RBD-IgG levels correlate with protection in Residents Facing SARS-CoV-2 B.1.1.7 Outbreaks

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Abstract

Background Limited information exists on nursing home (NH) residents regarding BNT162b2/Pfizer vaccine efficacy in preventing SARS-CoV-2 and severe Covid-19, and its association with post-vaccine humoral response. **Methods** 396 residents from seven NHs suffering a SARS-CoV-2 B.1.1.7 (VOC-α) outbreak at least 14 days after a vaccine campaign were repeatedly tested using SARS-CoV-2 real-time reverse-transcriptase polymerase chain reaction on nasopharyngeal swab test (RT-PCR). SARS-CoV-2 Receptor-Binding Domain (RBD) of the S1 subunit (RBD-IgG) was measured in all residents. Nucleocapsid antigenemia (N-Ag) was measured in RT-PCR-positive residents, and serum neutralizing antibodies in vaccinated residents from one NH. **Results** The incidence of positive RT-PCR was lower in residents vaccinated by two doses (22.7%) vs one dose (32.3%) or non-vaccinated residents (43.7%)($p<0.01$). Covid-19-induced deaths were observed in 10.4% of the non-vaccinated residents, in 6.4% of those who had received one dose, and in 0.9% with two doses ($p=0.0007$). Severe symptoms were more common in infected non-vaccinated (21.0%) vs vaccinated residents (47.6%, $p=0.002$). Higher levels of RBD-IgG ($n=325$) were associated with a lower SARS-CoV-2 incidence. No in vitro serum neutralization activity was found for RBD-IgG levels below 1,050 AU/mL. RBD-IgG levels were inversely associated with N-Ag levels, found as a risk factor of severe Covid-19. **Conclusions** Two BNT162b2/Pfizer doses are associated with a 48% reduction of SARS-CoV-2 incidence and a 91.3% reduction of death risk in residents from NHs facing a VOC-α outbreak. BNT162b2/Pfizer efficacy was partly predicted by post-vaccine RBD-IgG levels.

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ABSTRACT

Background

Limited information exists on nursing home (NH) residents regarding BNT162b2/Pfizer vaccine efficacy in preventing SARS-CoV-2 and severe Covid-19, and its association with post-vaccine humoral response.

Methods

396 residents from seven NHs suffering a SARS-CoV-2 B.1.1.7 (VOC-α) outbreak at least 14 days after a vaccine campaign were repeatedly tested using SARS-CoV-2 real-time reverse-transcriptase polymerase chain reaction on nasopharyngeal swab test (RT-PCR). SARS-CoV-2 Receptor-Binding Domain (RBD) of the S1 subunit (RBD-IgG) was measured in all residents. Nucleocapsid antigenemia (N-Ag) was measured in RT-PCR-positive residents, and serum neutralizing antibodies in vaccinated residents from one NH.

Results

The incidence of positive RT-PCR was lower in residents vaccinated by two doses (22.7%) vs one dose (32.3%) or non-vaccinated residents (43.7%)($p<0.01$). Covid-19-induced deaths were observed in 10.4% of the non-vaccinated residents, in 6.4% of those who had received one dose, and in 0.9% with two doses ($p=0.0007$). Severe symptoms were more common in infected non-vaccinated (21.0%) vs vaccinated residents (47.6%, $p=0.002$). Higher levels of RBD-IgG (n=325) were associated with a lower SARS-CoV-2 incidence. No in vitro serum neutralization activity was found for RBD-IgG levels below 1,050 AU/mL. RBD-IgG levels were inversely associated with N-Ag levels, found as a risk factor of severe Covid-19.

Conclusions

Two BNT162b2/Pfizer doses are associated with a 48% reduction of SARS-CoV-2 incidence and a 91.3% reduction of death risk in residents from NHs facing a VOC-α outbreak. BNT162b2/Pfizer efficacy was partly predicted by post-vaccine RBD-IgG levels.

