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Cellular and Humoral Immune Response to SARS-CoV-2 mRNA Vaccines in Patients Treated with either Ibrutinib or Rituximab

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Abstract:

Patients treated with B-cell targeting therapies like Rituximab or Ibrutinib have decreased serological response to various vaccines. In this study, we tested serological and cellular response to SARS-CoV-2 mRNA vaccines in 16 patients treated with Ibrutinib, 16 treated with maintenance Rituximab, 18 patients with chronic lymphocytic leukaemia (CLL) with watch and wait status and 21 healthy volunteers. In comparison to the healthy volunteers, where serological response was achieved by 100% subjects, patients on B-cell-targeting therapy (Ibrutinib and Rituximab) had their response dramatically impaired. The serological response was achieved in 0% of Rituximab treated, 18% of Ibrutinib treated and 50% of untreated CLL patients. Cell-mediated immunity analysed by the whole blood Interferon- γ Release immune Assay developed in 80% of healthy controls, 62% of Rituximab treated, 75% of Ibrutinib treated and 55% of untreated CLL patients. The probability of cell-mediated immune response development negatively correlates with disease burden mainly in CLL patients. Our study shows that even though the serological response to SARS-CoV-2 vaccine is severely impaired in patients treated with B-cell-targeting therapy, the majority of these patients develop sufficient cell-mediated immunity. The vaccination of these patients therefore might be meaningful in terms of protection against SARS-CoV-2 infection.

Key words:

SARS-COV-2, Vaccination, Ibrutinib, Rituximab, Cellular Immune Response

Abbreviations:

BTKi - Bruton tyrosine kinase inhibitor

CBC – complete blood count

CLL - Chronic lymphocytic leukemia

mRNA - messenger ribonucleic acid

mAbs – monoclonal antibodies

SARS-COV-2 - Severe acute respiratory syndrome coronavirus 2

RB – Rituximab-Bendamustine

RFC - Rituximab -Fludarabine- Cyclophosphamide

MCL – Mantle cell lymphoma

FL – Follicular lymphoma

R-CHOP - Rituximab and Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone

APL - Acute promyelocytic leukemia

AML - Acute myeloid leukemia

HL – Hodgkin lymphoma

DLBCL - Diffuse large B-cell lymphoma

WaW – watch and wait

Statements and Declarations

The research has been performed in accordance with the Declaration of Helsinki and was approved by local ethical authorities (Ethical Committee of the Faculty Hospital Kralovske Vinohrady, decision EK-VP/24/0/2021 dated 5th May 2021).

The work has not been published previously and it is not under consideration for publication elsewhere.

The authors declare that they have no competing interests.

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Author contributions statement:

Study conception and design: J.N.

Acquisition of data: B.B., Z.K., I.Z., L.G., P.K., T.H., M.M., J.N.

Analysis and interpretation of data: J.N., B.B., Z.K.

Drafting of manuscript: J.N.

Critical revision: B.B., Z.K., I.Z., L.G., P.K., T.H., M.M., J.N.

All authors read and approved the final manuscript.

Introduction:

Haematological malignancies induce immune disturbances.¹ The immune defects are commonly worsened by treatment with fludarabine, rituximab and other agents that induce long-term paralysis of the immune system.^{2,3} Those patients not only have decreased immune response against infections, but also their ability to develop sufficient serological response to vaccination is decreased. This immune deficiency has been well documented in patients treated with B-cell-targeting therapies (either rituximab or ibrutinib). Both groups of patients show insufficient serological response to both bacterial (pneumococcal) and viral (hepatitis B, influenza) vaccines.⁴⁻⁹

In this study, both serological and cell-mediated immune response to SARS-CoV-2 mRNA vaccines^{10,11} in patients treated with B-cell-targeting therapy was investigated. Based on previous research, we anticipated the impairment of serological response to vaccinations.^{12,13} In addition to antibodies, the vaccine generates virus-specific T cells that are required for protective immunity to SARS-CoV-2 and that might play crucial role in patients with B-cell-targeting therapies.¹⁴⁻¹⁷ The capacity of the vaccine to generate cell-mediated immunity in these patients was yet to be systematically investigated.

