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SARS-CoV-2-Omicron-variant Induced COVID-19-Infection in Unvaccinated and Vaccinated Patients: Impact on Immune Response, Symptomatology, and Risk of POST-COVID Syndrome

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Abstract

Worldwide, the SARS-CoV-2-omicron-variant (B.1.1.529) is highly infectious, even in vaccinated patients. Little is known about the duration of acute SARS-CoV-2-omicron-infection, severity of symptoms, immunologic response and development of POST-COVID-syndrome in unvaccinated compared to vaccinated patients after acute SARS-CoV-2 omicron infection.

Methods: In 24 patients suffered from acute SARS-CoV-2-omicron infection (10 unvaccinated participants, 5 participants twice vaccinated and 9 participants getting three mRNA vaccinations), symptoms and duration of disease, SARS-CoV-2-omicron immune response, 25(OH)-vitamineD3 and vitamine C were determined in blood 4 weeks after acute infection. In addition, POST-COVID-symptoms and autoantibodies against G protein-coupled receptors in blood were determined 16 weeks after acute infection. Pearson correlations and one-way ANOVA were used. $p \leq 0.05$ was assumed to be significant, and $p \leq 0.01$ was assumed to be highly significant, respectively.

Results: All 24 patients (age: 54 ± 12 years; 16 females - 8 males) complained of mild symptoms during acute omicron-COVID-19 infection. In vaccinated versus (vs) unvaccinated patients the disease duration was significantly prolonged (3.4-times vs 3.8-times). POST-COVID symptoms were reported by 50% of unvaccinated patients, 60%, and 78% of vaccinated patients, respectively. Vaccinated vs unvaccinated patients showed significantly larger number of POST-COVID-symptoms. Vitamin C deficiency was detectable in all patients 4 weeks after acute COVID-19 infection. The 25(OH) Vitamin D3 concentration was within the normal range in all patients. In the SARS-CoV2-immunoblot, the unvaccinated patients showed the same antibody level against the virus core protein (NP) virus compared with the vaccinated patients, but no antibody level against the linking protein (RBD) or the spike protein (S1). Also, unvaccinated vs vaccinated patients showed no immunological response in building neutralizing antibody level IgG-S1 [neutralizing antibody IgG-S1 (norm < 34 BAU/ml) at 0-vaccination vs 2-vaccination vs 3-vaccination = <34 vs 14584 ± 12012.4 vs 15116.7 ± 10997.9 BAU/ml]. 4 weeks after omicron-COVID-19-infection, patients getting 2-vaccination vs 3-vaccination before infection showed a significant 5.4fold vs 14.9fold activation of neutralizing antibody level IgG-S1 (neutralizing antibody IgG-S1 (norm < 34 BAU/ml) in 2-vaccination vs 3-vaccination patients - before / after infection = 2678.5 ± 3454.1 / 14584 ± 12012.4 vs 1012.9 ± 918.5 vs 15116.7 ± 10997.9 BAU/ml). The level of neutralizing antibody IgG-S1 after acute Omicron-COVID-19 infection correlates significantly with the duration of disease. All patients showed a cellular immune response against the SARS-CoV-2-omicron variant in the immune tolerance test (ITT) being significantly more robust in vaccinated vs. unvaccinated patients (ITT-Omicron IL2 / IFN at 0-vaccination vs. 2-vaccination vs. 3- vaccination = 14.8 ± 23.4 / 4.0 ± 4.8 vs. 34.4 ± 28.8 / 21.2 ± 13.0 vs 59.9 ± 103.8 / 29.3 ± 40.0 pg/ml). Autoantibodies level against G-protein coupled receptors are twice as strong in 3-dose vaccinated vs unvaccinated patients. Autoantibody level of ß1-adrenergic receptor or M3-muscarinergic-choline-receptor and the level of cellular immune response ITT-omicron-IL2 significantly correlate with the risk of developing POST-COVID syndrome.

Conclusion: Acute SARS-CoV-2-omicron infection causes mild disease in both unvaccinated and vaccinated patients. However, vaccinated patients experienced prolonged disease with each vaccination. Unvaccinated patients showed no level of neutralizing antibodies to IgG-S1 spike compared with vaccinated patients after acute Omicron-COVID-19 infection, which is led back to multiple mutations in the region of the spike genome in the Omicron variant. Acute Omicron-COVID-19 infection after mRNA vaccination increases the risk of a strong immune response (neutralizing antibodies IgG-S1 spike, antibodies IgG-RBD, T-cellular response in IL2 and IFN, Autoantibodies to G-protein coupled receptor ß1-ß2-M2-M3) which correlates with the risk of POST-COVID syndrome. A deficiency of immune modulating micronutrients (vitamin C, vitamin D3, selenium, zinc) might be responsible. Further studies treating POST-COVID patients with high-dose micronutrient infusion are necessary proving to reduce or eliminate the symptoms in POST-COVID patients.

