A prospective observational study for justification, safety, and efficacy of a third dose of mRNA vaccine in patients receiving maintenance hemodialysis.

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A prospective observational study for justification, safety, and efficacy of a third dose of mRNA vaccine in patients receiving maintenance hemodialysis.

**Methods**

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**Cohorts**

- French national registry of maintenance hemodialysis (MHD) patients (REIN)

**Period:** Feb 1st to May 18th 2021

**Groups:** not vaccinated vs 1D vs 2D

**Outcomes:** COVID-19 incidence and severity

**Results**

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**Conclusions**

Reduced incidence of COVID-19 but persistence of 11% mortality after 2D

Good tolerability of 3D and increase of immune responses in patients with positive IGRA and/or low anti RBD IgG

CONCLUSION: Standard (2 doses) scheme of vaccination provides insufficient protection to some MHD patients. A 3rd dose of vaccine increases the response rate, especially for patients with a sub-optimal antibody titer and/or specific CD4+ T cell (IGRA) after the 2nd dose.
A prospective observational study for justification, safety, and efficacy of a third dose of mRNA vaccine in patients receiving maintenance hemodialysis.

Running head: Third dose of mRNA vaccine in maintenance hemodialysis patients

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Abstract (249 words)

The level of protection achieved by the standard two doses of COVID-19 mRNA vaccines in patients receiving maintenance hemodialysis (MHD) remains unclear. To study this we used the French Renal Epidemiology and Information Network (REIN) Registry to compare the incidence and severity of 1474 cases of COVID-19 diagnosed in patients receiving MHD after none, one or two doses of vaccine. Vaccination significantly reduce COVID-19 incidence and severity but 11% of patients infected after two doses still died. Lack of vaccinal protection in patients naïve for SARS-CoV-2 could be due to defective Tfh response [38% patients with negative spike-specific CD4+ T cell interferon gamma release assay] and failure to generate viral neutralizing titers of anti-spike receptor binding domain (RBD) IgGs (63% patients with titer at or under 997 BAU/ml, defining low/no responders) after two doses of vaccine. To improve protection, a third dose of vaccine was administered to 75 patients [57 low/no responders, 18 high responders after two doses] from the ROMANOV cohort that prospectively enrolled patients receiving MHD vaccinated with BNT162b2 (Pfizer). Tolerance to the third dose was excellent. High responders to two doses did not generate more anti-RBD IgGs after three doses but had more side effects. Importantly, 31 (54%) of low/no responders to two doses reached neutralizing titers of anti-RBD IgGs after three doses. A positive interferon gamma release assay and/or suboptimal titer of anti-RBD IgGs after two doses were the only predictive variables for response to three doses in multivariate analysis. Thus, the standard scheme of vaccination insufficiently protects patients receiving MHD. Anti-RBD IgG and specific CD4+ T cell response after two doses can guide personalized administration of the third dose, which improves the humoral response of SARS-CoV-2 naïve patients receiving MHD.
Introduction

Among the various alarms raised by the COVID-19 pandemic was its impact on the population of patient with end stage renal disease \(^1,2\), particularly those requiring in center hemodialysis. The logistical aspects of the technique indeed increase the risk of SARS-CoV-2 infections \(^3\), which on the highly co-morbid profile of MHD patients then translates into a high rate of COVID-19-related death \(^1,4–6\).

Aiming at protecting this vulnerable population, French health authorities prioritized MHD patients for vaccination \(^7\). However, while two doses (2D) administered intramuscularly three weeks apart of BNT162b2, a lipid nanoparticle-encapsulated mRNA-based vaccine, induced both strong humoral and cellular immune responses against the spike protein of SARS-CoV-2 in the general population \(^8\), our group \(^9\) and others \(^10–14\) have recently reported that MHD patients, particularly those that were naïve for SARS-Cov-2 virus, generated weaker responses as compared with healthy volunteers after this “standard” scheme of vaccination, raising question about their actual level of protection.

The prospective observational ROMANOV-II (Response Of heModialyzed pAtieNts to cOvid-19 Vaccination) study, aimed at comparing the severity of COVID-19 disease in MHD patients according to their vaccination status, and at evaluating whether a 3\(^{rd}\) dose (3D) of BNT162b2 vaccine was safe and efficient to increase the generation of immune effectors.
Patients and methods

Epidemiological study

The Renal Epidemiology and Information Network (REIN) is the French national registry of all patients being treated by renal replacement therapy. Clinical, demographic, and laboratory data are collected at the start of renal replacement therapy along with dialysis modalities and are updated annually. Events such as death, transfer, withdrawal from dialysis, placement on a transplant waiting list, and kidney transplantation (from living or deceased donors), as well as COVID-19 diagnosis and severity are systematically reported in real time. Interrogation of REIN registry was made on June 18th, 2021 on the period from the February 1st to May 18th, 2021.

In order to estimate the cumulative incidence of COVID-19 in MHD patients, data from the REIN registry were cross-referenced with those of the “caisse nationale d’assurance maladie” (CNAM), which collects each week the cumulative number of MHD patients that had received their first and second dose of mRNA vaccine.

Because protection of a vaccine dose was previously reported to be efficient from the tenth day following injection onward, patients were considered as “not vaccinated” until the tenth days after the first dose and remained in the group “1 dose of vaccine” until the tenth day after the second dose.

Severity of COVID-19 was graded as asymptomatic, mild, moderate, severe, critical or death following the WHO recommendations.

The ROMANOV-II prospective observational study

In line with the French health authority recommendations, a third vaccine injection of mRNA BNT162b2 COVID-19 vaccine was proposed to all patients on MHD in the
two centers of Lyon University Hospital (France) that already received two doses of mRNA BNT162b2 and did not have any of the following contra-indications: diagnosis of COVID-19 within the last 3 months, organ transplantation within the last 3 months, Rituximab injection within the last 3 months, ongoing flare of vasculitis, acute sepsis, major surgery within the last 2 weeks. Before the third injection, patients were informed of their serological status after two doses.