Material and methods:

Subjects:

71 adult subjects with completed SARS-CoV-2 (64 Comirnaty by Pfizer/BioNTech or 7 Spikevax by Moderna) vaccination were recruited into the study (age 26-86 years, median 69 years) between May 2021 and August 2021. Median time between the last vaccine dose and study examination was 36 days. The time did not significantly differ between groups of patients. The study included 16 patients treated with Ibrutinib (age 59-78 years, median 72 years), 16 patients treated with maintenance Rituximab (age 38-86 years, median 64 years), 18 patients with yet untreated, active chronic lymphocytic leukaemia - watch and wait status (age 52-80 years, median 71 years) and 21 healthy vaccinated volunteers (age 26-81 years, median 57 years). All subjects signed informed consent approved by local ethical authorities (Ethical Committee of the Faculty Hospital Kralovske Vinohrady, decision EK-VP/24/0/2021 dated 5th May 2021). All subjects filled in a questionnaire focused on side effects of vaccination and underwent blood sampling for CBC with differential, blood biochemistry, serology testing for COVID-19, flow cytometry analysis and Quantiferon analysis. Further patient data is described in the Results section.

Serology

Antibody response was measured in the clinical laboratory using a commercial sandwich immunoassay, IgG antibodies to the Spike protein were measured by the quantitative Atellica IM SARS-CoV-2 IgG (sCOVG) (REF 11207386) test in a Siemens Atellica Solution analyser (Siemens Healthcare GmbH, Germany).

Cellular immunity

SARS-CoV-2-specific T cell response was assessed in the clinical immunology laboratory by a whole blood Interferon-Gamma Release immune Assay (IGRA) that uses two Qiagen proprietary mixes of SARS-CoV-2 S protein (Ag.1 and Ag.2) selected to activate both CD4⁺ and CD8⁺ T cells, following manufacturer's instructions. Briefly, venous blood samples were collected directly into the Quantiferon® tubes containing Spike peptides as well as positive and negative controls. Whole blood was incubated at 37°C for 16-24 hours and centrifuged to separate plasma. IFN-γ (IU/ml) was measured in these plasma samples using ELISA (QuantiFERON Human IFN-γ SARS-CoV-2, Qiagen) test.

Flow cytometry

Fresh peripheral blood was analysed by flow cytometry. Surface staining was performed with the following mAbs: anti-CD4 (clone OKT4), anti-CD8 (clone RPA-T8), anti-CD19 (HIB19), anti-CD45RO (clone UCHL1) and anti-CD69 (clone FN50) were purchased from Thermo Fisher Scientific; anti-CD161 (clone HP-3G10) and anti-TCR V α 7.2 (clone 3C10) were purchased from Sony Biotechnology and anti-CD3 (clone UCHT1) was purchased from BD Biosciences. The cell populations were defined as follows. T cells: CD3⁺CD19⁻; B cells: CD3⁻CD19⁺, NK cells: CD3⁻CD19⁻CD161⁺, MAIT cells: CD3⁺CD161⁺⁺TCRV α 7.2⁺. Cells were acquired with a Navios EX Flow Cytometer (Beckman Coulter) and analysed using Kaluza Analysis Software (Beckman Coulter).

Statistical analysis

The data was analysed using a Student's unpaired T-test, the Mann-Whitney test was used for comparisons between the particular groups of subjects (healthy, CLL WaW, Ibrutinib treated, Rituximab treated and pools of subjects described further) and the analysis of the dependence of the quantitative variables was calculated by Pearson correlation coefficient.

Results:

1. The vaccinated subjects.

71 subjects with completed SARS-CoV-2 vaccination were recruited; 21 healthy volunteers, 18 patients with yet untreated CLL, 16 patients treated with Ibrutinib and 16 patients treated with Rituximab (Table 1).

