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Research paper

Cellular and humoral immunogenicity of a SARS-CoV-2 mRNA vaccine in patients on haemodialysis

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ABSTRACT

Background: Patients with chronic renal insufficiency on maintenance haemodialysis face an increased risk of COVID-19 induced mortality and impaired vaccine responses. To date, only a few studies have addressed SARS-CoV-2 vaccine elicited immunity in this immunocompromised population.
Methods: We assessed immunogenicity of the mRNA vaccine BNT162b2 in at-risk dialysis patients and characterised systemic cellular and humoral immune responses in serum and saliva using interferon γ release assay and multiplex-based cytokine and immunoglobulin measurements. We further compared binding capacity and neutralization efficacy of vaccination-induced immunoglobulins against emerging SARS-CoV-2 variants Alpha, Beta, Epsilon and Cluster 5 by ACE2-RBD competition assay.
Findings: Patients on maintenance haemodialysis exhibit detectable but variable cellular and humoral immune responses against SARS-CoV-2 and variants of concern after a two-dose regimen of BNT162b2. Although vaccination-induced immunoglobulins were detectable in saliva and plasma, both anti-SARS-CoV-2 IgG and neutralization efficacy was reduced compared to a vaccinated non-dialysed control population. Similarly, T-cell mediated interferon γ release after stimulation with SARS-CoV-2 spike peptides was significantly diminished.
Interpretation: Quantifiable humoral and cellular immune responses after BNT162b2 vaccination in individuals on maintenance haemodialysis are encouraging, but urge for longitudinal follow-up to assess longevity of immunity. Diminished virus neutralization and interferon γ responses in the face of emerging variants of concern may favour this at-risk population for re-vaccination using modified vaccines at the earliest opportunity.
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1. Introduction

Since its emergence in late 2019, SARS-CoV-2 has become a global pandemic with more than 166 million confirmed cases and 3·46 million deaths (as of 24.05.21) [1]. Vulnerable populations such as the elderly, immunocompromised or those suffering from chronic

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Research in context

Evidence before this study

Patients on dialysis tend to have a reduced immune response to both infection and vaccination. We searched PubMed and medRxiv for studies including search terms such as "COVID-19", "vaccine", and "dialysis" but no peer-reviewed studies to date simultaneously assessed both SARS-CoV-2 specific B- and T-cell responses, mucosal immunoglobulins, and considered the impact of SARS-CoV-2 variants of concern in this at-risk population.

Added value of this study

We provide a comprehensive functional characterisation of both T- and B-cell responses following a two-dose regimen of BNT162b2 in at-risk patients on maintenance haemodialysis. More importantly, to the best of our knowledge, we assess for the first time binding and neutralization capacity of vaccination-induced circulation and mucosal antibodies towards emerging SARS-CoV-2 variants of concern in an immunocompromised population.

Implications of all the available evidence

Patients on maintenance haemodialysis develop a substantial cellular and humoral immune response following the BNT162b2 vaccine. These findings should encourage patients on maintenance haemodialysis to receive the vaccine. However, we suggest continuing additional protection measures against variants of concern in this at-risk population until longevity of the vaccine response is fully evaluated.

conditions or requiring continual medical intervention such as dialysis are at-risk of severe COVID-19 disease and associated death [2]. Although a series of vaccines have been developed, tested and approved at unprecedented speed, only one vaccine study for NVX CoV2373 has enrolled patients with chronic diseases such as chronic kidney disease to assess efficacy and safety of SARS-CoV-2 vaccination within this vulnerable population [3]. Patients on maintenance haemodialysis are a particularly high-risk group, as renal disease has been identified as a key risk factor for severe COVID-19 [4-7], while at the same time their regular need for therapy does not allow them to self-isolate and reduce contacts to avoid infection. A recent study also suggested that seroreversion following natural SARS-CoV-2 infection is faster in dialysis patients compared to the general population further increasing the risk of re-infection [8]. Other studies have also demonstrated an impaired humoral immune response in dialysis patients, as seen by the attenuation of antibody titres following vaccination with SARS-CoV-2 vaccines [9-15]. Both, COVID-19 and BNT162b2-induced effective immunity appear to result from functional T-follicular helper (TfH) cell responses and Th1 cytokines [16,17]. In contrast, end-stage kidney failure is associated with pro-inflammatory markers (including IFNγ, TNFα, IL-8, CCL-2, and others), exhausted T-cell phenotype, and perturbed TfH-cells [18]. Additional data on the efficiency of SARS-CoV-2 vaccination is urgently needed not only for risk mitigation but also to assess whether additional protective measures during therapy must be put in place as seen by the impaired infection and vaccination-induced responses for influenza A and hepatitis B [19-21]. While antibody titres have already been characterised within vaccinated dialysis patients, little is known about the ensuing cellular immune response [22] or about the neutralization potential of vaccine-induced antibodies [23], particularly in light of the increasingly appearing SARS-CoV-2 variants of concern (VoC) which threaten the success of vaccination programs [24].

To assess efficacy of SARS-CoV-2 vaccination in dialysis patients, we characterised cellular and humoral immune responses in serum and saliva of dialysed and non-dialysed individuals after vaccination with the mRNA vaccine BNT162b2. Considering the increasing presence of mutated SARS-CoV-2 strains, we further compared binding capacity and neutralization efficacy of vaccination-induced immunoglobulins against emerging variants of concern such as B.1.1.7 (Alpha), B.1.351 (Beta), B.1.429 (Epsilon) and Cluster 5 (Mink).