Key words: SARS-CoV-2; Covid-19; symptoms; nursing homes; residents; BNT162b2/Pfizer vaccine; efficacy; antibody response; nucleocapsid antigenemia; neutralizing antibodies

INTRODUCTION

Nursing Home (NH) residents are at high risk of serious illness and death from coronavirus disease 2019 (Covid-19) [1]. Vaccination is safe and effective in adults, but less documented in NH residents. [2-4] Vaccine monitoring is partly based on post-vaccine IgG response against SARS-CoV-2 Receptor-Binding Domain (RBD) of the S1 subunit (RBD-IgG). [5] Based on RBD-IgG levels, [6-8] it is recommended in France to

administer one mRNA vaccine dose in adults with prior Covid-19 and a third dose in immunocompromized patients. The interest of a third vaccine dose also arises in NH residents without prior Covid-19.[2,9,10,11] It remains however unclear as to whether blood levels are predictive of SARS-CoV-2 infection and Covid-19 severe symptoms.

The recent emergence of SARS-CoV-2 'variants of concern' (VOC) has transformed the epidemic, [12] possibly because RBD-IgG produced by vaccinated individuals is less effective in binding and neutralizing *in vitro* VOCs than the "wild type" SARS-CoV-2.[13,14-17]

Between January and March 2021, all French NH residents, including those of the Occitanie region, were offered two BNT162b2/Pfizer doses at a three-week interval. Between March and April 2021, 7 NHs of the Montpellier area (Occitanie) faced a SARS-CoV-2 B.1.1.7 (VOC-α) outbreak after the vaccine campaign.

The primary aim of this study was to assess the incidence of positive SARS-CoV-2 real-time reverse-transcriptase polymerase chain reaction (RT-PCR) results according to the vaccination status. Secondary aims were to compare Covid-19-related deaths and RBD-IgG levels according to the vaccination status. Other secondary aims were to assess the links in RT-PCR-positive residents between symptom severity, blood RBD-IgG levels and SARS-CoV-2 nucleocapsid antigenemia (N-Ag). Exploratory objectives were to analyze the link between RBD-IgG levels and serum neutralization activity against VOC-α and SARS-CoV-2 wild-type in one NH, and the links between biological markers with Covid-19 severity in RT-PCR-positive residents (N-Ag, C-reactive protein, and glomerular filtration rate).[18,19]

Methods

Design of the study

Between March and April 2021, among the 122 NHs (6 241 residents) followed by the Montpellier Covid-19 support platform after vaccination,[20] 13 had at least one RT-PCR-positive resident (747 residents; 124 positive RT-PCR residents). Among these 13 NH, a prospective cohort study was carried out in all seven NHs with more than five RT-PCR-positive residents tested at least 14 days after the end of the vaccination campaign, considering that the exposure risk of residents should be sufficient to study the effect of the vaccination on Covid-19 incidence. Clinical characteristics of all residents present at the time of the first positive RT-PCR were studied, regardless of the date of their arrival in the NH. As for other Occitanie NHs, when a first RT-PCR-positive resident was diagnosed, the same infection prevention and control (IPC) measures were implemented and no new resident entry was permitted until the end of the outbreak (Online supplement 1) [21,22]. Blood was drawn on the day of positive RT-PCR testing in the first infected residents and within the 5 following days in all the others. Blood collection within this delay was possible in 6/7 NHs. Blood sample allowed to measure IgG-RBD and nucleoprotein IgG levels and, in RT-PCR-positive residents, N-Ag. Serum neutralizing antibodies were assessed in a subsample of 36 vaccinated residents from the first NH facing a SARS-CoV-2 outbreak, chosen to have a wide range of IgG-RBD values.

Participants

When a first resident had a positive RT-PCR, residents and their family or legal representative were informed of the possibility to assess RDB-IgG and nucleocapsid IgG in order to assess humoral immunity.

All residents of the 6 NH in which the blood sample was offered were tested, except those in palliative medical situation and those for whom informed consent for the blood collection and the use of anonymized data was not obtained. History of previous RT-PCR testing and the Charlson comorbidity index were determined based on residents' medical files.[23]

The study was approved by the Montpellier University Hospital institutional review board (IRB-MTP_2020_-06_202000534 and IRB-MTP

Outcomes

Main outcome: Incidence of SARS-CoV-2 infection

The diagnosis of SARS-CoV-2 infection was performed using RT-PCR on nasopharyngeal swab (Allplex 2019-nCoV assay, Seegene) (Online supplement 2). [24] An RT-PCR multiple variant assay was used for the detection of α and β/γ variants (ID SARS-CoV-2/UK/SA Variant Triplex (id Solutions) and for genome sequencing.

