CLINICAL SCIENCE

Immunogenicity of BNT162b2 vaccine against the Alpha and Delta variants in immunocompromised patients with systemic inflammatory diseases

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ABSTRACT

Objectives The emergence of strains of SARS-CoV-2 exhibiting increase viral fitness and immune escape potential, such as the Delta variant (B.1.617.2), raises concerns in immunocompromised patients. We aimed to evaluate seroconversion, cross-neutralisation and T-cell responses induced by BNT162b2 in immunocompromised patients with systemic inflammatory diseases.

Methods Prospective monocentric study including patients with systemic inflammatory diseases and healthcare immunocompetent workers as controls. Primary endpoints were anti-spike antibodies levels and cross-neutralisation of Alpha and Delta variants after BNT162b2 vaccine. Secondary endpoints were T-cell responses, breakthrough infections and safety.

Results Sixty-four cases and 21 controls not previously infected with SARS-CoV-2 were analysed. Kinetics of anti-spike IgG after BNT162b2 vaccine showed lower and delayed induction in cases, more pronounced with rituximab. Administration of two doses of BNT162b2 generated a neutralising response against Alpha and Delta in 100% of controls, while sera from only one of rituximab-treated patients neutralised Alpha (5%) and none Delta. Other therapeutic regimens induced a partial neutralising activity against Alpha, even lower against Delta. All controls and cases except those treated with methotrexate mounted a SARS-CoV-2 specific T-cell response. Methotrexate abrogated T-cell responses after one dose and dramatically impaired T-cell responses after two doses of BNT162b2. Third dose of vaccine improved immunogenicity in patients with low responses.

Conclusion Rituximab and methotrexate differentially impact the immunogenicity of BNT162b2, by impairing B-cell and T-cell responses, respectively. Delta fully escapes the humoral response of individuals treated with rituximab. These findings support efforts to improve BNT162b2 immunogenicity in immunocompromised individuals (ClinicalTrials.gov number, NCT04870411).

Key messages

What is already known about this subject?
- The course of COVID-19 is less favourable in patients with systemic inflammatory diseases.
- Rituximab and methotrexate decrease seroprotection rate following vaccination against influenza, pneumococcus, and ancestral and Alpha variants of SARS-CoV-2.
- Sensitivity of Delta variant to antibody neutralisation is reduced in vitro.

What does this study add?
- This study describes that 95% of sera from patients treated with rituximab did not neutralise Alpha and Delta variants after two doses of BNT162b2.
- In contrast, these patients have similar SARS-CoV-2 specific T-cell response that controls.
- Methotrexate completely abrogated T-cell responses after one dose and dramatically impaired T-cell responses after two doses of BNT162b2.
- Third dose improved immunogenicity in patients with low responses after two doses but had no effect in those with no responses.

How might this impact on clinical practice or future developments?
- This differential impairment of immunogenicity after BNT162b2 vaccine according to the treatments received is critical to identify patients in which optimisation of vaccine strategies should be evaluated.
- The administration of a third dose of mRNA-based vaccine should be proposed in patients with low responses after two doses.
- Other strategies should be considered in patients with no response after two doses.

INTRODUCTION

The course of COVID-19 is less favourable in patients with systemic inflammatory diseases. Older age, male gender, cardiovascular disease and obesity are risk factors of severe forms and COVID-19-related death in this immunocompromised population,[1–4] as it is in the general population.[5 6] Disease-specific factors including disease activity and treatments, especially glucocorticoids,

mycophenolate mofetil and rituximab, are additional risk factors.[1–3]

BNT162b2 and mRNA-1273 COVID-19 vaccines have been developed using a novel liposomal mRNA-based delivery platform. These vaccines have a good safety profile, induce strong and persistent B-cell and T-cell responses,[7 8] and are highly effective to prevent SARS-CoV-2 infection, hospitalisation and death with the ancestral strain and the Alpha (B.1.1.7) variant.[9]

The efficacy of vaccine has been recently questioned by variants of SARS-CoV-2 exhibiting increase viral fitness and immune escape potential. Among them, the Delta variant (B.1.617.2) was first identified in India in October 2020 and rapidly became the predominant strain across the globe.[10] While in vitro data indicate reduced sensitivity of Delta variant to antibody neutralisation,[11] only modest differences in vaccine effectiveness are noted with Delta as compared with Alpha.[12] In patients with systemic inflammatory diseases, the use of rituximab and methotrexate, commonly used to induce and maintain remission, decreases seroprotection rate after vaccination against influenza, pneumococcus, and ancestral and Alpha variants of SARS-CoV-2.[13–16] Yet, how the different immunosuppressive or immunomodulatory drugs tune humoral and cellular responses, and how the Delta variant impacts vaccine effectiveness in this population remains unclear.

In this study, we measured seroconversion, cross-neutralisation of Alpha and Delta variants and T-cell responses induced by BNT162b2 in immunocompromised patients with systemic inflammatory diseases according to the treatments received.

METHODS

Study design

The prospective COVADIS study (NCT04870411) included patients with systemic inflammatory diseases managed in Cochin Hospital, University of Paris (Paris, France). Healthcare immunocompetent workers from the same hospital were included as controls. Patients with a positive COVID-19 serology at baseline were excluded from the main analysis. Cases and controls received two doses of BNT162b2 vaccine 28 days apart. Four groups of patients receiving different immunosuppressive or immunomodulatory drugs were defined: patients receiving rituximab ('rituximab' treatment group), methotrexate ('methotrexate' group), immunosuppressive drugs such as mycophenolate mofetil or azathioprine ('immunosuppressive drugs' group), and those receiving other strategies described to have limited impact on vaccine immunogenicity ('other' treatment group).