2. Methods

2.1. Study design and sample collection

Following written informed consent, heparinized blood samples from haemodialysis patients (n=81) were taken before start of dialysis using a vascular access which was either an arterio-venous fistula or a central venous catheter or by venipuncture from health care workers (n=34), who served as non-dialysed control population. Participants had to be over the age of 18 and able to give written informed consent. All study participants were either patients or worked at the dialysis centre Eickenhof and received the vaccine on the same occasion. 10 of the 81 dialysis patients were on immunosuppressive medication such as prednisolone, tacrolimus, mycophenolatmofetil, hydrocortisone, or a combination. Four of those patients had received a kidney transplant, and one of them additionally a liver transplant. Other reasons for immunosuppressive therapy included polymyositis, polyarthritis, vasculitis and chronic obstructive pulmonary disease. Further details about the study population can be found in Table 1, Table S2 and S3. One haemodialysis patient had previously been tested positive for SARS-CoV-2 by routine PCR screening. As subsequent antibody testing was negative for SARS-CoV-2 IgG, this patient received BNT162b2 and was not excluded from the study. All participants received the standard two-dose regimen of BNT162b2 21 days apart, followed by blood collection for analysis 21 days after the second dose. Plasma was obtained from lithium heparin blood (S-Monovette Plasma, Sarstedt, Germany). Whole blood samples were used immediately for interferon γ release assay (IGRA). For saliva collection, all individuals spat directly into a collection tube. To

Table 1
Characteristics of vaccinated study participants: IQR - Inter Quartile Range. BMI - Body Mass Index. n – absolute numbers per group. NA - Information not available. n. a. - not applicable.

Characteristics	Non-dialysis control group (n=34)	Haemodialysis group (n=81)	p-value for difference between groups
Age (years), median (IQR)	54·5 (15)	69 (18)	$2{\cdot}91 * 10^{-10}$
Gender (female, n, %)	28 (82·35)	34 (41·98)	$1{\cdot}71 * 10^{-4}$
Days since start of haemodialysis (median, IQR)	n. a.	1371 (1664)	n. a.
Immunosuppressive medication (n, %)	0 (0)	10 (12·34)	$7{\cdot}48 * 10^{-2}$
Co-morbidities			
Obesity (BMI, >30)	8 (23·53) (1 NA)	18 (22·22)	$1 * 10^{0}$
Diabetes mellitus (n, %)	1 (2·94)	22 (27·16)	$6{\cdot}78 * 10^{-3}$
Cardiovascular disease (n, %)	0 (0)	39 (48·15)	$1{\cdot}92 * 10^{-6}$

inactivate replication-competent SARS-CoV-2 virus particles potentially present in saliva samples, Tri(n-butyl) phosphate (TnBP) and Triton X-100 were added to final concentrations of 0·3% and 1%, respectively [25]. Both plasma and saliva samples were frozen and stored at −80°C until further use.

2.2. Ethics statement

Ethical approval of the study was obtained from the relevant authority - the Internal Review Board of Hannover Medical School (MHH, approval number 8973_BO-K_2020, amendment Dec. 2020). Written informed consent was obtained from all participants prior to starting the study.

2.3. Bead coupling

Coupling of antigens to spectrally distinct MagPlex beads (Cat #MC10XXX-01, Luminex Corporation, USA) was done by EDC/s-NHS coupling for all standard MULTICOV-AB antigens [26]. Receptor-binding domains (RBD)s from VoC were coupled using Anteo coupling (Cat #A-LMPAKMM-10, Anteo Tech Reagents, Australia) following the manufacturer's instructions [27].

2.4. MULTICOV-AB

Antibody titres and binding was analysed using MULTICOV-AB, a multiplex immunoassay which simultaneously analyses 20 antigens, as previously described [26]. The full list of antigens included in this study can be found in Table S1. Plasma samples were diluted 1:400, while saliva samples were diluted 1:12 [27]. Briefly, antigens were immobilised on spectrally distinct populations of MagPlex beads (as above) and combined into a single bead mix. Samples were combined with the bead mix, incubated for 2 h at 21°C and then washed using a microplate washer to remove unbound antibodies. Bound antibodies were detected following a 45 min incubation at 21°C with R-phycoerythrin labeled goat-anti-human IgG (Jackson ImmunoResearch Labs, United Kingdom, Cat #109-116-098, Lot #148837, RRID: AB_2337678, used at 3 μg/mL) or IgA (Jackson ImmunoResearch Labs, Cat #109-115-011, Lot #143454, RRID: AB_2337674, used at 5 μg/mL) as secondary antibodies. Following another washing step, beads were re-suspended and then measured using a FLEXMAP 3D instrument (Luminex Corporation, Texas, USA) using the following settings: Timeout 80 sec, Gate: 7500-15000, Reporter Gain: Standard PMT, 40 events. Each sample was measured once. Three quality control (QC) samples were included on each plate to monitor MULTICOV-AB assay performance. Raw median fluorescence intensity (MFI) values or normalised values (MFI/MFI of QC samples [27]) are reported.

2.5. ACE2-RBD competition assay

To determine neutralization, an ACE2-RBD competition assay was carried out as previously described [27]. Briefly, biotinylated ACE2 was added to the assay buffer to a final concentration of 500 ng/mL for all samples. Samples were then mixed with MULTICOV-AB bead mix (see above) and incubated for 2 h at 21°C, 750 rpm. After washing, ACE2 was detected using Streptavidin-PE (2 μg/mL, Cat #SAPE-001, Moss, Maryland, US) by incubating the sample for 45 min at 750 rpm. After an additional wash step and resuspension, samples were measured on a FLEXMAP 3D instrument (same settings as MULTICOV-AB). As control, 500 ng/mL ACE2 was used. For analysis, MFI values were normalised against the control wells. All samples were measured once.

2.6. SARS-CoV-2 QuantiVac ELISA

To further validate plasma IgG levels measured by MULTICOV-AB, samples were further analysed using the Anti-SARS-CoV-2-QuantiVac-ELISA IgG (Cat #EI 2606-9601-10G, Euroimmun, Germany) according to the manufacturer's instructions. Plasma samples were diluted 1:400 to achieve assay linearity.

2.7. Interferon γ release assay

SARS-CoV-2-specific T-cell responses were determined by measuring IFNγ production upon SARS-CoV-2 antigen stimulation using the SARS-CoV-2 Interferon Gamma Release Assay (Cat #ET-2606-3003, Euroimmun, Germany). Briefly, 0·5 mL of full blood was stimulated with peptides of the SARS-CoV-2 S1 domain of the Spike protein for a period of 20-24 h. Negative and positive controls were carried out according to the manufacturer's instruction. Following stimulation, supernatants were isolated through centrifugation and IFNγ measured using ELISA (Cat #EQ-6841-9601, Euroimmun, Germany). The remaining supernatant was stored at -80°C. Background signals from negative controls were subtracted and final results calculated in mIU/mL using standard curves. IFNγ concentrations >200 mIU/mL were considered as reactive. The upper limit of reactivity was 2000 mIU/mL.