The “healthy volunteers” group contained 7 disease free haematological patients treated more than 5 years ago with curative intent for curable diagnosis (APL, AML, HL, DLBCL).

The Ibrutinib group contained 15 CLL and 1 MCL patients. The majority of the patients previously received several lines of cytotoxic therapies (mainly RB and RFC) and the Ibrutinib represented 2.5th line of treatment (median).

The Rituximab group contained 13 patients with FL and 3 patients with MCL. In all of the patients, the Rituximab was maintenance therapy of the first line treatment (mainly R-CHOP and RB). The median time between last dose of chemotherapy and the study examination was 16 months in this group.

The flow cytometry analysis revealed significant differences in the groups reflecting the original disease activity and the effect of the treatments. Obviously, the B-cell count was the most striking difference among the groups.

All 71 patients completed the vaccination with mRNA vaccines, either Pfizer/BioNTech (64 subjects) or Moderna (7 subjects). The time between the last vaccine and study examination was shorter in Rituximab group but did not differ among other groups. The vaccines induced mild either local (typically local pain) or general (typically fatigue) side effects in the majority of the subjects in similar frequency within all groups. None of the subjects developed serious side effects.

2. Humoral response to the vaccination

The vaccine induced serological response (defined as S-anti-SARS-CoV-2 IgG exceeding cut-off value according to laboratory practice) in 21 of 21 studied healthy subjects. Expectedly, none of maintenance Rituximab treated patients developed serological response. The response rate was also very low in Ibrutinib group (3 of 16). The serological response was observed in 9 of 18 subjects in “WaW CLL” group (Figure 1A).

3. Cellular response to the vaccination

The cellular response was assessed by using a commercial QuantiFERON SARS-CoV-2 kit by Qiagen. The kit analyses CD4⁺ (Ag1) and CD4⁺/CD8⁺ (Ag2) specific immunity upon to the stimulation of whole blood with the mix of SARS-CoV-2 peptides. Using the recommended cut-off of 0.2 IU/ml, the positive response of CD4⁺ cells was detected in 80% (17/21) of the healthy subjects, 44% (8/18) subjects in the “WaW CLL” group, 56% (9/16) in the Rituximab and 68% (11/16) in the Ibrutinib group (Figure 1B).

In agreement with a previous report ¹⁸, the Ag1 and Ag2 response showed a high concordance rate. Using the cut-off of 0.2 IU/ml the positive response of both CD4⁺ and CD8⁺ cells was detected in 80% (17/21) of the healthy subjects, 50% (9/18) subjects in the “WaW CLL” group, 62% (10/16) in the Rituximab and 68% (11/16) in the Ibrutinib group (Figure 1C).

Positive cellular response defined as positivity of either Ag1 or Ag2 or both was achieved in 80% (17/21) of the healthy subjects, 55% (10/18) subjects in the “WaW CLL” group, 62% (10/16) in the Rituximab and 75% (12/16) in the Ibrutinib group.

4. Factors associated with positive cellular response to SARS-CoV-2 mRNA vaccines

The study also tested factors that predict positive cellular response to the SARS-CoV-2 vaccine. The subjects were divided according to the cellular response to the negative (“Cellular negative”) and positive (“Cellular positive”) groups and compared in various clinical and laboratory parameters. Due to the low sample size, the comparison of the “Cellular negative” versus “Cellular positive” patients was performed not only within WaW, Rituximab and Ibrutinib groups separately, but also in a pooled analysis. Data of Rituximab and Ibrutinib patients were pooled, data of CLL patients from Ibrutinib and WaW groups were analysed as CLL group etc. (Table 2).

The pooled data of all patients showed that positive cellular response to SARS-CoV-2 vaccine was associated with younger age (72 years in “Cellular negative” versus 66 years in “Cellular positive” subjects, $p=0.01$) and previous COVID-19 status. The time between vaccination and study examination was significantly shorter in “Cellular positive” subjects across various subject groups (Table 2).

