence, approximately 10–20 % of people experience various mid- and long-term complaints after recovery from the initial illness [2].

Most published studies to date on post-COVID symptoms found that 50–70 % of hospitalized patients exhibit several chronic symptoms up to 3 months after hospital discharge. The British Medical Association defines a syndrome “as a set of medical signs and symptoms which are correlated with each other and associated with a particular disease” [3].

Neopterin is a well-established immune activation biomarker whose concentration is elevated in the early phase of disorders associated with inflammatory response [4, 5]. Immune system activation may lead to elevated levels of neopterin and degradation of tryptophan to kynurenine production via indoleamine 2, 3-dioxygenase, and thus, kynurenine/tryptophan ratio increase. Recent studies of COVID-19 pneumonia show a positive correlation of these analytes with other inflammatory biomarkers, e.g. C-reactive protein or interleukin-6, and with the disease severity [6–9]. In 2005, it was reported that neopterin levels in patients with severe acute respiratory syndrome (SARS) are elevated earlier than commonly used inflammatory biomarkers, such as C-reactive protein [10]. Thus, the exploration of neopterin and kynurenine/tryptophan ratio use as reliable inflammatory biomarkers, which could give similar or superior information as commonly used biomarkers remains in the forefront if research [7, 11]. Other analytes investigated include urinary 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine, which have been established as essential biomarkers of DNA/RNA damage due to the hydroxyl radical attack of the nucleobase guanine or its nucleosideguanosine [12, 13]. 8-Hydroxy-2-deoxyguanosine and 8-hydroxyguanosine levels were studied not only as important biomarkers of DNA/RNA damage during carcinogenesis, aging, or degenerative diseases but also in viral infections and pulmonary diseases [14, 15].

Vitamins are another class of substances studied with regard COVID-19. Some vitamins have been found to decrease the expression of the indoleamine 2, 3-dioxygenase gene (alpha-tocopherol), and the aryl hydrocarbon receptors gene (calcitriol-active vitamin D metabolite). Aryl hydrocarbon receptors are activated after coronavirus entry into cells and are involved in several pathological conditions [16].

Tocopherol and retinol are important antioxidants with multiple immunomodulatory effects such as lymphocyte proliferation [4]. Insufficient tocopherol intake may contribute to increased susceptibility to COVID-19 infection and may promote the severity of this disease.

Neuropilin is a co-receptor that promotes the entry of SARS-CoV-2 into the cell [17–19]. The neuropilins are presented as neuropilin-1 and neuropilin-2. Neuropilin-1 has been associated with cell proliferation, immunity, and physiological as well as pathological angiogenesis. It has also been reported that this protein plays an essential role in axon and neuronal development [20] and it may be involved in the pathogenesis of SARS-CoV-2 [21]. In the previous study, we have demonstrated that serum and urinary neopterin, kynurenine and kynurenine/tryptophan ratios predict prognosis and the need for oxygen therapy in patients hospitalized for SARS-CoV-2 infection [9].

Using laboratory data from longitudinal follow up of the same cohort of patients the aim of the present study was to evaluate the changes in inflammatory biomarkers during the hospital stay, analysis of the relation of these biomarkers with the status of COVID-19 vaccination and therapeutic administration of anti-SARS-CoV-2 monoclonal antibodies. Another aim of the study was investigation of other potential prognostic biomarkers including neuropilin, 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine in patients hospitalized with SARS-CoV-2 infection and an assessment of the above mentioned biomarkers and vitamins A, E and D in post-COVID syndrome.

Materials and methods

Samples were obtained from consecutive patients aged 18 years or older hospitalized at University Hospital Hradec Králové, Czech Republic, between November 2021 and April 2022 with COVID-19 (omicron and delta variant). The data on prognostic significance of serum and urinary neopterin, kynurenine and kynurenine/tryptophan ratio in this cohort have been reported earlier [9]. The patient characteristics are shown in Table 1. The study protocol was approved by the Institutional Ethics Committees (No 202011P04), and all patients signed informed consent.

Serum and urine samples were obtained on the 1st to the 4th day (sample 1) and 4th to 7th day (sample 2) after hospital admission, and in post-COVID patients' group we continued the sampling 3rd, 6th and 9th month after positive PCR SARS-CoV-2 test. Ultra-high performance liquid chromatography tandem fluourescence, photodiode array and mass spectrometry detection ((U)HPLC-FLD-PDA-MS/MS) methods were used to measure serum neopterin, kynurenine, tryptophan, retinol, alpha-tocopherol and 25-OH D_3 vitamin and urinary 8-hydroxy-2-deoxyguanosine, 8-hydroxyguanosine, neopterin, kynurenine, tryptophan and urinary creatinine to correct for urine dilution. Serum and urine samples were protected from light and transported to the laboratory immediately after collection. Serum neopterin, kynurenine, and tryptophan concentrations were obtained using HPLC-FLD/PDA method [22], and urinary neopterin, kynurenine, tryptophan and creatinine levels were determined using HPLC-FLD/PDA [23] and UHPLC-MS/MS method (Stationary phase Kinetex Polar C18 100 × 4.6 mm, 2.6 µm protected with security guard column Kinetex EVO C18 3 mm ID. The mobile phase was composed of 5 mmol/L ammonium formate buffer and methanol with 0.2 % formic acid (at a ratio of 65/35) with a flow rate 0.6 mL/min). Urinary 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine were measured by UHPLC-MS/MS [24]. Briefly, to urine samples the internal standard was added, and then they were filtered via 96-well filter plates, which represents a slight modification in the sample preparation procedure in comparison with above mentioned published article. Vitamins A, E, and D were analysed by HPLC-PDA and UHPLC-MS/MS method [25,26].