History of COVID-19 was defined as a positive PCR test in nasopharyngeal swab. The screening for infection was performed in patients in the presence of symptoms or because the patient had contact with a positive case. The same detection strategy was applied to MHD patients and HV.

All adult patients who received a third vaccine injection (within 3 months after the second vaccine injection) with BNT162b2 vaccine and gave consent for the use of their blood were enrolled in this study. The samples were collected 10 to 14 days after the 2nd and after the 3rd vaccine injection for analysis of the post-vaccinal immune response. This timing was selected based on previous reports demonstrating that both cellular and antibody responses are at their peak at this time point 8.

Post-vaccinal immune responses of MHD patients were compared after 2D and 3D to those of a cohort of healthy volunteers (HV), with blood sample collected at the same time point after the 2D of BNT162b2 as MHD patients.

ROMANOV-II study was conducted in accordance with the French legislation on biomedical research and the Declaration of Helsinki, and the protocol was evaluated by a national ethical research committee (ID-RCB 2021-A00325-36) and registered on clinicaltrial.gov as NCT04881396. The French national commission for the protection of digital information (CNIL) authorized the conduction of the study.
Assessment of the tolerability and safety of vaccine injections

Local and systemic adverse events and use of anti-pyretic medications were collected retrospectively, based on a self-assessment questionnaire. Data collected correspond to adverse events within 7 days after the 2D and 3D, respectively.

As previously described, pain at the injection site was assessed according to the following scale: mild, does not interfere with activity; moderate, interferes with activity; severe, prevents daily activity; and critical, emergency department visit or hospitalization. Redness and swelling were measured according to the following scale: mild, 2.0 to 5.0 cm in diameter; moderate, >5.0 to 10.0 cm in diameter; severe, >10.0 cm in diameter; and critical, necrosis or exfoliative dermatitis (for redness) and necrosis (for swelling). Fever categories were mild, 38.0°C to 38.4°C; moderate > 38.4°C to 38.9°C; severe, >38.9°C to 40° and critical, >40°C. Medication use was not graded. Additional scales were as follows: fatigue, headache, chills, new or worsened muscle pain, new or worsened joint pain (mild: does not interfere with activity; moderate: some interference with activity; or severe: prevents daily activity), vomiting (mild: 1 to 2 times in 24 hours; moderate: >2 times in 24 hours; or severe: requires intravenous hydration), and diarrhea (mild: 2 to 3 loose stools in 24 hours; moderate: 4 to 5 loose stools in 24 hours; or severe: 6 or more loose stools in 24 hours); critical for all events indicated an emergency department visit or hospitalization.

Assessment of anti-SARS-CoV2 humoral response

In-vitro neutralization assay

SARS-CoV-2 [BetaCoV/France/IDF0571/2020 virus (GISAID Accession ID = EPI_ISL_411218)] was isolated in Vero E6 from a nasal swab of one of the first COVID-19-positive patient in France and was kindly provided by Dr Olivier Terrier and the
Virpath lab (CIRI-Lyon). To generate virus stocks, Vero E6 cells were inoculated with virus at an MOI of 0.01. Supernatant fluid was harvested at 72 h post-infection, clarified by low-speed centrifugation, aliquotted, and stored at −80°C. Virus stock was quantified by classic limiting dilution plaque assay on Vero E6 cells (kindly provided by Dr F-L. Cosset, CIRI-Lyon).

Two-fold dilutions of serum in 50 μl of Dulbecco’s modified Eagle medium (DMEM), containing 2X penicillin/streptomycin, were incubated with 200 plaque-forming units (PFU) of SARS-CoV-2 in 50 μl of DMEM for 15 min at room temperature. Aliquots of 100 μl of DMEM + 4% FBS containing 2.5 × 10^4 Vero E6 cells were added to achieve a final dilution of sera from 1:100 to 1:12,800 (4 wells per dilution). Cells were incubated for 5 days at 37°C, 5% CO2. After 15 min of fixation in PFA4% in PBS1X, cytopathic effect was revealed by crystal violet staining and scored by a researcher (CM) blinded to the study design and sample identity. Neutralization endpoint titers were expressed as the value of the last serum dilution that completely inhibited virus-induced cytopathic effect.

**Anti-RBD IgG response**

The IgG antibodies directed against the Receptor Binding Domain (RBD) of the Spike glycoprotein of the SARS-Cov2 were detected by a chemiluminescence technique, using the Maglumi® SARS-CoV-2 S-RBD IgG test (Snibe Diagnostic, Shenzen, China) on a Maglumi 2000® analyser (Snibe Diagnostic), according to the manufacturer’s instructions. Briefly, 10μL of serum were incubated in the appropriate buffer with magnetic microbeads covered with Spike RBD recombinant antigen, in order to form immune complexes. After precipitation in a magnetic field and washing, ABEI (N-(4-Aminobutyl)-N-ethylisoluminol)-stained anti-human IgG antibodies were added to the
samples. After a second magnetic separation and washing, the appropriate reagents were added to initiate a chemiluminescence reaction. When necessary, sera were diluted sequentially up to 1:1000.

As recommended by the WHO, the titers are expressed as binding arbitrary units/mL (BAU/mL).

**Assessment of the anti-SARS-CoV2 Spike cellular immune responses**

*Enumeration of SARS-CoV-2 spike-specific T CD4+, T follicular helper (Tfh) and CD8+ cytotoxic cells*

Peripheral Blood Mononuclear Cells (PBMC) were collected and isolated by centrifugation on a Ficoll density gradient. The cells were then frozen in fetal calf serum supplemented with 10% dimethylsulfoxide (DMSO, Sigma).

