Clinical outcomes, immunogenicity, and safety of BNT162b22 Vaccine in Primary Antibody Deficiency

Tomas Milota[1], Jitka Smetanova[1], Aneta Skotnicova[1], Michal Rataj[1], Jan Lastovicka[1],
Hana Zelena[2], Zuzana Parackova[1], Martina Fejtkova[1], Veronika Kanderova[1], Eva
Fronkova[1], Katerina Rejlova[1], Anna Šedivá[1], and Tomas Kalina[1]

[1]Univerzita Karlova 2 lekarska fakulta
[2]Zdravotni ustav se sidlem v Ostrav

June 27, 2022

Abstract

Background: Common variable immunodeficiency (CVID) is characterized by an impaired post-vaccination response, high susceptibility to respiratory tract infections, and a broad spectrum of non-infectious complications. Thus, patients with CVID are at high risk of coronavirus disease 2019 (COVID-19), and vaccination's role in prevention is questionable. The main aim of this study was to evaluate the clinical outcomes, safety, and dynamics of humoral and T-cell immune responses induced by the mRNA vaccine BNT162b2 in CVID. **Methods**: This prospective observational cohort study focused on the clinical outcomes (proportion of infected patients, disease severity), safety (adverse-event incidence, laboratory-parameter changes), and dynamics of humoral (specific post-vaccination and virus-neutralizing-antibody assessment) and T-cell immune responses (anti-SARS-CoV-2 specific T-cell detection) in 21 patients with CVID after a two-dose administration of BNT162b2. The patients were followed for 6 months. **Results**: Humoral response was observed in 52% (11/21) of patients at month 1 post-vaccination but continuously decreased to 33.3% (5/15) at month 6. Nevertheless, they had a remarkably lower anti-SARS-CoV-2 neutralizing antibody titer than healthy controls. The T-cell response was measurable in 33% (6/17) of patients with CVID at month 1, and it persisted for the study period. Mild infection occurred in three patients (14.3%) within the follow-up period. The vaccine also exhibited a favorable safety profile. **Conclusions**: The BNT162b2 vaccine elicited a measurable antibody response in a high proportion of patients, but it was limited by low titer of the virus-neutralizing antibodies and rapid waning of anti-RBD SARS-CoV-2 specific antibodies. T-cell response was detected in one-third of the patients and remained stable within the follow-up period. Vaccination has favorable safety and clinical-related outcomes in preventing severe COVID-19.

Clinical outcomes, immunogenicity, and safety of BNT162b22 Vaccine in Primary Antibody Deficiency

Running title: **Anti-SARS-CoV-2 Vaccination in Primary Antibody Deficiency**

Tomas Milota, PhD[1,#], Jitka Smetanova, MSc[1#*], Aneta Skotnicova, MSc[2#], Michal Rataj, MSc[1], Jan Lastovicka, MSc[1], Hana Zelena, PhD[3], Zuzana Parackova, PhD[1], Martina Fejtkova, MSc[2], Veronika Kanderova, PhD[2], Eva Fronkova, PhD[2], Katerina Rejlova, PhD[2], Anna Sediva, PhD[1], Tomas Kalina, PhD[2]

#These authors contributed equally to this work.

[1]Department of Immunology, Second Faculty of Medicine Charles University and Motol University Hospital, Prague, Czech Republic

[2]Childhood Leukemia Investigation Prague, Second Faculty of Medicine Charles University and Motol University Hospital, Prague, Czech Republic

1

[3]Department of Virology, Public Health Institute, Ostrava, Czech Republic

*Corresponding author:Tomas Milota, PhD., MD.

Department of Immunology, Second Faculty of Medicine Charles University, Motol University Hospital, V Uvalu 84, Prague 15006, The Czech Republic

Phone: +420 224435961; fax: +420 224435962; email:

tomas.milota@fnmotol.cz

Abstract

Background : Common variable immunodeficiency (CVID) is characterized by an impaired post-vaccination response, high susceptibility to respiratory tract infections, and a broad spectrum of non-infectious complications. Thus, patients with CVID are at high risk of coronavirus disease 2019 (COVID-19), and vaccination's role in prevention is questionable.

The main aim of this study was to evaluate the clinical outcomes, safety, and dynamics of humoral and T-cell immune responses induced by the mRNA vaccine BNT162b2 in CVID.

Methods : This prospective observational cohort study focused on the clinical outcomes (proportion of infected patients, disease severity), safety (adverse-event incidence, laboratory-parameter changes), and dynamics of humoral (specific post-vaccination and virus-neutralizing-antibody assessment) and T-cell immune responses (anti-SARS-CoV-2 specific T-cell detection) in 21 patients with CVID after a two-dose administration of BNT162b2. The patients were followed for 6 months.

Results : Humoral response was observed in 52% (11/21) of patients at month 1 post-vaccination but continuously decreased to 33.3% (5/15) at month 6. Nevertheless, they had a remarkably lower anti-SARS-CoV-2 neutralizing antibody titer than healthy controls. The T-cell response was measurable in 33% (6/17) of patients with CVID at month 1, and it persisted for the study period. Mild infection occurred in three patients (14.3%) within the follow-up period. The vaccine also exhibited a favorable safety profile.