Clinical and laboratory data

Clinical data were collected at baseline and during follow-up until month 6. To evaluate vaccine immunogenicity, blood samples were collected before the first dose of vaccine (M0), 1 month later just before the second dose (M1), at 3 months (M3) and 6 months (M6).

Outcomes

Primary endpoints were BNT162b2 immunogenicity and cross-neutralisation of Alpha and Delta variants at 3 months, that is, after two vaccine doses, defined by neutralisation titre (median of the half maximal effective dilution, ED50) for both virus with ED50 above 30. Secondary endpoints were the proportion of patients with positive anti-SARS-CoV-2 antibodies (define as an antibody binding unit (BU) above 1.1 for IgG and 0.2 for IgA) at M1, M3 and M6, cross-neutralisation of Alpha and Delta variants at 6 months, T-cell response defined by the number of circulating SARS-CoV-2-spike-specific interferon-γ (IFNγ)-producing T cells at M1, M3 and M6, breakthrough infections and safety.

T and B cell immunophenotyping

Extended B cell and T cell immunoprofiling were performed on whole blood as described in the online supplemental appendix 1 and online supplemental figures 1 and 2.

S-Flow assay

The S-Flow assay was used to detect antibodies bound to 293T cells stably expressing the spike protein (S) at their surface using *flow* cytometry. This assay is highly sensitive and allows quantification of antibodies through a standardised mean fluorescence intensity (MFI, referred to as binding unit, BU), which is calculated using an anti-spike monoclonal antibody as reference. The cut-off value of 1.1 BU was established using pre-pandemic sera. The method is described in the online supplemental appendix 1 and online supplemental figure 3.

Virus strains

The Alpha (B.1.1.7) variant originated from an individual returning from the UK. The Delta (B.1.617.2) variant originated from a hospitalised patient returning from India. The variant strains were isolated from nasal swabs using Vero E6 cells and amplified by two passages. Additional information is described in the online supplemental appendix 1.

S-Fuse neutralisation assay

The S-Fuse neutralisation assay was used to assess the neutralising activity of sera against emerging variants. The method is described in the online supplemental appendix 1.

T-cell response using enzyme-linked immunospot (ELISpot)

SARS-CoV-2-specific IFNγ-producing T cells were identified by using commercially available pools derived from a peptide scan through SARS-CoV-2 N-terminal (pool S1) and C-terminal (pool S2) fragments of spike glycoprotein (JPT Peptide Technologies GmbH, BioNTech AG, Berlin, Germany). Results are expressed as spot forming unit (SFU)/10^6 CD3+ T cells after subtracting background values from wells with non-stimulated cells. The method is described in the online supplemental appendix 1.

Statistical analysis

No statistical methods were used to predetermine sample size. The experiments were performed in blind regarding to the allocation groups. Flow cytometry data were analysed with FlowJo V.10 software (TriStar). Calculations were performed using Excel V.365 (Microsoft). Figures were drawn using GraphPad Prism V.9. Statistical analyses were conducted using GraphPad Prism V.9. Statistical significance between different groups was calculated using the tests indicated in each figure legend. Detailed statistical analysis is described in the online supplemental appendix 1.

RESULTS

Patients characteristics

Between January and April 2021, 77 cases and 28 controls were included in the study. Twenty participants (13 cases and 7 controls) with positive SARS-CoV-2 serological tests at baseline were excluded from the main analysis (figure 1). Finally, 64 cases and 21 controls were analysed. One patient and two controls were not sampled before the second dose of BNT162b2 vaccine.