The flow cytometry analysis showed that responding subjects had lower total lymphocyte count and lower percentage of B cells at the expense of the percentage of T cells. Unlike the absolute cellularity of T cells, which was identical in both groups (Cellular positive and Cellular negative respectively), the total amount of B cells was significantly higher in “Cellular negative” subjects in Ibrutinib-treated and CLL patients (Table 2).

Discussion:

The pandemic of SARS-CoV-2 killed nearly 5 million of people worldwide and has significant impact on the lives of the whole planet. The most powerful weapon against the pandemic is the vaccination that significantly reduces the mortality and morbidity caused by the virus.¹⁹ The effect of the vaccination is routinely monitored by the examination of the antibodies (anti-Spike protein). Immunocompromised and haematologic patients (mainly those receiving B cell-depleting therapies) are unable to produce antibodies thus making the assessment of vaccination efficacy complicated. This was confirmed by our work that showed that none of anti-CD20 treated patients and only 18% of Ibrutinib treated patients (3 from 16) produced anti-S antibodies. This data is identical with what has been observed by Herishanu and colleagues (none of the patients exposed to anti-CD20 antibodies < 12 months before vaccination and 16% patients treated with BTKi serologically responded)¹³ and similar to data of Roeker and colleagues (32% of the patients exposed to anti-CD20 antibodies < 12 months before vaccination and 32% patients treated with BTKi serologically responded).¹²

Experimental data however show that an important role in the defence against viruses including SARS-CoV-2 is played by T cells that are also abundantly generated by the vaccines.^{14–17} To analyze the cellular immune response, we have chosen commercially available whole blood Interferon- γ Release immune Assay that uses mixes of SARS-CoV-2 S proteins selected to activate both CD4⁺ and CD8⁺ T cells. This test showed certain concordance with anti-S antibody response in healthy subjects.^{18,20} Our analysis showed that significant fraction of both Ibrutinib and Rituximab treated patients develop cellular response to the vaccination (75% and 62% respectively). This is not completely surprising, as similar data was reported either by case reports or by small patient series. For example, Hueso and colleagues²¹ analyzed T-cell response by ELISPOT in 3 anti-CD20 treated patients and reported reactivity in all of them. Malard and colleagues²² used ELISPOT to show that the hematological patients develop a T-cell response to the BNT162b2 vaccine. The anti-B-cell treatment did not affect the probability of developing the response. One should emphasize that the studies vary by methods used to analyze T-cell response and peptide pool selection. Their comparison is complicated but the similarities are striking.

The probability of developing the cellular response increased with higher percentage of T cells. It seems intuitive, as larger T cell pool would have higher probability of containing SARS-CoV-2 specific T cells and generating IFN- γ upon specific stimulation. However, the

cellular response to vaccination does not correlate with absolute amount of T cells. Instead, it inversely correlates with the amount of B cells in Ibrutinib-treated and CLL groups suggesting that the generation of the cellular immune response to vaccine is hampered by the disease burden. It is not surprising as malignant B cells (typically CLL) inhibit cellular immunity.²³ Moreover, the analysis of CLL patients (WaW + Ibrutinib) showed that the cellular immune response inversely correlates with the fraction and total amount of CD69 positive B-cells (not shown). CD69 overexpression significantly correlates with disease burden and prognosis (advanced Rai stages, larger lymphadenopathy, splenomegaly, shorter progression free survival and overall survival).²⁴ Finally, common markers of disease activity, such as LDH and beta-2-microglobulin, were both increased (not statistically significantly) in non-responding CLL patients.