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Introduction

The SARS-CoV-2 -omicron variant (B.1.1.529) is considered as a WHO Variant of Concern (VOC) due to its high transmission rate and its large number of mutations and has become the predominant viral strain worldwide in early 2022 [1]. Its 34 mutations in the spike protein, 15 of them occur within the receptor-binding domain

(RBD) interacting with ACE2 receptor, are responsible reducing the neutralizing effect of serum antibodies in convalescent patients infected with previous SARS-CoV-2-strains and the neutralizing serum antibodies of 2 or 3 times-vaccinated individuals who had been vaccinated with BNT162b2 (Pfizer-BioNTech), mRNA-1273 (Moderna), Ad26.COV2.S (Johnson & Johnson), ADZ1222 (Astra Zeneca), Sputnik V, or BBIBP-CorV (Sinopharm) [2-7]. Although triple mRNA-vaccination with BNT162b2 or mRNA1273 or a combination of Wuhan- or delta-SARS-CoV-2-infection followed by mRNA-vaccination increased serum levels of neutralizing antibodies compared to double-vaccinated or convalescent patients, the neutralizing effect against omicron- was dramatically lower than Wuhan or delta SARS-CoV-2. This fact explains the high transmission rate of the omicron variant despite high vaccination rates. SARS-CoV-2-omicron infection is asymptomatic in 25% of cases, and 98% of symptomatic patients complained mild flu-like symptoms [8]. Although the Omicron variant can bypass immunity from previous COVID-19 infections, little is known about the immunological response after acute SARS-CoV2-omicron infection [9]. This study aims to compare duration of disease, severity of symptoms, immunological response and risk of POST-COVID syndrome in unvaccinated vs vaccinated patients after acute SARS-CoV-2-omicron infection. Pfizer and Moderna proposed a fourth mRNA booster dose (within 12 months of the third dose), possibly followed by other doses to combat SARS-CoV-2-infections [9]. Knowing the immunological response to omicron-SARS-CoV2 infection, is this vaccination strategy the right way to go?

Methods

24 PCR-confirmed SARS-CoV2 Omicron infected patients (10 unvaccinated participants, 5 participants with two mRNA vaccinations and 9 participants with three mRNA vaccinations [mRNA-1273 (Moderna), BNT162b2 (Pfizer/BioNTech)]) were included in the study for retrospective evaluation of symptoms, immune response, and risk of POST-COVID syndrome. Participants with previous SARS-CoV2-Wuhan or alpha- to delta variant infection were excluded. All patients gave their written informed consent.

A questionnaire was used to assess symptom severity (total = 0-15; mild course = 0-5, moderate course = 6-10, severe course = 11-15) of acute SARS-CoV2-omicron infection after 4 weeks and POST-COVID symptoms after 16 weeks. To assess the severity of acute disease, symptoms of fever/chills - sore throat/headache - muscle/joint pain - fatigue and cough/chest tightness each achieved 1 score point. Hospitalization was assigned 5 score points and intensive care unit treatment was assigned an additional 5 score points. A POST-COVID syndrome was diagnosed if severe fatigue and at least one other symptom (chest pain/exertional dyspnea - cardiac palpitations/resting tachycardia – dysphasia – memory loss – attention disorder – alopecia diffusa - muscle/joint pain) were detectable 12 weeks after SARS-CoV-2-omicron infection (score: 0-8). SARS-CoV-2 omicron immune status, 25(OH) Vitamin D3 and Vitamin C were assessed in all participants 4 weeks after acute omicron COVID-19 infection. In addition, autoantibodies to G protein-coupled receptors were evaluated in blood 16 weeks after infection.

25(OH) Vitamin D3

The quantitative determination of total 25-OH vitamin D3 in serum was performed according to the manufacturer's instructions using a commercial, CE-compliant measuring method of the company Abbott GmbHCo.KG, Wiesbaden at the laboratory machine alinity, I-module by direct competitive chemiluminescence microparticle immunoassay (CMIA, article no. 8P4532)

Vitamin C in Frozen Plasma

The determination of vitamin C in plasma was carried out by chromatography. The samples were processed immediately after thawing using a commercial CE-labeled reagent kit of the company RECIPE (ClinRep® HPLC Complete Kit) according to the manufacturer's specifications and measured on an HPLC system of the company Shimatzu.

SARS-CoV-2 Blot Antibodies (NP, RBD, S1)

The semi-quantitative detection of IgG antibodies against the SARS-CoV2 spike protein S1, the receptor binding site (RBD) of the spike protein and the nucleocapsid protein of SARS -CoV2 were determined according to the manufacturer with a commercial CE strip immunoassay of Mikrogen, Neuried (Recom Line SARS-CoV-2 IgG, article-no.7374) with recombinantly produced antigens. The evaluation of the results on the strips were determined automatically with the Recom-Scan software of the same manufacturer according to the manufacturer's specifications.

