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Original article

Humoral and cellular immunity in convalescent and vaccinated COVID-19 people with multiple sclerosis: Effects of disease modifying therapies

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ABSTRACT

Objectives: To determine anti-SARS-Cov2 antibodies and T-cell immunity in convalescent people with multiple sclerosis (pwMS) and/or pwMS vaccinated against Covid-19, depending on the disease modifying therapy, and in comparison to healthy controls (HC).
Methods: 75 participants were enrolled: Group 1—29 (38.7%) COVID-19 convalescent participants; Group 2—34 (45.3%) COVID-19 vaccinated; Group 3—12 (16.0%) COVID-19 convalescent participants who were later vaccinated against COVID-19. Cellular immunity was evaluated by determination of number of CD4+ and CD8+ cells secreting TNFα, IFNγ, and IL2 after stimulation with SARS-CoV-2 peptides.
Results: pwMS treated with ocrelizumab were less likely to develop humoral immunity after COVID-19 recovery or vaccination. No difference was observed in the cellular immunity in all studied parameters between pwMS treated with ocrelizumab compared to HC or pwMS who were treatment naïve or on first line therapies. These findings were consistent in convalescent, vaccinated, and convalescent+vaccinated participants. COVID-19 vaccinated convalescent pwMS on ocrelizumab compared to COVID-19 convalescent HC who were vaccinated did not show statistically difference in the rate of seroconversion nor titers of SARS-CoV-2 antibodies.
Conclusion: Presence of cellular immunity in pwMS on B-cell depleting therapies is reassuring, as at least partial protection from more severe COVID-19 outcomes can be expected.

1. Introduction

Several studies have indicated an association between B-cell depleting disease modifying therapy (DMTs) and higher probability of a more serious clinical course of COVID-19 (M.P. Sormani et al., 2021; Stastna et al., 2021). Furthermore, more and more data suggest an attenuated humoral response after SARS-COV-2 infection and after COVID-19 vaccination in people with MS (pwMS) who are using ocrelizumab (Habek et al., 2021; Achiron et al., 2021). In light of these data, it is of paramount importance to know how B-cell depleting agents affect the development of humoral and cellular immunity after the infection, and whether there is an impact on the vaccine response in different populations of pwMS.

The present study aims to determine the development of anti-SARS-Cov2 antibodies and development of T-cell mediated immunity in convalescent or/and vaccinated COVID-19 pwMS depending on the DMTs they use and in comparison to healthy controls (HC).

2. Objectives

The primary objective was to investigate the differences in presence of humoral and cellular immunity in:

a) convalescent COVID-19 pwMS treated with ocrelizumab compared to treatment naïve pwMS or pwMS on 1st line therapies (TN/1st pwMS) and HC.

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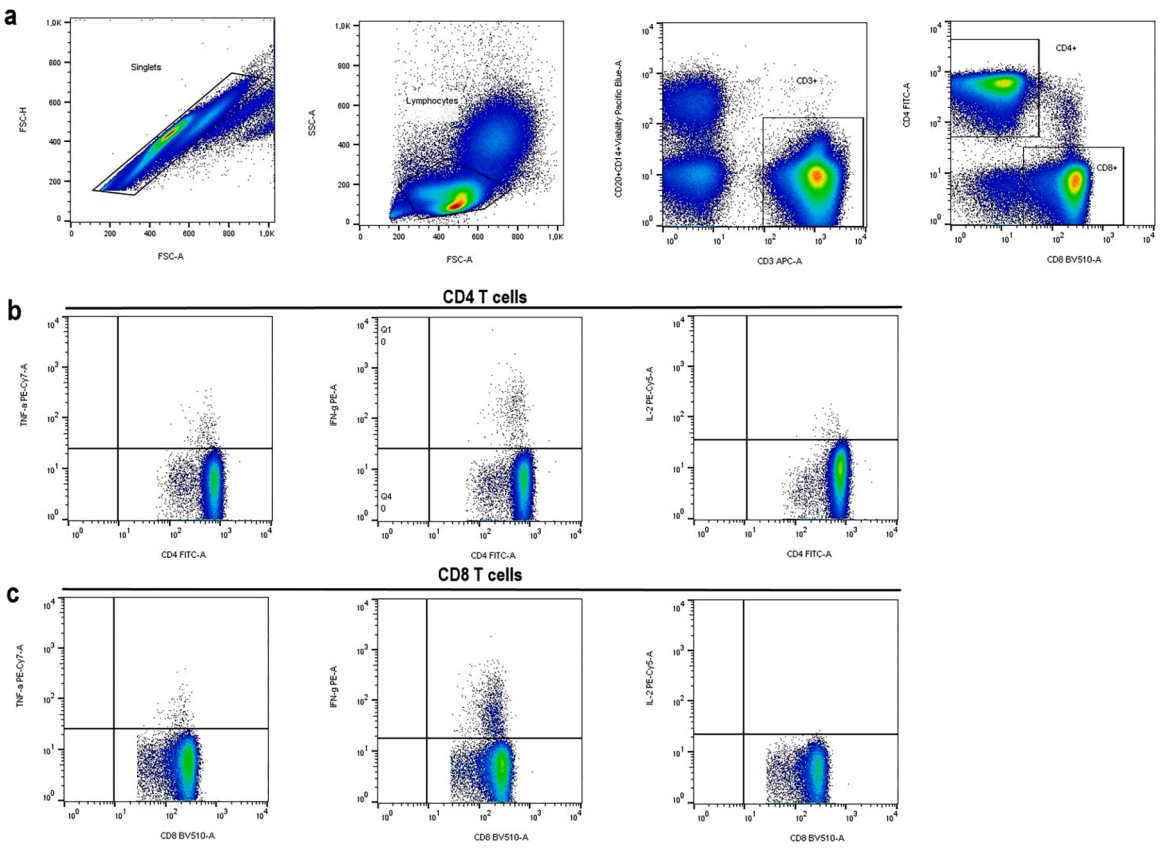