Secondary outcomes

Covid-19 severity: Symptoms were those recorded in residents' files by NH staff between 7 days before and 14 days after RT-PCR testing, according to previous studies [21,25] (Online supplement 3). Covid-19 was considered to be severe when residents had either (i) respiratory symptoms including shortness of breath, respiratory rate > 24/min, oxygen saturation < 90% or when oxygen therapy was used, or (ii) when they displayed other symptoms whose intensity was considered by the NH coordinating practitioner as sufficient to justify an hospitalization or an examination by the resident's General Practitioner. Other symptomatic residents had mild symptoms.

SARS-CoV-2 RBD and Nucleoprotein immunoglobulin levels: RBD-IgG levels were measured using the SARS-CoV-2 IgG II Quant assay (Abbott Diagnostics). Results were expressed as arbitrary units per mL (AU/mL; positive threshold: 50 AU/mL; upper limit: 40,000 AU/mL). According to the manufacturer and to previous studies, a first threshold [?] 1,050 AU/mL was considered as a significant response, [26] and a second one [?] 4,160 AU/mL indicated a high neutralizing effect.[27] Nucleoprotein IgG (N-IgG) levels were measured using the SARS-CoV-2 IgG assay (Abbott Diagnostics) (Online supplement 4).

Nucleocapsid antigenemia

N-Ag was quantified in all RT-PCR-positive residents seronegative for N-IgG, using the COV-QUANTO® kit (AAZ, France), a CE-IVD marked immunoassay. [28]

Exploratory outcomes

Neutralizing antibodies : Micro-neutralization assays were performed in 36 residents (online supplement 5). [27]

Inflammatory and kidney markers: C-reactive protein (hsCRP) and creatinine levels were measured (online supplement 6).

Statistical analysis

In NH 4 (Table S1), blood sampling was not possible, and residents' data were used only to assess the link between vaccination status and SARS-CoV-2 infection and severe Covid-19 incidence. Due to the very low number of missing data in the 6 NHs in which blood was collected, and to the fact that residents who did ou did not contribute to blood collection did not differ for age, gender, and comorbidity status, no assumptions were made for missing data et missing data were not replaced.

Categorical variables were described with frequency and proportions for each category. The description of continuous variables was performed using mean and standard deviation and/or median, with interquartiles, according to the distribution.

The statistical analysis plan was pre-specified according to previous studies from our group. [6,7], with the following primary, secondary, and exploratory outcomes

The primary outcome (incidence of positive RT-PCR) was estimated in the entire cohort with 95% confidence interval (CI), and compared according to the residents' vaccination status (non-vaccinated, one vaccine dose, or two vaccine doses) using the Chi-2 test. Death was not considered as a competing risk as all deaths were related to Covid-19 during the study period. Covid-19-related deaths were considered as incident cases.

Secondary outcomes were compared in the whole cohort according to the vaccination status using the Fisher exact test for deaths and the Kruskal-Wallis (KW) test for RBD-IgG levels.

Other secondary outcomes according to the vaccination status were analyzed in RT-PCR-positive residents: Covid-19 symptom severity using the Fisher exact test, RBD-IgG and N-Ag using the KW test. The relationship between levels of RBD-IgG and risk of incident SARS-CoV-2 was analyzed using the Fisher exact test.

Exploratory analyzes on the relationship between RBD IgG levels and serum neutralization in RT-PCR-positive residents of one NH were performed using Fisher's exact test. Wilcoxon–Mann–Whitney 2-sided tests were used to compare N-Ag in asymptomatic, mild, and severe Covid-19 residents.

Holm's correction was applied for the post-hoc comparisons for each outcome. The statistical significance threshold was set at 5%.