SARS-CoV-2 Neutralizing Antibodies IgG-S1

The quantitative detection of neutralizing IgG antibodies against SARS-CoV-2 were determined using a commercial, CE-labeled assay of Diasorin, Dietzenbach (SARS CoV-2 TrimericS IgG, article no. 311510) on their laboratory machine LIAISON® according to manufacturer's specifications.

T-Cell-Response (ITT) Omicron IL2 / IFN

Heparinized whole blood of the patient was diluted 1:1 with RPMI medium and incubated unstimulated (negative control), with influenza antigen (positive control) and with validated concentrations of commercially available SARS CoV-2 Omicron Spike S1 full antigen (amino acids 14-681) for 24 hours at 37 ° C. The supernatant of the whole blood cultures was taken and the concentration of the cytokines interferon-gamma (IFN) and interleukin 2 (IL2) was determined using the Luminex technology and the commercially available CE-labeled reagent kits for interleukin 2 (Art.No.171-B5003M) or interferon gamma (Art.No.171-B5019M) from Biorad Laboratories, Feldkirchen according to the manufacturer's specifications.

Autoantibodies against G Protein Coupled Receptor (ß1-ß2-M2-M3)

The quantitative analysis of the corresponding circulating antibodies of the isotype IgG were determined according to the manufacturer's specifications using an enzymatic color reaction of a commercial, CE-compliant enzyme immunoassay of the company Cell Trend, Luckenwalde.

Statistical Analysis

For descriptive purposes, we illustrate the means and standard deviation. A Pearson correlation table was used to measure the strength and direction of the relationship between different variables of interest. The two samples (i.e., vaccinated n = 14 vs. non-vaccinated n = 10) were compared using a one-way ANOVA (F-test) analysis. All statistical analyses were conducted using STATA 16, and the results are interpreted in an exploratory manner at 5 % level of significance (two-sided).

Results

Twenty-four patients (age: 54 ± 12 years; 16 females - 8 males) who had PCR-confirmed acute COVID-19 infection with the omicron variant but no previous COVID-19 infection with the Wuhan to Delta variants were included to the study. Before acute

SARS-CoV-2-omicron infection ten patients were unvaccinated, 5 patients were vaccinated twice and 9 patients three times with an mRNA vaccine [mRNA-1273 (Moderna), BNT162b2 (Pfizer/BioNTech)]. All patients complained of mild symptoms during acute omicron COVID-19 infection (symptom score: 2.5 ± 0.7 vs 2.0 ± 0 vs 2.4 ± 0.5 – 0-vaccination vs 2-vaccination vs 3-vaccination) [Table.1]. No patient required hospitalization. The duration of disease in vaccinated vs unvaccinated patients was significantly prolonged by 3.4 vs 3.8 times (duration of disease: 2-vaccination vs 3-vaccination vs 0-vaccination = 10.8 ± 3.6 vs 12.2 ± 10.6 vs 3.2 ± 1.9 days) [Figure.1].

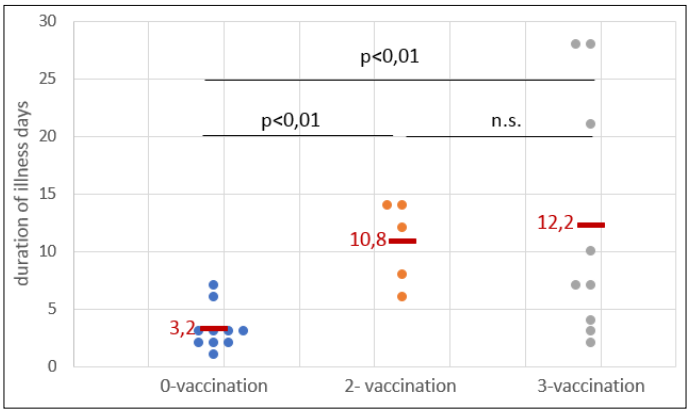

Figure 1: Duration of Disease of Acute Omicron COVID-19 Infection in Days in Unvaccinated Patients (0-vaccination), In Patients with 2 Vaccinations or 3 Vaccinations Before COVID-19-Infection. Red Bar = Mean Value. n.s.= not Significant

POST-COVID symptoms were present in 50% of unvaccinated patients, in 60% and 78% of vaccinated patients (POST-COVID symptoms 0-vaccination vs 2-vaccination vs 3-vaccination = 5/10 (50%) vs 3/5 (60%) vs 7/9 (78%). Vaccinated patients showed more symptoms compared to unvaccinated patients (score: 0-vaccination vs 2-vaccination vs 3-vaccination = 2.6 ± 1.8 vs 3.7 ± 1.9 and 4.3 ± 1.7). Vitamin C deficiency was detectable in all patients 4 weeks after acute COVID-19 infection but significantly lower in unvaccinated patients (Vitamin C (norm: 9-20 mg/l) 0-vaccination vs 2-vaccination and 3-vaccination = 2.0 ± 1.1 vs 4.3 ± 2.7 vs 4.5 ± 1.8 mg/l). The 25(OH) Vitamin D3 concentration was within the normal range 4 weeks after acute infection in all patients and did not differ significantly (Table 1).

