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T-cell mediated response after primary and booster SARS-CoV-2 mRNA vaccination in nursing homes residents.

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Running Title: Monitoring of T-cell immunity to COVID-19 vaccine

Key words: SARS-CoV-2, COVID-19 vaccines, nursing homes, cell-mediated immunity; vaccine booster.

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Figures: 3
Brief summary
Nursing home residents showed a durable cell-mediated immunity after the receipt of mRNA COVID-19 vaccine. The benefit of the third booster dose was especially evident in residents without a previous history of infection.

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ABSTRACT

Objectives. Nursing home (NH) residents have been significantly affected by the COVID-19 pandemic. Studies addressing the immune responses induced by COVID-19 vaccines in NH residents have documented a good post-vaccination antibody response and the beneficial effect of a third booster vaccine dose. Less is known about vaccine-induced activation of cell mediated immune response in frail elderly subjects in the long term. The aim of the present study is to monitor mRNA SARS-CoV-2 vaccine-induced T-cell responses in a sample of Italian NH residents who received primary vaccine series and a third booster dose and to assess the interaction between T-cell responses and humoral immunity.

Design. Longitudinal cohort study.

Setting and Participants. Thirty-four residents vaccinated with BNT162b2 mRNA SARS-CoV-2 vaccine between February and April 2021 and who received a third BNT162b2 booster dose between October and November 2021 were assessed for vaccine-induced immunity (pre-booster) and (post-booster) months after the first BNT162b2 vaccine dose.

Methods. Pre- and post-booster cell-mediated immunity was assessed at by intracellular cytokine staining of peripheral blood mononuclear cells stimulated in vitro with peptides covering the immunodominant sequence of SARS-CoV-2 Spike protein. The simultaneous production of IFN-γ, TNF-α and IL-2 was measured. Humoral immunity was assessed in parallel by measuring serum concentration of anti-trimeric Spike IgG antibodies.

Results. Before the booster vaccination, 31 out of 34 NH residents had a positive cell-mediated immunity (CMI) response to Spike. Post-booster, 28 out of 34 had a positive response. Residents without a previous history of SARS-CoV-2 infection who had a lower response prior the booster administration, showed a greater increase of T-cell responses after the vaccine booster dose Humoral and cell-mediated immunity were, in part, correlated but only before booster vaccine administration.

Conclusions and Implications. The administration of the booster vaccine dose restored Spike-specific T-cell responses in SARS-CoV-2 naïve residents who responded poorly to the first immunization, while a previous SARS-CoV-2 infection had an impact on the magnitude of vaccine-induced cell-mediated immunity at earlier
time-points. Our findings imply the need for a continuous monitoring of the immune status of frail NH residents to adapt future SARS-CoV-2 vaccination strategies.

INTRODUCTION

Older adults have been significantly affected by the coronavirus disease 2019 (COVID-19) pandemic. Among them, nursing home (NH) residents who often present with a high burden of comorbidities and clinical complexities, suffered the greatest impact of the pandemic. Epidemiological data indicate that during the first pandemic wave, up to 50% of deaths from COVID-19 may have occurred within NH facilities. For this reason, SARS-CoV-2 vaccination of NH residents has been a priority in most countries including Italy to reduce the risk of COVID-19-related morbidity and mortality.

Several studies have been performed to determine the quality and duration of immune responses induced by SARS-CoV-2 vaccines in NH residents. As a general feature, a good early post-vaccination antibody response followed by a decline over time and the beneficial effect of a third booster vaccine dose has been documented. Less is known about vaccine-induced activation of T-cell mediated immune response in frail elderly subjects and about its interaction with humoral response. T cell immunity plays a central role in the control of SARS-CoV-2 and increasing evidence now supports a potential role in both preventing initial infection and, more importantly, limiting the extent of disease following infection. Data collected so far have highlighted an impaired T-cell response in older individuals as compared to younger adults after two vaccine doses, with a greater response in previously infected older subjects, and the immune-potentiating effect of a third booster dose in previously unresponsive older adults.