CD8+ and CD4+ T cells specific for SARS-CoV2 spike protein were enumerated using the technique reported by Grifoni et al. SARS-CoV2 spike-specific Tfh were enumerated according to a technique developed by our team and previously published. Briefly, after thawing, cells were concentrated at 10^7 cells/mL in RPMI complete medium and left to rest overnight at 37°C and 5% CO2 in a 96-well round-bottom plate, 10^6 cells/well. The next day, the RPMI medium was changed, and the cells were cultured for 24 hours in the presence of a pool of overlapping peptides covering the entire sequence of the spike protein of SARS-CoV-2 (PepMixTM, JPT Peptides Technologies GmbH, Berlin, Germany). The final concentration of the peptides was 1µg/mL. Cells cultured with DMSO (Sigma) alone (1/250) were used as negative controls. Cells were then rinsed and incubated at room temperature with a Fixable Viability Dye (eBiosciences) and one of the two following fluorescent antibodies panels for 30 minutes. **Panel 1**: CD3 (UHCT1), CD8 (SK1), from BD Biosciences; CD4
(SK3), CD69 (FN50), CD137 (4B4-1), CD134 (OX-86) from Biolegend. **Panel 2:** CD4 (SK3) from Biolegend, CD3 (UHCT1) CXCR5 (RF8B2), CD25 (2A3), from BD Biosciences. Cells were fixed with 2% methanol-free formaldehyde. Sample acquisitions were made on a BD LSR Fortessa 4L flow cytometer (BD Biosciences). The gating strategies used for these analyses are shown in **Supplementary Figure 1A-C.**

*Interferon-gamma release assay (IGRA)*

Spike specific CD4+ T cells responses were quantified in the circulation of the HV and MHD patients using the QuantiFERON® SARS-CoV2 test (Qiagen, Netherlands), a commercially available Interferon Gamma Release Assay (IGRA), according to the manufacturer’s instructions.

Briefly, 1mL blood was distributed in each tube of the assay: (i) uncoated tube: negative control/background noise, (ii) tube coated with mitogen: positive control, (iii) tube coated with HLA-II restricted 13-mers peptides derived from the entire SARS-CoV2 Spike glycoprotein used to stimulate CD4+ T cells. After 20 hours of culture at 37°C, tubes were centrifuged 15 minutes at 2500g, and stored at 4°C before INFγ quantification in the supernatant by ELISA.

The CD4+ T cell assay value was the difference between tube (iii) and the negative control (i).

**Statistical analysis**

All the analyses were carried out using R software version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria, 2021, https://www.R-project.org) and or GraphPad Prism v8.0 (San Diego, California USA). Categorical variables were
expressed as percentages and compared with the chi-squared test. Continuous variables were expressed as mean ± SD and compared using one-way ANOVA and multiple t-tests post-hoc analyses or as median ± IQR and compared using Mann Whitney test for variables with non-normal distribution.

Logistic regression models were used in both univariate and multivariate analyses. All the explanatory variables significantly associated with outcomes in univariate analyses (p-value < 0.10) were included in multivariate models. Stepwise regression analyses with bidirectional elimination were then performed, using Aikake Information Criterion to select the most fitting final multivariate models.
Results

*MHD patients are insufficiently protected against COVID-19 after 2 doses of mRNA vaccine*

In order to evaluate the level of protection conferred by COVID-19 mRNA vaccination to naïve MHD patients the French national registry REIN [Renal Epidemiology and Information Network 23] was interrogated to identify all the cases of COVID-19 diagnosed in MHD patients from February 1st to May 18th 2021, the period during which MHD population was prioritized for vaccination in France (Figure 1A). During this period, the virus circulation rate in France was moderate (estimated ~200 cases/week/100 000) and the large majority of COVID-19 cases were related to either the original coronavirus strain detected in Wuhan, or the alpha variant (Supplementary Figure 2) 24.

The cumulative incidence of COVID-19 at 28 days was 1.98% in naïve non-vaccinated MHD patients. Although vaccination reduced this number to respectively 0.65% after the 1st dose and to 0.25% after the 2nd dose (Figure 1B; log rank<0.0001), this level of protection remains largely inferior to what reported in the general population 17,25.

Over the study period a total of 1474 cases of COVID-19 were reported. For the 1439 (97.6%) infected MHD patients for whom the information was available, the severity of disease was analyzed according to whether the diagnosis of COVID-19 was made before vaccination (not vaccinated, black; n=1122), 10 days after the 1st dose (1D, red; n=192) or the 2nd dose (2D, green; n=125) of vaccine. The characteristics of the population are presented in Table 1. Patients’ characteristics were similar in the 3 groups with exception of age, cardiopathy and time in MHD, which were all three higher in patients who developed COVID-19 after 2D of vaccine, probably because the
patients with the more comorbid profile were vaccinated with the highest priority (Table 1).

The distribution of patients across the 5 stages of severity of COVID-19 defined by the WHO [Asymptomatic, Mild, Severe, Critical or Death; 26] was statistically different between the 3 groups (Figure 1C). However, despite an increased proportion of less severe forms (asymptomatic or mild/moderate) of COVID-19 in vaccinated patients, 11% of MHD patients that had received 2D of mRNA vaccine still died from COVID-19 (Figure 1C). The latter result is drastically different from what reported in the pivotal studies conducted in the general population 17, and demonstrates that vaccination with the 2D “standard” scheme is insufficient to protect all MHD patients.