Neuropilin was determined by ELISA immunoassay by Biomedica Medizinprodukte GmbH (Vienna, Austria).

The values in patients who survived or died during hospitalization were compared. None of the patients was discharged with the fatal infection (i.e. all patients with terminal COVID-19 treated with palliative intent died in the hospital). The vaccinated group was classified as patients with minimally 2 doses of vaccine.

Table 1: Patients characteristics.

	Vaccinated	Not vaccinated
n	55	53
Male/female	31/24	34/19
BMI (median), kg/m^2	29.30	27.10
Age, years		
Median	75	68
Range	34–93	19–88
Delta/omicron variant (not known)	27/20 (8)	32/18 (3)
Dead, n	17	13
Delta variant	9	8
Omicron variant	5	5
Oxygen therapy (no/yes)	20/35	19/34
Diabetes mellitus	26	15
Arterial hypertension	43	30
Cancer	17	8
Renal insufficiency	13	12
Hepatopathy	5	5
Pulmonary disease	8	13
Cardiovascular disease	27	19
Neurological disease	6	4
Remdesivir	28	31
Administration of anti-SARS-CoV-2 monoclonal antibodies	17	23
Dexamethasone	32	36
Budesonide	10	24
Vitamin A/E/D[a]	4/4/22	6/8/22

[a]Number of patients supplemented by vitamins during hospitalization. BMI, body mass index.

Table 2: Characteristics of patients without and with post-COVID syndrome.

	No post-COVID	Post-COVID
n	8	14
Age, years (median)	64	65.5
Male/female	5/3	7/7
CFS (median)	3	3
BMI, kg/m^2 (median)	27	31
Variant (delta/omicron/not known)	4/1/3	10/3/1
Hospitalization duration days (median)	6	19.5
Comorbidities		
Diabetes mellitus	2	2
Arterial hypertension	3	6
Cancer	1	2
Hepatopathy	1	1
Pulmonary disease	2	3
Cardiovascular disease	0	5
Vaccination		
Yes/no	2/6	7/7
Therapy (during hospitalization)		
Remdesivir	2	11
Anti-SARS-CoV-2	2	7
Dexamethasone	4	11
Budesonide	3	4
Oxygen therapy (yes/no)	3/5	11/3

CFS, clinical frailty scale; BMI, body mass index; Anti-SARS-CoV-2, anti-SARS-CoV-2 monoclonal antibodies.

From the cohort of 108 hospitalized patients, 22 patients were followed for 3, 6, and 9 months. The patients were divided into 2 subgroups according to the presence or absence of the post-COVID syndrome defined as subjective complaints or objective findings related to the COVID-19 disease [27]. Both subgroups are characterised in Table 2.

The examination included a structured interview with a physician and a complete physical examination, and the patients underwent a complete pulmonary function test, including measurement of pulmonary diffusion, a 6-min walking test (6-MWT), and lung imaging (X-ray/computed tomography).

Vitamin D levels were evaluated as 25OH D metabolites [28–30] and results were divided into 5 subgroups:

Levels below 25 nmol/L were defined as severe deficiency, 25–49 nmol/L as deficiency, 50–74 as nmol/L mild deficiency 75–150 nmol/L as physiological range, and values above 500 nmol/L as intoxication risk.

Healthy volunteers (n=56), 31 males and 25 females, median age 44 years served to establish normal range.

Statistical analysis

Data were processed by NCSS (Kaysville, UT, USA) statistical software for the correlation analysis, nonparametric Mann-Whitney test, and Wilcoxon test for paired analysis were used. A p-value of 0.05 or lower was generally considered statistically significant.

Results

Acute changes in inflammatory biomarkers during the hospital stay

During the course of hospitalization initial sampling on 1st to 4th day of hospitalization and subsequent sampling on the 4th to 7th day of hospitalization a statistically significant decrease of serum and urinary biomarkers (neopterin and kynurenine and kynurenine/tryptophan ratio) during the hospitalization and an increased serum tryptophan levels were observed in the whole patient cohort.

This trend was evident among survivors. In patients who died only urinary kynurenine/tryptophan ratio, serum neopterin and kynurenine/tryptophan ratio decreased, and urinary tryptophan/creatinine ratio and serum tryptophan increased significantly (Tables 3 and 4).

