



Humoral response to BNT162b2 mRNA COVID-19 vaccine in peritoneal and hemodialysis patients: A comparative study

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Abstract

Introduction: Maintenance dialysis patients (MDP) are at higher risk of exposure with increased mortality from COVID-19 with generalized immunization becoming the cornerstone in prevention. This study aims to compare humoral response between hemodialysis (HD) and peritoneal dialysis (PD) patients.

Materials and Methods: Observational prospective study following HD and PD programs from a Portuguese Center receiving BNT162b2 vaccine. Specific anti-Spike IgG quantification to compare both for absolute value and non-responders (NR) between modalities and against risk factors.

Results: Of 67 MDP, 42 were HD and 25 PD patients. PD developed higher antibody titers after both first (median 5.44 vs. 0.99 AU/ml, $p < 0.01$) and second dose (median 170.43 vs. 65.81 AU/ml; $p < 0.01$). HD associated with NR after the first dose ($p < 0.01$).

Conclusion: This study demonstrated improved humoral immunogenicity with BNT162b2 in PD compared to HD patients. These differences are attributed to comorbidity burden and age differences, rather than dialysis modality.

KEYWORDS

BNT162b2 (Pfizer BioNTech), Charlson comorbidity index, COVID-19, hemodialysis, peritoneal dialysis, vaccination

1 | INTRODUCTION

Worldwide vaccination against coronavirus 2019 (COVID-19) has become the cornerstone in prevention of Severe Acute Respiratory Syndrome Coronavirus 2 (Sars-CoV-2) associated with this pandemic. Several vaccines with different acting mechanisms have been developed, namely BNT162b2 (Pfizer BioNTech) [1] and mRNA-1273 (Moderna) [2]—mRNA-based vaccines; ChAdOx1 nCov-19 (AstraZeneca) [3] and Ad26.COV2.S (Johnson & Johnson/Janssen) [4]—recombinant adenovirus vectors encoding Sars-CoV-2 Spike glycoprotein, among others.

The need for mandatory regular contact with health care services in maintenance dialysis patients (MDP), coupled with worse disease severity and increased mortality [5–7], establish MDP as a high-risk population. Following this assessment and international recommendations, the Portuguese government implemented vaccination for MDP early in the immunization plan, starting on February 2021. A known challenge in end-stage kidney disease (ESKD) then reemerged: impaired immune system function translating in low immunogenicity from vaccination [8]. Influenza, pneumococcal and Hepatitis B immunization have systematically shown some level of weakened



response compared to the general population [9–13]. New protocols involving follow-up antibody measurements, adjuvant improvement, and repeated inoculations have minimized this problem, particularly well documented for Hepatitis B [14, 15].

Immunodeficiency in ESKD is multifactorial and affects both the innate and adaptative system [16]. Uremic toxins, malnutrition, chronic inflammation and dialysis technique contribute to this deficiency [17]. Cellularly, all immune cells show a disturbance in ESKD, both quantitative and qualitative, starting early as impaired response to stimuli by monocytes and lymphocytes [17–20]. Specific humoral response requires antigen presenting dendritic cells which are both reduced in number and dysfunctional in ESKD and is now proposed as one of the main mechanisms of immunodeficiency in this population [21–24]. Additionally, antigen-specific memory CD4 T-cell, responsible for lasting immunity, is also functionally defective [25–27]. On a molecular level, dysregulation of toll-like receptors and upregulation of inflammatory cytokines contribute to immune system stunning [28, 29].

Dialysis modality also affects the immune system differently. Peritoneal dialysis specific factors for immune dysfunction include: (a) intra-abdominal catheter; (b) high glucose/glucose degradation products or endotoxins on dialysate; (c) constitutive complement activation; (d) repetitive peritonitis and exit-site infection; (e) protein loss, including immunoglobulin in spent dialysate (controversial) [30, 31]. For the HD population: (a) central venous catheter as vascular access; (b) use of conventional hemodialysis over hemodiafiltration; (c) use of bioincompatible dialysis membranes; (d) complement activation during session secondary to loss of inhibitory molecules [32–34]. Immune system dysfunction in ESKD is still a matter of active investigation and, at this moment, is not clear if any modality is superior to the other.