Table 1: Demographic Characteristics, Duration and Symptom Score of Acute Infection, Frequency and Symptom Score of POST-COVID Syndrome, Laboratory Values of Immunological Humoral and Cellular Response, Vitamin C and 25(OH) VitaminD3 Levels 4 Weeks After Acute Infection

Vaccination (N)	**none (N=10)**	**2 (N=5)**	**3 (N=9)**
Age	50 ± 10,3	62 ± 11,4	53,1 ± 13
Acute symptoms (Duration in days)	3,2 ± 1,9	10,8 ± 3,6	12,2 ± 10,6
SYMPTOM-SCORE (0-15)	2,5 ± 0,7	2,0 ± 0	2,4 ± 0,5
BLOT-ANTIBODIES (value: 0 – 3+)			
SARS-CoV2-NP	2,1 ± 0,9	2,4 ± 0,6	2,4 ± 0,9
SARS-CoV2-RBD	0	3 ± 0	3 ± 0
SARS-CoV2-S1	0	3 ± 0	3 ± 0
neutralizing antibodies IgG-S1 (< 34 BAU/ml)			
before infection	< 34	2678,5 ± 3454,1	1012,9 ± 918,5
after infection	< 34	14584 ± 12012,4	15116,7 ± 10997,9
ITT Omicron (> 3 pg/ml)			
IL2	14,8 ± 23,4	34,4 ± 28,8	59,9 ± 103,8
IFN	4,0 ± 4,8	21,2 ± 13,0	29,3 ± 40,0

LONG- / POST-COVID-Symptom N / N (%)	5 / 10 (50)	3 / 5 (60)	7 / 9 (77,7)
SYMPTOM-SCORE LONG/POST-COVID (0-8)	2,6 ± 1,8	3,7 ± 1,9	4,3 ± 1,7
AUTOANTIBODY against G protein coupled receptor			
ß1-adrenergic receptor-AB (< 8 U/ml)	17 ± 8,7	14,7 ± 4,8	28,0 ± 12,4
ß2-adrenergic receptor-AB (< 8 U/ml)	22,3 ± 10,1	14,1 ± 4,7	29,3 ± 12,0
M2 muscarinic choline receptor AB (< 9 U/ml)	11,7 ± 7,1	15,6 ± 2,7	22,4 ± 17,0
M3 muscarinic choline receptor-AB (< 6 U/ml)	11,7 ± 8,1	10,6 ± 7,1	18,4 ± 11,9
Vitamin C (norm: 9 – 20mg/l)	2,0 ± 1,1	4,3 ± 2,7	4,5 ± 1,8
25(OH) Vitamin D3 (norm: 30-80 ng/ml)	67,4 ± 35,2	74,4 ± 34,8	62,0 ± 25,7

NP = nucleus protein - RBP = receptor-binding protein - S1 = spike protein-1 - ITT = immune tolerance test - IL2 = interleukin 2 - IFN = interferon - AB = antibody - N = number.

In the SARS-CoV2 immunoblot, the unvaccinated patients build the same level of antibody against the core proteins (NP) of the virus compared to the vaccinated (SARS-CoV2-NP antibody IgG (norm:0-3) at 0-vaccination vs 2-vaccination vs 3-vaccination = 2.1 ± 0.9 vs 2.4 ± 0.6 vs 2.4 ± 0.9), but no antibody level against the receptor binding protein (RBD) or the spike protein (SARS-CoV-2-RBD antibody IgG or -S1 antibody IgG (norm: 0-3) at 0-vaccination vs 2-/3-vaccination respectively 0 vs 3 ± 0) [Tab.1]. Also, unvaccinated patients did not build neutralizing antibody IgG-S1 level 4 weeks after acute omicron COVID-19 infection (neutralizing antibody IgG-S1 (norm < 34 BAU/ml) at 0-vaccination vs 2-vaccination vs 3-vaccination = <34 vs 14584 ± 12012.4 vs 15116.7 ± 10997.9 BAU/ml). 4 weeks after omicron COVID-19 infection, 2- and 3-dose vaccine patients showed a significant 5.4-fold vs 14.9-fold immune activation response of neutralizing antibody IgG-S1 (neutralizing antibody IgG-S1 (norm < 34 BAU/ml) at 2-vaccination vs 3-vaccination before/after = 2678.5 ± 3454.1 / 14584 ± 12012.4 vs 1012.9 ± 918.5 vs 15116.7 ± 10997.9 BAU/ml) [Figure.2].