Studied biomarkers were also compared in the samples obtained 4th to 7th day of hospitalization in the group of survivors and patients who died, and statistically significantly high levels were observed in non-survivors in the serum (neopterin $p<0.0001$, kynurenine $p<0.0001$, and kynurenine/tryptophan $p<0.0001$) and in urine (neopterin $p<0.0001$, and kynurenine/tryptophan $p<0.0168$).

Table 3: Levels of neopterin, kynurenine and tryptophan in serum during hospitalization. Wilcoxon test for paired analysis was used.

	Serum sample, n	Neopterin, nmol/L		Kynurenine, μmol/L		Tryptophan, μmol/L		Kynurenine/tryptophan ratio, μmol/mmol	
Entire cohort	1st (92)	Median/mean (range)	**p<0.0001**	Median/mean (range)	**p=0.0002**	Median/mean (range)	**p=0.0019**	Median/mean (range)	**p<0.0001**
		44.46/ 78.46 (7.45–735.14)		4.54/5.01 (1.68–15.47)		42.26/42.71 (6.52–76.54)		106.44/146.95 (30.23–692.77)	
	2nd (92)	Median/mean (range)		Median/mean (range)		Median/mean (range)		Median/mean (range)	
		22.50/50.49 (5.70–797.21)		3.71/4.41 (1.71–20.37)		48.59/49.36 (16.78–134.62)		75.83/105.31 (31.46–535.71)	
Survived	1st (70)	Median/mean (range)	**p<0.0001**	Median/mean (range)	**p<0.0001**	Median/mean (range)	**p<0.0001**	Median/mean (range)	**p<0.0001**
		34.00/58.18 (7.45–735.14)		4.05/4.65 (1.68–13.48)		44.29/43.01 (15.91–76.54)		97.88/127.55 (30.23–689.27)	
	2nd (70)	Median/mean (range)		Median/mean (range)		Median/mean (range)		Median/mean (range)	
		18.84/31.26 (5.70–544.01)		3.35/3.87 (1.71–12.83)		51.20/50.26 (19.75–96.56)		62.33/86.14 (31.46–407.54)	
Died	1st (22)	Median/mean (range)	**p=0.0030**	Median/mean (range)	p=0.9133	Median/mean (range)	**p=0.0024**	Median/mean (range)	**p=0.0475**
		107.23/142.98 (15.36–640.04)		6.46/6.32 (2.43–15.47)		36.37/35.43 (6.52–54.66)		190.55/217.99 (76.18–692.77)	
	2nd (22)	Median/mean (range)		Median/mean (range)		Median/mean (range)		Median/mean (range)	
		60.13/111.67 (12.81–797.21)		5.98/6.37 (3.61–20.37)		44.70/46.49 (16.78–134.62)		120.74/175.56 (42.51–535.71)	

1st to 4th day of hospitalization – 1st sample; 4th to 7th day of hospitalization – 2nd sample; the entire cohort are patients with positive SARS-CoV-2 PCR test with omicron, delta, and not defined variant. Bold value means statistically significant result ($p \leq 0.05$).

When evaluating neopterin kynurenine and tryptophan in relation to prior COVID-19 vaccination and therapeutic administration of anti-SARS-CoV-2 monoclonal antibodies no statistically significant difference was observed in the concentrations of neopterin, kynurenine and tryptophan between the vaccinated and unvaccinated patients (data not shown). The only biomarker with a statistically significantly lower level in the vaccinated group was urinary neopterin (p=0.031). As shown in Table 1, number of patients among vaccinated and not vaccinated and patients with or without oxygen therapy was comparable.

Similar comparison was made in the subgroup of patients, who were treated with anti-SARS-CoV-2 monoclonal antibodies early in the course of infection (patients only with delta virus variant), and no statistically significant differences in concentrations of neopterin, kynurenine and tryptophan at admission and during hospitalization were evident between the patients treated with anti-SARS-CoV-2 monoclonal antibodies and those who did not receive this therapy.

Evaluation of neuropilin and 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine in patients hospitalized with SARS-CoV-2 infection

Neuropilin concentrations were not statistically different between patients who died and survivors and also no difference was evident between patients with or without subsequent oxygen therapy. The median and range of serum neuropilin in the whole patient cohort were 2.075 and 0.750–5.440 nmol/L, respectively. There were no differences also in the cohort with followed post-COVID syndrome.

8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine as biomarkers of DNA and RNA damage were evaluated in urine. The concentrations were significantly increased in patients hospitalized with COVID-19 compared to control group (Table 5). No differences were observed in urinary 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine between survivors and patients who died (data not shown).

Table 4: Concentrations of neopterin, kynurenine and tryptophan in urine during hospitalization.