Specifically, for SARS-CoV-2 vaccination, efficacy studies described above did not include MDP. Humoral response to BNT162b2 in HD patients has been a target in multiple studies [35–37]. Data on PD, however, is still lacking, and differences between modalities have not been studied. This study aims to describe and compare the initial humoral response to the BNT162b2 between a group of PD and HD patients in a nephrology unit.

2 | MATERIALS AND METHODS

The study design consisted of an observational prospective study. It included a group of 46 HD and 27 PD patients who were scheduled to receive two doses of BNT162b2 in a 3-week interval, in accordance with pharmaceutical guidelines for administration, between February and March 2021.

The assessment of humoral vaccination response was done as part of the internal policy of the center's contingency protocol and informed consent was obtained from each participant, regarding the use and access to these analytical results, as well as to the remaining clinical and demographic information for scientific research. Blood collection and analysis was made at two distinct phases: (1) 3 weeks after the administration of the first dose and (2) 3 weeks after the administration of the second dose. Because the recommended dosing interval is 3 weeks, the first collection was coincidentally performed with the second dose administration.

Humoral response, measured as IgG anti-Spike for SARS-CoV-2, in addition to IgM anti-Spike and anti-Nucleocapside for tracking possible contacts, even if asymptomatic. Titers were measured as arbitrary units per milliliter (AU/ml) using the MAGLUMI® SARS-CoV-2 S-RBD IgG chemiluminescence kit. Response was considered significant for titers superior to 1 AU/ml, in accordance with manufacturer's indications. Inclusion criteria included capacity to understand and provide informed consent and no significant increase in specific IgM anti-N for SARS-CoV-2 during follow-up.

Primary end point was established as comparison between the median of achieved titers to both the first and second dose and rate of non-responders (NR) for each modality. Secondary end points focused on comparison of clinical and demographic data including age, sex, Charlson comorbidity index (CCI) and dialysis vintage. Further subgroup exploratory analysis was performed based on age (under 70) and factors for weak response (IgG anti-Spike below percentile 25 for each modality) to vaccination.

2.1 | Statistical analysis

Statistical analysis was carried out using Microsoft Excel 2016 and IBM SPSS Statistics 25 software.

Study variables, divided in demographic/clinical and humoral, are summarized in Table 1. Descriptive analysis was performed using frequencies and percentages for categorical variables, whereas continuous variables are presented using means with standard deviations, if normal distributed, or medians with interquartile range, for skewed distribution.

Demographic and clinical data, anti-Spike IgG levels and NR rate were compared between both modality groups. Differences in continuous variables between groups were analyzed with Student's *t* test for means (parametric) and Mann–Whitney *U* test for medians (non-parametric). Response rate was analyzed between modalities using Fisher's exact test and Phi correlation

coefficient for association strength. Subgroup analysis using the same tests and Spearman's correlation test between antibody levels and continuous variables were also performed in an exploratory basis to further understand the primary outcome results. A binary logistic regression was performed using age, CCI and dialysis vintage to predict the likelihood weak response across the entire sample.

3 | RESULTS

Of the 46 HD patients enrolled, 42 were eligible for the study: (1) two patients discontinued dialysis before the administration of the second dose; (2) one patient died from an unrelated cause, and (3) one patient, despite asymptomatic, showed a significant increase in IgM titer raising the possibility of contact with the virus. Similarly, two patients from the PD group showed significant increase in specific IgM and were considered to have asymptomatic infection, resulting in 25 PD patients being enrolled into the statistical analysis, a total of 67 MDP.