We displayed the positive predicted values (PPVs) and negative predictive values (NPVs) of a positive RT-PCR testing for the accepted thresholds of IgG-RBD values (positive threshold set at 50 AU/mL, significant threshold set at 1,050 AU/mL, and 4,160 AU/mL, indication a high neutralizing effect). [26,27]

Analyses were performed and illustrated using SAS Enterprise Guide, v7.3 (SAS Institute Inc) and GraphPad Prism 9.1.1 (GraphPad Software, Inc., San Diego, CA).

Results

characteristics of the residents

In the seven NHs, non-vaccinated residents ranged from 3.9% to 32.6%, and RT-PCR-positive residents from 9.8% to 54.9%. Residents' vaccination coverage was not significantly different across the NH sites (Table S 1). A total of 396 residents (57 to 103 years, mean ± sd: 87.33 ± 9.17 years), with 312 females (78.8%), were included. The Charlson comorbidity index ranged from 2 to 15 (median, percentiles: 6.0, 5.0-7.0). Fifty-one residents had prior-Covid-19, determined by a positive RT-PCR in 2020 or 2021, and only residents without prior positive RT-PCR had detectable N-protein-IgG in the blood collected for this study. There were 48 (12.2%) non-vaccinated residents, 31 (7.8%) with one vaccine dose, and 317 (80.0%) with two doses. Age, sex, Charlson co-morbidity index, or previous RT-PCR results were comparable in the groups (Table 1).

Blood sampling could not be performed in residents from NH number 4 (Table 1, Table S1, and Figure 1). In the 6 NHs in which a blood sample was offered, residents who were (N = 325) or were not (N = 25) sampled were similar in age, gender, and Charlson index (data not shown).

Covid-19 incidence depending on vaccination status

RT-PCR was positive in 103 residents with an incidence of 26% (95% CI: 21.7%-30.3%). RT-PCR cycle thresholds (Ct) were comparable in vaccinated and non-vaccinated residents (Table 1). The VOC-α variant was found in all NHs. Positive RT-PCR results were obtained in 22.7% of residents with two vaccine doses, 32.3% with one dose, and 43.7% in non-vaccinated subjects.

Covid-19-related deaths depending on vaccination status

Incident deaths, all due to Covid-19, were higher in non-vaccinated (10.4%) than in vaccinated residents (6.4% and 0.9% for those vaccinated with one or two doses, Table 1). In RT-PCR-positive residents, the non-vaccinated ones died more frequently (25% vs 4.3%)(Table 2). Two non-vaccinated residents received monoclonal antibodies and recovered completely.

Covid-19-related symptoms depending on vaccination status

In RT-PCR-positive residents, the non-vaccinated developed significantly more severe symptoms than the vaccinated (47.6% vs 21.1%) (Table 2).

Clinical outcomes depending on RBD IgG levels

Serum samples were available for 325/350 residents from 6 NHs in which the blood collected could be organized (92.9%). Causes of missing data are displayed in Figure 1.

By comparison with residents who received two vaccine doses, the RBD-IgG level was lower in non-vaccinated residents and in those with one dose (Table 1).

Residents with higher levels of RBD-IgG had a lower risk of developing SARS-CoV-2 during the outbreak (Table 3). PPV and NPV of a positive RT-PCR by RBD-IgG levels over 1,050 AU/mL (significant response[26]) were 0.86 and 0.24, respectively, with a sensitivity and specificity of 0.63 and 0.54 (Table S2).

Among the 48 RT-PCR-positive vaccinated residents with blood results, RBD-IgG levels tended to be higher in asymptomatic residents than in those with mild or severe symptoms (median (IQR)[range] 1249 AU/mL (337 ; 3027) [11.00 ; 25,453.00] vs 517 AU/mL (150 ; 1,289) [3 ; 14,631] vs 358 AU/mL (128 ; 1,339) [93 ; 5,824], respectively).

No *in vitro* serum neutralization activity was found for RBD-IgG levels under 1,050 AU/mL for both SARS-CoV-2 and VOC-α. Above this threshold, the RBD-IgG levels were associated with serum neutralization activity (Figure 2).

nucleocapside antigenemia

Among the 49 RT-PCR-positive residents with N-Ag measurement, median N-Ag levels were higher in non-vaccinated residents than in those having received 2 vaccine doses (Table 2), and higher in residents with severe symptoms (Fig. S1A). RBD-IgG level was predictive of N-Ag in SARS-CoV-2-infected residents (Fig. S1B).