An interesting observation is provided by the comparison of WaW and Ibrutinib treated patients. The proportion of responding patients is slightly higher in the Ibrutinib group suggesting that Ibrutinib can overcome the negative impact of CLL on the cellular immunity. It has indeed been shown that Ibrutinib restores T cell number and function in CLL patients by various mechanisms including targeting of PD-1 or CTLA-4.^{25,26}

Our analysis across practically all groups showed that responding patients have shorter time from vaccination to analysis than non-responding patients. It suggested that the cellular response weakens through the time as it was raised by epidemiologic and immunological studies.²⁷⁻³⁰

In conclusion our work shows that humoral immune response to SARS-CoV-2 vaccine is severely impaired in patients treated with Rituximab and Ibrutinib. Significant fraction of these patient mounts cellular immune response to the vaccine. The capacity to develop cellular response is compromised by disease burden in CLL patients.

Figure legends:

Figure 1. Humoral and cellular immunity to SARS-CoV-2 m-RNA vaccine

The immune response was evaluated 1-192 days (median 38 days) after the second dose of mRNA vaccine. Each bar corresponds to one subject in the study. (A)

Serological response to vaccination is defined as S-anti-SARS-CoV-2 IgG exceeding cut-off index $< 1,00$, with uppermost value measurable in clinical laboratory being cut-off index 150. According to internal laboratory standard cut-off value 1 is equal to 1,00U/ml. Negative subjects are schematically marked by white color below the line.

IFN- γ response (IU/ml) to SARS-CoV-2 peptides by CD4⁺ T cells (B) and CD4⁺ plus CD8⁺ T cells (C) measured by ELISA. Cut-off value of 0.2 IU/ml was used for determination of positivity, with negative subjects schematically marked by white color below the line.

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Reference list:

1. Forconi, F. & Moss, P. Perturbation of the normal immune system in patients with CLL. *Blood* **126**, 573–581 (2015).
2. Bergmann, L., Fenchel, K., Jahn, B., Mitrou, P. S. & Hoelzer, D. Immunosuppressive effects and clinical response of fludarabine in refractory chronic lymphocytic leukemia. *Annals of Oncology* **4**, 371–375 (1993).
3. McLaughlin, P. *et al.* Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. <https://doi.org/10.1200/JCO.1998.16.8.2825> **16**, 2825–2833 (2016).
4. van der Kolk, L. E., Baars, J. W., Prins, M. H. & van Oers, M. H. J. Rituximab treatment results in impaired secondary humoral immune responsiveness. *Blood* **100**, 2257–2259 (2002).
5. Zhuang, W.-H. & Wang, Y.-P. Analysis of the immunity effects after enhanced hepatitis B vaccination on patients with lymphoma. *Leukemia & Lymphoma* **61**, 357–363 (2020).
6. Mauro, F. R. *et al.* Response to the conjugate pneumococcal vaccine (PCV13) in patients with chronic lymphocytic leukemia (CLL). *Leukemia* **2020 35:3** **35**, 737–746 (2020).
7. B, A. *et al.* Lack of adequate pneumococcal vaccination response in chronic lymphocytic leukaemia patients receiving ibrutinib. *British journal of haematology* **182**, 712–714 (2018).
8. Sun, C. *et al.* Seasonal Influenza Vaccination in Patients With Chronic Lymphocytic Leukemia Treated With Ibrutinib. *JAMA Oncology* **2**, 1656–1657 (2016).
9. Douglas, A. P. *et al.* Ibrutinib may impair serological responses to influenza vaccination. *Haematologica* **102**, e397–e399 (2017).
10. Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. <https://doi.org/10.1056/NEJMoa2034577> **383**, 2603–2615 (2020).
11. Baden, L. R. *et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. <https://doi.org/10.1056/NEJMoa2035389> **384**, 403–416 (2020).
12. Roeker, L. E. *et al.* COVID-19 vaccine efficacy in patients with chronic lymphocytic leukemia. *Leukemia* **2021 35:9** **35**, 2703–2705 (2021).
13. Y, H. *et al.* Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood* **137**, 3165–3173 (2021).
14. Sahin, U. *et al.* COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature* **2020 586:7830** **586**, 594–599 (2020).
15. C, R. M. *et al.* Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. *Cell* **183**, 996–1012.e19 (2020).