The aim of the present study is to monitor over a 12-month period mRNA SARS-CoV-2 vaccine-induced T-cell responses in a sample of Italian NH residents who received the primary mRNA vaccine series and a booster dose and to assess the interaction between T-cell responses and humoral immunity.
METHODS

Study design

The present study has been conducted in the framework of the GeroCOVID VAX project, aimed at investigating effects of anti-SARS-CoV-2 vaccine use in NH residents in Italy\textsuperscript{24}. Fifty-one NH residents belonging to the the GeroCOVID VAX cohort, were selected for assessment of T-cell mediated immunity and followed longitudinally up to one year after the administration of the first vaccine dose.

Residents were vaccinated with the Pfizer BNT162b2 mRNA SARS-CoV-2 vaccine between February and April 2021. Following recommendations issued by the Italian Ministry of Health, residents who experienced SARS-CoV-2 infection in the 6 months before vaccination received a single vaccine dose, while other residents were administered a second dose one month after the first one. A booster vaccination with lower dose BNT162b2 was administered to NH residents between October-November 2021 (7 to 9 months from the first dose). Blood samples were collected at after primary vaccine series (6 months after first vaccine dose – pre-booster) and after booster dose (12 months after first vaccination – post-booster). SARS-CoV-2 Spike-specific IgGand Spike-specific T-cell responses were assessed concomitantly pre- and post-booster. A diagram of the study design is shown in Supplementary Figure 1.

Demographic and clinical data were collected in a dedicated electronic form and included sex, age, prior SARS-CoV-2 infection, co-morbidities and time of vaccination.

SARS-CoV-2 IgG immunoassays

Blood samples (5 ml) were collected in Serum Separator Tubes (BD Diagnostic Systems, Franklin Lakes, NJ, USA) and centrifuged at room temperature at 1600 rpm for 10 min. Serum aliquots were transferred to 2ml polypropylene, screw cap cryo tubes (Nunc™, Thermofisher Scientific, Waltham, MA USA) and immediately frozen at -20 °C. Sera were evaluated by the DiaSorin Liaison SARS-CoV-2 trimericS IgG assay (DiaSorin, Saluggia, VC, Italy), a two-step CLIA assay for the detection of IgG antibodies against the Spike (S) protein of SARS-CoV-2 in its trimeric native form. The assay was performed on the LIAISON® XL chemiluminescence analyser. The analyser automatically calculates SARS-CoV-2 trimeric S IgG antibody concentrations expressed as Binding Antibody Units (BAU/mL) and grades the results. The assay range is up to 2080 BAU/mL. According
to manufacturer’s instructions, values ≥ 33.8 BAU/mL were interpreted as positive. If the results were above the assay range, samples were automatically diluted 1/20 and testing was repeated.

**PBMC isolation**

Whole blood (5 ml) was collected from patients in sodium heparin Vacutainer tubes (BD Biosciences, San Jose, CA, USA). Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare), washed twice with cold wash buffer (2% FCS), and resuspend at 2.0–2.5 × 10^6 cells/ml in R-10 medium (RPMI 1640 supplemented with 10% FBS, sodium pyruvate, penicillin, streptomycin and nonessential amino acids; all from Sigma-Aldrich, Saint Louis MO, USA). Freshly isolated PBMCs were then frozen in 90% FCS and 10% DMSO and stored in −196 °C liquid nitrogen for later experiments.