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RT-PCR-positive residents with elevated CRP (>10 mg/L, S1C) or reduced glomerular filtration rates (GFR) (<60 ml/min/1.73m2) had higher N-Ag levels (Fig.S1D).

Discussion

Among 396 residents from 7 NHs facing a VOC-α outbreak over 14 days after the end of a BNT162b2/Pfizer vaccination campaign, 103 had a positive RT-PCR test. After two vaccine doses, the risk of incident VOC-α infection was reduced by 48% when compared to non-vaccinated residents, and by 26% in residents with one dose. In RT-PCR-positive residents, two vaccine doses reduced the risk of severe symptoms by 56% (from 47.6% to 21.1%), and Covid-19 death by 82.4% (from 23.8% to 4.2%). Post-vaccine RBD-IgG levels under 1,050 AU/mL were associated with (i) an increased risk of incident VOC-α infection, (ii) a low serum neutralizing effect against SARS-CoV-2 wild type and VOC-α, and (iii) high levels of N-Ag that were associated with a higher risk of severe Covid-19 (severe symptoms, high C-reactive protein, low glomerular filtration rate).

The 48% reduction of SARS-CoV-2 infection after two vaccine doses found in the present study is consistent with NH studies reporting a VOC-α outbreak, even if the percentage of infected residents differed among the vaccinated and non-vaccinated. [2,4,9] The reduction in Covid-19 risk was estimated at 65% after a single BNT162b2/Pfizer vaccine dose in a national data study in England. [29]The strong reduction of severe symptoms and death found in vaccinated residents was also reported. [4,9]

In contrast to two previous studies, [2,30] we did not find any difference in viral load, estimated by the mean Ct. Because Ct values, vaccine regimen, and Covid-19 severity strongly differed between the three studies, further investigations are needed to determine the reduction of the viral load in infected residents after vaccination. This information is crucial to minimize the duration and consequences of isolation in vaccinated infected residents.

A novel result of the present study is that post-vaccine RBD-IgG levels can partly predict the efficacy of the vaccine in to prevent incident SARS-CoV-2 and severe symptoms. This is consistent with This result is also consistent with he lack of neutralizing effect observed in the serum against SARS-CoV-2 *in vitro* under a threshold of 1,050 AU/mL and the association between RBD-IgG levels and serum neutralization activity found above this threshold. In addition, lower RBD-IgG levels were significantly associated with

higher N-Ag levels which were associated with a higher risk of Covid-19 severity (more severe symptoms, higher C-Reactive Protein levels and lower glomerular filtration rate - two biological markers of Covid-19 severity)[18,19] which is in line with previous studies conducted in the general population. [31] Our results are consistent with studies in the general population showing a correlation between RBD-IgG and serum neutralization, [32,33] These studies combine to suggest that (i) low RBD-IgG levels following vaccination may not necessarily possess the key footprints required to block viral infection, and (ii) a minimal post-vaccine RBD-IgG level, possibly over 1,050 AU/mL, may be required to block VOC-α infection and/or prevent severe symptoms.

The strengths of this study include the sample size, and all studied NHs followed the same IPC guidance, making it possible to compare the results.[21,22]