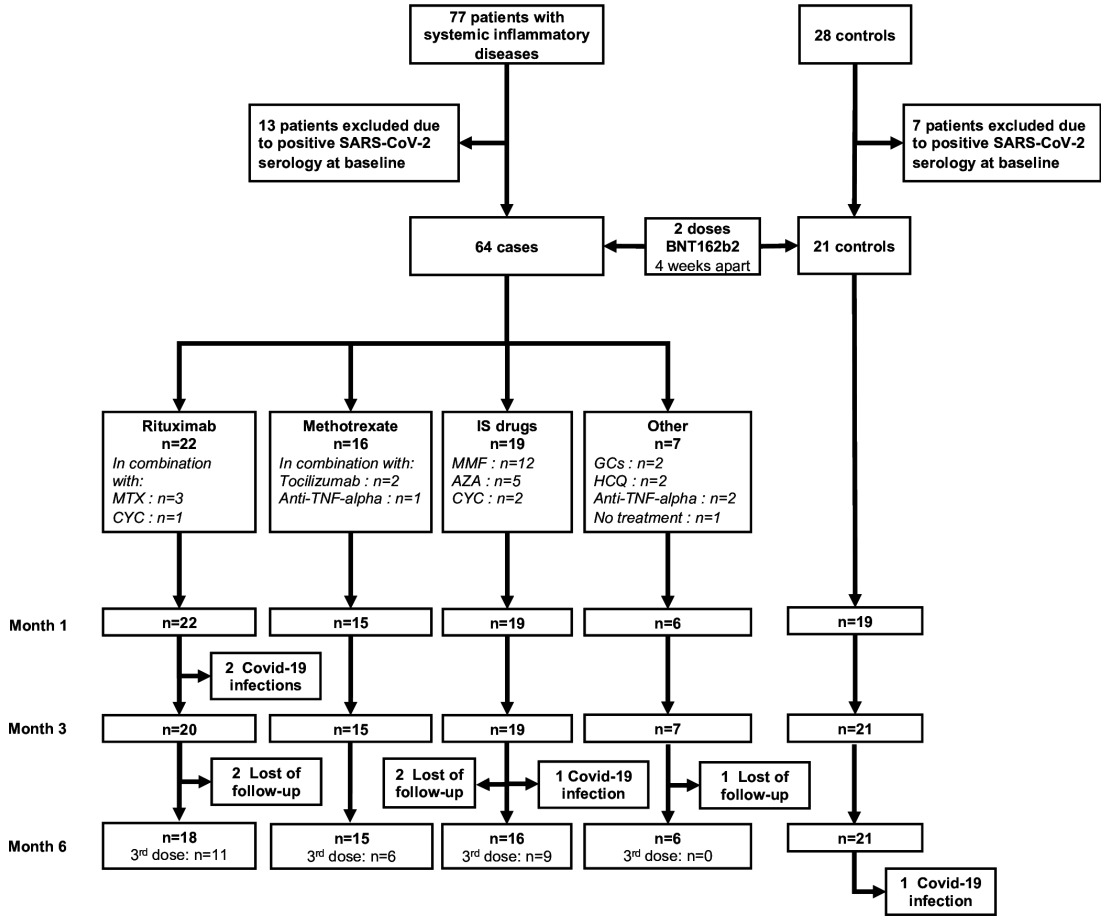

Figure 1 Flowchart of the study. AZA, azathioprine; CYC, cyclophosphamide; GCs, glucocorticoids; HCQ, hydroxychloroquine; IS, immunosuppressive; MMF, mycophenolate mofetil; MTX, methotrexate.

Baseline characteristics of patients are shown in table 1. Median age in controls and cases was 56 (39.5–59.5) and 52 (37.8–66.3) years, respectively. In patients in the 'rituximab' group, median time since the last infusion was 13.5 (0–117.5) days. The immunological characteristics are shown in the online supplemental table 1 and online supplemental figures 4 and 5. Compared with controls, cases showed lower total lymphocytes count. As expected, in the 'rituximab' treatment group, circulating B cells were not detected (except in one patient) and levels of IgG, IgA and IgM were significantly lower.

Induction of anti-spike antibodies after BNT162b2 vaccine

First, we analysed the kinetics of induction of anti-spike IgG in patients' sera after the first and second dose of BNT162b2 vaccine. We observed a delayed response in cases compared with controls. Anti-spike IgG inductions were detectable mainly after the second dose in cases, whereas it was noted from the first dose in controls (online supplemental figure 6). On samples collected after the two doses, at 3 months, all treatment groups except the 'other' group showed significantly lower anti-spike IgG levels than controls (figure 2A). The 'rituximab' group showed the lowest response. Then, we categorised individuals who seroconvert in IgG at M3 as 'responders'. All controls and cases from the 'other' treatment group seroconverted in IgG (figure 2B). 'Rituximab' showed again the lowest response, with only 50% of individuals who seroconverted at M3 (figure 2B). 'Methotrexate' and 'immunosuppressive drugs' treatment groups showed intermediate levels of anti-spike'IgG levels at 3 months, with 93% and 68% of individuals who seroconverted, respectively (figure 2A,B). A large interindividual variability was observed in these two groups. The use of azathioprine or mycophenolate mofetil did not discriminate between responders and non-responders in the immunosuppressive drugs group. Analysis of the circulating follicular helper CD4+ T cells after the first and second dose showed a delayed increase in cases compared with controls, occurring mainly after the second dose in patients treated by methotrexate and immunosuppressive drugs and detected after the first dose in controls. No difference was observed in the proportion of plasmablast and memory B cells (online supplemental figure 7).

Neutralisation of Alpha and Delta variants by sera after BNT162b2 vaccine

We next examined whether BNT162b2 vaccine-elicited antibodies at month 3 neutralised the Alpha and Delta variants in cases and controls (figure 2C,D). Median ED50 for Alpha in controls and in cases from the 'rituximab', 'methotrexate', 'immunosuppressive drugs' and 'other' treatment groups were 1942, <7.5, 199, 65 and 2173, respectively; and 539, <7.5, 31, <7.5 and 270 for Delta (figure 2C). Delta was fourfold less sensitive to neutralisation than Alpha in the controls, confirming previous observation.[11] Among cases, titres were reduced by sixfold between Delta and Alpha in the 'methotrexate' group, ninefold in the 'immunosuppressive drugs' group, eightfold in the 'other' group. The lack of neutralisation in the 'rituximab' group impaired the calculation of a fold decrease.