2.8. Cytokine measurements

Supernatants of SARS-CoV-2 antigen stimulated blood cells were prepared and isolated as explained for the detection of IFNγ by SARS-CoV-2 Interferon Gamma Release Assay and analysed by LEGENDplex™ using the Human Essential Immune Response Panel (Cat #740930, Bio Legend, California, US) for L-4, IL-2, CXCL-10 (IP-10), IL-1β, TNFα, CCL-2 (MCP-1), IL-17A, IL-6, IL-10, IFNγ, IL-12p70, CXCL-8 (IL-8), TGFβ1) according to manufacturer's instructions. Analysis was performed using an LSR II flow cytometer (Becton Dickinson, Germany) and data analysed using the LEGENDplex™ Data Analysis Software Suite.

2.9. Data analysis and statistics

RStudio (Version 1.2.5001), with R (version 3.6.1) was used for data analysis and figure generation. Additionally, the R add-on package "beeswarm" was utilised to visualise data as stripcharts with overlaying boxplots and to create non-overlaying data points. A second R add-on package "RcolorBrewer" was used to generate specific colours for plots. The type of statistical analysis performed (when appropriate) is listed in the figure legends. Figures were exported from Rstudio and then edited using Inkscape (Inkscape 0.92.4). Spearman's rho coefficient was calculated in order to determine correlation between IGRA results and antibody responses or neutralization using the "cor" function from R's "stats" library. Mann-Whitney-U test was used to determine difference between signal distributions between dialysed and non-dialysed groups using the "wilcox.test" function from R's "stats" library. To assess differences in the study population, Pearson's Chi-squared test with Yates' continuity correction was used for categorical characteristics using the "chisq.test" function from R's "stats" library and Mann-Whitney-U test as above was used for difference in age. Pre-processing of data such as matching sample metadata and collecting results from multiple assay platforms was performed in Excel 2016.

2.10. Role of the funders

This work was financially supported by the Initiative and Networking Fund of the Helmholtz Association of German Research Centres (grant number SO-96), the EU Horizon 2020 research and

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3. Results

3.1. Dialysed patients have reduced antibody titres following vaccination

To characterise the vaccination response in patients on maintenance haemodialysis, we measured immunoglobulin levels 21 days after the second dose of Pfizer BNT162b2 using MULTICOV-AB, a multiplex immunoassay containing antigens from Spike and Nucleocapsid proteins of both SARS-CoV-2 and the endemic human coronaviruses (hCoVs) [26]. As a control group, 34 samples from healthcare workers vaccinated at the same time points as the 81 patients on haemodialysis were used. (Detailed information on the study population can be found in Table 1, Table S2 and S3). As indicated by the lack of a significant anti-Nucleocapsid (N) IgG or IgA response at the time of blood draw 21 days after the second vaccination, none of the study participants had been previously infected or seroconverted after a SARS-CoV-2 infection (data not shown). IgG responses towards the original B.1 isolate in vaccinated dialysis patients were significantly reduced ($p<0{\cdot}0001$, Mann-Whitney-U test) and more variable (Fig. 1a) than in the control group, which reached the upper limit of detection of the assay, as seen previously [27]. Interestingly, plasma IgA responses in the dialysis group were comparable to the control group (Fig. 1b, p=0·38, Mann-Whitney-U test). While all participants in the control group seroconverted, within our dialysis population, four from 81 vaccinated individuals (4·92%) were classified as serologic non-responders with antibody titres below the cut-off. As an additional control, S1 IgG titres were measured using a commercial assay (Fig. S2), which identified the same pattern of a significantly diminished antibody response in dialysed patients (272·3 RU/mL) compared to non-dialysed individuals (456·8 RU/mL, $p<0{\cdot}0001$, Mann-Whitney-U test). Due to the small sample size, we were unable to confidently identify factors associated with reduced humoral immunity by analysis of variance. Dialysis patients receiving immunosuppressive therapy tended to have lower anti-spike IgG levels (median 65·68 RU/mL, IQR 285·14) compared to the remaining dialysis patients (median 112.3 RU/mL, IQR 323.6, p=0·09, Mann-Whitney-U test), which is consistent with a recent report [28].

As SARS-CoV-2 is a mucosal-targeted virus, we also collected saliva from our vaccination cohort and assessed IgG and IgA levels using MULTICOV-AB. When examining antibody titres found in saliva, dialysed individuals had significantly lower IgG titres (p=0·0007), but similar IgA titres (p=0·70) to the control group (Fig. 1c and d, p-values: Mann-Whitney-U test). To examine responses towards emerging VoC, Spike-RBDs of the B.1.1.7 (Alpha), B.1.351 (Beta), Cluster 5 (Mink) and B.1.429 (Epsilon) strain were included as part of MULTICOV-AB [27]. As expected [24,27], antibody