	Urine sample, n	Neopterin/creatinine ratio, nmol/mmol		Kynurenine/creatinine ratio, μmol/mmol		Tryptophan/creatinine ratio, μmol/mmol		Kynurenine/tryptophan ratio, μmol/mmol	
Entire cohort	1st (92)	Median/mean (range)	**p<0.0001**	Median/mean (range)	p=0.0736	Median/mean (range)	**p<0.0001**	Median/mean (range)	**p<0.0001**
		914.93/ 1,089.41(167.19–6,333.00)		2.57/4.63 (0.08–43.96)		5.63/7.21 (0.68–45.13)		534.32/778.83 (21.81–6,690.54)	
	2nd (92)	Median/mean (range)		Median/mean (range)		Median/mean (range)		Median/mean (range)	
		493.18/760.11 (161.34–4,069.41)		2.30/4.80 (0.14–54.19)		9.67/10.86 (0.51–65.16)		256.61/534.74 (31.81–9,627.30)	
Survived	1st (70)	Median/mean (range)	**p<0.0001**	Median/mean (range)	**p=0.0429**	Median/mean (range)	**p=0.0001**	Median/mean (range)	**p<0.0001**
		840.16/918.07 (167.19–2,871.32)		1.78/4.16 (0.08–14.54)		5.71/7.03 (0.83–29.22)		370.15/703.31 (21.81–6,690.54)	
	2nd (70)	Median/mean (range)		Median/mean (range)		Median/mean (range)		Median/mean (range)	
		440.21/558.82 (161.34–2,110.09)		1.84/4.39 (0.14–7.04)		9.10/10.73 (0.51–23.69)		216.60/521.50 (31.81–2,791.54)	
Died	1st (22)	Median/mean (range)	p=0.2234	Median/mean (range)	p=0.8710	Median/mean (range)	**p=0.0090**	Median/mean (range)	**p=0.0008**
		1,257.49/1,626.81 (306.93–6,333.00)		5.41/6.12 (0.13–43.96)		4.05/7.79 (0.67–45.13)		787.89/1,019.14 (93.30–4,206.21)	
	2nd (22)	Median/mean (range)		Median/mean (range)		Median/mean (range)		Median/mean (range)	
		1,120.53/1,391.45 (267.99–4,069.41)		3.46/6.08 (0.24–38.36)		10.26/11.25 (1.05–29.05)		305.95/442.26 (114.36–2,267.44)	

1st to 4th day of hospitalization – 1st sample; 4th to 7th day of hospitalization – 2nd sample; the entire cohort are patients with positive SARS-CoV-2 PCR test with omicron, delta, and not defined variant. For data comparison Wilcoxon test for paired test was used. Bold value means statistically significant result ($p \leq 0.05$).

In patients followed for post-COVID syndrome, we compared levels of neopterin, kynurenine, and kynurenine/tryptophan ratio at admission and after 3, 6, and 9 months after positive PCR SARS-CoV-2 test. The results were compared with patients from the initial cohort without known post-COVID syndrome. All subgroups are characterized in Table 2.

We observed a statistically significantly increased concentrations of neopterin, kynurenine, and kynurenine/tryptophan ratio and decreased tryptophan concentration in serum at admission in the patients who subsequently developed post-COVID syndrome compared with patients who did not developed post-COVID syndrome during subsequent follow up.

In contrast in the second sample obtained during the hospitalization only urinary neopterin was increased in patients with subsequent post-COVID syndrome. No differences between patients with or without post-COVID syndrome were observed during subsequent follow up. Results are shown in Supplementary Material, Figures S1 and S2. Levels of the biomarkers in all samplings during 9 months are shown in Tables 6 and 7.

Compares with controls concentrations of serum and urinary neopterin, serum kynurenine, kynurenine/tryptophan ratio were significantly increased during the course of follow up in patients with or without post-COVID syndrome (Tables 8 and 9).

Levels of vitamins in hospitalized COVID patients and patients with post-COVID syndrome

Initial concentrations of vitamins A, D, and E were not different between survivors and patients who died (data not shown). We compared the levels with controls healthy volunteers measured in our laboratory in the same year period (vitamin D) and reports in the literature (A and E) [31, 33]. Levels of vitamins at admission were lower than in controls (Table 5).

The levels of vitamin D in hospitalized patients were classified as insufficient in most cases (vitamin D deficiency; Figure 1).

Table 5: Concentration levels of vitamin A, E, D, 8-OH-2-deoxyguanosine, 8-OH-guanosine and neuropilin in COVID patients during hospitalization vs. controls.