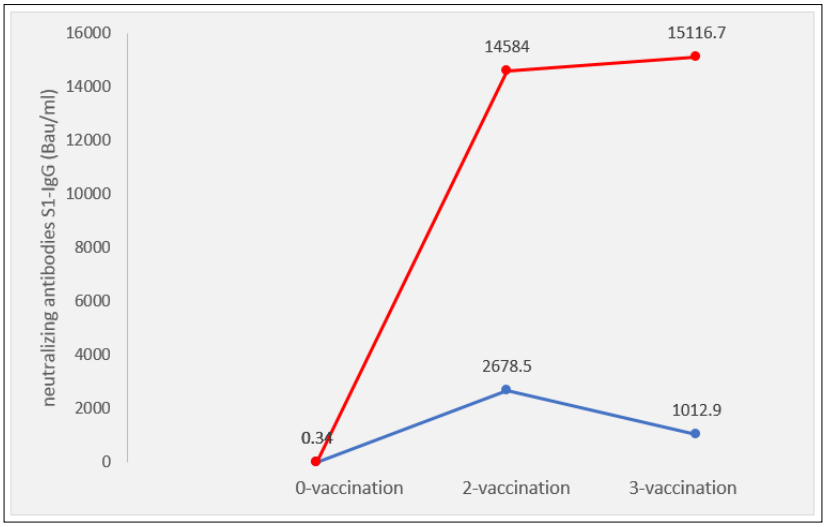

Figure 2: Mean Values of Neutralizing Antibodies S1-IgG Before (Blue Line) and 4 Weeks After (Red Line) Acute Omicron COVID-19 Infection in Unvaccinated Patients (0-Vaccination), in Patients with 2 Vaccinations, Respectively, 3 Vaccinations

p<0.001 = 0-Vaccination vs. 2 Vaccinations Respectively 3 Vaccinations (Red Line)
p<0.01 = Before (Red Line) vs. after (Blue Line) Disease for 2-Vaccinations Respectively 3 Vaccinations
Not Significant = 2 Vaccination vs. 3 Vaccination (Red Line)

Given the small group sizes we could only statistically compare the groups in terms of unvaccinated (n=10) and vaccinated (n=14). The results of a one-way ANOVA indicated that participants in the two conditions did not differ in terms of age (p=ns.) and gender (p=ns.). Therewith providing evidence that the groups are comparable and reducing the likelihood of additional confounds driving the results. Moreover, the groups did not statistically differ in terms of the strength of the experienced symptoms. Nonetheless, vaccinated vs. non-vaccinated patients experienced symptoms significantly longer ($p < 0.01$), more likely to develop POST-COVID symptoms ($p<0.01$). Unvaccinated vs. vaccinated patients had greater Vitamin C deficiencies after passing COVID-19 infection ($p<0.01$). Vaccinated vs non-vaccinated patience developed significantly more neutralizing antibody IgG-S1 ($p<0.001$). Finally, our analyses could not detect any statistically different results for 2- and 3-times vaccinated patients. Correlations are reported in (Table .2).

Table 2: Correlation of All Parameters

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
(1) Age	1															
(2) Gender	0.0100 (0.9629)	1														
(3) Number of vaccinations	0.1789 (0.4029)	0.1524 (0.4772)	1													
(4) Symptoms	-0.2514 (0.2360)	.3136 (0.1356)	-0.1024 (0.6339)	1												
5) Duration in days	-0.1818 (0.3952)	0.0847 (0.6939)	0.5465 (0.0057)	0.1208 (0.5741)	1											
(6) Post Covid	0.0967 (0.6532)	-0.0313 (0.8845)	0.4616 (0.0232)	0.3291 (0.1164)	0.3700 (0.0752)	1										
(7) BLOT-NP	0.3515 (0.0921)	0.0373 (0.8626)	0.2031 (0.3411)	0.2224 (0.2963)	0.2403 (0.2581)	0.4757 (0.0188)	1									
(8) BLOT-RBD	0.2635 (0.2135)	0.1195 (0.5780)	0.9627 (0.0000)	-.1874 (0.3805)	0.5486 (0.0055)	0.4005 (0.05249	0.2052 (0.3361)	1								
(9) neutralS1post	0.4850 (0.0163)	0.3721 (0.0734)	0.6555 (0.0005)	-0.1338 (0.5332)	0.4101 (0.0465)	0.1743 (0.4152)	0.2200 (0.3017)	0.6758 (0.0003)	1							
(10) ß1-R-AB	-0.3610 (0.1411)	0.0927 (0.7145)	0.3871 (0.1125)	0.6434 (0.0040)	0.4289 (0.0758)	0.5156 (0.0285)	0.1881 (0.4549)	0.2420 (0.3332)	0.1247 (0.6221)	1						
(11) ß2-R-AB	-0.3611 (0.1410)	-0.1701 (0.4999)	0.2023 (0.4209)	0.3843 (0.1154)	0.2421 (0.3330)	0.3253 (0.1878)	-0.1260 (0.6183)	0.0271 (0.9151)	-0.0382 (0.8804)	0.8257 (0.0000)	1					
(12) M2-R-AB	-0.1537 (0.5427)	-0.1736 (0.4908)	0.3797 (0.1201)	0.4106 (0.0905)	0.6359 (0.0046)	0.4185 (0.0839)	0.4580 (0.0560)	0.3205 (0.1947)	0.0131 (0.9590)	0.3890 (0.1106)	0.2435 (0.3303)	1				
(13) M3-R-AB	-0.3651 (0.1363)	-0.3402 (0.1672)	0.2664 (0.2852)	0.6247 (0.0056)	0.2964 (0.2323)	0.4454 (0.0640)	0.3333 (0.1765)	0.1715 (0.4962)	-0.1200 (0.6353)	0.7219 (0.0007)	0.6640 (0.0027)	0.4084 (0.0924)	1			
(14) ITT-Omi-IL2	0.0547 (0.8089)	0.1727 (0.4420)	0.2851 (0.1985)	0.3372 (0.1248)	0.1517 (0.5005)	0.4834 (0.0226)	0.2167 (0.3328)	0.2546 (0.2528)	0.3123 (0.1571)	0.5034 (0.0394)	0.3884 (0.1234)	-0.1753 (0.5011)	0.3658 (0.1488)	1		
(15) ITT-Om-Ifn	-0.1426 (0.5267)	-0.0305 (0.8928)	0.4112 (0.0573)	0.1323 (0.5573)	0.2585 (0.2454)	0.3383 (0.1236)	0.0244 (0.9142)	0.3951 (0.0688)	0.0551 (0.8076)	0.4784 (0.0520)	0.3920 (0.1196)	0.2523 (0.3285)	0.4747 (0.0542)	0.0280 (0.9014)	1	
(16) Vaccinated(all) vs Non-Vacinated(0)	0.2635 (0.2135)	0.1195 (0.5780)	0.9627 (0.0000)	-0.1874 (0.3805)	0.5486 (0.0055)	0.4005 (0.0524)	0.2052 (0.3361)	1 (0.0000)	0.6758 (0.0003)	0.2420 (0.3332)	0.0271 (0.9151)	0.3205 (0.1947)	0.1715 (0.4962)	0.2546 (0.2528)	0.3951 (0.0688)	1