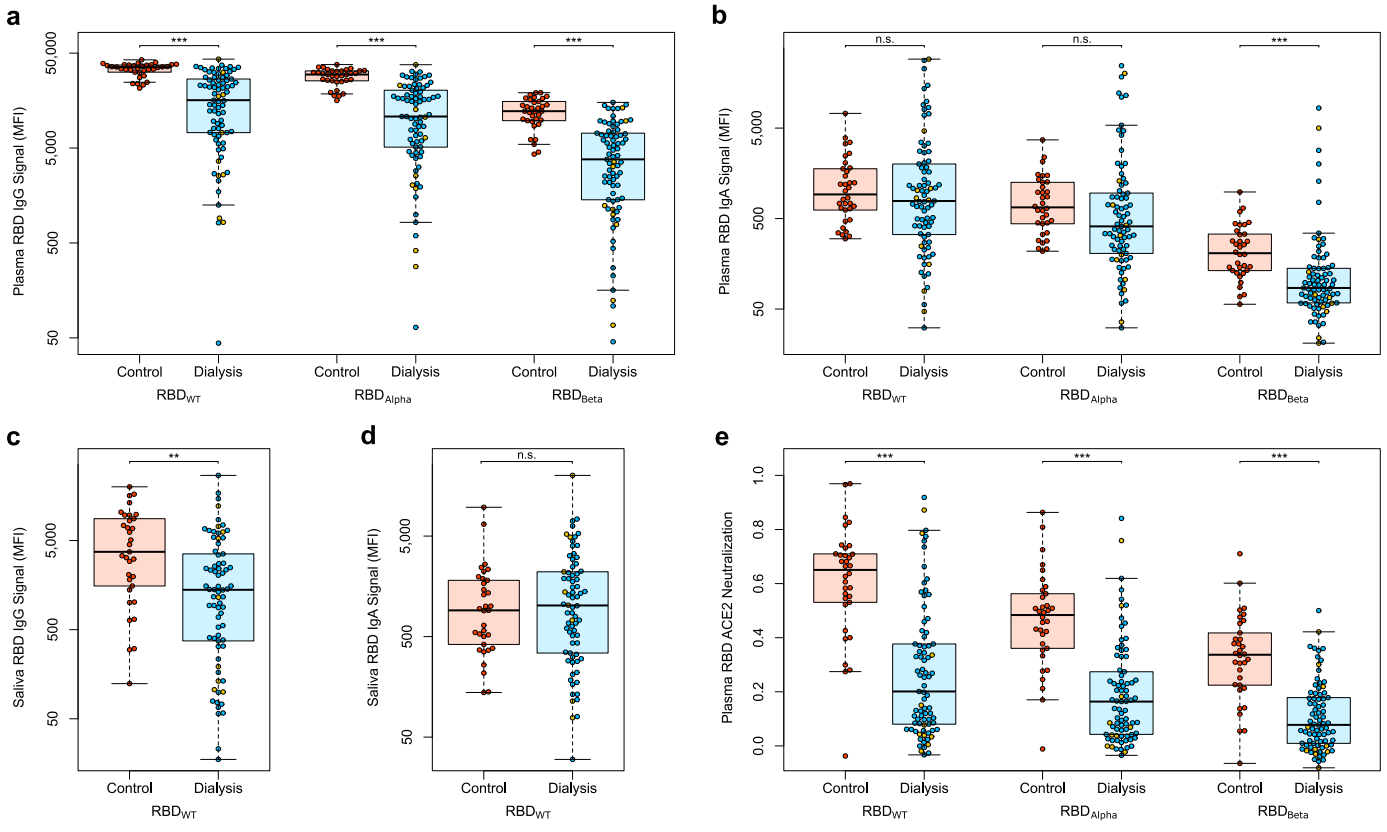

Fig. 1. Humoral immune response in haemodialysed individuals after vaccination with Pfizer BNT162b2.

IgG (a, c), IgA response (b, d) and neutralizing capacity of IgG (e) towards the indicated SARS-CoV-2 WT (B.1), Alpha (B.1.1.7), Beta (B.1.351) in plasma (a, b, e) or saliva (c, d) from controls (red circles, n=34), individuals on maintenance haemodialysis (blue circles, n=71) and haemodialysed individuals on immunosuppressive medication (yellow circles, n=10) 21 days post second vaccination was measured using MULTICOV-AB (a, b, c, d) or an ACE2-RBD competition assay (e). Data is displayed as median fluorescence intensity (MFI) (a-d). Neutralization capacity is displayed as ratio where 1 indicates maximum neutralization and 0 no neutralization (e). Saliva (c, d) was collected from 33 controls (red circles), 65 individuals on maintenance haemodialysis (blue circles) and from 9 individuals on maintenance haemodialysis and immunosuppressive medication (yellow circles). Boxes represent the median, 25th and 75th percentiles, whiskers show the largest and smallest non-outlier values. Outliers were determined by 1·5 times IQR. Statistical significance was calculated by Mann-Whitney-U (two-sided). Significance was defined as $^{*}<0{\cdot}01$, $^{**}<0{\cdot}001$, $^{***}<0{\cdot}0001$ or n.s.$>0{\cdot}01$.

binding towards B.1.1.7 in both dialysed and non-dialysed individuals was comparable to B.1 (original isolate), while binding was clearly reduced for B.1.351 (Fig. 1a). Antibody binding for Cluster 5 and B.1.429 was similar to the B.1 isolate for both groups (Fig. S1). As part of the MULTICOV-AB antigen panel, we also analysed the humoral response towards endemic CoV S1 and N protein, but found no general significant differences between control group and dialysis patients (Fig. S3).

3.2. Neutralization is reduced in dialysis patients after vaccination

To assess neutralizing potency of plasma towards both the original B.1 isolate and VoC RBDs, we used a previously described ACE2-RBD competition assay [27]. Neutralization across both wild-type and all VoCs measured was significantly reduced in dialysed compared to non-dialysed individuals (all $p<0{\cdot}0001$, Mann-Whitney-U test) (Fig. 1e, Fig. S4). As expected, there were differences between the VoCs themselves, with B.1.351 having the lowest neutralization for both control and dialysed individuals. However, responses were comparably low for all VoCs tested for patients on maintenance haemodialysis and additional immunosuppressive medication in the dialysis group further reduced neutralizing potency with the majority of samples located in the 25th quartile (Fig. 1e).

3.3. T-cell response to SARS-CoV-2 vaccination is diminished in dialysed individuals

As clinical studies have suggested that both cellular and humoral response can confer protection from a SARS-CoV-2 infection [29], we also assessed vaccination-induced T-cell responses by IFNγ release assay and characterised cytokine and chemokine responses after stimulation with a Spike S1-derived peptide pool by multiplex