	1st sampling (1st – 3rd day of hospitalization) n – 108	2nd sampling (4st – 7th day of hospitalization) n – 92	Control group n-56
Serum vitamin A, µmol/L median (range)	0.71 (0.16–3.75)	1.19 (0.22–5.29)	1.71[a] NA
Serum vitamin E, µmol/L median (range)	18.13 (9.65–37.07)	17.76 (7.95–40.59)	27.8[a] NA
Serum vitamin D, nmol/L (25-OH-vitamin D_3) median (range)	33.49 (0.75–142.50)	41.54 (1.10–121.39)	52.35 (14.32–122.75)
Urine 8-OH-2-deoxyguanosine/creatinine, ng/mg median (range)	6.80 (0.80–49.20)	6.86 (1.81–27.76)	3.13 (1.76–7.39)
Urine 8-OH-guanosine/creatinine, ng/mg median (range)	7.56 (1.73–53.06)	9.80 (0.49–131.00)	3.03 (1.88–7.22)
Neuropilin, nmol/L median (range)	2.075 (0.750–5.440)	xxx	2[b]

Concentration levels are expressed as medians and ranges are given in the brackets. [a][31], [b][32].

Vitamin D in post-COVID syndrome patients was not significantly decreased at admission and in the second sampling in comparison to the controls. There were no differences in the vitamin D levels between patients with or without post-COVID syndrome p=0.41277 (Table 10).

Discussion

In the present study, we demonstrate a decrease in urinary and serum concentrations of neopterin, kynurenine and kynurenine/tryptophan ratio in patients hospitalized for SARS-CoV-2 infection [9]. This expands the results of a prior study on the same cohort of patients that demonstrated an association of these biomarkers with short-term prognosis and the need for oxygen therapy. In this prior report neopterin, kynurenine and kynurenine/tryptophan ratio were markedly elevated in patients who died during the hospital stay and in patients who subsequently needed oxygen therapy. Interestingly, in the present analysis the levels of these biomarkers either remained unchanged or decreased to a lesser extent in patients who died.

Table 6: Concentration levels of measured biomarkers neopterin, kynurenine, tryptophan, 8-OH-2-deoxyguanosine, 8-OH-guanosine in patients without post-COVID syndrome.

	1st sampling (1st – 3rd day of hospitalization)	2nd sampling (4th – 7th day of hospitalization)	3rd sampling (3 months)	4th sampling (6 months)	5th sampling (9 months)
Serum neopterin, nmol/L median (range)	25.66 (9.66–31.80)	14.78 (5.78–16.30)	10.66 (7.47–22.38)	12.06 (6.87–20.09)	10.63 (5.68–20.07)
Serum kynurenine, µmol/L median (range)	2.48 (2.20–6.30)	2.69 (1.86–3.48)	2.71 (1.58–5.04)	2.65 (2.04–4.28)	3.01 (1.99–5.05)
Serum tryptophan, µmol/L median (range)	48.49 (37.67–76.54)	56.21 (42.19–58.78)	63.11 (59.08–72.99)	68.56 (59.50–75.76)	69.96 (46.13–86.06)
Serum kynurenine/tryptophan, µmol/mmol median (range)	55.39 (32.06–127.35)	54.44 (32.43–59.48)	41.55 (25.06–76.54)	36.13 (26.89–62.01)	43.13 (28.66–71.17)
Urine neopterin/creatinine, nmol/mmol median (range)	591.39 (167.19–1,031.25)	215.28 (161.34–353.05)	189.00 (91.92–295.25)	196.18 (130.00–468.00)	224.00 (167.00–282.00)
Urine kynurenine/creatinine, µmol/mmol median (range)	1.24 (0.20–3.14)	0.87 (0.14–1.42)	0.34 (0.19–0.79)	0.31 (0.13–0.75)	0.26 (0.06–0.89)
Urine tryptophan/creatinine, µmol/mmol median (range)	7.12 (2.31–12.82)	6.94 (3.24–8.60)	4.78 (4.47–5.80)	5.05 (2.70–6.89)	4.56 (2.30–9.55)
Urine kynurenine/tryptophan, µmol/mmol median (range)	191.19 (52.60–336.95)	121.52 (42.16–199.05)	58.68 (33.68–171.30)	68.23 (26.56–157.33)	57.53 (26.89–149.46)
Urine 8-OH-2-deoxyguanosine/creatinine, ng/mg median (range)	6.28 (2.80–12.62)	5.97 (2.91–12.11)	3.79 (2.31–6.19)	4.04 (2.08–12.15)	5.66 (3.31–13.22)
Urine 8-OH-guanosine/creatinine, ng/mg median (range)	6.03 (1.89–13.37)	4.87 (3.06–9.82)	4.08 (2.35–5.30)	3.19 (1.15–5.10)	3.10 (2.07–5.54)

Concentration levels are expressed as medians and ranges are given in the brackets.

Table 7: Concentration levels of measured biomarkers neopterin, kynurenine, tryptophan, 8-OH-2-deoxyguanosine, 8-OH-guanosine in patients with post-COVID syndrome.