Table 1 Patients' characteristics at vaccination

	All n=64	Rituximab n=22	Methotrexate n=16	Immunosuppressive drugs n=19	Others n=7
Age, years					
Median (IQR)	52 (37.8–66.3)	58.5 (48.3–67.8)	50 (38.5–72.3)	34 (30–53.5)	51 (44–58.5)
>50 year, n (%)	35 (54.7)	16 (72.7)	8 (50)	7 (36.8)	4 (57)
Female, n (%)	48 (75)	15 (68.2)	11 (68.8)	15 (79)	7 (100)
Diagnosis					
Vasculitis					
ANCA-associated vasculitis	18 (28.1)	18 (81.8)	0	0	0
Behçet's	2 (1.6)	0	0	2 (10.5)	0
Cryoglobulinemia vasculitis	2 (1.6)	2 (9.1)	0	0	0
Large vessel vasculitis	4 (6.3)	0	4 (25)	0	0
Connective tissue disease					
Systemic lupus erythematosus	15 (23.4)	0	4 (25)	9 (47.4)	2 (28.6)
Systemic sclerosis	7 (10.9)	1 (4.5)	0	4 (21.1)	2 (28.6)
Sjogren syndrome	2 (1.6)	1 (4.5)	1 (6.3)	0	0
Myositis	5 (7.8)	0	3 (18.8)	2 (10.5)	0
Inflammatory rheumatic diseases*	3 (4.7)	0	2 (12.5)	0	1 (14.3)
Sarcoidosis	3 (4.7)	0	1 (6.3)	1 (5.3)	1 (14.3)
Others	3 (4.7)	0	1 (6.3)	1 (5.3)	1 (14.3)
Disease duration (years), mean (SD)	9.5 (9)	9.2 (9.1)	10.1 (8)	8.4 (8.9)	12 (11.9)
Disease activity status					
Active disease, n (%)	17 (26.5)	4 (18.2)	6 (37.5)	7 (36.8)	0 (0)
Renal involvement, n (%)	19 (29.7)	9 (41)	3 (18.8)	6 (31.6)	1 (14.3)
Ongoing treatments, n (%)					
Prednisone	45 (70.3)	13 (59.1)	12 (75)	17 (89.5)	3 (42.9)
Median, mg/day (IQR)	7.5 (5–15)	5 (5–13.8)	7.5 (5–13.8)	10 (5–25)	5 (5–12.5)
cDMARDs					
Methotrexate	19 (29.7)	3 (13.6)	16 (100)	0	0
Azathioprine	5 (7.8)	0	0	5 (26.3)	0
Mycophenolate mofetil	12 (18.8)	0	0	12 (63.2)	0
Cyclophosphamide	3 (4.7)	1 (4.5)	0	2 (10.5)	0
Biological therapies					
Anti-TNF-α	6 (9.4)	0	1 (6.3)	3 (15.8)	2 (28.6)
Rituximab	22 (34.4)	22 (100)	0	0	0
Tocilizumab	3 (4.7)	0	3 (18.8)	0	0
Belimumab	1 (1.6)	0	1 (6.3)	0	0
Hydroxychloroquine	15 (23.4)	2 (9.1)	4 (25)	7 (36.8)	2 (28.6)
No DMARDs, biologics or prednisone	1 (1.6)	–	–	–	1 (14.3)
Number of lines of previous treatments, n, median (IQR)	2 (1–3.8)	2 (1–4.3)	2 (1–4)	2 (1–3)	2 (1–2)

*Inflammatory rheumatic diseases: rheumatoid arthritis (n=2), spondyloarthritis (n=1).
cDMARDs, conventional disease-modifying antirheumatic drugs; TNF-α, tumour necrosis factor.

Then, we arbitrarily classified individuals as neutralisers according to the detection of neutralising antibodies at a serum dilution of 1:30 and non-neutralisers. Administration of two doses of BNT162b2 generated a neutralising response against the Alpha and Delta variants in 100% of controls. Only one individual in the 'rituximab' group neutralised Alpha (5%) and none neutralised Delta (figure 2C,D). Of note, despite a seroconversion in 50% of vaccinated individuals, IgG levels were particularly low and probably insufficient to display any detectable neutralising activity. Sera of 87% of patients in the 'methotrexate' group neutralised Alpha, dropping to 57% against Delta (figure 2D). Sera from patients in the 'immunosuppressive drugs' group neutralised Alpha and Delta in 53% and 42%, respectively. Nine (14%) cases neutralised Alpha but not Delta, including five patients treated with methotrexate, two with immunosuppressive drugs, one with rituximab and one with anti-TNF-α therapy. Correlation between Alpha and Delta neutralisation titres, and between IgG production and ED50 of Alpha variant was strong in all participants except for those receiving rituximab and immunosuppressive drugs (online supplemental figure 8).

The lack of neutralisation of Delta was associated with active disease ($p<0.001$), the use of rituximab ($p<0.001$), glucocorticoids ($p=0.007$) and low IgM ($p=0.047$) and IgG2 ($p=0.05$) levels (online supplemental table 3). In multivariate analysis, ED50 of Delta remained negatively associated with rituximab ($p<0.001$), methotrexate ($p<0.001$) and immunosuppressive drugs ($p<0.001$) (table 2).

Overall, B-cell response to BNT162b2 vaccine was impaired in immunocompromised patients at different levels depending on the treatments received. The effect was further amplified when evaluating the efficacy of sera to neutralise the Delta variant.