**T cell stimulation assay and cytokine detection**

A total of 2 × 10^6 PBMCs were cultured for 20 hours in R-10 medium at 37°C with 5% CO2 in FACS tubes with 0.6 nmol of a pool of peptides encompassing the immunodominant sequence domain of SARS-CoV-2 Spike protein (Peptivator, Miltenyi, Bergisch Gladbach, Germany). Unstimulated PBMCs were used as negative control. As a positive control, the non-specific superantigen SEB was added at 100 ng/ml (Sigma-Aldrich). Brefeldin-A (Sigma-Aldrich) was added during the last 18 h of incubation, at 10 μg/mL, to inhibit cellular secretion. After overnight stimulation, PBMCs were stained with Live/Dead Fixable Violet Dead Cell Stain Kit (Thermo Fisher Scientific, Waltham MA, USA) to exclude dead cells from the analyses. Cells were washed twice with FACS buffer and then fixed with BD Cytofix/Cytoperm buffer (BD Biosciences, Franklin Lakes, NJ USA) for 20 min at 4°C. Following fixation and permeabilization, cells were washed twice with 1× BD Perm/Wash buffer and stained with a predetermined optimal concentration of fluorochrome-conjugated Abs: anti-CD3-APC-H7, anti-IL-2-FITC, anti-TNFα-PE-Cy7 (all from BD Biosciences), anti-IFN-γ-PerCP-Cy5.5 (Biolegend, San Diego, CA, USA), anti-CD8-APC (eBiosciences, Thermo Fisher Scientific). Cells were fixed in 200 μL of 1× PBS/formaldehyde (2% v/v), acquired by flow cytometry in a Gallios flow cytometer and then analysed using Kaluza software (Beckman Coulter, Brea, CA, USA). This gating strategy allowed to identify CD8^+ (CD3^+/CD8^+) and CD4^+ (CD3^+/CD8^-) T cells. Stained samples were acquired with a standard stopping gate set at 50,000 CD3^+ T-cells. Frequencies of cytokine producing cells were calculated after subtraction of
cytokine positive cells in the relative negative control tube, i.e., unstimulated sample. A frequency above
0.04% was considered a positive cell-mediated immunity (CMI) response.

**Statistical analysis**

Wilcoxon’s test was used to compare median values of cytokine positive T cells pre- and post-booster.

Given the differences in vaccination strategy, analyses of T-cell mediated immune response were
performed not only in the whole sample, but also after stratification by history of former COVID-19. Mann
Whitney’s test was used to compare median values of cytokine positive T cells of previous SARS-CoV-2
infection and COVID-19 naive residents pre- and post-booster. McNemar’s tests was used to investigate the
change in percentage of cytokine positive T-cells before and after the booster vaccine dose. Given the
nonnormal distribution of SARS-CoV-2 trimeric S IgG antibody concentrations, analyses were performed
using log-transformed values and the distribution of IgG levels pre- and post-booster was expressed as
Geometric Mean Titre (GMT). The Student's t test was used to compare antibody titer values between non-
low CMI responders, producing 0-1 cytokine upon Spike stimulation and high CMI responders, producing 2-
3 cytokines upon Spike stimulation. Correlation analyses were performed using Spearman’s rank
correlation coefficient. Statistical analysis was carried out with STATA Software Version 17.0 (Stata
Cooperation, College Station, TX, USA) and Prism 8.1 (GraphPad Software, San Diego, CA, USA). P < 0.05
was considered statistically significant. Data plotted in logarithmic scales were expressed as median. T-cell
data have been calculated as background subtracted data.

**Ethical approval**

The study was approved by the Italian National Ethical Committee with the permission number 264/2021
(January 26, 2021).

**RESULTS**

**Study sample**

The initial study sample consisted of 51 residents. During the 12 months follow-up period 10 subjects
dropped out of the study due to death (N=5), discharge (N=3) or transfer to another facility (N=2). Two
subjects were excluded from the study due to ongoing SARS-CoV-2 infection at time of 12-month blood sampling. Samples from 5 subjects could not be analysed due to scarce PBMCs yield from whole blood samples. Hence, the final study sample consisted of 34 subjects. Demographic and clinical characteristics of the study sample are shown in Table 1. Among the 24 residents with prior SARS-CoV-2 infection, 21 (87.5%) received a single vaccine dose and 3 (12.5%) two doses. Mean follow up time between booster dose and post-booster assessment was 5 months.