**Standard 2 doses scheme of vaccination induces flawed humoral immune responses in naïve MHD patients**

Among the 150 MHD patients dialyzing at Lyon University Hospital, 38 (25.3%) refused the vaccine or had contra-indications. Of the 112 who received 2D of BNT162b2 mRNA vaccine, 106 (14 of whom had a previous history COVID-19, black circle) gave consent for analysis of the post-vaccinal immune response and were enrolled in ROMANOV study (Figure 2). In order to understand why naïve MHD patients were insufficiently protected by COVID-19 mRNA vaccination, the humoral and cellular immune responses of the latter were compared to that of 30 healthy volunteers (HV, 4 of whom had a previous history COVID-19; black triangle).

Enumeration of SARS-Cov-2 spike-specific CD8+ T cells was made after 2D by flow cytometry using the activation induced marker technique 21 (Figure 3A). Although the percentage of circulating spike-specific CD8+ T cells was more heterogeneous and slightly reduced in MHD patients as compared with HV (0.15 [0.05-0.57] vs 0.31 [0.21-0.31]).
0.45], p=0.042, **Figure 3B**), this mild difference was unlikely to be the sole explanation to the major difference in protection against COVID-19 observed in the 2 vaccinated populations.

We next went on analyzing the viral neutralizing capacity of patients’ sera after 2D using an *in vitro* functional assay (**Figure 3C**). While the serum of MHD patients with previous history of COVID-19 (**Figure 3D**, black circles) had similar viral neutralizing capacity as the sera of HV, this serum characteristic was profoundly depressed in vaccinated MHD patients naïve for the virus (**Figure 3D**, open circles).

Viral neutralization capacity of serum depends upon the presence of high titers of IgGs directed against the spike protein. The generation of IgGs against a protein antigen requires a particular subset of CD4+ T cells, the T follicular helper (Tfh) cells, which are specialized in providing the help to B cells and necessary to their differentiation into antibody-producing plasma cells. Enumeration of SARS-Cov-2 spike-specific CD4+ T cells and Tfh was performed in the circulation after 2D using two distinct techniques. In contrast with MHD patients that developed neutralizing IgG titers (Neutral+), MHD patients whose serum lacks viral neutralizing capacity (Neutral-) had reduced levels of both spike-specific CD4+ T cells (**Supplementary Figure 1D-E**) and spike-specific Tfh in their circulation (**Figure 3F**).

**Surrogate assays to monitor neutralizing antibodies and spike-specific Tfh in routine clinical practice**

Viral neutralizing antibodies, the generation of which depends upon spike-specific Tfh, seems important to provide protection against COVID-19 after vaccination. Monitoring of these immune effectors after vaccination could therefore be interesting to identify MHD patients insufficiently protected. Unfortunately, neither *in vitro* neutralizing assay,
nor the enumeration of spike-specific Tfh in the circulation can be performed in routine clinical practice.

Antigen-binding assays are convenient and widely used in routine for monitoring of antibody-response. Comparing the titer of IgG directed against the Receptor Binding Domain (RBD) of the spike glycoprotein of the SARS-CoV2 (anti-RBD IgG) measured with chemoluminescence assay and the neutralizing capacity of the serum, we observed a highly significant (p<0.0001) and strong ($r^2=0.67$) positive correlation (Figure 3G). Hence, we could establish that a titer of anti-RBD IgGs ≥ 997 BAU/mL (Figure 3G, vertical dashed line) was systematically associated with viral neutralizing capacity of the serum. This threshold was therefore used in the rest of the study to define “high” (anti-RBD IgG ≥ 997 BAU/ml; n=39/106, 36.8%) versus “low/no” (anti-RBD IgG < 997 BAU/ml n=67/106, 63.2%) response to vaccine (Figure 2). An indirect validation of this functional threshold is provided by the fact that almost all healthy volunteers, which are efficiently protected against COVID-19 by the vaccination, had anti-RBD IgG titers ≥ 997 BAU/ml after 2D of vaccine (Figure 3D & 3G).

Interferon Gamma Release Assay (IGRA) are already used in clinical practice to monitor the T cell response against Mycobacterium tuberculosis. The results obtained with a commercially available SARS-CoV-2 CD4+ T cells IGRA were compared to the enumeration of antigen-specific Tfh by flow cytometry (Figure 3H). The highly significant (p<0.0001) and strong ($r^2=0.65$) positive correlation observed suggests that CD4+ T cells IGRA can be used as a surrogate assay to flow cytometry for the monitoring of spike-specific Tfh response.

*Prospective observational study on the 3rd dose of mRNA vaccine in MHD patients*
In an attempt to improve vaccine protection against COVID-19 of MHD patients, France health officials authorized the administration of a third dose (3D) of vaccine in this population from mid-April 2021 onward 19.

A 3rd dose of BNT162b2 mRNA vaccine was therefore offered to all 67 MHD patients of ROMANOV study with low/no anti-RBD IgG response and effectively administered to 57 (85.1%) of them (Figure 2). In absence of clear consensus, the administration of the 3D of vaccine was not limited to MHD patients with low/no anti-RBD IgG titers, and 18/39 (46.2%; p<0.0001) MHD patients with high IgG response also accepted a 3D of vaccine (Figure 2). The characteristics of the 75 MHD patients that received 3D of vaccine are presented Table 2.

Reactogenicity to the 3rd dose of mRNA vaccine in maintenance hemodialysis patients

Among included MHD patients, tolerability data were available for 82/106 patients after 2D and 63/75 after 3D. Overall tolerance to the 3D of BNT162b2 mRNA vaccine was good in MHD patients (Figure 4A-B). No patients developed critical side effects requiring hospitalization. Forty percent (25/63) developed local sides effects, the most frequently reported being pain at the injection site (40%). Forty-six percent (29/63) reported systemic side effects, including fatigue (32%), chills (16%) and soreness (16%). In almost all cases (74/83, 89%) the intensity of the symptoms was mild or moderate (Figure 4A-B).