NP = nucleus protein - RBP = receptor-binding protein - neutralS1post = neutralizing antibody S1-spike protein-1 four weeks after acute infection - *ß*1-R-AB = ß1-adrenergic-receptor-antibody - *ß*2-R-AB = *ß*2-adrenergic-receptor-antibody - M2-R-AB = M2-muscarinic-choline-receptor-antibody - M3-R-AB = M3-muscarinic-choline-receptor-antibody - ITT = immune tolerance test - omi = omicron variant - IL2 = interleukin 2 - IFN = Interferon-*ß*.(p-level)

The level of neutralizing antibody IgG-S1 after acute Omicron-COVID-19 infection positively correlates significantly with disease duration (the higher the neutralizing antibody IgG-S1 the longer the acute disease duration r=0.4101, p<0.05 (Table.2). All patients showed a cellular immune response against the Omicron variant in the immune tolerance test (ITT) being significantly stronger in vaccinated vs. unvaccinated patients (ITT-Omicron IL2 / IFN at 0-vaccination vs. 2 vaccination vs. 3 vaccination = 14.8 ± 23.4 / 4.0 ± 4.8 vs. 34.4 ± 28.8 / 21.2 ± 13.0 vs 59.9 ± 103.8 / 29.3 ± 40.0 pg/ml) [Table .1]. Level of autoantibodies against G-protein coupled receptors is twice as strong in triple vaccinated compared to unvaccinated patients (Table.1). Autoantibody level of ß1-adrenergic receptor or M3-muscarinergic choline receptor and the level of cellular immune response ITT-omicron-IL2 significantly and positively correlates with the risk of POST-COVID syndrome (the higher the levels the more the risk of developing POST-COVID syndrome: ß1-adrenergic receptor antibody r=0.5156 - p<0.05; M3-muscarinic choline receptor antibody r=0.4454 - p=0.064; ITT-omicron-IL2 r=0.4834 - p<0.05) [Table .2].

Discussion

Although only 12.8% of the SARS-CoV-2 genome encodes the spike protein, this region contains 60% of all mutations that distinguish the Omicron variant from the original Wuhan Hu-1 SARS-CoV-2 strain [10]. The spike protein is the key determinant of virus transmission and immune evasion [11, 12]. In addition to determining infectivity, tropism, and transmission fitness, the spike protein is the primary target of SARS-CoV-2 vaccines and antibodies from convalescents [10]. Numerous recent studies show that the Omicron variant escapes the neutralizing antibodies induced by vaccination or previous SARS-CoV-2 infection [3, 7, 13-17]. Initial epidemiologic findings and experimental studies suggested a 40-fold reduction in the efficacy of neutralizing sera against the Omicron variant [13, 14]. The immunologic response after acute Omicron COVID-19 infection in unvaccinated patient vs vaccinated patients confirmed the lack of efficacy of neutralizing antibodies IgG spike and IgG-RBD in our study. 4 weeks after acute Omicron-COVID-19 infection the unvaccinated patients developed antibodies only to the core protein (NP) of SARS-CoV-2 virus but not to the receptor-binding domain (RBD) or spike protein S1. In vaccinated patients, no antibodies to NP but antibodies to RBD and S1 were present before contact with Omicron-COVID-19-infection.