**SARS-CoV-2 S-specific T-cell response**

Six months after vaccination (pre-booster) most of 34 NH residents displayed a T-cell mediated immunity to Spike, indeed, 31 subjects (91.2%) had a positive response to Spike antigenic stimulation in the CD4+ or in the CD8+ compartment to at least one of the cytokines analysed. More in details, 75% of the subjects had CD4+ T cells and 65% of them had CD8+ T cells producing at least one cytokine. At post-booster assessment, 28 residents (82.3%) had a positive response to Spike antigenic stimulation in the CD4+ or in the CD8+ compartment to at least one of the cytokines analysed. The magnitude of T-cell response showed no significant variation as compared to pre-booster assessment (Figure 1, A). In the CD8+ compartment an increase was observed in the median frequencies of cytokine positive cells as compared to pre-booster, but this difference only reached a borderline significance for IL-2 (p=0.066). Polyfunctional CD4+ and CD8+ T cells, simultaneously producing more than one cytokine, were detected in stimulated PBMCs from NH residents pre- and post-booster (Figure 1, B). A slight decrease in the percentage of subjects with polyfunctional CD4+ T cells after booster administration compared to pre-booster assessment was noticed, while subjects with polyfunctional CD8+ T cells were increased (Figure 1, B).

**Effect of previous SARS-CoV-2 infection**

To investigate the effect of a previous infection on the immune response to vaccination, we stratified the sample population based on a history of SARS-CoV-2 infection received before the vaccination. As shown in Figures 2 and 3, it was evident that the previous infection promoted a superior T-cell response up to 6 months after vaccination. Pre-booster median frequencies of CD4+ IFN-γ, CD8+ IFN-γ and CD8+ TNF-α T cells were significantly lower in the naïve group (p=0.0057, p=0.0003, p=0.0011; previous SARS-CoV-2 infection
vs SARS-CoV-2 naïve residents; Wilcoxon’s test) and lesser subjects displayed polyfunctional CD4+ and CD8+ T cells producing more than one cytokine simultaneously.

Median frequencies of polyfunctional CD4+ and CD8+ T cells in the previous SARS-CoV-2 infection group at T12 showed minimal variations as compared to pre-booster (Figure 2, A). Importantly, the effect of the booster vaccine dose on T-cell responses was more evident in the naïve population. Indeed, the median frequencies of CD4+ IFN-γ T cells and CD8+ IFN-γ T cells significantly increased (Figure 3). Considering the frequencies of polyfunctional T cells, a shift to a more polarized CD8 response was observed in both groups (Figures 2 and 3), albeit the benefit of the booster dose on the poly-functionality of T cells could not be statistically demonstrated, possibly because the number of studied residents is too small.

**Correlation between humoral and cell-mediated immune response to vaccination**

The geometric mean titre (GMT) of anti-trimeric Spike IgG, before the booster dose in the sample population was 892 BAU/ml. The post-booster GMT was markedly increased to 4397 BAU/ml. A significant positive correlation was found at pre-booster assessment between serum levels of anti-trimeric Spike IgG and the percentage of CD4+ T cells producing IFN-γ, CD8+ T cells producing IFN-γ and CD8+ T cells producing TNF-α (Supplementary Table 1). To analyse these data in more depth, the sample population was divided based on CD4+ and CD8+ T cell cytokine production in two groups: no/low CMI responders (0 or 1 cytokine produced) and CMI responders (2 or 3 cytokines produced). Confirming the correlation data, before booster administration CMI responders had higher antibody levels than no/low CMI responders, reaching the statistical significance in the CD8+ compartment (Table 2). At the following time-point, after booster administration, high antibody titres were measured both in non/low CMI responders and in CMI responders and no significant differences were found.