Demographic and clinical data is summarized in Table 2, both for descriptive and comparative analyses. PD

group was younger (60.5 vs. 75.1 years; t [65] = 5.1; $p < 0.01$), with shorter dialysis vintage (23.9 vs. 41.1 months; $p = 0.02$) and lower CCI (5.2 vs. 7.8, $p < 0.01$). There was no statistical difference for gender or diabetes between both groups. Humoral response data is summarized in Table 3 with boxplot graph representation in Figures 1 and 2. Overall, IgG titers were higher in PD patients both for the first (5.44 vs. 0.99; $U = 844$; $p < 0.01$) and second doses (170.43 vs. 65.81; $U = 766$; $p < 0.01$). HD was weakly associated with NR for the first dose (Phi 0.383; $p < 0.01$) and showed no association with the second dose ($p = 0.08$) when compared to PD. However, after complete vaccination schedule, there were no NR patients in the PD group, an outcome that was observed in six patients (14%) in the HD group.

Additional exploratory analysis was performed to better clarify the influence of these factors in humoral response: (1) correlation analysis between CCI, age and achieved humoral titers after both doses was performed using Spearman's test. CCI correlated moderately ($\rho = -0.62$, $p < 0.001$), and age weakly ($\rho = -0.38$, $p = 0.001$) with humoral titers after both doses. Separately, however, this correlation was still verified in the HD subgroup (CCI: $\rho = -0.66$, $p < 0.001$; age: $\rho = -0.3$, $p = 0.05$), but not for PD (CCI: $p = 0.4$; age: $p = 0.49$). (2) A sub-analysis was performed for the same outcomes while restricting investigation to patients under 70 years of age. Differently from the global analysis, age and CCI means were not statistically different between subgroups ($p = 0.12$ and $p = 0.22$, respectively), while dialysis vintage remained shorter in the PD subgroup (21.6 vs. 45.5 months, $p = 0.04$). Humoral response was not significant after complete vaccination (173.3 vs 89.9 AU/ml, $p = 0.08$). (3) A binary logistic regression was performed to ascertain the effects of age, dialysis vintage and CCI on the likelihood that patients develop a low response to vaccination, defined by IgG anti-Spike under percentile 25 after both doses in each modality. The model was statistically significant, χ^2 (3) = 19, $p < 0.001$ and explained 37% of the variance in low response (Nagelkerke R^2) while also correctly classifying 82.1% of cases. Increasing CCI was associated with increased likelihood of low response (OR 1.56; 1.11–2.18 95% CI,

TABLE 1 Humoral and demographic/clinical variables in the study

Continuous	Categorical
Humoral response associated variables	
IgG anti-Spike S1 levels 1st dose (AU/ml)	1st dose NR (<1 AU/ml) 2nd dose NR (<1 AU/ml)
IgG anti-Spike S1 levels 2nd dose (AU/ml)	
Demographic/clinical variables	
Age	Sex
CCI	Diabetes
Dialysis vintage	

Abbreviations: AU/ml: arbitrary units per milliliter; CCI: Charlson comorbidity index; NR, non-responders.

	HD	PD	Comparison p value
Age, years, mean (σ)	75.1 (11.7)	60.5 (10.7)	<0.001 (t test)
Sex, female, n (%)	17 (40.5)	7 (28)	0.43 (Fish)
Diabetes, n (%)	19 (45.2)	7 (28)	0.2 (Fish)
Dialysis vintage, mo, Med (IQR)	35 (44.8)	18 (19.5)	0.02 (MW-U)
CCI, mean (σ)	7.8 (2.4)	5.2 (2.4)	<0.001 (MW-U)

Abbreviations: CCI, Charlson comorbidity index; Fish, Fisher's exact test; HD, hemodialysis; IQR, interquartile range; mo, months; Med, median; MW-U, Mann-Whitney U test; PD, peritoneal dialysis; t test, Student's t test.