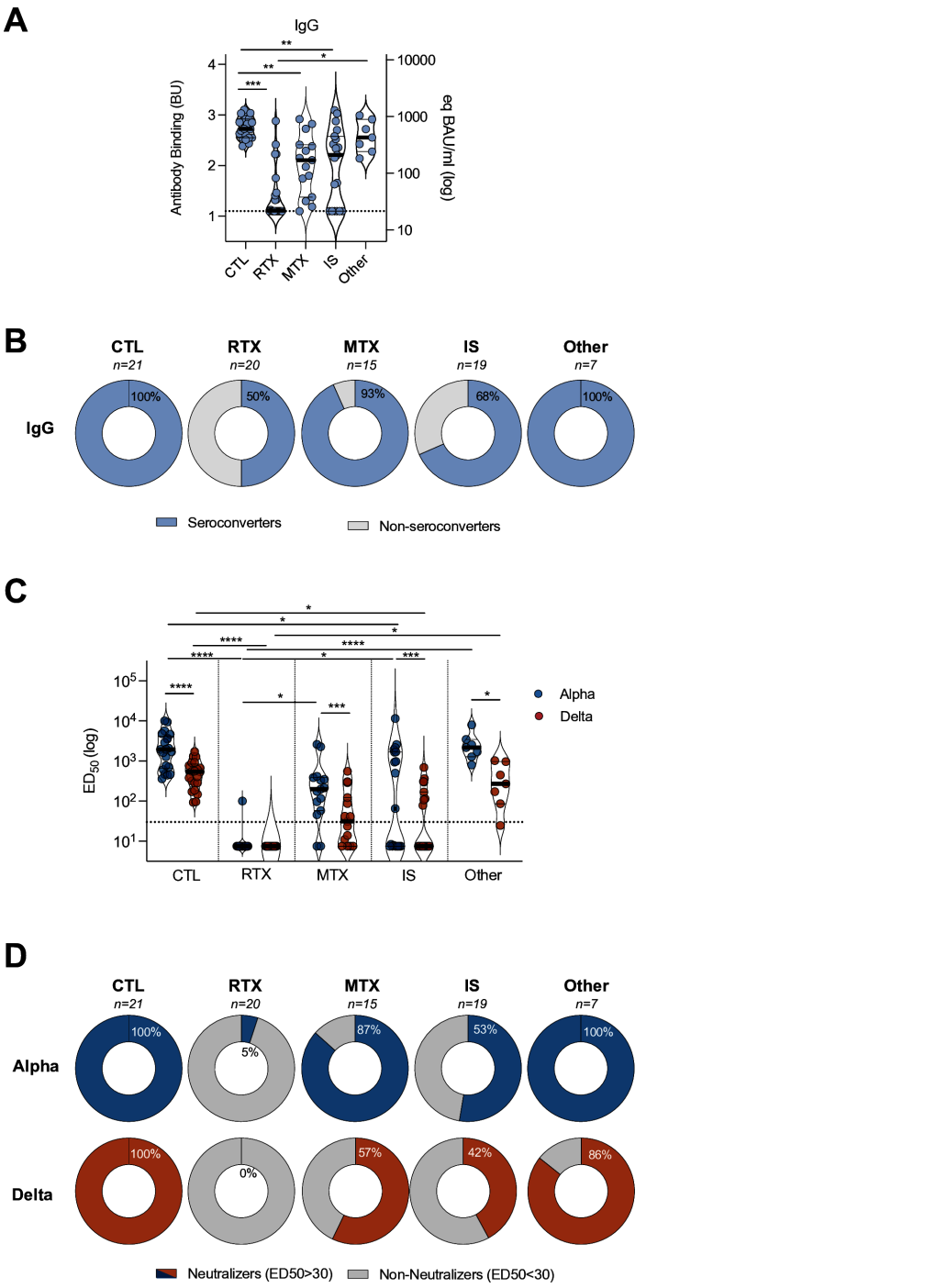

Figure 2 Humoral immune response to SARS-CoV-2 3 months after BNT162b2 vaccine. (A) Levels of anti-S IgG antibodies in the indicated groups after full vaccination at 3 months (M3) as determined by the S-Flow assay. The binding unit (BU), in a log scale, is calculated using a serially diluted anti-S monoclonal antibody as standard. Dotted lines indicate threshold of positivity (BU=1.1). Two-sided Kruskal-Wallis test with Dunn's test for multiple comparisons were performed. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. (B) In each group, individuals were defined as a 'seroconverter' (blue) if antibodies were detected above the threshold or 'non-responders' (grey) otherwise. Numbers of individuals in each group and percentages of responders are indicated. (C) Neutralising titres of sera against Alpha and Delta variants are expressed as ED50 values, in a log scale. Dotted line indicates the limit of detection (ED50=30). Data are mean of two independent experiments. In each group, Wilcoxon paired t-test was performed to compared ED50 of Alpha vs Delta variants. Two-sided Kruskal-Wallis test with Dunn's test for multiple comparisons between group of treatment was performed. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. (D) In each group, individuals were defined as a 'neutralisers' (blue for Alpha; orange for Delta) if neutralisation was detected at the dilution 1:30 or 'non-neutralisers' (grey) otherwise. Numbers of individuals in each group and percentages of neutralisers are indicated. CTL, controls; IS, immunosuppressive; MTX, methotrexate; RTX, rituximab.

Seroconversion and neutralisation of Alpha and Delta variants in convalescent vaccinated individuals

We then quantified anti-spike IgG and neutralisation activity 3 months after vaccination in the 7 controls and 13 cases who had been previously infected with SARS-CoV-2 and excluded from the main analysis (online supplemental figure 9). In convalescent

Table 2 Multivariate linear regression models assessing the association between patient's characteristics and quantitative humoral and cellular response

	ED50 alpha		ED50 delta		SARS-CoV-2-specific IFNγ-producing T cells	
	β coefficient(95% CI)	P value	β coefficient(95% CI)	P value	β coefficient(95% CI)	P value
Age, years	7.49 (–25.17 to 40.14)	0.649	2.16 (–1.91 to 6.22)	0.294	–1.61 (–4.41 to 1.18)	0.253
Treatment group controls	Ref		Ref		Ref	Ref
Immunosupressants	–1809.56 (–3590.36 to to 28.77)	0.047	–434.85 (–669.67 to 200.03)	<0.001	26.40 (–126.56 to 179.35)	0.731
Methotrexate	–2729.50 (–4485.78 to 973.23)	0.003	–462.83 (–701.35 to 224.31)	<0.001	–70.95 (–227.87 to 85.97)	0.370
Rituximab	–3153.98 (–4823.90 to 1484.06)	<0.001	–583.41 (–803.88 to 362.95)	<0.001	77.73 (–62.81 218.26)	0.273
Other	–398.73 (–2351.02 1553.55)	0.685	–190.32 (–440.08 59.43)	0.133	–35.00 (–198.04 to 128.04)	0.669
Glucocorticoids (%)	–50.01 (–1332.51, 1232.50)	0.938	–48.87 (–207.06 to 109.31)	0.540	–44.34 (–153.91 to 65.23)	0.4222
IgA, g/L	31.61 (–279.05 to 342.27)	0.840	0.10 (–41.14 to 41.34)	0.996	45.96 (14.67 to 77.25)	0.005
IgG2, g/L	189.43 (–183.08 to 561.95)	0.314	246.59 (–29.18 to 522.36)	0.079	–11.57 (–38.53 to 15.38)	0.394

controls, vaccination boosted levels of anti-spike IgG as well as neutralising antibody titres against both variants, as compared with the uninfected vaccinated control group. In previously infected cases under immunosuppressive or immunomodulatory drugs, a low response remained after vaccination.