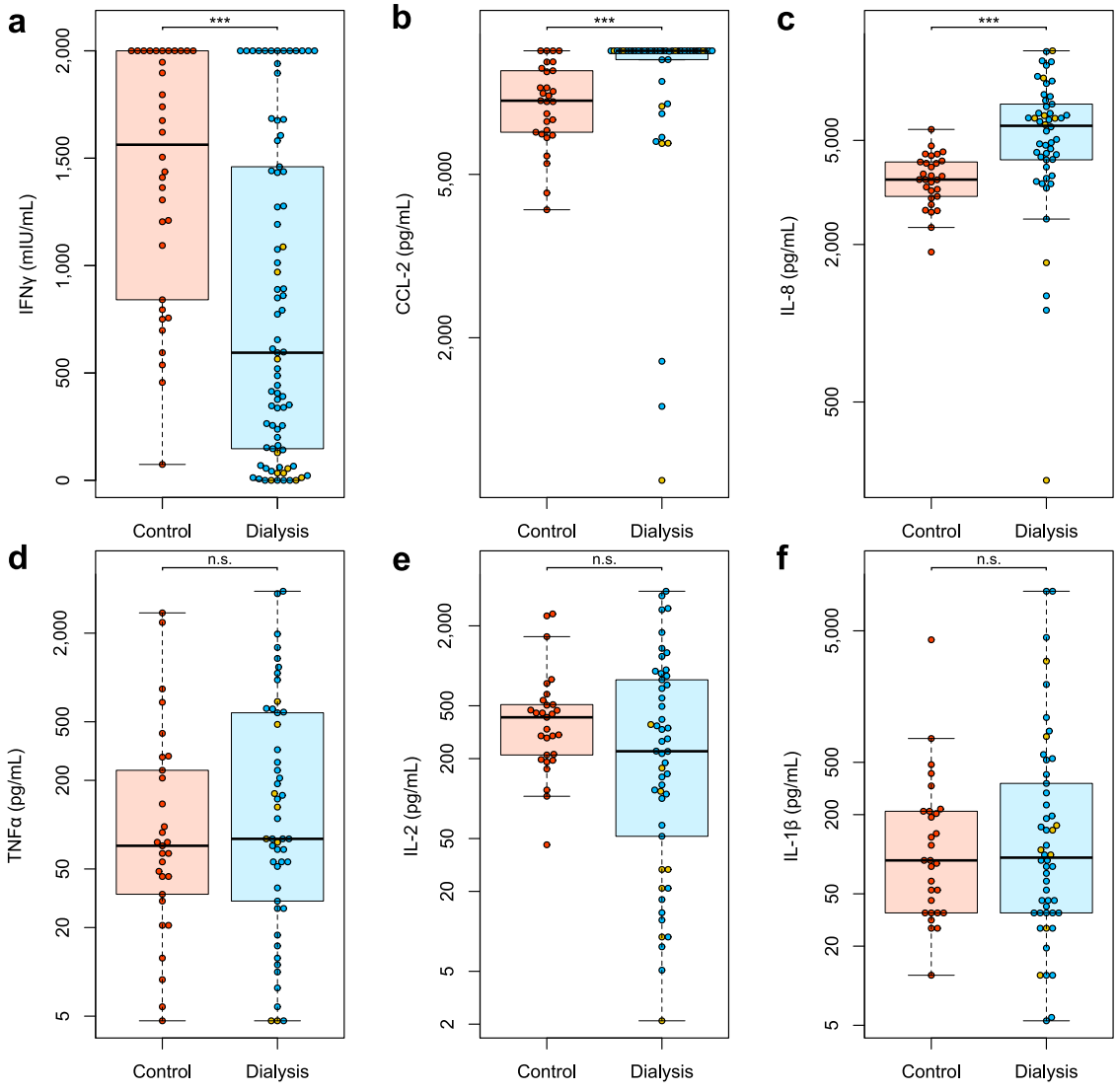

Fig. 2. Cellular immune response in haemodialysed individuals after vaccination with Pfizer BNT162b2.

Whole blood from vaccinated controls (red circles, n=34), individuals on maintenance haemodialysis (blue circles, n=71) and haemodialysed individuals on immunosuppressive medication (yellow circles, n=10) 21 days post second vaccination was *ex vivo* stimulated using a SARS-CoV-2 Spike S1-specific peptide pool. Supernatant fractions were analysed by interferon γ release assay (IGRA, a) or bead-based multiplex-cytokine assay for CCL-2 (b), IL-8 (c), TNFα (d), IL-2 (e) and IL-1β (f). Data is shown in mIU/mL for IGRA or pg/mL for the multiplex-cytokine assay. T-cells were classified as reactive if IFNγ was >200 mIU/mL. IGRA (a) was carried out with samples from all study participants. Bead based-cytokine measurements (b-f) were performed with samples from 29 control, 42 haemodialysed and 8 haemodialysed individuals on immunosuppressive medication. Samples that were classified as above upper or below the lower limit of detection of the cytokine assay or the IGRA are shown at the respective limit. Boxes represent the median, 25th and 75th percentiles, whiskers show the largest and smallest non-outlier values. Outliers were determined by 1·5 times IQR. Statistical significance was calculated by Mann-Whitney-U (two-sided). Significance was defined as $^{*}<0{\cdot}01$, $^{**}<0{\cdot}001$, $^{***}<0{\cdot}0001$ or n.s.$>0{\cdot}01$.

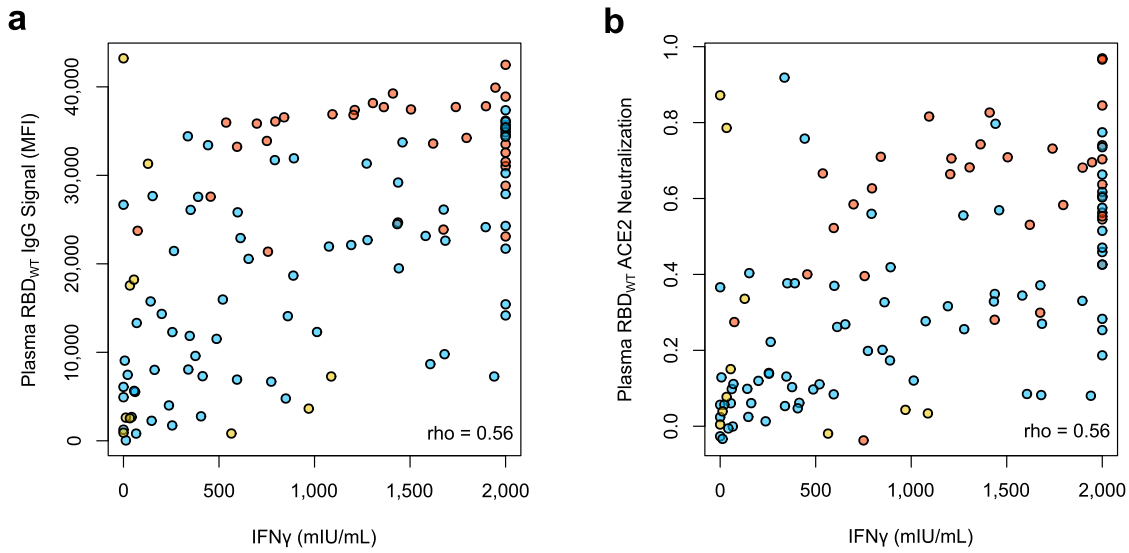

Fig. 3. Relationship of cellular and humoral immune response after vaccination with Pfizer BNT162b2.