	1st sampling (1st – 3rd day of hospitalization)	2nd sampling (4th – 7th day of hospitalization)	3rd sampling (3 months)	4th sampling (6 months)	5th sampling (9 months)
Serum neopterin, nmol/L median (range)	34.16 (10.50–95.12)	18.66 (6.65–63.41)	10.81 (5.42–19.21)	12.31 (6.40–52.49)	10.35 (7.33–26.81)
Serum kynurenine, μmol/L median (range)	4.31 (2.63–8.07)	3.55 (1.72–9.68)	2.80 (1.75–3.92)	2.91 (1.89–4.90)	2.84 (2.09–4.55)
Serum tryptophan, μmol/L	37.36 (20.04–60.74)	41.83 (25.88–73.50)	57.18 (36.24–104.12)	57.31 (47.76–91.88)	65.32 (46.83–97.45)
Serum kynurenine/tryptophan, μmol/mmol median (range)	101.92 (64.04–313.78)	75.87 (31.46–374.11)	47.05 (30.15–75.28)	46.85 (36.07–84.08)	48.78 (30.21–77.68)
Urine neopterin/creatinine, nmol/mmol median (range)	1,046.31 (316.46–2,547.45)	513.92 (274.89–2,213.11)	239.39 (131.45–411.00)	262.50 (183.15–662.02)	250.50 (137.00–660.00)
Urine kynurenine/creatinine, μmol/mmol	1.68 (0.47–10.05)	2.33 (0.22–54.19)	0.29 (0.06–0.98)	0.28 (0.06–0.91)	0.27 (0.09–0.69)
Urine tryptophan/creatinine, μmol/mmol median (range)	6.13 (1.42–29.22)	10.68 (4.22–65.16)	4.44 (1.78–7.65)	3.33 (1.33–8.19)	4.11 (1.58–7.75)
Urine kynurenine/tryptophan, μmol/mmol median (range)	376.57 (73.05–2,017.14)	186.42 (53.23–3,026.76)	71.49 (31.13–151.48)	81.80 (30.35–270.99)	73.50 (28.40–249.74)
Urine 8-OH-2-deoxyguanosine/creatinine, ng/mg median (range)	7.68 (3.47–18.38)	6.48 (2.77–16.88)	2.81 (1.68–10.96)	4.22 (1.63–16.76)	4.89 (2.26–15.52)
Urine 8-OH-guanosine/creatinine, ng/mg	8.00 (3.56–21.74)	9.99 (2.34–31.16)	4.79 (2.04–8.71)	3.47 (2.21–7.86)	4.50 (1.72–5.85)

Concentration levels are expressed as medians and ranges are given in the brackets.

Table 8: Comparison of inflammatory biomarkers neopterin, kynurenine and tryptophan in serum in patients with post-COVID syndrome, without post-COVID syndrome, and control group.

Serum	3rd month			6th month			9th month		
Neopterin, nmol/L	Nonpost-COVID median	10.66	**p=0.0016**	Nonpost-COVID median	12.06	**p=0.0016**	Nonpost-COVID median	10.63	**p=0.0019**
	Controls	6.90	**p=0.0002**	Controls	6.90	**p=0.0001**	Controls	6.90	**p<0.0001**
	Post-COVID median	10.53		Post-COVID median	11.22		Post-COVID median	10.05	
Kynurenine, μmol/L	Nonpost-COVID median	2.71	p=0.1730	Nonpost-COVID median	2.65	**p=0.0021**	Nonpost-COVID median	3.01	**p=0.0013**
	Controls	2.19	**p=0.0045**	Controls	2.19	**p=0.0004**	Controls	2.19	**p<0.0001**
	Post-COVID median	2.78		Post-COVID median	2.91		Post-COVID median	2.76	
Tryptophan, μmol/L	Nonpost-COVID median	63.11	p=0.4165	Nonpost-COVID median	68.56	p=0.3180	Nonpost-COVID median	69.96	p=0.5823
	Controls	66.20	**p=0.0127**	Controls	66.20	**p=0.0437**	Controls	66.20	p=0.4109
	Post-COVID median	57.56		Post-COVID median	57.43		Post-COVID median	66.36	
Kynurenine/tryptophan, μmol/mmol	Nonpost-COVID median	41.55	p=0.0516	Nonpost-COVID median	36.13	**p=0.0316**	Nonpost-COVID median	43.13	**p=0.0026**
	Controls	33.19	**p<0.0001**	Controls	33.19	**p<0.0001**	Controls	33.19	**p<0.0001**
	Post-COVID median	46.43		Post-COVID median	45.23		Post-COVID median	48.30	

Bold value means statistically significant result ($p \leq 0.05$).

The decrease in the levels of these biomarkers indicates that patients the inflammation was suppressed due to the treatment or natural evolution in the course of the disease. No differences in baseline concentrations of neopterin, kynurenine, and kynurenine/tryptophan ratio were observed in patients previously vaccinated or unvaccinated. However, it should be kept in mind, that vaccination markedly reduces the risk of hospital admission [34] and hospitalized patients with prior vaccinations were a selected subgroup that is probably not representative of vaccinated patients with subsequent infection.

Gustine et al. [35] reported that elevated inflammatory markers can relate to the cytokine storm in which the immune system is activated, but does not function properly,

Table 9: Comparison of inflammatory biomarkers neopterin, kynurenine and tryptophan in urine in patients with post-COVID syndrome, without post-COVID syndrome, and control group.