Fig. 1. Gating strategy to detect SARS-CoV-2 reactive CD4+ and CD8+ T cells after in vitro stimulation for 8 h with surface glycoprotein, matrix and nucleoprotein overlapping peptide pools. Representative gating of a single live CD4 and CD8 T cells in the upper panel (a), and further enumerated in respect to intracellular TNFα, IFNγ and IL2 (from left to right) cytokine production.

b) vaccinated COVID-19 pwMS treated with ocrelizumab compared to treatment naïve pwMS or pwMS on 1st line therapies (TN/1st pwMS) and HC.
c) convalscent and vaccinated COVID-19 pwMS treated with ocrelizumab compared to HC.

The secondary objectives were to investigate the differences in the titers of SARS-CoV-2 IgG antibodies and absolute values of CD4 and CD8 cells expressing INFγ, TNFα, and IL2 in same groups as above. Finally, differences in humoral and cellular immunity were investigated between convalescent, vaccinated, and convalescent+vaccinated pwMS on ocrelizumab.

3. Materials and methods

3.1. Participants

This single-center, case-control study was performed at University Hospital Center, Zagreb, Croatia, and was approved by the Ethics Committee of the University Hospital Center Zagreb. All pwMS, who came on their regular follow-up visit between July 15th and Aug 15th 2021, were invited to participate in the study. Inclusion criteria included: 1) recovery from COVID-19 and/or full vaccination against COVID-19 in the period of 12 months before blood sampling, 2) treatment naïve pwMS or pwMS on first line DMTs—interferons, glatiramer acetate, teriflunomide or dimethyl fumarate—or pwMS on ocrelizumab. Exclusion criteria were: 1) all other DMTs except the aforementioned, 2) any other off-label MS therapy, 3) pwMS who were non-compliant to DMTs. Age and sex matched convalescent or vaccinated healthy controls (HC) were enrolled as well.

3.2. Humoral immunity

Testing for humoral immunity was performed in the Clinical Institute for Laboratory Diagnostics, University Hospital Center Zagreb, Zagreb, Croatia, using Elecsys® Anti-SARSCoV-2 S assay (Roche Diagnostics Int, Rotkreuz, Switzerland). The assay was performed per the manufacturer's instructions, using Cobas e 801 analytical unit for immunoassay tests (F. Hoffmann-La Roche Ltd.). (https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2.html) Antibody titer ≥0.8 U/mL was considered positive, as recommended by the manufacturer.

3.3. Cellular immunity

Virus reactive cytokine producing T cells were enumerated using the human SARS-CoV-2 T Cell Analysis Kit (PBMC, Miltenyi Biotech) following manufacturer protocol, with some modifications.