RBD- and S1-antibodies could not protect against acute Omicron COVID-19 infection. Also, IgG-S1 spike neutralizing antibodies present at sufficient levels in vaccinated patients before Omicron-variant infection, did not protect against acute Omicron COVID-19 infection. Four weeks after acute Omicron COVID-19 infection unvaccinated patients did not develop neutralizing antibody IgG-S1 spikes. In contrast, vaccinated patients developed a 5.4-fold (patients with 2 mRNA vaccinations) or a 14.9-fold (patients with 3 mRNA vaccinations) increase in neutralizing antibody IgG-S1 spikes. Bongiovanni et al. confirmed that antibodies to nucleocapsids (NP) were only present in the case of infection by COVID-19 (patients with 2x mRNA vaccination and COVID-19 infection (N=10) vs without COVID-19 infection (N=26) - antibodies to nucleocapsids (NP) (norm > 1 UI/ml): 49.2 vs 0 UI/ml, p<0.0001) and that their levels were not significantly affected by vaccine administration [18].

This finding was easily predictable because the mRNA (BNT162b2) vaccine offers only one spike antigen and consequently only RBD and spike antibodies are produced. Furthermore, the vaccinated study participants with passed COVID-19 infection also showed a strong activation response of neutralizing spike antibodies (patients with 2x mRNA vaccination and COVID-19 infection (N=10) vs without COVID-19 infection (N=26) - antibodies against spike protein (norm > 13 AU/ml): >800 vs 611 AU/ml, p<0.0001) and of RBD antibodies (patients with 2x mRNA vaccination and COVID-19 infection (N=10) vs without COVID-19 infection (N=26) - antibodies against RBD (norm> 0.8 UI/ml): >2500 vs 1601 UI/ml, p<0.0001) [18].

Despite the lack of vaccine protection, acute infections with the Omicron variant are mild in both unvaccinated and vaccinated patients. In our study, vaccinated patients experienced symptomatic disease 3.4-fold respectively 3.8-fold longer than unvaccinated patients (duration of disease: 2-vaccination vs 3-vaccination vs 0-vaccination = 10.8 vs 12.2 vs 3.2 days; p<0.01). The duration of disease correlates significantly with the concentration of neutralizing antibody IgG-S1 spikes after Omicorn-COVID-19 infection (the higher the neutralizing antibody IgG-S1 spikes the longer the acute duration of disease r=0.4101, p<0.05). A review by Yu et al. [8] confirmed the mild course of Omicron-COVID-19 infection: 25% of all patients had an asymptomatic course and 98% of patients with symptomatic infection showed a mild disease. No data are available in the literature to the duration of symptomatic infection comparing unvaccinated vs vaccinated patients and the dependence of disease duration to concentration of neutralizing antibody IgG-S1 spike.

The T-cellular immune response to ITT-omicron-Interleukin-2 (IL2) is lower in unvaccinated patients. It increases 2.3-fold respectively 4-fold in vaccinated patients (O- vaccination vs. 2-vaccination vs. 3-vaccination = ITT-Omicron-IL2 (norm > 3pg/ml): 14.8 vs 34.4 vs 59.9 pg/ml) and 5.3-fold vs 7.3-fold to ITT-omicron-Interferon (IFN) response (O-vaccination vs 2-vaccination vs 3-vaccination = ITT-Omicron-IFN (norm > 3pg/ml): 4.0 vs 21.2 vs 29.3 pg/ml). In literature no data is available.

Many patients take a long time recovering from acute COVID-19 infection. Symptoms range from a moderate decrease in performance to total exhaustion or fatigue, as well as persistent symptoms of illness. Symptoms occur after severe as well as moderate and mild diseases persisting a few weeks to several months [19]. Incidence data of LONG-/POST-COVID syndrome vary from 2.3% to 89% [20, 21]. In our study, 12 weeks after acute Omicron-COVID-19 infection, 50-77.7% of participants suffered from POST-COVID symptoms: patients with 3 mRNA vaccinations more frequently than unvaccinated patients (0-vaccination vs 3-vaccinations - POST-COVID syndrome: 50% vs 77.7%). Unvaccinated patients showed a mild disease, patients with 3 mRNA vaccinations a moderate disease (0-vaccination vs 3-vaccinations - score POST-COVID syndrome (0-8): 2.6 vs 4.3 score points).