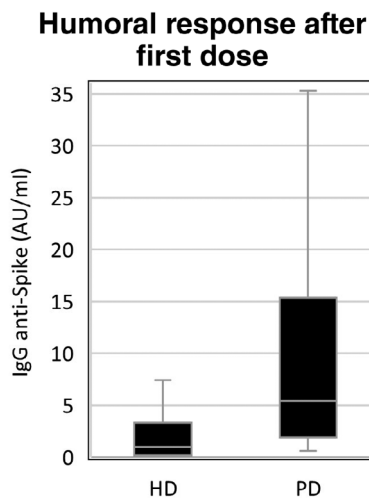
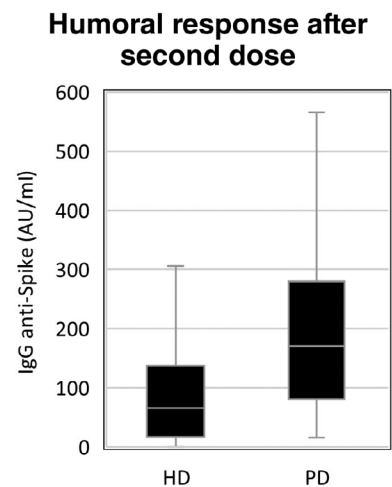
TABLE 2 Description and comparison of clinical and demographic data by modality

TABLE 3 Humoral response titers and rate of non-responders by modality

	HD	PD	Comparison <i>p</i> -value
First dose			
IgG anti-Spike			
Med (IQR)	0.99(3.16)	5.44 (13.45)	<0.01 (MW-U)
Min/Max	0.1/49.1	0.6/68.53	
IgM			
Med (IQR)	0.62 (0.15)	0.66 (0.15)	
Non-responders			
<i>N</i> (%)	21 (50%)	3 (12%)	<0.01 (Fish)
OR (95% CI)			7.35 (1.9–28.57)
Second dose			
IgG anti-Spike			
Med (IQR)	65.81 (120.56)	170.43 (199.06)	<0.01 (MW-U)
Min/Max	0.1/411.6	16.04/912	
IgM			
Med (IQR)	0.53 (0.22)	0.62 (0.24)	
Non-responders			
<i>N</i> (%)	6 (14%)	0 (0)	0.08 (Fish)
OR (95% CI)			N/A
Sub-analysis			
Under 70 years old			
<i>N</i>	14	22	
Age, mean (σ)	61.9 (8.7)	57.8 (8.1)	0.12 (<i>t</i> test)
CCI, mean (σ)	6.1 (2.2)	5.0 (1.9)	0.22 (<i>t</i> test)
IgG, Med (IQR)	89.9 (189.9)	173.3 (167.4)	0.08 (MW-U)

Note: Humoral titers are expressed in arbitrary units per milliliter (AU/ml).

Abbreviations: Fish, Fisher's exact test; HD, hemodialysis; IQR, interquartile range; Med, median; Min, minimum value; Max, maximum value; MW-U, Mann-Whitney *U* test; OR, odds ratio; PD, peritoneal dialysis; St *t*: Student's *t* test.

**FIGURE 1** Boxplot graph for first dose humoral response between modalities. AU/ml, arbitrary units per milliliter; HD, hemodialysis group; PD, peritoneal dialysis group**FIGURE 2** Boxplot graph for second dose humoral response between modalities. AU/ml: arbitrary units per milliliter; HD, hemodialysis group; PD, peritoneal dialysis group

$p = 0.01$), whereas increasing dialysis vintage was protective (OR 0.96; 0.93–0.99 95% CI, $p = 0.03$). Age alone did not contribute significantly to this model ($p = 0.56$).

4 | DISCUSSION

Humoral response to BNT162b2 was significant in this MDP sample with only 9% of the total sample failing to achieve measurable response, coherent with current studies [37, 38]. All of these non-responders were from the HD group, but, nevertheless, the difference in NR rate was non-significant between modalities, illustrating the most important limitation of this study: a small sample size. Additionally, coupling this limitation with the non-parametric distribution of resulting humoral titers impaired statistical analysis and restricted feasible testing, namely regression analysis.