Urine	3rd month			6th month			9th month		
Neopterin/creatinine, nmol/mmol	Nonpost-COVID median	188.32	p=0.0777	Nonpost-COVID median	185.00	p=0.1557	Nonpost-COVID median	224.00	**p=0.0067**
	Controls	166.00	**p=0.0078**	Controls	166.00	**p<0.0001**	Controls	166.00	**p=0.0002**
	Post-COVID median	234.52		Post-COVID median	257.00		Post-COVID median	249.00	
Kynurenine/creatinine, µmol/mmol	Nonpost-COVID median	0.34	p=0.1832	Nonpost-COVID median	0.20	p=0.4912	Nonpost-COVID median	0.26	p=0.7960
	Controls	0.23	p=0.6965	Controls	0.23	p=0.6557	Controls	0.23	p=0.3057
	Post-COVID median	0.26		Post-COVID median	0.21		Post-COVID median	0.25	
Tryptophan/creatinine, µmol/mmol	Nonpost-COVID median	4.82	p=0.4079	Nonpost-COVID median	4.76	p=0.8518	Nonpost-COVID median	4.56	p=0.8508
	Controls	4.41	p=0.3043	Controls	4.41	**p=0.0192**	Controls	4.41	p=0.1168
	Post-COVID median	4.29		Post-COVID median	3.59		Post-COVID median	3.61	
Kynurenine/tryptophan, µmol/mmol	Nonpost-COVID median	58.68	p=0.2430	Nonpost-COVID median	68.23	p=0.1803	Nonpost-COVID median	57.53	p=0.7161
	Controls	54.53	p=0.1290	Controls	54.53	**p=0.0061**	Controls	54.53	**p=0.0058**
	Post-COVID median	71.49		Post-COVID median	81.80		Post-COVID median	73.50	

Bold value means statistically significant result (p≤0.05).

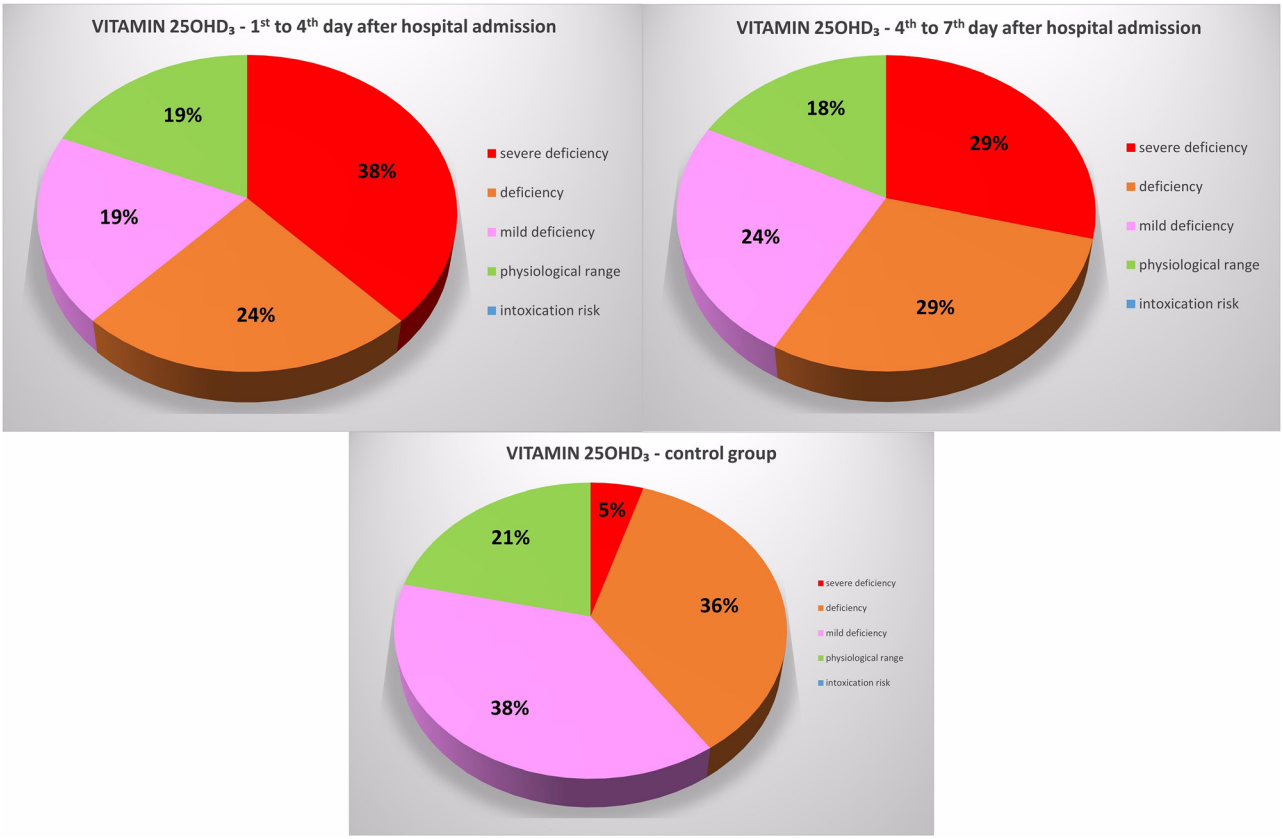

Figure 1: Levels of vitamin $25OHD_3$ in studied and control group.