**DISCUSSION**
In the present study we describe T-cell immune responses in a population of NH residents before and after the administration of the booster vaccine dose in a 12-month follow-up study. A robust and durable T-cell response to recall Spike stimulation was measured pre- and post- the booster vaccination in most residents. The immune response induced by COVID-19 vaccines in older people was shown to be significant, especially when considering immune impairments of this population, and to significantly prevent severe COVID-19. In this regard, T-cell responses after two doses of BNT162b2 vaccine have been described in older people, although impaired, accordingly we found that 6 months after vaccination most NH residents had detectable cytokine-producing Spike-specific T-cells and that higher responses were in those residents with a previous history of SARS-CoV-2 infection. A positive correlation between humoral and cellular immune response was found before booster vaccination, indeed lower anti-Spike IgG levels were detected in no/low CMI responders, an observation similar to previously published data. Yet, we extended our observation up to one-year post-vaccination. The effect of the third booster dose, administered to NH residents in the timeframe between the 6- and 12-month blood withdrawals, on the magnitude of the humoral immune response was evident. Spike-specific IgG levels increased substantially, confirming our previous results in NH settings. Worth of note, a significant increase of Spike-specific T-cell responses were observed for naïve SARS-CoV-2 residents but not for previously infected residents. Overall, pre- and post-booster CMI data show that a previous SARS-CoV-2 infection represents a first immunizing event that built a T-cell memory pool efficiently expanded by the primary vaccination. Only 1 out of 24 previously infected residents did not show any CD4$^+$ or CD8$^+$ positive response at the pre-booster assessment, while 3 out of 10 were CD4$^+$ and CD8$^+$ non-responders among naïve COVID-19 residents. The receipt of the booster dose induced a marked increase of serum IgG levels, measured 12 months after the first vaccine shot, and a strong induction of cell-mediated immunity in low-responding NH residents. These findings confirm previous data on the effect of an additional vaccine dose in unresponsive older adults and show that repeated immunization might lead to a sustained T-cell immune response even in immune impaired frail elderly subjects.
Conclusions and Implications

Albeit the current study has some limitations, such as the limited number of participants and lack of a control group, it allows to draw important considerations. We show a robust CMI against the Spike protein of SARS-CoV-2 in frail elderly NH residents one year after the first COVID-19 mRNA vaccination, an observation that adds to data on antibody induction\textsuperscript{9-11}. The most important finding of the present study is that administration of the booster vaccine dose substantially increased Spike-specific T-cell responses in frail NH residents who responded poorly to the first immunization, suggesting the value of continuously monitoring the immune status of this frail population to adapt future SARS-CoV-2 vaccination strategies.

Our data regarding the effect of booster doses of mRNA vaccines to elderly NH residents show high levels of both the humoral and the cell-mediated components of the immune response one year after the immunization, in previously infected and in naive subjects. However, how immunity induced by COVID-19 vaccines tailored against the ancestral Spike protein translates into protection against SARS-CoV-2 variants in this population group needs to be assessed.

References


24. XXXXXXXXXXX


FIGURE LEGENDS

**Figure 1.** T-cell mediated immune response in NH residents 6 months (pre-booster, T6) and 12 months (post-booster, T12) after first immunization. The frequencies of CD4 and CD8 T cells producing IFN-\(\gamma\), TNF\(\alpha\) and IL-2 in response to in vitro stimulation with Spike are shown (Panel A). The percentages of subjects with non-responding CD4\(^+\) and CD8\(^+\) T cells or producing 1 to 3 cytokines pre- and post-booster are shown (Panel B). Differences between median frequencies of cytokine positive T cells at T6 and T12 were calculated by Wilcoxon’s test.

**Figure 2.** T-cell mediated immune response in NH residents with a history of SARS-CoV-2 infection 6 months (pre-booster, T6) and 12 months (post booster, T12) after first immunization. The frequencies of CD4 and CD8 T cells producing IFN-\(\gamma\), TNF-\(\alpha\) and IL-2 in response to Spike are shown in Panel A. The percentages of previously infected NH residents with non-responding CD4\(^+\) and CD8\(^+\) T cells or producing 1 to 3 cytokines pre- and post-booster are shown in Panel B. Differences between median frequencies of cytokine positive T cells at T6 and T12 were calculated by Wilcoxon’s test.

**Figure 3.** T-cell mediated immune response in SARS-CoV-2 naïve NH residents 6 months (pre-booster, T6) and 12 months (post-booster, T12) after first immunization. The frequencies of CD4 and CD8 T cells producing IFN-\(\gamma\), TNF-\(\alpha\) and IL-2 in response to Spike are shown in Panel A. The percentages of SARS-CoV-2 naïve NH residents with non-responding CD4\(^+\) and CD8\(^+\) T cells or producing 1 to 3 cytokines pre- and post-booster are shown in Panel B. Differences between median frequencies of cytokine positive T cells at T6 and T12 were calculated by Wilcoxon’s test.