Conclusions : The BNT162b2 vaccine elicited a measurable antibody response in a high proportion of patients, but it was limited by low titer of the virus-neutralizing antibodies and rapid waning of anti-RBD SARS-CoV-2 specific antibodies. T-cell response was detected in one-third of the patients and remained stable within the follow-up period. Vaccination has favorable safety and clinical-related outcomes in preventing severe COVID-19.

Key words: common variable immunodeficiency, COVID-19, mRNA vaccine, post-vaccination response

Abbreviations

ACE-2, angiotensin-converting enzyme 2

AE, adverse event

APRIL, a proliferation-inducing ligand

BAFF, B-cell-activating factor

COVID-19, coronavirus disease 2019

CVID, common variable immunodeficiency

ELISA, enzyme-linked immunosorbent assay

ESID, European Society for Immunodeficiencies

HCs, healthy controls

IB, immunoblot

ICON, International Consensus Document

IEI, inborn errors of immunity

IL, interleukin

IFN, interferon

IRT, immunoglobulin replacement therapy

NCP, nucleocapsid protein

PGA-VAS, Patient Global Assessment-Visual Analog Scale

RBD, receptor-binding domain

RT-PCR, reverse transcription polymerase chain reaction

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

INTRODUCTION

The coronavirus disease 2019 (COVID-19) outbreak has affected over 500 million individuals and caused over 6 million deaths worldwide since its emergence in 2019 . Severe COVID-19 infections with poorer outcomes have been reported in immunocompromised patients, such as those with inborn errors of immunity (IEI) . Conversely, Marcus et al. reported neither more severe disease nor excess hospital admissions in a cohort of patients with IEI and implied a high awareness level, extra precautions, and even self-isolation as possible explanations . However, preventive measures only provide short-term protection; therefore, vaccination may offer a long-term effective and safe solution.

At present, two mRNA (BNT162b2, also known as Comirnaty, Pfizer/BioNTech; mRNA-173, also known as Spikevax, Moderna), one recombinant-subunit (Novaxovid, Novavax), and two viral-vector (AZD1222, also known as Vaxzevria, AstraZeneca; Ad26.COV2.S, also known as COVID-19 Vaccine Janssen, Janssen/Johnson & Johnson) vaccines have been approved by the European Medicines Agency for COVID-19prevention. The vaccines induce high levels of immunogenicity and efficacy, ranging from 66.9% to 90.4%, and demonstrate a favorable safety profile, with a dominant prevalence of local reactions in the general population . However, severe AEs, such as thromboembolic events or peri- and myocarditis, have been reported . Additionally, evidence regarding their use in specific patient populations is limited, particularly those with heavily impaired antibody production, disturbed cellular immunity, and/or immune system dysregulation.

Common variable immunodeficiency (CVID) is the most prevalent IEI, characterized by impaired vaccination-induced specific antibody production , immune dysregulation, and partially impaired T-cell phenotype and function . Therefore, this study investigated the immunogenicity, safety, and clinical outcomes of the mRNA vaccine BNT162b2 in a cohort of patients with CVID.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Motol University Hospital Ethics Committee (nr. EK-753.1.3/21, issued 10th June 2020) and conducted according to the guidelines of the Declaration of Helsinki. Informed consent was obtained from all patients.

Study design

This prospective observational study focused on the immunogenicity, safety, and efficacy of the mRNA vaccine BNT162b2. The study followed STROBE recommendations (STrengthening the Reporting of OBservational studies in Epidemiology) . Patients received two standard doses (0.3 mL/30 μg mRNA) intramuscularly within the recommended 3-week interval and were followed up for 6 months post-vaccination. The follow-up

period was divided into six consecutive visits. The study design is illustrated in Fig 1. This study was conducted from March 2021 to November 2021.

Study population

This study included adult patients with CVID meeting the European Society for Immunodeficiency (ESID)/International Consensus Document (ICON) diagnostic criteria , and vaccination was indicated by the attending immunologist and performed by specialized vaccination centers. Patients with pre-vaccination reverse transcription polymerase chain reaction (RT-PCR)-confirmed SASRS-CoV-2 infection or severe non-infectious complications were excluded. Their results were compared with those of corresponding sex- and age-matched healthy controls (HCs).

B- and T-cell immunophenotyping

B-cell subpopulations (including CD21^{low}, naïve, transitional, marginal zone-like, class-switched cells, and plasmablasts) and T-cell subpopulations (recent thymic emigrants [RTE], naïve, central memory [CM], effector memory [EM], effector memory expressing CD45RA [TEMRA], and activated T cells) were analyzed using antibody-fluorochrome conjugates for fluorescence-activated cell sorting (FACS) (Supplementary Table 1).

Humoral-response assessment

Enzyme-linked immunosorbent assay (ELISA) COVID-19 receptor-binding domain (RBD) IgG (TestLine Clinical Diagnostics, Brno, Czech Republic) kits were used to measure anti- SARS-CoV-2 IgG titers (positive cutoff value > 18 U/mL), and anti-nucleocapsid (NCP), anti-Spike 2, anti-protein E, anti-ACE2, and RBD antibodies (positive cutoff value > 180 U/mL) were measured using the immunoblot assay (IB, Microblot-Array COVID-19 IgG, TestLine Clinical Diagnostics, Brno, Czech Republic).