This study also has several limitations: (i) RBD-IgG levels were measured using an immunoassay and results expressed in arbitrary units. However, the assay used in our study can probably be considered as a quantitative assay (<3.5% imprecision). [34](ii) RBD-IgG levels were measured within five days after the diagnosis of the first RT-PCR-positive resident of the NH. Even if this delay was as short as possible, RDB-IgG levels may also reflect a possible rapid anamnestic response to infection. This bias tends however to reinforce our results, since we found a relationship between low levels of RBD-IgG and a higher risk of SARS-CoV-2. (iii) Although the NH residents of the present study were comparable to the French NH population in terms of mean age, gender, and comorbidity status, our results obtained in residents exposed to a high risk of infection may not be extrapolated to all NH residents. (iv) Our results are only valuable for NHs facing a VOC-α outbreak. RBD-IgG produced by vaccinated residents seems less effective for binding and neutralizing *in vitro* β, δ, ϰ and ε variants [13] [14-17,35] than the SARS-CoV-2 "wild type". [14-17,35,36] (v) The results obtained are restricted to the BNT162b2/Pfizer vaccination in NH residents. Further studies conducted in NH residents facing other variants and different vaccines are therefore necessary. In this study, 8.7% of residents with RBD-IgG levels of over 4,160 AU/mL developed a positive RT-PCR. This suggests that vaccinated residents, even with high levels of S-protein IgG after two vaccine doses, may participate in SARS-CoV-2 transmission while most often being asymptomatic or pauci-symptomatic. These results suggest that vaccinated residents should be included in the wide-facility testing strategy when a resident is infected.

Conclusions

These results confirm the effectiveness of two BNT162b2/Pfizer doses to reduce significantly the incidence and the severity of Covid-19 in NH residents facing a VOC-α outbreak. They tend to validate the quantification of the vaccine antibody response as an estimate of SARS-CoV-2 prevention efficacy.

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Conflicts of Interest:

The authors declare no conflicts of interest/competing interests.

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Table 1. Characteristics of the residents depending on their vaccination status

	Non-vaccinated (a) (n=48)	Vaccinated	Vaccinated	P value
		1 vaccine dose (b) (n=31)	2 vaccine doses (c) (n=317)	
Age, mean (SD), yr*	87.2 (9.2)	85.1 (9.2)	87.6 (9.2)	0.22
Sex, n (%)** Female Male	35 (72.9) 13 (27.1)	25 (80.7) 6 (19.3)	252 (79.5) 65 (20.5)	0.56
Charlson index (median, IGR) [range] *	6.0 (5.0-7.0) 2-15	6.0 (5.0-8.0) 3-15	6.0 (5.0-7.0) 2-15	0.41
Prior SARS-CoV-2 positive RT-PCR, n (%)**	5 (10.4)	3 (9.7)	43 (13.6)	0.82
Positive RT-PCR during outbreak, n (%)**	21 (43.7)	10 (32.3)	72 (22.7)	0.0059 0.005 (a vs c)
N-protein IgG > 0.8 signal to cutoff ratio, n (%)***	5 (20.0)	5 (17.9)	38 (14.0)	0.57
RBD-IgG level, AU/mL, median (IQR) *	n = 26 2 (1-415)	n =28 534.00 (201.50 ; 2166.50)	n =271 1522.00 (444.00 ; 5389.00)	0.000001 0.001 (a vs b) 0.01 (a vs c) 0.08: (b vs c)
Covid-19-related deaths, n (%)***	5 (10.4)	2 (6.4)	3 (0.9)	0.0007 0.004 (a vs c)

*Analysis using Kruskal-Wallis test; ** Analysis using chi-2 test; ***Analysis using Fisher test

Table 2. Characteristics of the residents with RT-PCR positive results

	Non-vaccinated (a) (n =21)	Vaccinated	Vaccinated	P value
		1 vaccine dose (b) (n =10)	2 vaccine doses (c) (n =72)	
Age, mean (SD), yr**	90.3 (7.4)	83.8 (8.4)	89.1 (7.8)	0.06
Sex, n (%)**** Female Male	15 (71.4) 6 (28.6%)	8 (80.0) 2 (20.0)	56 (77.8) 16 (22.2)	0.87
Charlson index (median, IQR) [range] **	6 (6.0-8.0) [4-15]	7.0 (5.0-8.0) 5-9	7.0 (6.0-8.0) 3-15	0.96