PBMC were isolated from heparinized whole blood by density gradient centrifugation (Ficoll-Paque Plus from Cytiva). After collection of PBMC ring, cells were washed twice with RPMI 1640 medium and resuspended in RPMI 1640 media with 10% of human AB serum from healthy, SARS-Cov-2 naive donor with undetectable virus specific antibodies and undetectable virus specific T cells. PBMC were stimulated with PepTivator SARS-CoV-2 (Miltenyi Biotec) for complete spike glycoprotein covering the complete protein coding sequence aa 5–1273 (GenBank MN908947.3, Protein QHD43416.1), matrix and nucleoprotein overlapping peptides, at concentration 1 μg/ml of each for 8 h at 37 °C, 5% CO_2. Positive control PBMC were stimulated with CytoStim™ whereas negative control PBMC were cultivated in complete medium with 10% DMSO. Brefeldin A at concentration 2 μg/ml was added for the last 6 h. Upon harvest, PBMC were labeled with fluorophore-conjugated

Table 1
Baseline and COVID-19 related characteristics and results of humoral in cellular immunity in three groups of participants.

Group 1				
	HC (*N* = 4)	**TN/1st pwMS (*N* = 9)**	**OCR pwMS (*N* = 16)**	**p value**
Baseline characteristics				
Sex (females)	4 (100.0%)	6 (66.7%)	6 (37.5%)	0.09
Age (years)	50.1 ± 6.8	39.8 ± 6.5	38.9 ± 11.7	0.202[%]
Disease duration (years)	NA	1.3 (0.3–15.5)	6.3 (2.5–17.5)	0.121
EDSS (median, range)	NA	1.0 (0–3.5)	1.75 (0–6)	0.276
COVID-19 characteristics				
Time between COVID-19 and blood sampling (days)	238 (233–239)	217 (48–335)	159 (62–255)	0.034
Humoral response				
Presence of SARS-CoV2 IgG antibodies (N)	4 (100.0%)	6 (100.0%)[*]	7 (43.8%)	0.02
Cellular response				
CD4 cells				
INFγ	4 (100.0%)	8 (88.9%)	15 (93.8%)	1.000
TNFα	2 (50.0%)	4 (44.4%)	7 (43.8%)	1.000
IL2	2 (50.0%)	4 (44.4%)	8 (50.0%)	1.000
CD8 cells				
INFγ	2 (50.0%)	1 (11.1%)	8 (50.0%)	0.127
TNFα	2 (50.0%)	0 (0.0%)	7 (43.8%)	0.05
IL2	3 (75.0%)	0 (0.0%)	1 (6.3%)	0.004
Group 2				
	HC (*N* = 11)	**TN/1st pwMS (*N* = 10)**	**OCR pwMS (*N* = 13)**	**p value**
Baseline characteristics				
Sex (females)	8 (72.7%)	5 (50.0%)	8 (61.5%)	0.551
Age (years)	43.3 ± 7.0	46.7 ± 15.3	42.3 ± 10.3	0.629
Disease duration (years)	NA	12.4 ± 8.9	12.1 ± 7.9	0.923
EDSS (median, range)	NA	2.5 (0–6.0)	4.5 (1.0–6.5)	0.101
COVID-19 characteristics				
Type of vaccine				0.286
Comirnaty®	11 (100%)	6 (60%)	9 (69.2%)	
Spikevax®	0	2 (20%)	2 (15.4%)	
Vaxzevria®	0	2 (20%)	2 (15.45)	
Time between COVID-19 vaccination and blood sampling (days)	149.1 ± 38.8	52.6 ± 38.1	57.4 ± 25.8	<0.001
Humoral response				
Presence of SARS-CoV2 IgG antibodies (N)	10 (100.0%)[#]	10 (100.0%)	5 (38.5%)	< 0.001
Cellular response				
CD4 cells				
INFγ	9 (81.8%)	6 (60.0%)	10 (76.9%)	0.620
TNFα	8 (72.7%)	5 (50.0%)	5 (38.5%)	0.242
IL2	3 (27.3%)	4 (40.0%)	5 (38.5%)	0.817
CD8 cells				
INFγ	5 (45.5%)	3 (30.0%)	6 (46.2%)	0.748
TNFα	5 (45.5%)	2 (20.0%)	4 (30.8%)	0.482
IL2	3 (27.3%)	1 (10.0%)	1 (7.7%)	0.478
Group 3				
	HC (*N* = 7)		**OCR pwMS (*N* = 5)**	**p value**
Baseline characteristics				
Sex (females)	7 (100.0%)		4 (80.0%)	0.417
Age (years)	40.2 ± 12.1		43.5 ± 7.9	0.755[&]
Disease duration (years)	NA		10.4 (4.6–14)	–
EDSS (median, range)	NA		3 (1–5)	–
COVID-19 characteristics				
Type of vaccine				–
Comirnaty®			5 (100%)	
Spikevax®				
Vaxzevria®	7 (100%)			
Time between vaccine and blood sampling (days)	115 (35–167)		34 (21–61)	0.030
Humoral response				
Presence of SARS-CoV2 IgG antibodies (N)	7 (100.0%)		4 (80.0%)	0.417
Cellular response				
CD4 cells				
INFγ	7 (100.0%)		5 (100.0%)	1.000
TNFα	3 (42.9%)		3 (60.0%)	0.205
IL2	4 (57.1%)		5 (100.0%)	
CD8 cells				
INFγ	6 (85.7%)		4 (80.0%)	1.000
TNFα	2 (28.6%)		3 (60.0%)	0.558
IL2	1 (14.3%)		0 (0.0%)	1.000