The virus neutralization test (VNT) was performed according to a previously published protocol using the SARS-CoV-2 strain extracted from a clinical sample (hCoV-19/Czech Republic/NRL_9640/2020|EPI_ISL_-626593) and CV-1 cells (African green monkey kidney fibroblasts). Serum samples were diluted to a final serum concentration of 1/10-/2560. Thereafter, uninfected cells were stained with neutral red dye. VNT results were expressed in the form of a virus-neutralization titer, representing an inverted value of the highest sample dilution neutralizing the virus's cytopathic effect by > 50%. Positivity was determined using a titer [?] 20.

Responders were defined as individuals in whom anti-RBD SARS-CoV-2 specific antibodies exceeding the positive cutoff level were detected by IB and ELISA at month 1. The humoral response was also determined by measuring serum B-cell-activating factor (BAFF), a proliferation-inducing ligand (APRIL), and interferon-α (IFN-a) levels using ELISA (BAFF, R&D Systems, Minneapolis, MN, USA; APRIL, Abcam, Cambridge, UK; and IFNa, Thermo Fisher Scientific).

Anti-RBD SARS-CoV-2 specific antibody presence was also tested in immunoglobulin replacement therapy (IRT) solutions used for immunoglobulin substitution in the enrolled patients with CVID (Kiovig and HyQvia, Takeda Manufacturing, Vienna, Austria; Hizentra, CSL Behring, Marburg, Germany). All solutions were diluted with a 5% bovine serum albumin (BSA, lyophilized IgG-free powder, Merck KGaA, Darmstadt, Germany) solution to a 1% concentration, corresponding to the IgG concentration in human plasma.

T-cell response

The T-cell response was assessed as previously described . Briefly, peripheral blood mononuclear cells (PBMCs) were stimulated with 4 µL of BD Fast ImmuneTM CD28/CD49d (BD Biosciences, San Jose, CA, USA) and PepMix^{TM} SARS-CoV-2 S-RBD/ NCAP (JPT Peptide Technologies, Berlin, Germany) or anti-human CD3 low endotoxin as a positive control. After incubation, PBMCs were stained with antibody-fluorochrome conjugates for FACS. Subsequently, cells were stained for viability with LIVE/DEAD Violet Viability Dye (Invitrogen, Waltham, MA, USA). Finally, samples were measured on a BD LSR II flow cytometer to detect intracellular production of the cytokines interleukin 2 (IL-2), interferon α (IFNγ), and

tumor necrosis actor α (TNFα) in CD4+ T cells, and data were analyzed using FlowJo software (version 10.6.1; BD Biosciences).

Only patients who responded to non-specific anti-CD3 stimulation were considered for further analysis of antigen-specific responses. A positive response required a >1.5-fold increase above the non-stimulated controls and detection of >20 responding cells, as previously described for sensitive CMV-specific T-cell detection . All combinations of IL-2, IFNγ, and TNFα production upon stimulation were analyzed for the total CD4+ T-cell response. The gating strategy is illustrated in Supplementary Fig 1.

Safety assessment

Adverse events (AEs) were reported using the Patient Clinical Questionnaire, focusing on local (injection-site reactions) and systemic (fever, headache, myalgia, and arthralgia) reactions and emergency medication (e.g., analgesic/antipyretics drugs). Pain intensity was self-assessed by patients on a 100-point visual analog scale (PGA-VAS). Severe AE (SAEs) was defined as acute conditions requiring hospital admission or urgent medical intervention followed by vaccination. Further, hematological, immunological, and biochemical parameters were measured using routine and standardized laboratory tests for safety assessment. All assessed parameters are listed in Supplementary Table 2.

Clinical outcomes

The primary outcome of vaccination was defined as the proportion of patients with CVID in whom SARS-CoV-2 infection was not confirmed by RT-PCR. RT-PCR testing was indicated in cases where respiratory-tract-infection symptoms were present or following risk contact with a SARS-CoV-2-positive person. Secondary efficacy was defined as COVID-19 severity in RT-PCR-positive patients with CVID, who were divided into four groups: asymptomatic, mild (symptomatic treatment only), moderate (antiviral drugs and/or anti-SARS-CoV-2 monoclonal antibodies), and severe (hospital admission).