When local and systemic side effects of vaccine were compared between 2D and 3D, no significant difference was found, neither regarding the frequency nor severity of symptoms (Figure 4A-B). However, when the profile of tolerance was compared between MHD patients according to the intensity of the humoral response after 2D of
vaccine, a significant trend for more side effects was observed in patients with high response (Figure 4C-D).

**Impact of the 3rd dose of mRNA vaccine on humoral response of MHD patients**

When the whole cohort of MHD patients (n=75) was considered, a significant increase in the median titer of anti-RBD IgG was observed after 3D of vaccine (309.8 [36.5 – 996.3 vs 2212 [394.9 – 3247] BAU/mL after the 2D and 3D respectively; p<0.0001; Figure 5A). This global positive result hides however major inter-individual heterogeneity.

MHD patients with high humoral response after 2D of vaccine (n=18), all maintained high levels of anti-RBD IgG after 3D but without significant increase of their titer (2757 [1869 – 4365] vs 3619 [2127 – 11035] BAU/mL after the 2D and 3D respectively; p=0.154; Figure 5B). In contrast, MHD patients with low/no humoral response after 2D experienced a significant increase of anti-RBD IgG after the 3D: 10.5 [1.05 – 69.9] vs 353.1 [36.2 – 2592] BAU/mL (Figure 5C; p<0.0001). However, there was again significant inter-individual heterogeneity in the response and only 31/57 (54.4%) of low/no responders to 2D reached optimal titer (i.e. ≥ 997 BAU/ml; Figure 5C, dashed line) of anti-RBD IgG after 3D.

**Impact of the 3rd dose of mRNA vaccine on cellular response of MHD patients**

The impact of 3D on antigen-specific Tfh response was indirectly monitored using spike-specific CD4+ T cells IGRA. Globally, the 3D of vaccine did not result in a significant increase in spike-specific Tfh response in the circulation of MHD patients neither when the amount of IFNγ: 0.127 [0.014 – 1.040] vs 0.261 [0.025 – 0.820] IU/mL, p=0.517 nor the proportion of MHD patients with positive IGRA (57% vs 64%; p=0.50) were considered (Figure 5D). The result remained unchanged when the analysis was
made within the subpopulations of MHD patients with high versus low/no humoral response after 2D of vaccine (Figure 5E & 5F).

Unexpectedly, we noticed that some MHD patients experienced a reduction in their CD4+ T cells IGRA result between 2D and 3D (Figure 5D-F). This proportion was not different between high and low/no responders to 2D (8/18, 44% vs 24/57, 42%, p>0.99). Although we do not have definitive explanation for this observation, it could be due to technical limitations of the assay and/or indicate inter-individual heterogeneity in the durability of the CD4+ T cell response.

**Defining the subpopulation of MHD patients that should receive a 3rd dose of vaccine**

Because it was less well tolerated in these patients (Figures 4C-D) and did not improve their immune response (Figures 5B, 5F), we concluded that the subpopulation of MHD patients with already high humoral response after 2D should not receive a 3D of vaccine.

In order to identify among the MHD patients with low/no response after the standard scheme of vaccination those who would benefit from 3D of vaccine, the characteristics of the MHD patients, who reached high titer of anti-RBD IgG after 3D (responders to 3D; n=31/57; 54.4%) were compared to that of the rest of the cohort (non-responders to 3D; n=26/57; 45.6%). The former group was less exposed to immunosuppressive drugs, and had more often detectable anti-RBD IgG and positive spike-specific CD4+ T cells IGRA after 2D (Table 3). In multivariate analysis, the only variables that predicted an immune response to 3D was the presence of low titers of anti-RBD IgG (OR=10.1 [1.3-216.5], p=0.054) and a positive spike-specific CD4+ T cells IGRA (OR=9.25 [2.44–40.7], p=0.002) after 2D (Figure 5G). Furthermore, combining these
two information, we observed that the probability to respond to D3 in MHD that were low/no responders to 2D was the highest in patients positive for both tests (82%, Figure 5H). The response rate decreased to 41% in MHD with only low anti-RBD IgG after D2 and dropped to 0% in those in whom both tests were negative after D2 (Figure 5H).
Discussion

This prospective observational study demonstrates that, in contrast with what reported in the general population, the “standard” 2D scheme with BNT162b2 vaccine provides insufficient protection against the severe forms of COVID-19 in MHD patients naïve for the virus.

This problem could be due to the fact that MHD patients develop flawed humoral response after 2D of vaccine, as illustrated by the very limited viral neutralizing capacity of their serum as compared with healthy volunteers. This could be the consequence of the deleterious impact of uremic toxins on the generation of spike-specific Tfh, a crucial subset to generate high titer of IgGs that was detected in reduced number in MHD patients who fail to respond to the vaccination. These conclusions are in line with recent studies, which reported that MHD patients naïve for SARS-Cov-2 develop impaired humoral and cellular immune responses after 2D of BNT162b2.

Based on the observations that i) MHD patients with a previous history of COVID-19 had a response to vaccine indistinguishable from that of HV, and ii) previous studies with protein-based vaccine (such as hepatitis B vaccine) reported acceptable response rates when dosing and/or number of administrations were increased, a 3D of BNT162b2 vaccine was offered to MHD patients in France. Although, the safety of the 3D of BNT162b2 vaccine was excellent and comparable to that of the 2D with no critical local or systemic side-effect reported, the tolerance was worst in patients with already high humoral response, who did not improve significantly their immune response against the spike protein of SARS-Cov-2 after this additional injection. Furthermore, while not identified in our cohort, some cases of (re)activation of auto-
immune disorders have been reported after mRNA vaccines in the literature. This threat further supports avoiding useless additional vaccine injection in patient already protected after 2D.