T-cell response to BNT162b2 vaccine

We next investigated whether controls and cases mounted a SARS-CoV-2-specific T-cell response following the first and second doses of BNT162b2 vaccine (figure 3 and online supplemental figure 10). All controls (except one) and cases except those from the 'methotrexate' treatment group had similar levels of specific T-cells in response to S1 pool (figure 3A). Methotrexate completely abrogated T-cell responses after one dose and dramatically impaired T-cell responses after two doses of BNT162b2 compared with controls and cases from other treatment groups (figure 3A,B). Similar results, but less pronounced, were observed for S2 peptide pool (figure 3A and online supplemental figure 10). Importantly, despite the absence of neutralising activity in response to BNT162b2, patients receiving rituximab showed increased levels of specific T-cell responses that reached after a delay the same levels as controls (figure 3A,B). No correlation between B-cell and T-cell responses within the rituximab and the methotrexate groups was observed. The relationship between humoral and cellular immune responses against SARS-CoV-2 is shown in online supplemental figure 11, highlighting the impact of the different treatment groups on both humoral and cellular responses. The lack of T-cell response was associated with the use of methotrexate (p=0.045) and glucocorticoids (p=0.012) (online supplemental table 4). In multivariate analysis, no variable correlated with SARS-CoV-2-specific IFNγ-producing T cells (table 2). Also, no significant differences in the proportion of circulating CD4+ memory T cells and Th1 T cells after the first and second dose of BNT162b2 were found in all groups (online supplemental figure 7).

Overall, T-cell responses to S1 and S2 peptide pools were similar in cases compared with the controls except methotrexate treated patients showing significantly decreased T-cell responses.

Impact of booster vaccination at 6 months

Lastly, we evaluated in controls and cases how B-cell and T-cell responses persisted at 6 months after the two first doses of vaccine and the impact of a third booster vaccination in some of the patients (figures 1 and 4). In controls who did not receive a third dose, anti-spike IgG levels were stable at 6 months, and neutralisation titres against Alpha and Delta waned by 3.5-fold and 5-fold, respectively (figure 4A,B). A similar dynamic of anti-S antibodies and neutralisation was observed in patients from the 'other group' who were not eligible for a booster dose in France (figure 4A,B). A third dose was administered in 26 cases (all from RTX, MTX and immunosuppressive drugs groups) after a median time since the first dose of 102 (88–127) days. This third injection had no effect on humoral response in patients treated with rituximab but significantly increased anti-spike IgG levels and neutralisation against both variants in patients with methotrexate and immunosuppressive drugs compared with those that received only two doses of vaccine (figure 4A,B). Number of circulating B cells in the 'rituximab group' at the time of the third dose was not available. Conversely, booster vaccination increased levels of specific T-cells in the 'rituximab group', whereas methotrexate still dramatically impaired T-cell responses after three doses (figure 4C).

At 6 months of follow-up, one control and three patients from the cohort developed symptomatic COVID-19. Two individuals belonged to the 'rituximab' and one to 'immunosuppressive drugs' treatment groups. Four patients (6.3%) experienced a disease flare within the 3 months after the first dose of vaccine, two patients with systemic lupus erythematosus and two with systemic vasculitis, leading to modification of immunosuppressive regimen.

DISCUSSION

As the Delta variant spreads across the globe, aggregating data on the effectiveness of COVID-19 vaccines in specific immunocompromised populations is a critical issue. Data from solid organ transplant recipients, patients with malignant hemopathy or with chronic inflammatory arthritis suggested that risk factors for reduced SARS-CoV-2 vaccine immunogenicity included older age and treatments with glucocorticoids, rituximab, mycophenolate mofetil and abatacept.[15 17–19] However, levels of anti-spike antibodies were mainly measured and few studies used neutralisation assay or assessed T-cell response.

Additional studies specifically reported that B cell depletion by rituximab blocked humoral but not T cell response to vaccination, using anti-RBD IgG measurement and IFNγ ELISpot T-cell response. The time since the last infusion of rituximab and the number of circulating B cells are major predictive factors of humoral response.[20] SARS-CoV-2 antibody response was reported in 0% –39% of the vaccinated B-cell-depleted patients, whereas T cell responses were noted in 58%–100%.[20 21] This early assessment showed that humoral immunity to one or two doses of BNT162b2 was also impaired by methotrexate treatment.[22–24]