[*] for three patients data on SARS-CoV2 IgG antibodies was not available.
[#] for one HC data on SARS-CoV2 IgG antibodies was not available.
[%] Kruskal-Wallis test, but presented in the form of mean±st.dev for better clinical understanding.
[&] Mann-Whitney test, but presented in the form of mean±st.dev for better clinical understanding.

antibodies for surface markers CD3 APC, CD4 Vio®Bright B515, CD8 VioGreen™, CD14 VioBlue® and CD20 VioBlue® in the presence of Tandem Signal Enhancer and FcR Blocking Reagent.

After washing in PBS, PBMC were stained with Viobility 405/452 Fixable Dye, and fixed with InsideFix buffer overnight at 4 °C. The next day, PBMC were washed with PEB buffer and permeabilized with InsidePerm buffer. For intracellular staining cells were incubated in a mixture of Tandem Signal Enhancer, FcR Blocking Reagent and anti-IFN-γ PE, anti-TNF-α PE-Vio® 770 and anti-IL-2 PE-Vio® 615 antibodies. Finally, PBMC were resuspended in PEB buffer and analyzed on 3-laser equipped BD LSR II flow cytometer (BD Biosciences). A live CD3 T cell acquired ranged from 250k-450k per tube.

Data analysis was performed using FlowJo 10.7.1 (TreeStar Inc/FlowJo LLC). Doublets, debris, and dead cells as well as CD14+ and CD20+ cells were excluded. After pregating on live CD3 as well as CD4 and CD8 subtypes, cytokine expression for CD4+ T cells and CD8+ T cells were assessed. Fluorescence minus one (FMO) controls were performed for all fluorescence and used for gating setting (Fig. 1).

Percentages of cytokine producing cells was assessed after deduction of values from unstimulated cells, with the threshold of 0.02%, based on FMO.

3.4. Statistical analysis

Statistical analysis was performed with the IBM SPSS v25 software. The data distribution was tested with the Kolmogorov-Smirnov test. The differences between qualitative variables were tested with the Chi-square test. The differences between the quantitative variables were tested with the parametric independent sample *t*-test and non-parametric Mann-Whitney test for the comparison between the two groups, and ANOVA test and Kruskal-Wallis test for the comparison between the more than two groups. P-values less than 0.05 were considered significant.

4. Results

Altogether, 75 participants were enrolled: 29 (38.7%) COVID-19 convalescent participants – group 1, 34 (45.3%) COVID-19 vaccinated - group 2, and 12 (16.0%) COVID-19 convalescent participants who were later vaccinated against COVID-19 – group 3. Demographic characteristics of the cohort are presented in the Table 1.

Results regarding the presence or absence of humoral and cellular

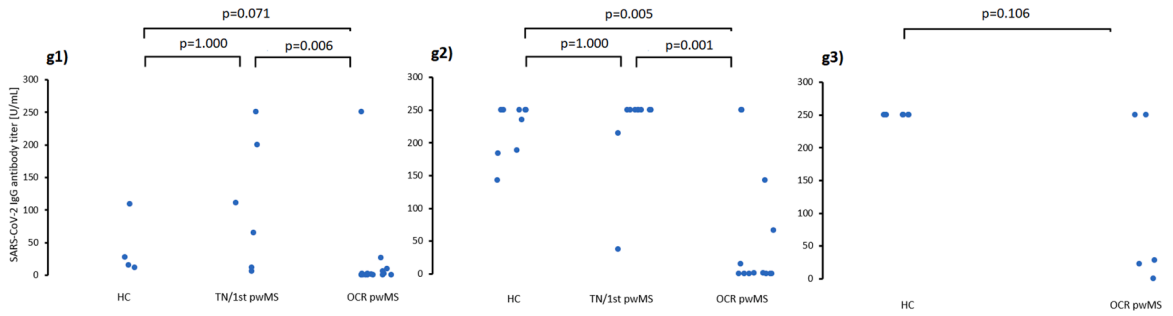

Fig. 2. Titers of IgG against spike protein of SARS-CoV-2 in three groups of participants.