In contrast, after a 3D, 91% of naïve MHD patients with low/no response after 2D, experienced an increase of anti-RBD-IgG titer, 54% of whom up to optimal (≥ 997 BAU/mL) level, associated with viral neutralization. This latter subgroup could be identified after 2D (preemptively) by the fact that they had a positive CD4+ T cells IGRA (a surrogate for the presence of spike-specific Tfh) and/or suboptimal titres (detectable but <997 BAU/mL) of anti-RBD IgGs in antigen-binding assay. Importantly, both assays are commercially available and easy to implement in routine clinical practice, which could pave the way for the personalization of the vaccination strategy in MHD.

Our study has however some limitations. Even if the size of ROMANOV cohort is at least comparable to what currently reported in the literature, our observations were made on a limited number of patients from a single university hospital. Furthermore, all these patients were receiving in-center maintenance hemodialysis and were therefore characterized by a highly comorbid profile (Table 2). This shall be kept in mind since this bias may limit the generalizability of our conclusions.

Based on the findings presented above, we propose the following strategy to optimize the protection of MHD patients naïve for SARS-Cov-2. All these vulnerable patients should be offered the standard scheme of vaccination in priority. Anti-RBD IgG and spike-specific CD4+ T cells should be monitored in their circulation 10 to 14 days after 2D, resulting in the definition of 3 subgroups with distinct needs: i) patients with high anti-RBD IgG titer (≥ 997 BAU/mL) do not require further intervention, ii) patients with low/no anti-RBD IgG titer but positive CD4+ T cells IGRA, whom are the most likely to
respond, should be offered a 3D of vaccine, and iii) patients with neither detectable anti-RBD IgG nor positive CD4+ T cells IGRA after 2D, whom will not respond to 3D, might rather receive infusion of monoclonal antibodies as a mean to induce passive immunization. Future prospective studies are urgently needed to confirm the validity of such personalized anti-COVID-19 vaccination strategy in MHD patients naïve for SARS-Cov-2.
Disclosers

All authors declared no conflicts of interest.
References


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ME is supported by the Hospices civils de Lyon (année médaille d’or) and by INSERM (Poste accueil). XC is supported by the Société Française de Transplantation. OT is supported by Fondation pour la Recherche Médicale (PME20180639518) and the "Etablissement Français du Sang".

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### Tables

**Table 1 – Characteristic of COVID-19 infected MHD patients, according to their vaccination status**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Whole cohort n=1439</th>
<th>Not vaccinated n=1122</th>
<th>1D n=192</th>
<th>2D n=125</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexe ratio (M/F)</td>
<td>1.37 (833/606)</td>
<td>1.32 (639/483)</td>
<td>1.49 (115/77)</td>
<td>1.72 (79/46)</td>
<td>0.338</td>
</tr>
<tr>
<td>Age (years)</td>
<td>69.6 ± 15.0</td>
<td>68.5 ± 15.2</td>
<td>71.7 ± 14.3</td>
<td>74.0 ± 13.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.4 ± 6.30</td>
<td>27.5 ± 6.24</td>
<td>27.0 ± 6.48</td>
<td>27.3 ± 6.52</td>
<td>0.676</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>732 (51)</td>
<td>581 (52)</td>
<td>89 (46)</td>
<td>62 (50)</td>
<td>0.364</td>
</tr>
<tr>
<td>Cardiopathy</td>
<td>572 (40)</td>
<td>420 (37)</td>
<td>84 (44)</td>
<td>68 (54)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>380 (26)</td>
<td>280 (25)</td>
<td>58 (30)</td>
<td>42 (34)</td>
<td>0.051</td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>269 (19)</td>
<td>204 (18)</td>
<td>42 (22)</td>
<td>23 (18)</td>
<td>0.477</td>
</tr>
<tr>
<td>Malignancy</td>
<td>148 (10)</td>
<td>108 (10)</td>
<td>25 (13)</td>
<td>15 (12)</td>
<td>0.289</td>
</tr>
<tr>
<td>HD parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time in HD (months)</td>
<td>5.5 ± 6.6</td>
<td>5.2 ± 6.2</td>
<td>6.4 ± 7.7</td>
<td>6.0 ± 7.6</td>
<td>0.046</td>
</tr>
<tr>
<td>Time HD/week (hours)</td>
<td>11.6 ± 1.63</td>
<td>11.6 ± 1.62</td>
<td>11.8 ± 1.70</td>
<td>11.7 ± 1.60</td>
<td>0.196</td>
</tr>
</tbody>
</table>

**Abbreviations are:** BMI, body mass index; MHD, maintenance hemodialysis.
Table 2 – Clinical and biological characteristic of MHD patients injected with a 3rd dose of BNT162b2.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Whole cohort n=75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>48 (64)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.8± 14.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.8± 6.4</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>35 (47)</td>
</tr>
<tr>
<td>Cardiopathy</td>
<td>36 (48)</td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Hepatic disease</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Cause of renal failure</td>
<td></td>
</tr>
<tr>
<td>Vascular</td>
<td>17 (23)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>27 (36)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>7 (9)</td>
</tr>
<tr>
<td>Hereditary</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Uropathy</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Others</td>
<td>21 (28)</td>
</tr>
<tr>
<td>Previous SOT</td>
<td>16 (21)</td>
</tr>
<tr>
<td>IS therapy</td>
<td>8 (11)</td>
</tr>
<tr>
<td>History of COVID-19</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Time in HD (months)</td>
<td>56± 69</td>
</tr>
<tr>
<td>HD parameters</td>
<td></td>
</tr>
<tr>
<td>Time HD/week (hours)</td>
<td>10.6± 2.77</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.56± 0.43</td>
</tr>
<tr>
<td>Biological characteristics</td>
<td></td>
</tr>
<tr>
<td>Hemoglobinemia (g/L)</td>
<td>106± 16</td>
</tr>
<tr>
<td>C reactive protein (mg/L)</td>
<td>13.4± 20.9</td>
</tr>
<tr>
<td>Albuminemia (g/L)</td>
<td>36.4± 6.7</td>
</tr>
</tbody>
</table>