	Non-vaccinated (a) (n =21)	Vaccinated	Vaccinated	P value
Prior SARS-CoV-2-positive RT-PCR, n (%)***	1 (4.8)	0 (0)	3 (4.2)	1.00
First positive RT-PCR cycle threshold, median (IQR) ****	26.0 (23.0-32.0)	24.85 (20.4 ; 28.0)	24.00 (19.2 ; 28.6)	0.74
Cycle threshold of the second RT-PCR*, median (IQR) ****	30.3 (27-35.2)	30.7 (23.1 ; 34.5)	27.00 (21.0 ; 31.7)	0.21
Symptoms, n (%)*** Asymptomatic Mild Severe	2 (9.5) 4 (19.1) 10 (47.6)	6 (60.0) 2 (20.0) 0	31 (43.7) 23 (31.0) 15 (21.1)	0.002 0.01 (a vs b), 0.002 (a vs c) 0.09 (b vs c)
Deaths, n (%)***	5 (23.8)	2 (20.0)	3 (4.2)	0.002 (a vs c)
SARS-CoV-2 IgG levels - N-protein IgG > 0.8 signal to cutoff ratio, n (%)** - SARS-RBD-IgG (AU/mL) ***** <50 51-1,050 1,051-4,160 >4,160	n =10 2 (20.0) 6 (60.0) 3 (30.0) 0 (0) 1 (10)	n =7 1 (14.3) 1 (14.3) 5 (71.4) 1 (14.3) 0 (0)	n =49 4 (8) 5 (10.2) 22 (44.9) 15 (30.6) 7 (14.3)	0.29 0.010.01 (a vs c)
Nucleocapide antigenaemia titer **, median (IQR)[range] ******	n = 7 43.0 (2.9-150.2) [0.7-555.2)	n =5 1.8 (0.5-3.0) [0.2-12.5]	n =37 1.5 (-0.4-16-6) [-1.5-328-4]	0.03 0.004 (a vs c)

* The second RT-PCR was performed 7 days after the first positive RT-PCR result in 47 residents; **Analysis using Kruskal-Wallis test; *** Analysis using Fisher test; ****Analysis using ANOVA; *****Analysis using Mann-Whitney test; ****** level of detection of the assay is 2.95 ng/mL. Samples with Nucleocapside Ag level over 180 pg/mL were diluted. Titer is the number of dilution possible, still reaching the detection threshold. According to the manufacturer of the SARS-CoV-2 IgG II Quant assay (Abbott Diagnostics), the positive threshold is of 50 AU/mL RBD-IgG levels. Based on previous studies, an RBD-IgG level [?] 1,050 AU/mL is considered as a significant antibody response to the vaccine,[26] and a level [?] 4,160 AU/mL indicates a high neutralizing effect against SARS-CoV-2 [27].

Table 3. Incidence rate of positive RT-PCR testing and serum neutralizing activity depending on SARS-CoV-2 RBD IgG levels

	SARS-CoV-2 RBD IgG levels, AU/mL*	SARS-CoV-2 RBD IgG levels, AU/mL*	SARS-CoV-2 RBD IgG levels, AU/mL*	SARS-CoV-2 RBD IgG levels, AU/mL*	
	< 50	51-1,050	1,051-4,160	> 4,160	P value
RT-PCR testing result, n (%) Positive Negative	n = 12 5 (41.67) 7 (58.33)	n = 97 22 (22.68) 75 (77.32)	n = 82 15 (18.29) 67 (81.71)	n = 80 7 (8.75) 73 (91.25)	0.01
Serum micro-neutralization titer against SARS-CoV-2 wild type, median (IQR)[range]**	n = 3 0 (0-0) [0-0]	n = 10 0 (0-0) [0-0]	n = 10 10.0 (0-10) [0-20]	n = 5 160 (40-320) [20-640]	0.005
Serum neutralization against SARS-CoV-2 B.1.1.7 (VOC α), median (IQR)[range]**	n = 3 0 (0-0) [0-0]	n = 10 0 (0-0) [0-0]	n = 10 10.0 (10-20) [0-40]	n = 5 320 (80-640) [40-640]	0.005

* Analysis using the Fisher test; ** Analysis using the Kruskal-Wallis test; Micro-neutralization titers are expressed as the serial dilution for which 50% neutralization is obtained.

Figure 1. Flow Diagram of the sample of residents having faced a COVID-19 outbreak at least 14 days after a BNT162b2/Pfizer vaccination campaign.