immunity across groups is presented in Table 1. To summarize the results, absence of humoral immunity was more frequent in ocrelizumab treated pwMS in convalescent and vaccinated groups compared to HC and TN/1st pwMS. No difference was observed in Group 3. On the contrary, there was no difference in cellular immunity between ocrelizumab treated pwMS, TN/1st pwMS, and HC in all three groups (Table 1).

A similar observation was made while interpreting data presented as the absolute values.

In convalescent groups, titer of SARS-CoV2 IgG was significantly lower in pwMS on ocrelizumab, compared to TN/1st pwMS (0.21 (0 −250) vs. 87.60 (5.25–250), $p = 0.006$) (Fig. 2). In the vaccinated group, titer of SARS-CoV2 IgG was significantly lower in pwMS on ocrelizumab, compared to healthy controls and TN/1st pwMS (0.42 (0–250) vs. 250 (143–250) vs. 250 (37.5–250), < 0.001; respectively), (Fig. 2). In Group 3, there was no statistically significant difference in titer of SARS-CoV2 IgG between the pwMS on ocrelizumab and healthy controls ($p = 0.106$).

While looking at the cellular immunity, there was no difference in CD4+ INFγ ($p = 0.370$), TNFα ($p = 0.639$), and IL2 ($p = 0.782$); or CD8+ INFγ ($p = 0.233$), TNFα ($p = 0.390$), and IL2($p = 0.175$) between ocrelizumab treated pwMS, TN/1st pwMS and HC in convalescent group (Figs. 3 and 4). Similarly, there was no difference in CD4+ INFγ ($p = 0.596$), TNFα ($p = 0.717$), and IL2 ($p = 0.930$); or CD8+ INFγ ($p = 0.869$), TNFα ($p = 0.449$), and IL2 ($p = 0.511$) between ocrelizumab treated pwMS, TN/1st pwMS and HC in vaccinated group (Figs. 3 and 4). No difference was observed in CD4+ INFγ ($p = 0.530$), TNFα ($p = 0.432$), and IL2 ($p = 0.106$) or CD8+ INFγ ($p = 0.202$), TNFα ($p = 0.268$), and IL2 ($p = 0.530$) between ocrelizumab treated pwMS and HC in group 3 (Figs. 3 and 4).

Finally, no differences were observed in the presence of humoral or cellular immunity between convalescent, vaccinated, and convalescent+vaccinated pwMS on ocrelizumab (all $p>0.05$).

5. Discussion

The results of our study have shown that although a significant proportion of pwMS on B-cell depleting therapy do not mount a humoral response after recovery from COVID-19 or vaccination against COVID-19, they are able to mount sufficient cellular immunity against COVID-19. Specifically, no difference was observed in both CD4 or CD8 positive INFγ, TNFα, and IL2 cells in pwMS on B-cell depleting therapy compared to healthy controls or treatment naïve pwMS and/or pwMS on 1st line therapies.

Our findings may have implications in mitigating the risk associated with treatment of pwMS with B-cell depleting therapy.

First, results from the MSBase registry, with largest cohort of pwMS with COVID-19, had demonstrated consistent associations of rituximab with increased risk of hospitalization, ICU admission, and requiring artificial ventilation, and ocrelizumab with hospitalization and ICU admission. (Simpson-Yap et al., 5) Further, several other studies have identified that pwMS who are receiving ocrelizumab are not able to mount protective humoral response. (Habek et al., 2021; Achiron et al., 9) Recent data also suggest that development of a humoral immune response remains rare in pwMS using B-cell depleting therapies, even after third dose of the homologous SARS-CoV-2 mRNA vaccine. (Achtnichts et al., 2021) Studies have shown a significant correlation between time since the last dose of B-cell depleting therapies and the development of humoral immunity after vaccination, with rituximab having longer intervals (mean=386 days) than ocrelizumab (mean=129 days). (König et al., 20; M.P. Sormani et al., 2021) It has been suggested that, in selected clinically and radiologically stable pwMS, delaying the cycle of B-cell depleting therapies by 3 to 6 months before vaccination could increase the probability of developing appropriate humoral responses. (Disanto et al., 2021) All these data indicate that pwMS treated with B-cell depleting therapies should be informed about the risk of more severe COVID-19 outcome and risk of reduced humoral immunity after vaccinations, if they are already on therapy.