T-cell responses assessed by IGRA for IFNγ (mIU/mL) and B-cell responses assessed by MULTICOV-AB IgG binding to WT (B.1) RBD (a) or ACE2-WT (B.1) RBD competition assay (b) were plotted for correlation analysis in the vaccinated control group (red circles, n=34), in the vaccinated haemodialysed group (blue circles, n=71) and in haemodialysed individuals on additional immunosuppressive medication (yellow circles, n=10). Correlation was calculated using Spearman's coefficient.

cytokine measurements. Consistent with reduced anti-Spike S1 IgG and anti-RBD IgG levels (Fig. 1a, Fig. S2), IGRA showed significantly lower levels of IFNγ released in the supernatants of stimulated T-cells from vaccinated patients on maintenance haemodialysis ($p<0{\cdot}0001$, Fig. 2a). In addition, all but one individual within the control group (97·1%) was classified as reactive by IGRA whereas only 71·6 % were within the haemodialysed group. Of the 12 analysed cytokines beyond IFNγ, only IL-8 and CCL-2 (both $p<0{\cdot}0001$, Mann-Whitney-U test) were significantly different between the two immunised groups, whereas no other Th1 type cytokines such as TNFα or IL-2 accompanied the IFNγ response (Fig. 2b-f, Fig. S5).

At this moment, there are no defined correlates of protection against a SARS-CoV-2 infection and the relative importance of cellular versus humoral response is equally undefined [30,31]. To shed light on this, we correlated B- and T-cell responses within our vaccination cohort. Comparable to other studies examining vaccination responses of BNT162b2 in a similar setting [32], we observed a moderate correlation between T-cell responses (measured by IGRA) and B-cell responses (determined by RBD B.1-specific IgG levels (Spearman's rho=0·56, Fig. 3a) or RBD B.1 IgG-neutralizing potency (Spearman's rho=0·56, Fig. 3b)). In addition, we observed a skew towards increased B-cell reactivity in both control group and individuals on haemodialysis.

4. Discussion

To control the ongoing COVID-19 pandemic, efficient vaccination to create herd immunity without the infection-induced mortality will be key. We initiated this study to further increase information, including neutralization and response to VoCs, on vaccination-induced immune responses in at-risk immunocompromised populations such as renal dialysis patients. Similar to other groups, we found robust antibody responses following vaccination within dialysed patients [9,13], confirming that our results are comparable to other studies. Overall, 95% of our dialysis patients showed a humoral immune response to vaccination, higher than what other studies with similar time points had found [10-12,14,32]. We can only speculate about potential reasons for those discrepancies such as differences in renal replacement therapies, composition of the patient cohort or co-morbidities. It should be noted that titres were significantly reduced in dialysis patients compared to control individuals, which could result in reduced vaccine efficacy within this group. We also observed differences in humoral IgG and cellular T-cell response in our dialysis group, with four (4·92%) non-humoral responders and 23 (28·4%) non-T-cell responders, respectively. Overall, it is apparent from our data that in both groups vaccination response was skewed towards secretory immunity. This is in line with exploratory studies using BNT161b1 and other mRNA vaccines, which induce a B-cell response peak around two weeks after the boosting dose to then decline before reaching a memory plateau phase [33, 34]. Future studies will be needed to determine the longevity and relative contribution of both T- and B-cell responses towards vaccination-induced protection. We found no significant differences in vaccination-induced IgA levels in both saliva and plasma between our study groups. Although several studies reported lower protective IgG titres over time following hepatitis B, influenza A or SARS-CoV-2 vaccinations, IgA levels were not analysed to determine if IgA vaccination responses are in general less affected in dialysis patients [9-14,19-21]. Interestingly, a monoclonal IgA antibody capable of recognizing both the SARS-CoV-1 and SARS-CoV-2 spike proteins and blocking ACE2 receptor interaction combined with an increased neutralization ability over its IgG equivalent has been described [35]. ·Some studies even report a higher neutralizing capacity of purified serum IgA monomers from early convalescent sera compared to IgG and increased saliva IgA titres and neutralization versus IgG in recovered hospitalised COVID-19 patients [36]. We identified a clearly reduced neutralizing capacity towards all VoC RBDs tested in our dialysed individuals compared to controls. Taking into account that SARS-CoV-2 infections were increased in vaccinated dialysis patients compared to vaccinated control individuals [11], further monitoring is urgently needed to determine if vaccine-induced protection prevents infection with increasingly circulating and diverse SARS-CoV-2 mutant strains, or if additional protection measures still need to be put in place throughout therapy session despite a completed vaccination scheme.

Individuals with kidney failure are at increased risk of infections and malignancies, with the uremic milieu potentially triggering a chronic inflammatory state, which promotes T-cell exhaustion and suppression of IFNγ production [37,38]. Indeed, patients with end-stage kidney disease (ESKD) are reported to have elevated serum levels of cytokines such as IFNγ, TNFα, IL-8, and CCL-2 compared to healthy controls. After mitogen stimulation, both $CD4^+$ and $CD8^+$ T-

cells in ESKD group demonstrated a pro-inflammatory phenotype, more exhausted and anergic $CD4^+$ and $CD8^+$ T-cells and a reduced frequency of follicular helper T-cells, which are important for humoral immunity [18]. In light of these immunological changes, our results about diminished SARS-CoV-2 specific T-cell responses and increased proinflammatory cytokine release may account in part for impaired vaccine-induced IgG responses in these patients. Specifically, CCL-2 and IL-8, which both were increased following restimulation with SARS-CoV-2 peptides in dialysis patients, could reflect a more pronounced innate immune response released by monocytes and acting on neutrophils and endothelial cells. We speculate these responses to be secondary to mechanisms triggered by spike-specific T-cells and their cytokines. Which mechanisms critically trigger and facilitate such cytokine patterns in dialysis patients and whether constitutively disturbed cytokine responses in dialysis patients are contributing to hamper humoral or cellular immunity after BNT161b2 vaccination remains to be studied with more careful approaches.