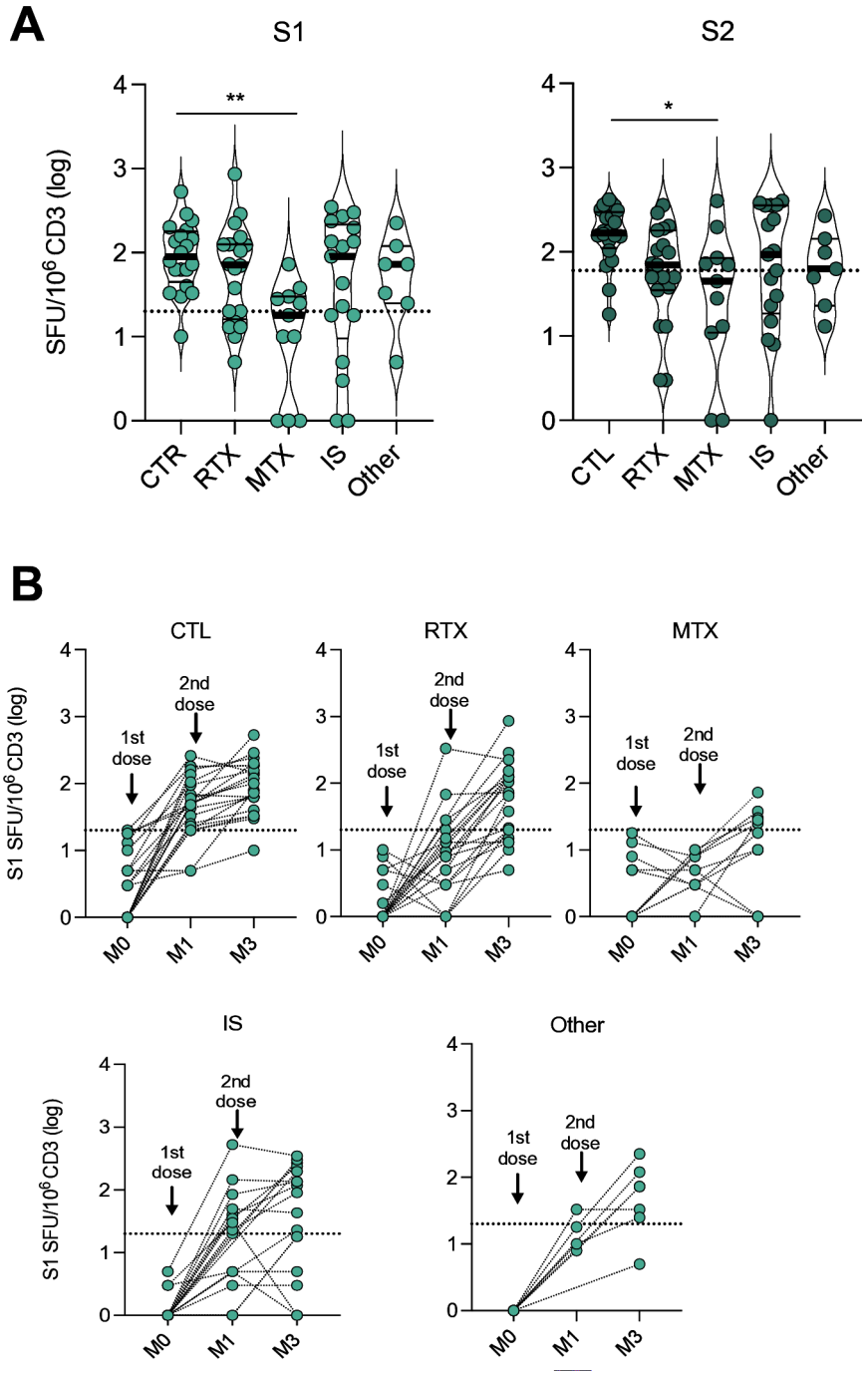

Figure 3 Cellular immune response to SARS-CoV-2 after BNT162b2 vaccine. (A) Quantification of SARS-CoV-2-specific T-cell responses using ELISpot at M3 in the indicated groups. Results were expressed as spot forming unit (SFU)/10^6 CD3+ T cells after subtraction of background values from wells with non-stimulated cells, in a log scale. Negative controls were PBMC in the culture medium. Positive controls were PHA-P and CEFX Ultra SuperStim Pool. SARS-Cov-2 peptide pools tested were derived from a peptide scan through SARS-CoV-2 Spike glycoprotein (left S1, N-terminal fragment, right: S2, C- terminal fragment). P values were determined with two-sided Kruskal-Wallis test with Dunn's test for multiple comparisons were performed. *p<0.05; **p<0.01; ***p<0.001. (B) Kinetic of specific T-cell responses against the SARS-CoV-2 S1 peptide before the first dose (M0), before the second dose (M1) and after full vaccination at 3 months (M3) according to the treatments received. Data indicate median. Each dot represents a single patient. CTL, controls; MTX, methotrexate; RTX, rituximab; IS, immunosuppressive.

However, conflicting results were found for cellular responses showing either preserved[22] or impaired T-cell activation.[24] Most of these studies assessed very early timepoints that may not allow an appropriate assessment of immune response after complete vaccination.

Sera from convalescent and vaccinated individuals neutralise less efficiently the Delta variant than the Alpha.[11] However, this was studied in the general population and assessing the sensitivity of the Delta variant to antibody neutralisation in immunocomprised populations is thus necessary.

In this study, we focused on patients with systemic inflammatory diseases that were receiving rituximab, methotrexate and/or other immunosuppressive drugs, and provided important data regarding sensitivity to Delta variant according to the treatments