This raises the question whether COVID-19 convalescent patients or patients who have been vaccinated against COVID-19 and do not develop an antibody response, will in fact have adequate immunity from subsequent SARS-Cov-2 infections. Although data on reinfection or infection after vaccination in pwMS on B-cell depleting therapies is currently lacking, several studies have emphasized T-cell immunity as an important factor in the protection against SARS-CoV-2. The first study compared B cell and T cell responses longitudinally in 20 pwMS on anti-CD20 antibody monotherapy with 10 HC after BNT162b2 or mRNA-1273 mRNA vaccination. In this study all pwMS treated with aCD20 therapy generated antigen-specific CD4 and CD8 T cell responses after vaccination. (Apostolidis et al., 14) Moreover, several subsequent studies confirmed that pwMS who were treated with ocrelizumab generated comparable SARS-CoV-2-specific T-cell responses with healthy controls and/or pwMS on other MS therapies. (Brill et al., 23; Sabatino et al., 14; Gadani et al., 16; N. Madelon et al., 2021)

The results of our study further expand the knowledge on T-cell immunity, not only on vaccinated, but also on COVID-19 convalescent pwMS. We have found no difference in any studied parameter of T-cell immunity in COVID-19 convalescent pwMS regardless of DMT and HC. Furthermore, COVID-19 convalescent pwMS on ocrelizumab who were vaccinated compared to COVID-19 convalescent HC who were vaccinated do not show statistically different rate of seroconversion or titers of SARS-CoV-2 antibodies. This finding is in the line with a previous study showing that humoral response to mRNA vaccine is significantly influenced by previous SARS-CoV-2 infection. (Pitzalis et al., 2021)

With the rapid spread of the SARS-CoV-2 variant Omicron, the question raised whether T-cell responses to Omicron variant are conserved in pwMS using B-cell depleting therapies after COVID-19 mRNA vaccination. Preliminary, non-peer reviewed data, suggest that in pwMS using B-cell depleting therapies the vaccine-induced T-cell responses are little affected by the mutations carried by Omicron, and a third vaccine dose improves cytotoxic T-cell responses. (N. Madelon et al., 2021)

Limitations of this study are small number of participants and lack of longitudinal data on both humoral and cellular immunity in the studied sample of participants.