Our study has several limitations. While we have a reasonable sample size (81 dialysis patients), which is similar or even larger compared to several other studies [12,13,32], our control group is not age- and gender-matched. Since others have described that both factors have independent influence on the immunogenicity of COVID-19 vaccines [39–42], our observed differences are likely to be influenced by age and sex to some extent. In addition, we evaluated only one of the currently approved SARS-CoV-2 vaccines with samples from a single dialysis centre and did not perform in-depth immune phenotyping or assessment of SARS-CoV-2 responsive T-cell frequencies. Thus, we cannot extrapolate that other COVID-19 vaccines or vaccine schedules will lead to reduced immune responses in dialysis patients as described here. We also lack paired saliva and plasma samples pre and post first dose to characterise B- and T-cell response kinetics or assess potential cross-reactivity of endemic CoV antibodies in immunocompromised individuals across the dosing scheme. However, all of our samples were collected at the same time with an identical dosing regimen which allows us to make a direct comparison between our two groups of interest (dialysed versus non-dialysed). Additionally, the lack of previously infected samples within our study groups limits us to only study vaccine-induced responses. Several groups independently report high antibody titres and neutralization activity after the first dose of Pfizer or Moderna RNA vaccine in individuals who already had SARS-CoV-2 infections [43]. This effect is likely to be repeated in dialysis patients with a single vaccination post COVID-19.

Taken together, we provide robust evidence that a completed two-dose regimen of BNT162b2 elicits both antibody and T-cell responses in patients on maintenance haemodialysis towards the SARS-CoV-2 B.1 isolate. Future studies are needed to assess the lifespan and long-term kinetics of the vaccination response. As neutralization is reduced in dialysed patients towards all VoCs examined, our data also highlights the need to monitor if infection with SARS-CoV-2 VoC occur more frequently in this vulnerable population compared to vaccinated healthy individuals.

Contributors

AD-J, GMNB, NSM, GL and MS conceived the study. MB, AD, MS, AD-J, GMNB, AC, NSM and MVS designed the experiments. NSM, MS, GMNB, AD-J, and GK procured funding. GMR, JG, JJ and MVS performed experiments. KL, AB, EW, GL, AC, and GMNB collected samples or organised their collection. PDK, BT and UR produced and designed recombinant assay proteins. MB, AD, MS, GMR, MVS and AC performed data collection and analysis. MB generated the figures. MB, MS, AD and GMNB verified the underlying data. MS wrote the first draft of the manuscript with input from MB, NSM, GMNB and AD. All authors critically reviewed and approved the final manuscript.

Declaration of Competing Interest

NSM was a speaker at Luminex user meetings in the past. The Natural and Medical Sciences Institute at the University of Tübingen is involved in applied research projects as a fee for services with the Luminex Corporation. The other authors declare no competing interest.

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Data Sharing Statement

Data relating to the findings of this study are available from the corresponding authors upon request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2021.103524.

References

[1] WHO Coronavirus (COVID-19) Dashboard, Accessed 24th May 2021, https://covid19.who.int/
[2] Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature 2020;584(7821):430–6.
[3] Windpessl M, Bruchfeld A, Anders HJ, Kramer H, Waldman M, Renia L, et al. COVID-19 vaccines and kidney disease. Nat Rev Nephrol 2021;17(5):291–3.
[4] Sim JJ, Huang CW, Selevan DC, Chung J, Rutkowski MP, Zhou H. COVID-19 and survival in maintenance dialysis. Kidney Med 2021;3(1):132–5.
[5] Council E-E, Group EW. Chronic kidney disease is a key risk factor for severe COVID-19: a call to action by the ERA-EDTA. Nephrol Dial Transplant 2021;36(1):87–94.
[6] Hsu CM, Weiner DE, Aweh G, Miskulin DC, Manley HJ, Stewart C, et al. COVID-19 among US dialysis patients: risk factors and outcomes from a national dialysis provider. Am J Kidney Diseas 2021;77(5):748–56 e1.
[7] Khatri M, Islam S, Dutka P, Carson J, Drakakis J, Imbriano L, et al. COVID-19 antibodies and outcomes among outpatient maintenance hemodialysis patients. Kidney360. 2021;2(2):263–9.
[8] Labriola L, Scohy A, Seghers F, Perlot Q, De Greef J, Desmet C, et al. A longitudinal, 3-Month serologic assessment of SARS-CoV-2 infections in a belgian hemodialysis facility. Clin J Am Soc Nephrol 2021;16(4):613–4.
[9] Anand S, Montez-Rath M, Han J, Garcia P, Cadden L, Hunsader P, et al. Antibody response to COVID-19 vaccination in patients receiving dialysis. J Am Soc Nephrol 2021 ASN.2021050611.
[10] Lacson E, Argyropoulos CP, Manley HJ, Aweh G, Chin AI, Salman LH, et al. Immunogenicity of SARS-CoV-2 vaccine in dialysis. medRxiv 2021.
[11] Yanay NB, Freiman S, Ma Shapira, Wishahi S, Hamze M, Elhaj M, et al. Experience with SARS-CoV-2 BNT162b2 mRNA vaccine in dialysis patients. Kidney Int 2021.
[12] Simon B, Rubey H, Treipl A, Gromann M, Hemedi B, Zehetmayer S, et al. Haemodialysis patients show a highly diminished antibody response after COVID-19 mRNA vaccination compared to healthy controls. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association. European Renal Association 2021:gfab179.
[13] Grupper A, Sharon N, Finn T, Cohen R, Israel M, Agbaria A, et al. Humoral response to the Pfizer BNT162b2 vaccine in patients undergoing maintenance hemodialysis. Clin J Am Soc Nephrol 2021.
[14] Agur T, Ben-Dor N, Goldman S, Lichtenberg S, Herman-Edelstein M, Yahav D, et al. Antibody response to mRNA SARS-CoV-2 vaccine among dialysis patients - a prospectivecohort study. Nephrol Dial Transplant 2021.
[15] Longlune N, Nogier MB, Miedougé M, Gabilan C, Cartou C, Seigneuric B, et al. High immunogenicity of a messenger RNA based vaccine against SARS-CoV-2 in chronic dialysis patients. Nephrol Dialysis Transplant 2021.
[16] Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021;371(6529):eabf4063.
[17] Lederer K, Castaño D, Gómez Atria D, Oguin TH, Wang S, Manzoni TB, et al. SARS-CoV-2 mRNA vaccines foster potent antigen-specific germinal center responses associated with neutralizing antibody generation. Immunity 2020;53(6):1281–95 e5.
[18] Hartzell S, Bin S, Cantarelli C, Haverly M, Manrique J, Angeletti A, et al. Kidney failure associates with T cell exhaustion and imbalanced follicular helper T cells. 2020;11(2390).