Statistical analysis

The mean and standard deviation (SD) were calculated for continuous data (age, body mass index, and laboratory parameters). Statistically significant differences in the means were assessed using the Mann–Whitney and Wilcoxon tests for non-normally distributed unpaired and paired data, respectively. Normality was tested using the Shapiro–Wilk normality test. Proportions were calculated for attributive data, and statistically significant differences in proportions were evaluated using Fisher's exact test. Correlation was determined using Spearman's rank correlation coefficient (r). Statistical significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

RESULTS

Patient characteristics

Twenty-one patients with CVID (14 women and 7 men) were enrolled in the study. The mean age was 46.3 years (±9.7 SD). The mean disease duration was 9.1 years (±7.49). All patients underwent regular IRT with intravenous (IVIG: 23.8%, n=5/21) or subcutaneous (SCIG: 76.2%, n=16/21) administration, with mean dose of 287.6 mg/kg/month (± 69.6). The mean serum IgG trough level was 6.13 g/L (±1.59). Antibiotic prophylaxis was indicated in three patients with CVID (14.3%), including cotrimoxazole and macrolides. Non-infectious complications were observed in 16 patients (76.2%), and the most prevalent complications were bronchial asthma (28.6%, n=6/21), autoimmune thyroiditis (23.8%, n=5/21), enteropathy (23.8%, n=5/21), chronic lung disease (19%, n=4/21), splenomegaly (19%, n=4/21), vitiligo (19%, n=4/21), immune thrombocytopenic purpura (9.5%, n=2/21), lymphadenopathy (9.5%, n=2/21), and sarcoid-like disease (9.5%, n=2/21). Other complications were present in <2 patients, and 6 patients exhibited >2 complications. Three patients (14.3%) were on active immunosuppression with glucocorticosteroids upon vaccination, and five (23.8%) had previously received immunosuppressive therapy. Patient characteristics are summarized in Table 1.

Humoral immune response

One month after the second BNT162b2 dose, anti-RBD SARS-CoV-2 specific antibodies were detected in 52.4% of patients (n=11/21) using the IB assay (574.4±454 U/mL, Fig 2A). The humoral immune response was confirmed using ELISA (Fig 2B). Other anti-SARS-CoV-2 specific antibodies, such as NCP and anti-Spike S2, were detected in two patients; however, they neither developed symptomatic infection nor had positive RT-PCR results for SARS-CoV-2, suggesting asymptomatic infection.

The humoral response was comparable to that of the HCs (870±225.0 U/mL). However, patients with CVID had significantly lower virus-neutralizing antibody titers (49.4±81.5 U/mL vs. 960±1093 U/mL,p <0.0001, Fig 2C), which persisted at month 3 in 44.4% (n=8/18) of patients; however, they were significantly lower than those of the HCs (436.3 ± 415.4 U/ml vs. 900.3 ± 232.7% U/mL,p =0.0002). On the other hand, the level of neutralizing antibodies correlated with the concentration of anti-RBD SARS-CoV-2 specific antibodies (r= 0.82, p= 0.0001). Three patients were lost to follow-up. The antibody titer decreased further at 6 months and was detected in only 33.3% (5/15) of patients. The mean anti-RBD-specific IgG level was 218.2 U/mL (±268.5) in patients with CVID and 1056 U/mL (±360.4) in HCs, with statistically significant differences (p <0.0001; Fig 2A). Three patients were excluded based on RT-PCR-confirmed SARS-CoV-2 infection during the study.

Responders and non-responders did not differ in sex, disease duration, proportion of ATB prophylaxis, IRT dose, or IgG trough levels; however, we observed significant differences between age and serum IgM concentration. The responder group comprised significantly younger patients with CVID (39.1±8.1 years vs. 51.36±8.13 years; p =0.003) who had significantly higher serum IgM levels (0.29±0.21 g/L vs. 0.1±0.06 g/L; p =0.002). The humoral-response level (serum anti-RBD SARS-CoV-2 specific antibody concentration) also negatively correlated with higher age (r=-0.61, p =0.003) as well as serum IgM (r=0.46, p =0.036). The humoral response was not influenced by ongoing or previous immunosuppression or the presence of non-infectious complications. We did not observe any significant differences in the T-cell phenotypes. However, responders had a significantly higher proportion of CD19+ class-switched (CS) B cells (7.58±3.09 vs. 3.39±2.09%; p =0.009; Table 1). The number of CD19+CS B cells correlated with the concentration of anti-RBD SARS-CoV-2 specific antibodies (r= 0.58, p = 0.04).

Additionally, we assessed APRIL, BAFF, and IFNa as potential markers of humoral response that contribute to B-cell maturation, survival, and class switch. However, we did not observe any significant differences in the serum BAFF concentration (1841±527 vs. 2496±1183 pg/mL;p =0.61), APRIL (8.4±5.39 vs. 7.54±10.7 pg/mL; p =0.21), and IFNa (25.06±8.41 vs. 21.72±6.22 pg/mL; p =0.3) between responders and non-responders at month 1.

We tested all used IRT products to exclude the presence of anti-RBD SARS-CoV-2 specific antibodies leading to a false interpretation of vaccination response. Specific-antibody levels did not exceed the positive cutoff value (>18 U/mL) in any of the products (Kiovig: 7.38±0.4; HyQvia: 6.22±1.1; and Hizentra: 7.95±0.66 U/mL).

T-cell immune response

One month after the second dose, CD4+ T cells in 33.3% (n=6/17) of patients responded to the S-RBD antigen in a short *ex vivo*stimulation and cytokine-production assays (Fig 3). Four patients were excluded due to low cell viability and/or unresponsiveness to CD3 stimulation. Among the HCs, 73% (n=8/11) responded (one was excluded); however, the proportion of responders and percentage of responding CD4+ T cells did not significantly differ. Fifty percent (3/6) of patients with CVID with T-cell immune responses also responded with specific antibody production.