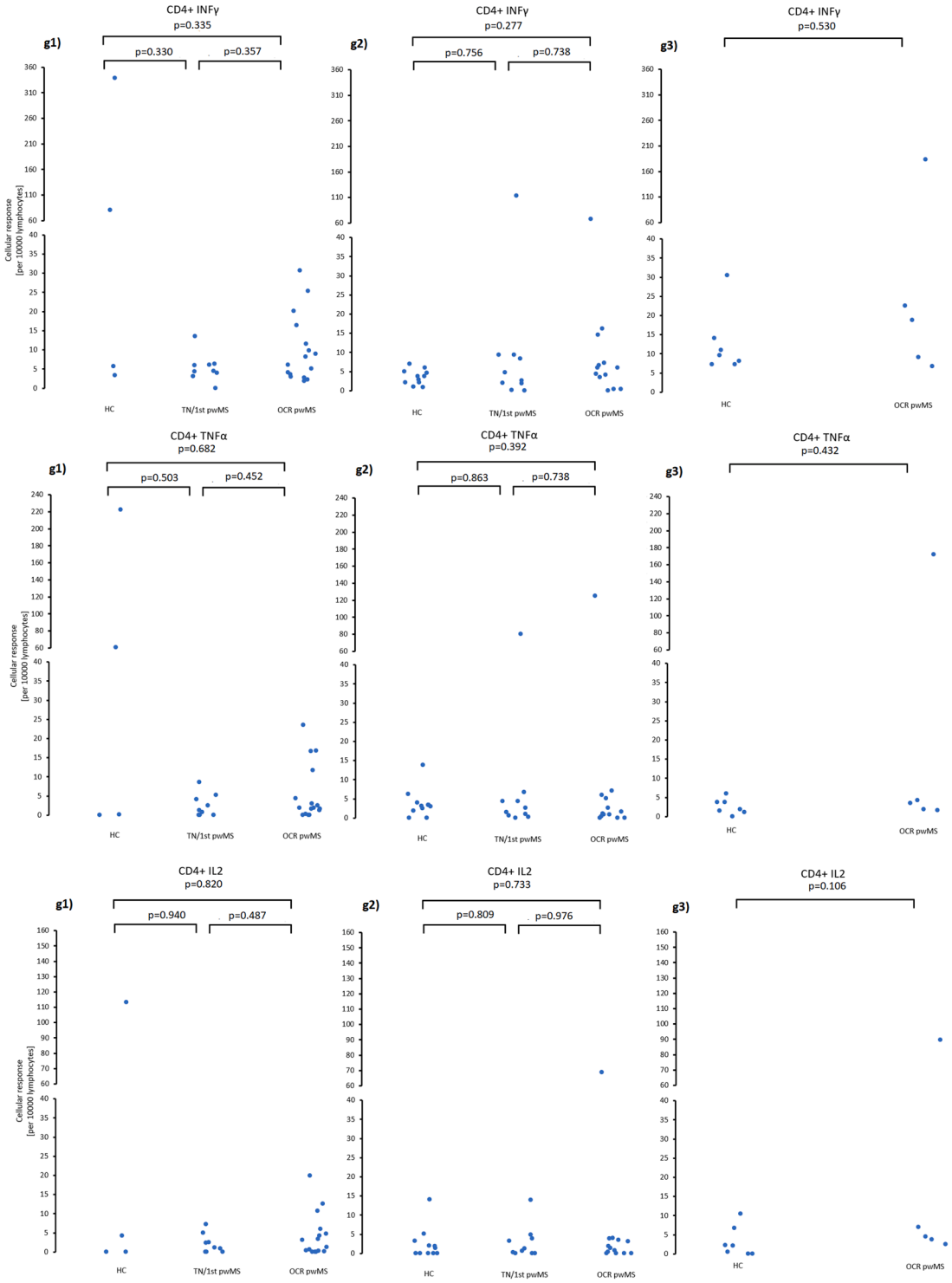

Fig. 3. Number of CD4+ TNFα, IFNγ and IL2 cells in three groups of participants.

In conclusion, presence of cellular immunity in pwMS on B-cell depleting therapies is reassuring, as at least partial protection from more severe COVID-19 outcomes can be expected. Our data on COVID-19 convalescent persons, who are later vaccinated against COVID-19, support the use of booster SARS-CoV-2 vaccinations in patients receiving B-cell depleting therapies.

Authors' contributions

Study concept and design: Habek. In vitro experimental set up and flow cytomeric acquisition: Cvetić, Bendelja. Flow cytometry data analysis: Savić Mlakar, Bendelja. Acquisition of data: Habek, Rogić, Adamec, Barun, Gabelić, Krbot Skorić. Analysis and interpretation of data: Habek, Cvetić, Savić Mlakar, Bendelja, Rogić, Adamec, Barun, Gabelić, Krbot Skorić. Drafting of the manuscript: Habek. Critical revision of the manuscript for important intellectual content: Habek, Cvetić, Savić Mlakar, Bendelja, Rogić, Adamec, Barun, Gabelić, Krbot Skorić. Administrative, technical, and material support: Habek, Cvetić, Savić Mlakar, Bendelja, Rogić, Adamec, Barun, Gabelić, Krbot Skorić.