Table 10: Levels of vitamins A, E, D in patients without and with post-COVID syndrome.

		1st sampling (1st – 3rd day of hospitalization)	2nd sampling (4th – 7th day of hospitalization)	3rd sampling (3 months)	4th sampling (6 months)	5th sampling (9 months)
Serum vitamin A, μmol/L median (range)	Without post-COVID syndrome	1.01 (0.70–1.81)	1.95 (1.04–2.70)	1.76 (0.99–2.20)	1.75 (1.24–2.20)	1.83 (1.08–2.27)
	Post-COVID syndrome	0.47 (0.29–1.83)	1.00 (0.51–2.84)	1.30 (1.08–2.56)	1.55 (0.97–2.34)	1.77 (0.74–2.74)
Serum vitamin E, μmol/L median (range)	Without post-COVID syndrome	15.42 (10.34–19.78)	18.17 (14.43–23.64)	17.37 (11.31–24.58)	14.07 (11.16–21.10)	13.54 (11.66–21.55)
	Post-COVID syndrome	17.64 (11.91–26.44)	21.12 (10.48–36.58)	20.53 (13.21–31.99)	15.89 (13.10–26.02)	16.80 (12.28–30.08)
Serum vitamin D (25-OH-vitamin D_3), nmol/L median (range)	Without post-COVID syndrome	61.77 (32.40–142.50)	65.82 (40.34–94.58)	54.20 (25.15–105.05)	82.17 (21.35–100.42)	82.02 (20.55–150.71)
	Post-COVID syndrome	38.98 (10.03–127.83)	50.72 (15.79–117.65)	61.77 (10.43–132.16)	74.98 (23.51–148.99)	73.99 (10.02–134.84)

and this may explain the negative prognostic significance of increase inflammatory biomarkers in acute infection.

Utilization of urine as sample matrix is of advantage as it allows for repeated measurements without the inconvenience to the patient that is associated with the need for venepuncture. Another advantage is that the concentrations of neopterin, kynurenine and tryptophan in the urine are expressed as ratios of urinary creatinine, correcting for a fluctuation of renal function in patients with critical state.

In contrast to neopterin, kynurenine, and kynurenine/tryptophan ratio, parameters of DNA and RNA damage and neuropilin, the receptor for viral antigen, were not associated with the outcomes.

Changes in neopterin, kynurenine, and tryptophan levels in serum and urine at admission were also observed in patients with post-COVID syndrome who were followed up for 9 months. In patients with subsequent post-COVID syndrome initial neopterin, kynurenine, and kynurenine/tryptophan levels were significantly higher compared to patients with subsequent follow up who did not develope post-COVID syndrome but, the differences were no longer evident later during the course of follow up, but both groups of patients had elevated levels compared to the control group of healthy subjects even after 9 months. One possibility may be the lower age of the control group as neopterin levels increase with age. Kynurenine concentration was reported to be age-independent; however, it may be elevated in the elderly due to comorbidities (Supplementary Table S1).

In the whole entire cohort of hospitalized patients and patients with post-COVID syndrome, we also evaluated vitamins, especially vitamin D, which is related to this disease [36, 37]. Vitamins A and E are important antioxidants and are related to the expression of the aryl hydrocarbon receptor gene [16]. Vitamins levels have not been shown to be potential predictors of disease severity or prognosis. However, during the treatment, the levels have not reached those of the healthy population. Therefore, supplementation may be considered in clinical practice. Of particular interest is the vitamin D, which despite supplementation did not reach satisfactory concentrations in most patients. Present data are in agreement with the results of the studies of Saldmann et al. [38] and Orchard et al. [39]. These data are of interest because of the discussion of potential of vitamin supplementation in the management of SARS-CoV-2 infection [40, 41]. It would still be interesting to investigate the severity of the disease and its prognosis with higher supplementation to reach physiological levels in hospitalized patients.

In conclusion, we demonstrate a decrease in urinary and serum concentrations of neopterin, kynurenine and kynurenine/tryptophan ratio in patients hospitalized for SARS-CoV-2 infection. The initial concentrations of these biomarkers were higher in patients who subsequently developed the post-COVID syndrome. In contrast, initial concentrations of neuropilin and 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine were not associated with prognosis. Low vitamin A, E and D concentrations were detected in patients hospitalized for SARS-CoV-2 infection.

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Research ethics: The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Data availability: The raw data can be obtained on request from the corresponding author.

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