Thereafter, we investigated the CD4+ T-cell response's persistence in 11 patients 6 months post-vaccination. The remaining patients were excluded due to loss to follow-up (n=3), or RT-PCR confirmed SARS-CoV-2 infection (n=3). Despite antibody-level decline, we observed a response in 50% (6/12) of patients, similar to that observed in month 1 (Fig 3). T cells, along with a humoral immune response, were observed in 5 of these patients (62.5%). The proportion of responders among the HCs remained constant (60%, n=9/15).

6

Clinical outcomes

Eighteen out of the 21 (85.7%) vaccinated patients neither developed COVID-19-infection symptoms nor tested positive following a risk contact with a SARS-CoV-2-infected person. COVID-19 infection was confirmed in three (14.3%) vaccinated patients with CVID (two females and one male; 38.33±7.57 years; range: 33–47 years) at the end of the follow-up period at month 6 (November 2021). Two of them had CVID-associated non-infectious complications (splenomegaly, chronic enteropathy, and autoimmune thyroiditis). The infection was mild in all three patients. The major symptoms were fever, arthralgia, and myalgia, which were present in all patients. Two patients reported cough, rhinitis, gastrointestinal symptoms, and fatigue. One patient developed a loss of smell. None of the patients required antiviral treatment or hospital admission. Two of them were classified as responders to vaccination; however, the humoral response persisted in a single patient at month 3. The three patients did not have a cellular response.

Safety

AEs were reported in 90% (n=19/21) of patients with CVID after the first and second doses. The most common event was local pain at the injection site (20/21), followed by fatigue (10/21), headache (7/21), fever (5/21), myalgia (3/21), and arthralgia (2/21) after the first dose. A similar AE spectrum was observed after the second dose. AE incidence is summarized in Fig 4. AE mean durations after the first and second doses were 3.55 (±2.19 SD) and 2.95 (±2.04) days, respectively. PGA-VAS-100 was 18.25 (±21.96) and 16.75 (±22.38) points. We also evaluated a broad spectrum of laboratory parameters, including biochemical, hematological, immunological, and inflammatory parameters. We detected significantly increased soluble CD25 levels after both vaccine doses. No changes in the total blood count, liver or renal function, and coagulation were observed, and no autoantibodies were detected during the follow-up period. Vaccination did not increase inflammatory markers, except for soluble IL-2R (sCD25) levels, which were significantly increased after both vaccine doses. A complete overview of these parameters is provided in Supplementary Table 1.

DISCUSSION

CVID is characterized by recurrent and chronic respiratory tract infections and a broad spectrum of non-infectious complications, including chronic lung disease. Therefore, patients with CVID are at high risk of severe COVID-19 associated with poor outcomes. Despite an impaired specific antibody response to protein as well as polysaccharide antigens in patients with CVID , T-cell-mediated immunity is intact in most patients with CVID . Therefore, patients with CVID may benefit from vaccination against COVID-19, which may induce a specific T-cell response .

Owing to the COVID-19 outbreak, issues regarding immunogenicity, safety, and efficacy of vaccination in patients with IEI have been raised. However, studies on the immunogenicity and safety of vaccination in a broad spectrum of IEI have shown encouraging results. Amodio et al. revealed humoral and cellular responses in 86% and 76% of 21 patients with IEI, respectively, with no correlation with patient age , which is in contrast to the study by Hagin et al. Both authors also reported a lower humoral response than that in the general population, and only 4 (out of 12 patients) did not develop a cellular response . Similar findings were reported by Delmonet et al., in which specific anti-SARS antibodies were detected in 63 of 74 patients with IEI (85.1%). Furthermore, Leuween et al. demonstrated a negative correlation between the presence of non-infectious complications and immunosuppression in a large study of 505 patients, including 196 patients with CVID; however, these patients were vaccinated with the mRNA-1273 vaccine . Another significant limitation of previously published studies is the lack of prospective follow-up and limited vaccine-safety data in patients with IEI.

In the present study, a specific antibody response was observed in 52.4% of patients 1-month post-vaccination, and anti-RBD SARS-CoV-2 antibody levels were comparable to those in HCs. Nevertheless, seemingly favorable humoral responses differed significantly in qualitative properties and persistence over time. The neutralizing-antibody titer, which is predictive of the protection level , suggested a qualitative insufficiency, which is consistent with a previous study reporting a reduced capacity to produce virus-neutralizing

7

antibodies in CVID . Additionally, the humoral response was not influenced by baseline or previous immunosuppression, or the presence of non-infectious complications. However, this might have been limited by the small number of patients included in our study. Responders were further characterized by a lower age (<40 years) and higher proportion of class-switched B cells, which is consistent with a previous study reporting an increased number of $CD21^{low}$ B cells, suggesting a possible dysregulation in the immune response to vaccination . Moreover, we demonstrated that a higher serum IgM concentration is a novel potential positive-response predictor. However, we did not observe differences in CD4 and CD8 subsets between responders and non-responders.