Developing POST-COVID syndrome correlated significantly with autoantibodies level of ß1-adrenergic receptor / M3-muscarinerger choline receptor and the level of T-cellular immune response ITT-omicron-IL2 (the higher the concentration the more the development of POST-COVID syndrome: ß1-adrenergic receptor antibody: r=0.5156 - p<0.05; M3-muscarinic choline receptor antibody: r=0.4454 - p=0.064; ITT-omicron-IL2: r=0.4834 - p<0.05). In our study the autoantibodies level of G-protein coupled receptors is twice as strong in 3-dose-vaccinated vs unvaccinated patients. Perrin et al [22]. Postulate that proinflammatory cytokines (interferon gamma, interleukin 7) cross the blood-brain barrier in the postinfectious phase and cause autonomic dysfunction that may manifest as dysregulation of sleep/wake rhythm, cognitive dysfunction and fatigue.

POST-COVID-19 fatigue has been compared with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) [23]. Symptoms common to both diseases ME/CFS and LONG-/POST-COVID include fatigue, neurologic symptoms, pain, neurocognitive and psychiatric, neuroendocrine, autonomic and immune symptoms. Both ME/CFS and LONG-/POST-COVID patients suffering from prolonged symptoms, reduced daily activity, and post-exertional malaise [23]. ME/CFS remains enigmatic but, like LONG-/POST-COVID syndrome, it is associated with elevated autoantibodies level of G protein-coupled receptor (ß1-ß2-M2-M3) as a causative agent [24]. Studies show that autoantibodies (AAB) are detectable in acute infection as well as in POST-COVID syndrome [25]. In an acute infection, AAB against cytokines, chemokines, complement components and cell surface proteins, which regulate the immune system and therefore may also interfere with the control of viral infection, are significantly more provable in infected patients vs. uninfected individuals [25].

A prominent example is AAB against type-1 interferons [26]. AAB against G protein-coupled receptors (GPCR), which have an impact on the control of the autonomic nervous system, have also been detected in POST-COVID syndrome confirming the association of diverse AAB and proinflammatory cytokines and the development of POST-COVID syndrome [24]. They found that the detection of antinuclear antibodies (ANAs), interferon-alpha AAB, and proinflammatory cytokines in acute COVID-19 infection correlated with respiratory symptoms typical of POST-COVID syndrome [24-26]. Vitamin C deficiency has been associated with post-viral fatigue syndromes and POST-COVID [27]. Clinically relevant vitamin C deficiency is a disease-causing condition because the water-soluble vitamin is one of the body's most important antioxidants and a cofactor in more than 150 metabolic functions [28]. Ascorbic acid, as an enzymatic cofactor, is essential for immunomodulation, synthesis of collagen and carnitine, the bioavailability of tetrahydrobiopterin and thus the formation of serotonin and dopamine, synthesis of norepinephrine, degradation of the transcription factor HIF-1 α, and hypomethylation of DNA [29-30]. Fatigue, pain, cognitive impairment, and depression-like symptoms are known symptoms of Vitamin C deficiency [31]. In our study, all patients had Vitamin C deficiency, with unvaccinated patients having significantly lower Vitamin C levels in frozen plasma compared to vaccinated patients (0-vaccination vs 2-vaccination vs 3-vaccination - vitamin C (norm: 9-20mg/l): 2.0 vs 4.3 vs 4.5). Comparable results are currently not available in the literature.

Conclusion

Both unvaccinated and vaccinated patients experience a mild course of their disease with acute Omicron-COVID-19 infection. However, vaccinated patients suffer prolonged from acute disease with each vaccination. Unvaccinated patients do not develop neutralizing antibodies to IgG-S1 spike compared to vaccinated patients after acute Omicron-COVID-19 infection, which is explained by the multiple mutations in the region of the spike genome in the Omicron variant and is thought to be responsible for infectivity of mRNA-vaccinated patients. A subsequent acute Omicron-COVID-19 infection after mRNA vaccination, increases the risk of a strong immune response (neutralizing antibodies IgG-S1 spike, antibodies IgG-RBD, T-cellular response in IL2 and IFN, Autoantibodies against G-protein coupled receptor ß1-ß2-M2-M3) which correlates with the development of POST-COVID syndrome. A deficiency of immunomodulatory micronutrients (Vitamin C, Vitamin D3, selenium, zinc) may be responsible. Further studies need to show whether optimal Vitamin C substitution, aiming a blood-Vitamin-C-level of 20-30 mg/l, can alleviate severe fatigue and, due to its neurotransmitter-forming and antioxidant and anti-inflammatory properties, eliminate the neurocognitive, proinflammatory or autoimmune symptoms in POST-COVID patients.

Vaccination with the mRNA is protective against fatal courses but does not prevent infection with an Omicron variant. Only NP-antibodies are effective and protect against further COVID-19 infections. Further research needs to show whether additional mRNA booster vaccinations are reasonable. However, they do not appear to elicit an immune response against the nucleoprotein (NP) of the SARS-CoV-2 virus, or whether booster vaccination with an inactivated vaccine developing NP-antibodies seems more useful and protects more effectively against further COVID-19 infections.

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