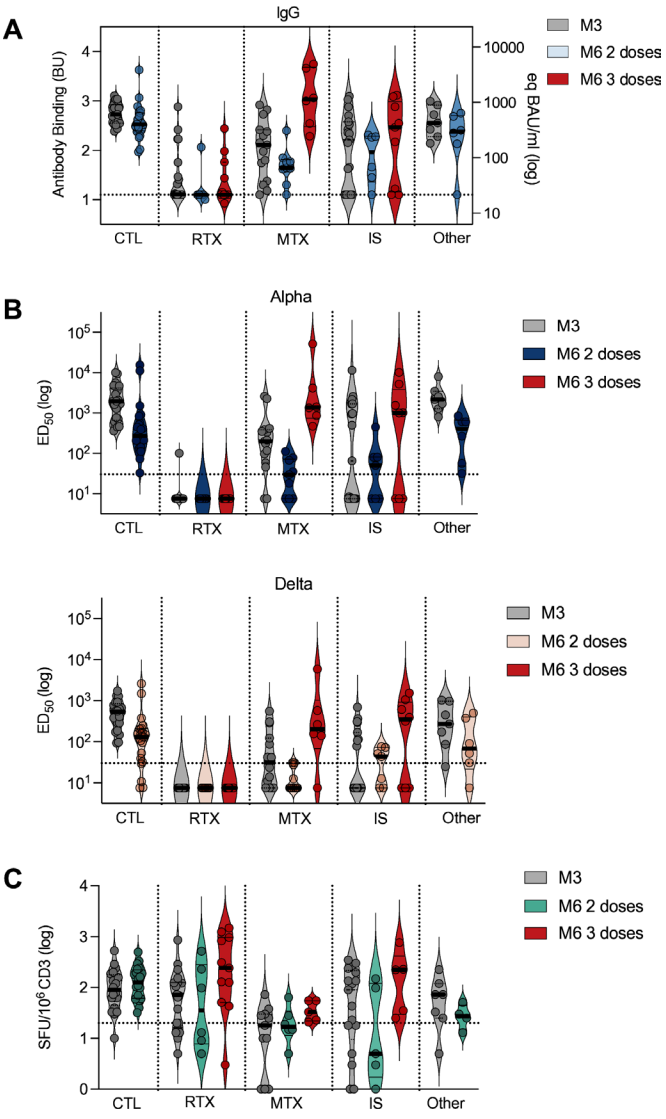

Figure 4 Impact of booster vaccination on immune response at 6 Months. (A) Levels of anti-S IgG antibodies in the indicated groups at 3 and 6 months (M3/M6) as determined by the S-Flow assay. (B) Neutralising titres of sera against Alpha and Delta variants at M3 and M6 are expressed as ED50 values, in a log scale. Dotted line indicates the limit of detection (ED50=30). Data are mean of two independent experiments. (C) Quantification of SARS-CoV-2-specific T-cells responses using ELISpot at M3 and M6 in the indicated groups. Results were expressed as spot forming unit (SFU)/10^6 CD3+ T cells. CTL, controls; MTX, methotrexate; RTX, rituximab; IS, immunosuppressive.

used. We analysed patients after the first and the second doses of the BNT162b2 vaccine. We report a delayed and lower induction of anti-spike IgG compared with controls, much more pronounced with rituximab. While two doses of BNT162b2 generated a neutralising response against Alpha and Delta variants in 100% of controls, 95% of sera from patients treated with rituximab did not neutralise these two variants. Of note, we observed that 50% of RTX-treated individuals have seroconverted despite an almost complete lack of neutralisation in this group. It is likely explained by our serological assay, which measures total anti-S antibodies (ie, targeting RBD and non-RBD epitopes). The hypothesis that RTX-treated seroconverters have an antibody response biased towards non-neutralising epitopes deserves further investigation. In contrast, SARS-CoV-2-specific T-cell response was similarly measured in controls and cases with the exception of methotrexate-treated patients. This differential impairment of immunogenicity after BNT162b2 vaccine according to the treatments received, mainly for rituximab and methotrexate, is critical to identify patients in which optimisation of vaccine strategies should be evaluated.

To counteract this impaired immunogenicity, the administration of a third dose of mRNA-based vaccine has been proposed. Recent data in solid-organ transplant recipients showed that a third dose of BNT162b2 vaccine increased the prevalence of seroconversion and antibody titres, without serious adverse events.[25–27] A third dose also increased specific cellular response even in patients who remained seronegative, but the impact of this cellular response remains to be determined.[27] We analysed B-cell and T-cell responses at 6 months in 40% of our immunocompromised patients having received a third dose of vaccine. A third dose of vaccine had no effect on B-cell response in patients treated with rituximab but it significantly increased anti-spike IgG levels and neutralisation activity against both variants in patients with methotrexate and cDMARDs compared with those receiving only two doses. In a cohort of 33 patients treated with rituximab who did not respond to two injection, only 21% harbour neutralising antibodies after a booster vaccination.[28] The discrepancy in response is most likely due to variation in the extent of B-cell depletion as suggested by other studies.[20 29 30] Our results are in line with these observations, and suggest that a third dose is needed, mainly in patients with low responses after two doses, but not sufficient, in most RTX-treated individuals. Finally, a third dose increased levels of specific T-cells in the 'rituximab group', whereas methotrexate still dramatically impaired T-cell responses after three doses.

Our study has several limitations. The findings are observational and based on small numbers and should be interpreted with caution. Differences in treatment groups were highly associated with the type of underlying inflammatory disease, and there may be differences among the populations. Especially, 82% of patients on rituximab were patients with antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, limiting the generalisation of the findings to patients with rheumatoid arthritis. However, except for more frequent renal involvement at diagnosis in the 'rituximab' group and younger age in the 'immunosuppressive drugs' group, patients' characteristics were comparable between treatment groups. Lastly, ELISpot is a less sensitive assay than intracellular staining and could have played a role if in the detection of T-cell response.

Overall, we found that rituximab and methotrexate differentially impact the immunogenicity of BNT162b2 vaccine, by impairing B-cell and T-cell responses, respectively. The Delta variant fully escapes the suboptimal humoral response of individuals treated with rituximab. Our findings support efforts to improve effectiveness of mRNA vaccines in this immunocompromised population.

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Contributors All authors contributed to manuscript preparation. BT is responsible for the overall content as the guarantor. JH and BT contributed to the study design. DP, IS and TB performed antibody measurement, neutralisation assay and data analysis. AO and LC contributed to cellular assays, data analysis and manuscript preparation. JH, BT, DP, TB, LD, YN, SB, OS and LC performed data analysis. LD and MCS performed lymphocytes phenotyping.

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Patient consent for publication Not applicable.

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