Additionally, although a specific T-cell response was detected at month 1 in less than half of the patients with CVID in our study, the proportion of responders was not significantly different than that observed in HCs (73%). Importantly, the proportion of both patients with CVID and HCs with persistently measurable Spike-specific T cell responses remained the same at month 6. However, we excluded four and five patients with CVID from T-cell response analysis at months 1 and 6, respectively, due to an absent anti-CD3 response and/or low viability which might have been related to T-cell abnormalities in CVID . Based on the immunophenotyping findings and central role of APRIL and BAFF in the survival and maturation of B cells and their dysregulation in CVID , we examined the serum concentration of both cytokines as potential response markers; however, we did not observe any significant differences in their levels between responders and non-responders at month 1. Additionally, no differences were found in serum IFNα concentrations, which can promote isotype switching .

Despite the promising antibody response rate at month 1, the proportion of responders rapidly decreased to 44.4% and 33.3% at 3 and 6 months, respectively. Anti-SARS-CoV-2 antibody titers were also significantly lower than those in HCs at the end of the study. The results were not influenced by IRT, and no specific anti-RBD antibodies were detected in IRT solutions. Noteworthily, previous studies used different methods to assess humoral and cellular responses. Therefore, our findings must be compared and interpreted with caution. Moreover, the specific anti-SARS antibody level that can predict immune protection remains unknown, and the efficacy of vaccination needs to be confirmed by long-term observation.

While a high vaccine-efficacy level has been observed in the general population, in our study, 3 of 21 patients (14.3%) tested positive for SARS-CoV-2 infection by RT-PCR. All patients were infected 6 months after second-dose administration when the SARS-CoV-2 Delta variant prevailed (November 21). The infected patients had a mild course and none developed a T-cell immune response, and the humoral response persisted in a single patient upon infection. Therefore, our data support booster vaccination in intervals shorter than 6 months for patients with CVID, as recommended for the general population .

Moreover, studies on vaccination safety in patients with IEI showed a favorable vaccination profile. In our study, AEs occurred in all patients with CVIDs, including injection-site reactions, fatigue, headaches, and fever. No SAE was reported. The spectra of the reactions after the first and second doses were comparable. Notably, we did not observe any changes in coagulation, including D-dimers, as a higher risk of thromboembolic events was described 15–21 days after BNT162b2 vaccine administration . Among the tested parameters, only soluble CD25 was significantly increased in patients with CVID post-vaccination.

In conclusion, to the best of our knowledge, this is the first study to investigate the long-term persistence of post-vaccination responses and clinical outcomes, providing data on a 6-month follow-up. We revealed that the anti-SARS-CoV-2 mRNA vaccine BNT162b2 induces a humoral response in a high proportion of patients with CVID. However, the vaccine induces lower anti-SARS-CoV-2 neutralizing antibody levels in patients with CVID than in the general population. Importantly, the antibody response was not persistent and continuously decreased 3 months after vaccination, whereas the CD4+ T-cell response persisted. We also demonstrated satisfactory clinical outcomes after vaccination in patients with CVID. No SARS-CoV-2 infections were reported within 5 months of the follow-up period, and only three patients (14.3%) tested positive for COVID-19; however, these patients had mild symptoms. Therefore, the BNT162b2 vaccine has a favorable safety profile in a proportion of patients with CVID, and this study supports booster vaccination in intervals shorter than 6 months for patients with CVID.

8

ACKNOWLEDGEMENTS

CONFLICTS OF INTERESTS

Tomas Milota, Jitka Smetanova, Aneta Skotnicova, Michal Rataj, Jan Lastovicka, Hana Zelena, Zuzana Parackova, Martina Fejtkova, Veronika Kanderova, Eva Fronkova, Krystof Seferna, Anna Sediva, and Tomas Kalina declare no conflict of interest relevant to this work.

REFERENCES

TABLES

Table 1. Patient characteristics

Characteristics	All	Responders	Non-resp
Demography	**Demography**	**Demography**	**Demogra**
Sex (females, %)	14/21 (66.7%)	6/10 (60%)	4/11 (36.4
Age (yrs., ± SD)	46.3 (9.7)	39.1 (8.10)	51.36 (8.13
Disease duration (yrs., ± SD)	9.1 (7.49)	7.4 (8.13)	10.8 (6.78)
Disease Characteristics	Disease Characteristics	Disease Characteristics	Disease Ch
Non-infectious complications (no. of pts., %)	16/21 (76.2%)	7/10 (70%)	9/11 (81.8
Baseline immunosuppression (no. of pts., %)	3/21 (14.3%)	1/10 (10%)	2/11 (18.2
Previous immunosuppression (no. of pts., %)	2/21 (9.5%)	0/10 (0%)	2/11 (18.2
Treatment Characteristics	**Treatment Characteristics**	**Treatment Characteristics**	**Treatmen**
IRT dose (mg/kg/month, ± SD)	288 (69.6)	262 (60.52)	310.9 (71.6
Serum IgG (trough g/L, ± SD)	6.13 (1.59)	6.28 (1.94)	6.0 (1.27)
ATB prophylaxis (no. of pts., %)	3/21 (14.3%)	1/10 (10%)	2/11 (18.2
Laboratory Parameters	**Laboratory Parameters**	**Laboratory Parameters**	**Laborato**
Serum IgA (g/L, ± SD)	0.1 (0.09)	0.13 (0.13)	0.08 (0.03)
Serum IgM (g/L, ± SD)	0.19 (0.18)	0.29 (0.21)	0.1 (0.06)
Lymphocytes (E9/L, ± SD)	1.49 (0.7)	1.52 (0.52)	1.42 (0.85)
CD4+ (% of CD3+ cells, ± SD)	57.69 (7.38)	59.20 (5.36)	56.75 (8.62
CD4+ naïve (% of CD4+ cells, ± SD)	15.51 (13.3)	22.44 (16.24)	11.18 (9.89
CD4+ TREG (% of CD4+ cells, ± SD)	12.27 (5.54)	15.04 (6.28)	10.54 (4.6
CD8+ (% of CD3+ cells, ± SD)	38.46 (8.17)	36.40 (6.07)	39.75 (9.41
CD8+ naïve (% of CD8+ cells, ± SD)	23.47 (9.58)	26.0 (7.42)	21.89 (10.8
CD8+ senescent (% of CD8+ cells, ± SD)	49.23 (16.44)	41.0 (18.75)	54.38 (13.5
CD19+ (% of lymphocytes, ± SD)	11.19 (6.79)	8.52 (3.06)	12.86 (8.09
CD19+ transitional (% of CD19 cells, ± SD)	7.48 (15.94)	2.34 (1.42)	10.7 (20.10