Hosted file

`image1.emf` available at https://authorea.com/users/343419/articles/538850-rbd-igg-levels-correlate-with-protection-in-residents-facing-sars-cov-2-b-1-1-7-outbreaks

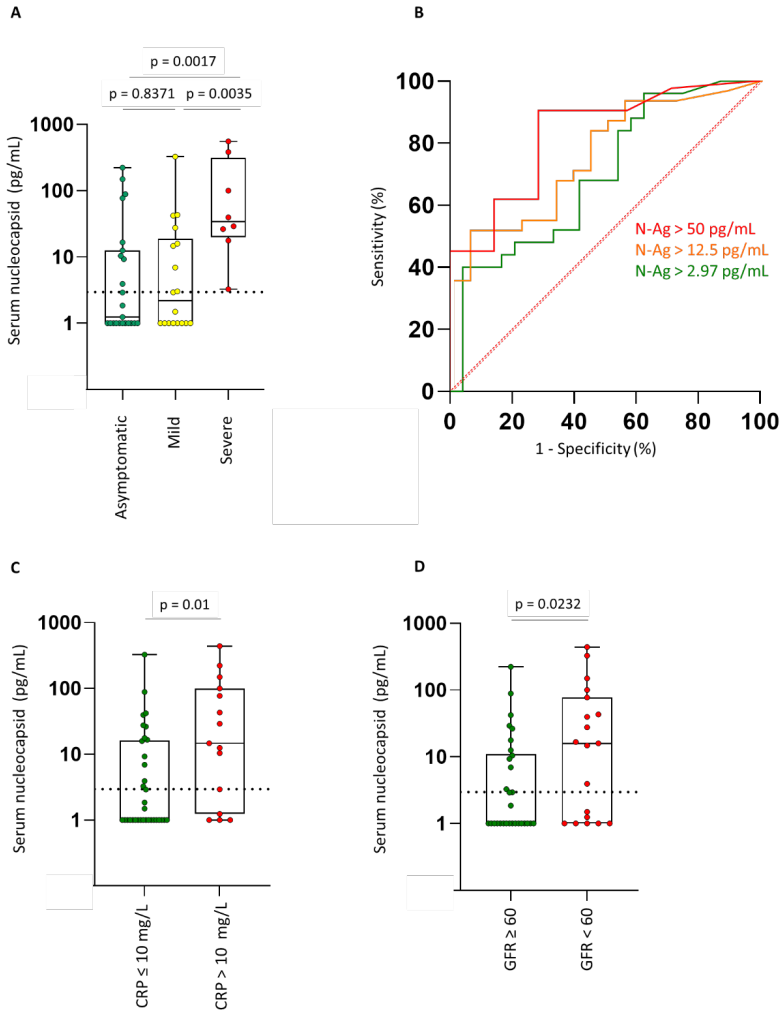
Figure 2. SARS-CoV-2-specific neutralizing antibody levels against SARS-CoV-2 wild type (WT) and VOV-alpha depending on RBD-IgG levels in 36 residents.

Legend. Micro-neutralization titers are expressed as the serial dilution for which 50% neutralization is measured.

Figure S1. Levels of nucleocapsid antigenemia (N-Ag) in 49 vaccinated residents with SARS-CoV-2 confirmed infection. A) N-Ag levels according to Covid-19 severity, green circles: asymptomatic; yellow circles: mild forms; red circles: severe forms. B) ROC evaluating RBD-IgG levels to predict undetectable N-Ag at the thresholds of 2.97 pg/mL, AUC: 0.70; 12.5 pg/mL, AUC: 0.76; 50 pg/mL, AUC: 0.83. C).

N-Ag levels according to C-reactive protein (CRP) level; green circles: CRP [?] 10 mg/L; red circle CRP > 10 mg/L. D) N-Ag levels according to glomerular filtration rates; green circles: GFR [?] 60 ml/min); red circles: GFR < 60 ml/min.

Figure 2



Legend. Covid-19 residents were considered as having severe symptoms when they had either (i) respiratory symptoms including shortness of breath, respiratory rate > 24/min, oxygen saturation $< 90\%$ or when oxygen therapy was used, or (ii) when they displayed other symptoms whose intensity was sufficient to lead to hospitalization or to justify medical examination. Residents with mild symptoms had no severe symptoms.