[19] Crespo M, Collado S, Mir M, Cao H, Barbosa F, Serra C, et al. Efficacy of influenza A H1N1/2009 vaccine in hemodialysis and kidney transplant patients. Clin J Am Soc Nephrol 2011;6(9):2208–14.
[20] Scharpé J, Peetermans WE, Vanwalleghem J, Maes B, Bammens B, Claes K, et al. Immunogenicity of a standard trivalent influenza vaccine in patients on long-term hemodialysis: an open-label trial. Am J Kidney Dis 2009;54(1):77–85.
[21] Udomkarnjananun S, Takkavatakarn K, Praditpornsilpa K, Nader C, Eiam-Ong S, Jaber BL, et al. Hepatitis B virus vaccine immune response and mortality in dialysis patients: a meta-analysis. J Nephrol 2020;33(2):343–54.
[22] Bertrand D, Hamzaoui M, Lemée V, Lamulle J, Hanoy M, Laurent C, et al. Antibody and T cell response to SARS-CoV-2 messenger RNA BNT162b2 vaccine in kidney transplant recipients and hemodialysis patients. J Am Soc Nephrol 2021 ASN.2021040480.
[23] Speer C, Göth D, Benning L, Buylaert M, Schaier M, Grenz J, et al. Early humoral responses of hemodialysis patients after COVID-19 vaccination with BNT162b2. Clin J Am Soc Nephrol 2021 CJN.03700321.
[24] Altmann DM, Boyton RJ, Beale R. Immunity to SARS-CoV-2 variants of concern. Science 2021;371(6534):1103.
[25] Rabenau HF, Biesert L, Schmidt T, Bauer G, Cinatl J, Doerr HW. SARS-coronavirus (SARS-CoV) and the safety of a solvent/detergent (S/D) treated immunoglobulin preparation. Biologicals 2005;33(2):95–9.
[26] Becker M, Strengert M, Junker D, Kaiser PD, Kerrinnes T, Traenkle B, et al. Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity. Nat Commun 2021;12(1):1152.
[27] Becker M, Dulovic A, Junker D, Ruetalo N, Kaiser PD, Pinilla YT, et al. Immune response to SARS-CoV-2 variants of concern in vaccinated individuals. Nature Commun 2021;12(1):3109.
[28] Deepak P, Kim W, Paley MA, Yang M, Carvidi AB, El-Qunni AA, et al. Glucocorticoids and B cell depleting agents substantially impair immunogenicity of mRNA vaccines to SARS-CoV-2. medRxiv : the preprint server for health sciences 2021 2021.04.05.21254656.
[29] Forni G, Mantovani A, Forni G, Mantovani A, Moretta L, Rappuoli R, et al. COVID-19 vaccines: where we stand and challenges ahead. Cell Death Different 2021;28(2):626–39.
[30] Krammer F. Correlates of protection from SARS-CoV-2 infection. The Lancet 2021;397(10283):1421–3.
[31] Sui Y, Bekele Y, Berzofsky JA. Potential SARS-CoV-2 immune correlates of protection in infection and vaccine immunization. Pathogens 2021;10(2):138.
[32] Schrezenmeier E, Bergfeld L, Hillus D, Lippert J-D, Weber U, Tober-Lau P, et al. Immunogenicity of COVID-19 Tozinameran vaccination in patients on chronic dialysis. Front Immunol 2021;12:2550.
[33] Feldman RA, Fuhr R, Smolenov I, Mick Ribeiro A, Panther L, Watson M, et al. mRNA vaccines against H10N8 and H7N9 influenza viruses of pandemic potential are immunogenic and well tolerated in healthy adults in phase 1 randomized clinical trials. Vaccine 2019;37(25):3326–34.
[34] Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 2020;586(7830):594–9.
[35] Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Viant C, Gaebler C, et al. Enhanced SARS-CoV-2 neutralization by dimeric IgA. Sci Translat Med 2021;13(577):eabf1555.
[36] Sterlin D, Mathian A, Miyara M, Mohr A, Anna F, Claër L, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. Sci Translat Med 2021;13(577):eabd2223.
[37] Lonnemann G, Novick D, Rubinstein M, Dinarello CA. Interleukin-18, interleukin-18 binding protein and impaired production of interferon-gamma in chronic renal failure. Clin Nephrol 2003;60(5):327–34.
[38] Lonnemann G, Bahlmann FH, Freise J, Hertel B, Dinarello CA. Low-flux hemodialysis suppresses interferon-gamma production: the possible role of beta2-microglobulin. Clin Nephrol 2009;72(3):170–6.
[39] Collier DA, Ferreira IATM, Kotagiri P, Datir R, Lim E, Touizer E, et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. Nature 2021.
[40] Bayart J-L, Morimont L, Closset M, Wieërs G, Roy T, Gerin V, et al. Confounding factors influencing the kinetics and magnitude of serological response following administration of BNT162b2. Microorganisms 2021;9(6).
[41] Abu Jabal K, Ben-Amram H, Beiruti K, Batheesh Y, Sussan C, Zarka S, et al. Impact of age, ethnicity, sex and prior infection status on immunogenicity following a single dose of the BNT162b2 mRNA COVID-19 vaccine: real-world evidence from healthcare workers. Israel 2021;26(6):2100096.
[42] Pellini R, Venuti A, Pimpinelli F, Abril E, Blandino G, Campo F, et al. Initial observations on age, gender, BMI and hypertension in antibody responses to SARS-CoV-2 BNT162b2 vaccine. EClinicalMedicine 2021;36:100928.
[43] Frieman M, Harris AD, Herati RS, Krammer F, Mantovani A, Rescigno M, et al. SARS-CoV-2 vaccines for all but a single dose for COVID-19 survivors. EBioMedicine 2021;68:103401.