9

CD19+ naïve (% of CD19+ cells, ± SD)	68.46 (22.86)	56.0 (18.41)	76.25 (22.8
CD19+ CS (% of CD19+ cells, ± SD)	5.0 (3.2)	7.58 (3.09)	3.39 (2.09)
CD19+ CD21^{low} (% of CD19 cells, ± SD)	15.0 (21.16)	11.64 (6.99)	17.1 (26.96
T-Cell Response	**T-Cell Response**	**T-Cell Response**	**T-Cell Re**
Cellular response at month 1 (no. of pts., %)	5/13 (38.5%)	3/8 (37.5%)	2/5 (40%)
Cellular response at month 6 (no. of pts., %)	8/12 (66.7%)	5/7 (80%)	3/5 (60%)

Statistically significant differences (p [?] 0.05) between responders and non-responders are marked in bold. no., number; yrs., years; pts, patients; SD, standard deviation; NS, non-significant; IRT, immunoglobulin replacement therapy; ATB, antibiotics

FIGURES

Figure 1. Study timeline and profile of the study participants. CVID, common variable immunodeficiency; HCs, healthy controls; AE, adverse events; SAE, severe adverse events; RT-PCR, reverse transcription polymerase chain reaction; pts., patients

Figure 2. Humoral immune response in patients with CVID and HCs. **A** : Serum concentrations of anti-RBD SARS-CoV-2 specific antibodies at months 1 (M1), 3 (M3), and 6 (M6) measured by the immunoblot (IB; positive cutoff value >180 U/mL marked as grid line, graph A) or **B** : enzyme-linked immunosorbent (ELISA; positive cutoff value >18 U/mL marked as grid line) assays.**C** : Virus-neutralizing antibody titer (Ab) at month 1 (positive cut-off value >1:20); * $p < 0.05$, **$p < 0.01$, *** $p < 0.001$, **** $p< 0.000$. NS, not significant; CVID, common variable immunodeficiency; HC, healthy controls; RBD, receptor-binding domain

Figure 3. T-cell immune response. The proportion of IFNγ/TNFα/IL-2 producing CD4+ T cells (total count) in patients with CVID and HC measured at months 1 and 6. CVID, common variable immunodeficiency; HC, healthy controls; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin

Figure 4. Summary of adverse events (AEs) observed in the study's participants. The incidence of AEs (as % of 21 enrolled patients) after the first and the second dose. No severe adverse events (SAEs) were reported.