The first studies of BNT162b2 in HD soon showed a reduce in humoral response, namely when compared to healthy cohorts [35–37]. While these outcomes were expected, our study supports a lower response in HD also when compared to PD in a real-world environment. The reason for this difference, however, does not appear to reside simply on modality. Age and use of immunosuppressive therapy have both been associated with low seroconversion and humoral titers in this population [35–39]. The analysis of clinical and demographic data favors PD patients as a younger, with less dialysis vintage and less comorbidity burden (as evaluated by CCI) when compared to HD. These differences were expected, given the nature and requirements of each modality. The performed sub analysis of humoral response in younger patients (under 70 years of age), was executed to normalize subgroups for comorbidity burden and age, since the difference for these variables that was verified for the primary analysis lost its significance in this subgroup. As a result, and differently from the primary comparison, this sub analysis showed no significant difference in IgG titers after complete vaccination, hence supporting a secondary role of dialysis modality with age and comorbidity burden taking primacy. The second part of sub analysis was to use binary logistic regression using low responders (IgG anti-S under percentile 25), rather than non-responders (IgG anti-S under 1 AU/ml) in order to identify common risk factors in low responders across both modalities. This resulted in a significant model, where CCI and dialysis vintage contributed, but age did not. Shorter dialysis vintage was associated with worse response in this model, even though PD patients have significant better quantitative responses and shorter dialysis vintage in the main analysis, suggesting CCI as stronger predictor. Still, early adaptation to dialysis, with lower dialysis adequacy and

uremic toxin removal could play a role in these results [40]. Comorbidity burden, measured by CCI, is suggested as the most complete form of assessment for predicting humoral response. It contributed significantly to the likelihood of low response in the regression model and, although both CCI and age correlated significantly with IgG titers, CCI showed a stronger correlation coefficient across the entire sample of MDP. These results were sustained in a sub analysis for HD after separation by modality, but was not verified for PD alone, suggesting that CCI is particularly important in HD and can be used as a better marker of likelihood of insufficient response to vaccination over age individually, for this modality.

Taken together, these results suggest a superior response to vaccination with BNT162b2 in PD compared to HD, most likely due to comorbidity and clinical status over modality-related factors, while also suggesting CCI as a better predictor of humoral response, over age alone, specifically in the HD population. It is licit to differentiate contingency protocols and to implement adequate policies for each modality, instead of a “one-fits-all” method for MDP, allowing for better allocation of resources and promoting the protection of those at higher risk of contagium and death. Consideration on a third or subsequent doses, similarly to other countries, is warranted, particularly in high-risk patients since several recent studies have shown a faster decline in humoral titers when compared to the general population [41]. A recent study from France involving humoral response to BNT162b2 after three doses in a small sample of MDP (both PD and HD) revealed beneficial in patients that had not achieved a response with two doses [42]. Further investigation relating comorbidity burden and immune dysfunction is required to better establish causality and evaluate the usefulness of CCI quantification as a surrogate marker of immune deficiency in ESKD.

It is worth noting that, during the follow-up, no patient developed clinically significant COVID-19 disease, neither was diagnosed with it by nasopharyngeal swab.

5 | CONCLUSION

This study suggests that humoral response in MDP is significantly different for BNT162b2 vaccination depending on modality, with PD patients achieving higher titers after both first and second doses when compared to their HD counterpart. Nevertheless, dialysis modality is not the crucial factor, with clinical and demographic differences between the two groups taking precedence, particularly age and comorbidity burden. CCI, as a quantitative measure of comorbidity burden, is a strong predictor for low response in MDP, and correlates with achieved humoral titers in HD patients, being suggested as a

surrogate marker of immunodeficiency in this modality. Additional investigation with larger study groups is required to further explore immunogenicity factors and their influence on overall humoral response.

Regardless of the abovementioned differences and limitations, our study supports a quick and expeditious vaccination of all MDP, irrespective of modality, for the yield of elicited immunity is high, and therefore immunization should not be discouraged or postponed for fear of a lack of response. The identification and signalization of higher risk subgroups in MDP for no response to vaccination is essential, providing institutions an opportunity to develop and establish protective and follow-up protocols in this pandemic, namely closer monitoring, adequate isolation, vigilance of symptoms and as a possible indication for booster doses.

To the best of our knowledge, this is the first study comparing humoral response to BNT162b2 between PD and HD patients.

CONFLICTS OF INTEREST

There are no relevant disclosures or conflicts of interest for any of the authors of this work.

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