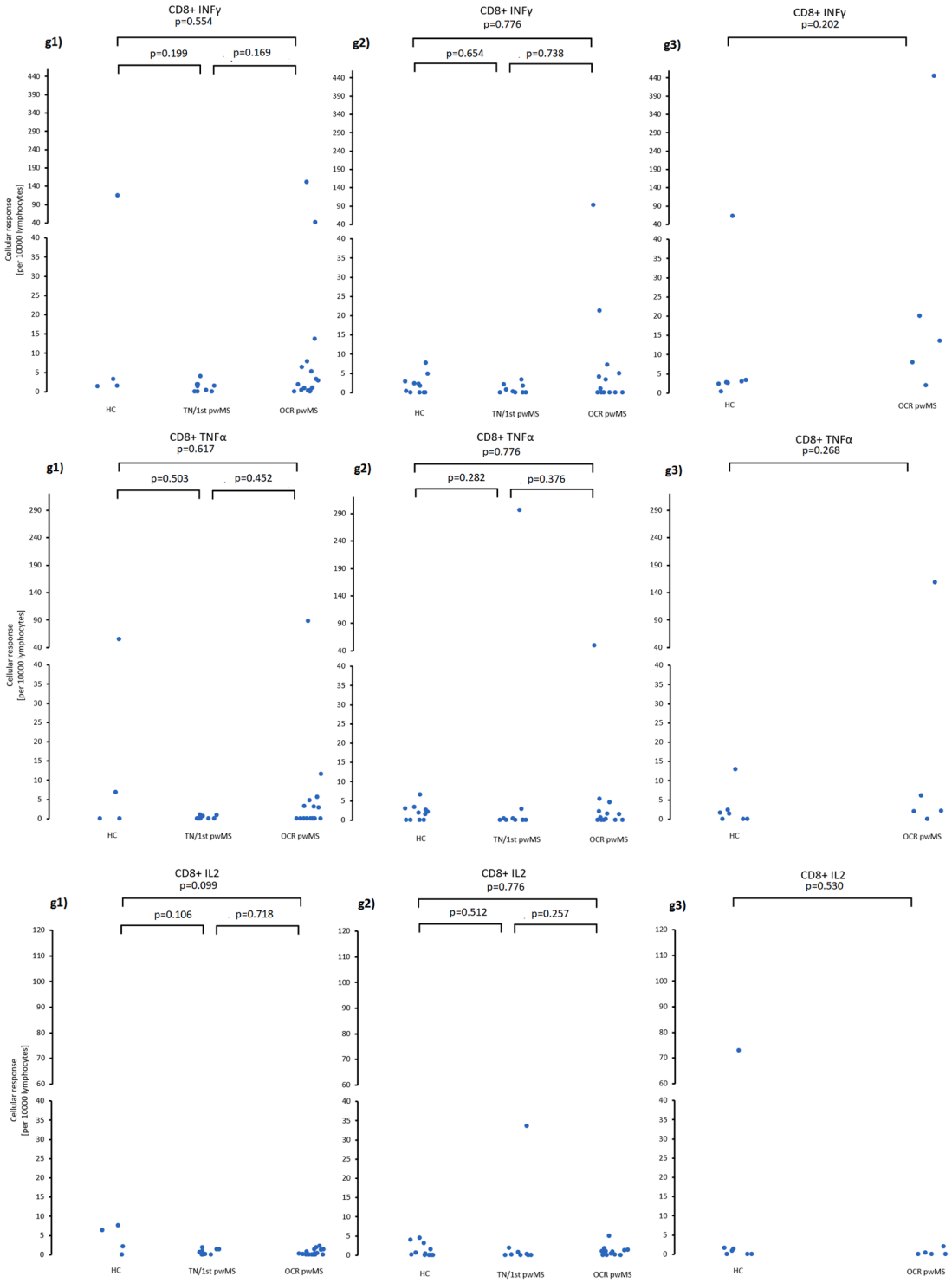

Fig. 4. Number of CD8+ TNFα, IFNγ and IL2 cells in three groups of participants.

Declaration of Competing Interest

MH: Participated as a clinical investigator and/or received consultation and/or speaker fees from: Biogen, Sanofi Genzyme, Merck, Bayer, Novartis, Pliva/Teva, Roche, Alvogen, Actelion, Alexion Pharmaceuticals, TG Pharmaceuticals.

ŽC: Reports no conflict of interest.

ASM: Reports no conflict of interest.

KB: Reports no conflict of interest.

DR: Reports no conflict of interest.

IA: Participated as a clinical investigator and/or received consultation and/or speaker fees from: Biogen, Sanofi Genzyme, Merck, Bayer, Novartis, Pliva/Teva, Roche, Alvogen, Actelion, Alexion Pharmaceuticals, TG Pharmaceuticals.

BB: Participated as a clinical investigator and/or received consultation and/or speaker fees from: Biogen, Sanofi Genzyme, Merck, Bayer, Novartis, Pliva/Teva, Roche, Alvogen, Actelion, Alexion Pharmaceuticals.

TG: Participated as a clinical investigator and/or received consultation and/or speaker fees from: Biogen, Sanofi Genzyme, Merck, Bayer,

Novartis, Pliva/Teva, Roche, Alvogen, Actelion, Alexion Pharmaceuticals.

MKS: received consultation and/or speaker fees from: Sanofi Genzyme, Roche.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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