Abbreviations are: BMI, body mass index; OR, odd ratio; CI95%, 95% confident interval; SOT, solid organ transplantation; IS, immunosuppressive; HD, hemodialysis; Kt/V was used for the quantification of dialysis adequacy by the formula: dialysis clearance of urea (K) multiplied by t (dialysis time) divided by the volume of distribution of urea (V); CRP, c reactive protein.
Table 3 – Uni and multivariate analysis used to identify predictive factors of response to the 3rd dose.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No-response to 3D n=26</th>
<th>Response to 3D n=31</th>
<th>OR univariate (CI95%, p)</th>
<th>OR multivariate (CI95%, p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>21 (81)</td>
<td>19 (61)</td>
<td>0.38 (0.10-1.22, p=0.115)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>69.2± 13</td>
<td>66.6± 14</td>
<td>0.99 (0.95-1.02, p=0.455)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8± 4.7</td>
<td>27.7± 7.3</td>
<td>1.06 (0.97-1.18, p=0.256)</td>
<td></td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>13 (50)</td>
<td>15 (48)</td>
<td>0.94 (0.33-2.67, p=0.903)</td>
<td></td>
</tr>
<tr>
<td>Cardiopathy</td>
<td>16 (62)</td>
<td>13 (42)</td>
<td>0.45 (0.15-1.29, p=0.143)</td>
<td></td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>2 (8)</td>
<td>3 (10)</td>
<td>1.29 (0.20-10.4, p=0.792)</td>
<td></td>
</tr>
<tr>
<td>Hepatic disease</td>
<td>3 (12)</td>
<td>1 (3)</td>
<td>0.26 (0.01-2.14, p=0.251)</td>
<td></td>
</tr>
<tr>
<td>Previous SOT</td>
<td>6 (23)</td>
<td>3 (10)</td>
<td>0.36 (0.07-1.52, p=0.179)</td>
<td>1.18 (0.10-13.2, p=0.890)</td>
</tr>
<tr>
<td>IS drug</td>
<td>6 (23)</td>
<td>2 (6)</td>
<td>0.23 (0.03-1.11, p=0.090)</td>
<td>1.72 (0.16-38.4, p=0.664)</td>
</tr>
<tr>
<td>History of COVID-19</td>
<td>1 (4)</td>
<td>2 (6)</td>
<td>1.72 (0.16-38.4, p=0.664)</td>
<td></td>
</tr>
<tr>
<td>Time in HD (days)</td>
<td>1135± 1022</td>
<td>1266± 1511</td>
<td>1.00 (1.00-1.00, p=0.705)</td>
<td></td>
</tr>
<tr>
<td>HD parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time HD/week (hours)</td>
<td>683± 94</td>
<td>685± 78</td>
<td>1.00 (0.99-1.01, p=0.926)</td>
<td></td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.5± 0.3</td>
<td>1.4± 0.5</td>
<td>0.74 (0.20-2.63, p=0.644)</td>
<td></td>
</tr>
<tr>
<td>Biological</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobinemia (g/L)</td>
<td>108± 10</td>
<td>107± 16</td>
<td>1.00 (0.96-1.04, p=0.940)</td>
<td></td>
</tr>
<tr>
<td>C reactive protein (mg/L)</td>
<td>19.0± 30.1</td>
<td>9.0± 8.4</td>
<td>0.97 (0.93-1.00, p=0.124)</td>
<td></td>
</tr>
<tr>
<td>Albuminemia (g/L)</td>
<td>34.6± 4.5</td>
<td>36.6± 5.7</td>
<td>1.08 (0.97-1.20, p=0.167)</td>
<td></td>
</tr>
<tr>
<td>Anti SARS-Cov-2 response after 2 doses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detectable anti RBD-IgG</td>
<td>15 (58)</td>
<td>30 (97)</td>
<td>22.0 (3.8-421.7, p=0.005)</td>
<td>10.1 (1.3-216.5, p=0.054)</td>
</tr>
<tr>
<td>CD4+ T cell IGRA (+)</td>
<td>5 (19)</td>
<td>24 (77)</td>
<td>14.4 (4.26-57.6, p&lt;0.001)</td>
<td>9.25 (2.44-40.7, p=0.002)</td>
</tr>
</tbody>
</table>

Abbreviations are: BMI, body mass index; OR, odd ratio; CI95%, 95% confidence interval; SOT, solid organ transplantation; IS, immunosuppressive; HD, hemodialysis; Kt/V was used for the quantification of dialysis adequacy by the formula: dialysis clearance of urea (K) multiplied by t (dialysis time) divided by the volume of distribution of urea (V); CRP, c reactive protein.
Figures legends

**Figure 1 – Severity of COVID-19 disease in MHD patients according to their vaccination status**

A/ Flow chart of the epidemiological study conducted through the REIN network.

B/ Cumulative incidence of the cases of COVID-19 that occurred over the study period in MHD patients before vaccination or up to 10 days after the first dose (Not vacc, black curve), from 10 days after the first dose to 10 days after the second dose (1D, red curve), or more than 10 days after he second dose of vaccine (2D, green curve). Log rank test; ****, p<0.0001.

C/ Severity of COVID-19 was color coded and the distribution was compared between the groups of MHD patients defined according to their vaccination status. Chi-square test; ****, p<0.0001.