Supplementary Figure 1. Gating of SARS-CoV-2 S-RBD-specific response of T cells. CD4+ or CD8+ T cells were gated from lymphocyte, and CD3+ T cells were gated after the removal of CD14 monocytes, CD20 B cells, and doublets. All combinations of cytokine production upon antigen stimulation (interferon-γ, interleukin-2, and tumor necrosis-α) were gated and reported.
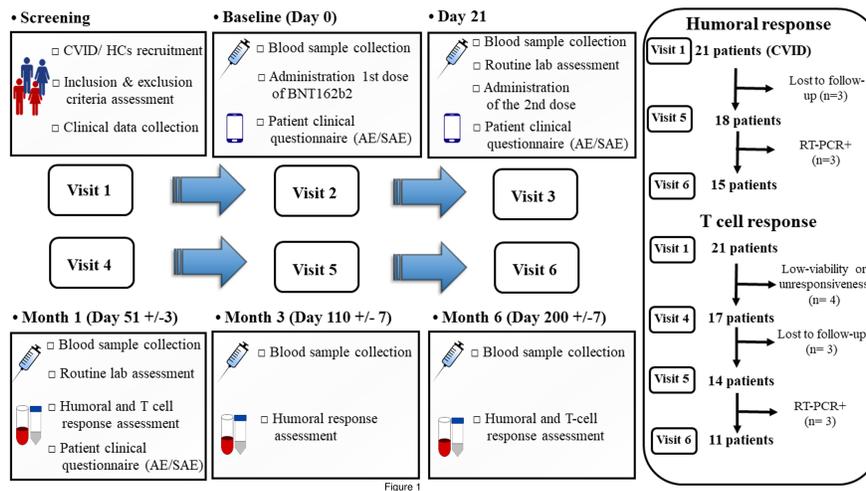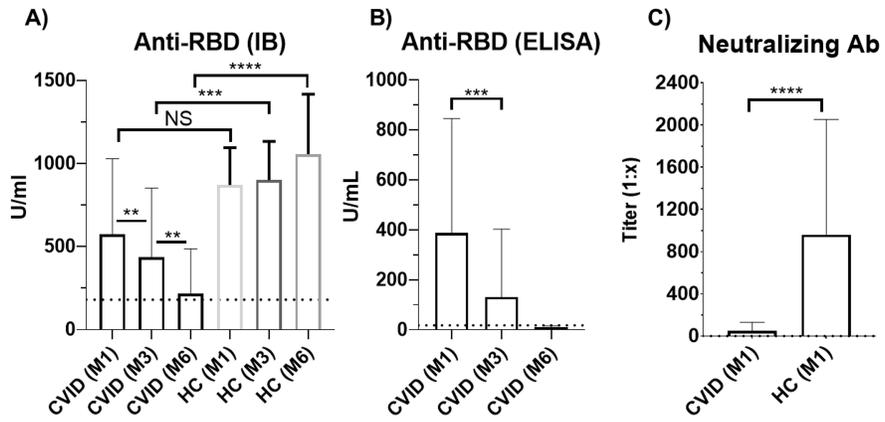

Figure 1

Figure 2

Figure 3

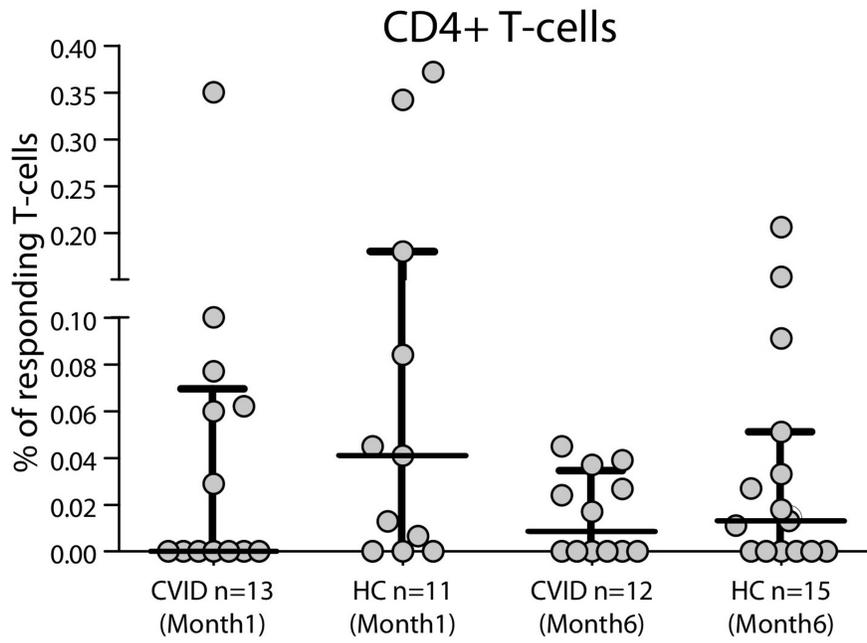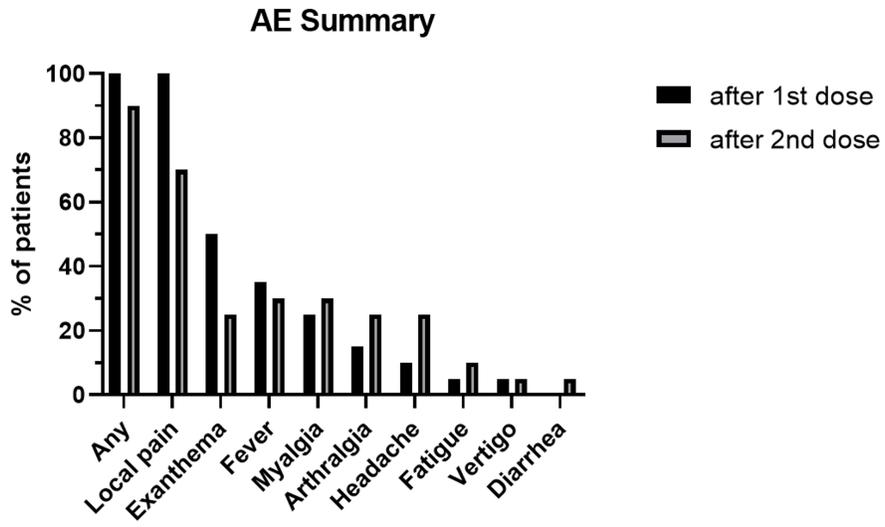11

AE Summary

Figure 4