16. Peng, Y. *et al.* Broad and strong memory CD4+ and CD8+ T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. *Nature Immunology* 2020 21:11 **21**, 1336–1345 (2020).
17. Bange, E. M. *et al.* CD8+ T cells contribute to survival in patients with COVID-19 and hematologic cancer. *Nature Medicine* 2021 27:7 **27**, 1280–1289 (2021).
18. Martínez-Gallo, M. *et al.* Commercialized kit to assess T-cell responses against SARS-CoV-2 S peptides. A pilot study in Health Care Workers. *medRxiv* 2021.03.31.21254472 (2021) doi:10.1101/2021.03.31.21254472.
19. JR, T. & DL, F. COVID-19 vaccines: modes of immune activation and future challenges. *Nature reviews. Immunology* **21**, 195–197 (2021).
20. van Praet, J. T., Vandecasteele, S., de Roo, A., de Vriese, A. S. & Reynders, M. Humoral and Cellular Immunogenicity of the BNT162b2 Messenger RNA Coronavirus Disease 2019 Vaccine in Nursing Home Residents. *Clinical Infectious Diseases* (2021) doi:10.1093/CID/CIAB300.
21. T, H. *et al.* Convalescent plasma therapy for B-cell-depleted patients with protracted COVID-19. *Blood* **136**, 2290–2295 (2020).
22. Malard, F. *et al.* Weak immunogenicity of SARS-CoV-2 vaccine in patients with hematologic malignancies. *Blood Cancer Journal* 2021 11:8 **11**, 1–8 (2021).
23. P, L. & B, E. Immune Dysfunction in Patients with Chronic Lymphocytic Leukemia and Challenges during COVID-19 Pandemic. *Acta haematologica* **144**, (2021).
24. Poeta, G. del *et al.* CD69 is independently prognostic in chronic lymphocytic leukemia: a comprehensive clinical and biological profiling study. *Haematologica* **97**, 279–287 (2012).
25. Long, M. *et al.* Ibrutinib treatment improves T cell number and function in CLL patients. *The Journal of Clinical Investigation* **127**, 3052–3064 (2017).
26. Solman, I. G. *et al.* Ibrutinib restores immune cell numbers and function in first-line and relapsed/refractory chronic lymphocytic leukemia. *Leukemia Research* **97**, 106432 (2020).
27. Goldberg, Y. *et al.* Waning immunity of the BNT162b2 vaccine: A nationwide study from Israel. *medRxiv* 2021.08.24.21262423 (2021) doi:10.1101/2021.08.24.21262423.
28. Naaber, P. *et al.* Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study A R T I C L E I N F O. (2021) doi:10.1016/j.lanepe.2021.100208.
29. Khoury, D. S. *et al.* Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature Medicine* 2021 27:7 **27**, 1205–1211 (2021).
30. RR, G. *et al.* mRNA Vaccination Induces Durable Immune Memory to SARS-CoV-2 with Continued Evolution to Variants of Concern. *bioRxiv : the preprint server for biology* (2021) doi:10.1101/2021.08.23.457229.

Figures

Figure 1

Humoral and cellular immunity to SARS-CoV-2 m-RNA vaccine The immune response was evaluated 1-192 days (median 38 days) after the second dose of mRNA vaccine. Each bar corresponds to one subject in the study. (A) Serological response to vaccination is defined as S-anti-SARS-CoV-2 IgG exceeding cut-off index < 1,00, with uppermost value measurable in clinical laboratory being cut-off index 150. According to internal laboratory standard cut-off value 1 is equal to 1,00U/ml. Negative subjects are schematically marked by white color below the line. IFN- γ response (IU/ml) to SARS-CoV-2 peptides by CD4+ T cells (B) and CD4+ plus CD8+ T cells (C) measured by ELISA. Cut-off value of 0.2 IU/ml was used for determination of positivity, with negative subjects schematically marked by white color below the line.