**Figure 2 – Flow chart of the ROMANOV prospective study**

**Figure 3 – Comparison of the immune responses of MHD and HV after 2 doses of BNT162b2**

Spike-specific cellular and humoral immune responses were evaluated 10 to 14 days after the second dose of vaccine in the circulation of 77 MHD patients (circles; among which 14 had a previous history of COVID-19, black circles) and 30 healthy volunteers (triangles; among which 4 had a previous history of COVID-19, black triangles).

A-B Enumeration of spike-specific CD8+ T cells by the Activation-Induced Markers (AIM) technique. A/ Gating strategy is shown on representative flow cytometry profiles. B/ Histogram showing individual values for HV and MHD.
**C-D** Evaluation of viral neutralization capacity of the serum by *in vitro* functional assay. **C/** Schematic representation of the methodology. **D/** Histogram showing individual values for HV and MHD.

**E-F** Enumeration of spike-specific CD4+ Tfh cells. **E/** Gating strategy is shown on representative flow cytometry profiles. **F/** Histogram showing individual values for HV and MHD, the latter being distributed in two groups (Neutral+ or Neutral-) according to the viral neutralization capacity of their serum.

Mann Whitney test; ns, p>0.05; *, p≤0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001.

**G/** Relation between the titers of anti-RBD IgG measured in antigen-binding assay and the viral neutralization capacities evaluated in the *in vitro* functional assay shown panel C was analyzed by linear regression. The threshold of anti-RBD IgG titer (997 BAU/ml) above which all sera had viral neutralization capacity is indicated by a vertical dashed line and was used to defined high vs low/no responders to 2D of vaccine.

**H/** Relation between the result of spike-specific CD4+ T cells IGRA and the percentage of spike-specific CD4+ Tfh cells enumerated as shown in panel E was analyzed by linear regression.

*Figure 4 - Reactogenicity to the 3rd dose of mRNA vaccine in MHD patients*

**A/** Proportion of MHD patients who developed local and systemic adverse events after 2D and after 3D dose of vaccine are represented. Severity of the adverse event is color-coded (0-4) according to the scale detailed in the material and method section.

**B/** The number and the severity of local and systemic adverse events that occurred after 2D and 3D of vaccine are compared. Chi-square test.
**C**/ Proportion of MHD patients who developed local and systemic adverse events after 3D of vaccine according to the viral neutralization capacity of their serum after the 2\textsuperscript{nd} injection (high: neutralization+ vs low/no: neutralization-).

**D**/ The number and the severity of local and systemic adverse events that occurred after 3D of vaccine were compared between high and low/no patients. Chi square test.

**Abbreviations are:** ns, p non-significant; ***, p<0.0001.

**Figure 5 – Evolution of anti-RBD IgG titers and the results spike-specific CD4+ T cells IGRA between 2D and 3D of vaccine in MHD patients**

**A-C**/ Anti RBD-IgG titers expressed in binding arbitrary units (BAU/mL) were measured 10-14 days after the second (2D) and third (3D) dose of vaccine. Upper dashed line represents the threshold (997 BAU/ml) above which all sera have viral neutralization capacity. This limit was used to define high vs low/no responders to 2D. Lower dotted line indicates the limit of detection (LOD) of the assay. A/ Results of the whole cohort of MHD patients are plotted. B-C/ Evolution of anti RBD-IgG titers between the 2D and 3D of vaccine were compared for high responders (n=18, B) and low/no responders (n=57, C) only. Wilcoxon test.

**D-F**/ Result of spike-specific CD4+ T cells IGRA were measured 10-14 days after the second (2D) and third (3D) dose of vaccine. Lower dashed line indicates the limit of positivity of the assay. D/ Results of the whole cohort of MHD patients are plotted. E-F/ Evolution of the results of spike-specific CD4+ T cells IGRA between the 2D and 3D of vaccine were compared for high responders (n=18, E) and low/no responders (n=57, F) only. Wilcoxon test. The proportion of positive IGRA is indicated in the pie chart.
**G**/ Forest plot of the results of the multivariate analysis conducted to identify the variables independently associated with the generation of anti RBD-IgG titers $\geq 997$ BAU/ml after 3D.

**H**/ The proportion of MHD patients that generated anti RBD-IgG titers $\geq 997$ BAU/ml after 3D is shown according to the presence of anti RBD-IgG and the result of spike-specific CD4+ T cells IGRA after 2D. Chi square test

**Abbreviations are:** ns, $p>0.05$; *, $p \leq 0.05$; ****, $p<0.0001$.  
Supplementary material:

Supplementary Figure 1 - Flow cytometry analyses of anti-spike T cell responses

Supplementary Figure 2 – Epidemiology of COVID-19 pandemic in France during the study period
A
Declared cases of COVID-19 between 1st February 2021 and 18th May 2021 in MHD patients, n=1474

No information concerning severity of the disease, n=35 (2.4%)

Population for severity analysis, n=1439 (97.6%)

B
Cumulative COVID-19 incidence (%)

- Not vacc
- 1D
- 2D

Days

C
Severity of COVID-19:
- Asympto
- Mild/Moderate
- Severe
- Critical
- Death

Not vaccinated
n=1122

1D
n=192

2D
n=125
Predictive factors of response to 3D

<table>
<thead>
<tr>
<th>Immunosuppressive drugs</th>
<th>Odds ratio (■) and 95% CI (—)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T cells IGRA(+) after 2D</td>
<td></td>
</tr>
<tr>
<td>Detectable anti RBD IgG after 2D</td>
<td></td>
</tr>
</tbody>
</table>

Response to 3D (%)

- Low anti RBD and IGRA+ (n=28)
- Low anti RBD IgG only (n=17)
- None (n=11)