Table 1. Demographic and clinical characteristics of the study sample
Table 1. Demographic characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Whole sample (N=34)</th>
<th>Previous Covid-19 (N=24)</th>
<th>Naïve (N=10)</th>
<th>p-value</th>
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<tbody>
<tr>
<td><strong>Age</strong></td>
<td>Mean 74.3</td>
<td>Mean 72.3</td>
<td>Mean 79.3</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>SD 9.1</td>
<td>SD 1.6</td>
<td>SD 3.1</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>Female 22 (65%)</td>
<td>Female 15</td>
<td>Female 7</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Male 12 (35%)</td>
<td>Male 9</td>
<td>Male 3</td>
<td></td>
</tr>
<tr>
<td><strong>Co-morbidities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td>21 (62%)</td>
<td>13</td>
<td>8</td>
<td>0.16</td>
</tr>
<tr>
<td>Arterial Hypertension</td>
<td>12 (35%)</td>
<td>8</td>
<td>4</td>
<td>0.71</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>8 (23%)</td>
<td>6</td>
<td>2</td>
<td>0.75</td>
</tr>
<tr>
<td>Cardiomyopathy ischaemic</td>
<td>7 (21%)</td>
<td>3</td>
<td>4</td>
<td>0.07</td>
</tr>
<tr>
<td>Perypheral arterial disease</td>
<td>7 (21%)</td>
<td>6</td>
<td>1</td>
<td>0.32</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (12%)</td>
<td>3</td>
<td>1</td>
<td>0.84</td>
</tr>
<tr>
<td>Obesity</td>
<td>4 (12%)</td>
<td>3</td>
<td>1</td>
<td>0.84</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>2 (6%)</td>
<td>1</td>
<td>1</td>
<td>0.51</td>
</tr>
<tr>
<td>Stroke</td>
<td>2 (6%)</td>
<td>1</td>
<td>1</td>
<td>0.51</td>
</tr>
<tr>
<td>Chronic Renal Failure</td>
<td>1 (3%)</td>
<td>1</td>
<td>0</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Table 2. Geometric mean titer (GMT) of Anti-S IgG (BAU/mL) before booster dose (T6), and 12 months (T12) after first dose of vaccine.

<table>
<thead>
<tr>
<th></th>
<th>T6</th>
<th>T12</th>
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<tbody>
<tr>
<td></td>
<td>GM</td>
<td>p-value</td>
</tr>
<tr>
<td>CD4 Low or non-responders</td>
<td>496.50</td>
<td><strong>0.047</strong></td>
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<tr>
<td>CD4 2+ cytokines</td>
<td>1282.50</td>
<td>0.7953</td>
</tr>
<tr>
<td>CD8 Low or non-responders</td>
<td>496.21</td>
<td><strong>0.0059</strong></td>
</tr>
<tr>
<td>CD8 2+ cytokines</td>
<td>1719.86</td>
<td>0.1415</td>
</tr>
</tbody>
</table>

Results of Student’s t-test for non-low and high CMI responders.
A

B

CD4⁺

CD8⁺

1-cytokine
2-cytokine
3-cytokine
No Responders
**Supplementary Table 1.** Correlation between CD4/CD8 IFN-γ, IL2, TFN-α and SARS-CoV-2 trimeric S IgG antibody concentrations

<table>
<thead>
<tr>
<th></th>
<th>Anti-S IgG in BAU/mL</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>T6</td>
<td>T12</td>
</tr>
<tr>
<td></td>
<td>rs</td>
<td>p-value</td>
<td>rs</td>
</tr>
<tr>
<td>CD4 IFNg</td>
<td>0.36</td>
<td><strong>0.036</strong></td>
<td>-0.07</td>
</tr>
<tr>
<td>CD4 IL2</td>
<td>0.21</td>
<td>0.24</td>
<td>0.03</td>
</tr>
<tr>
<td>CD4 TFNα</td>
<td>0.04</td>
<td>0.80</td>
<td>-0.10</td>
</tr>
<tr>
<td>CD8 IFNg</td>
<td>0.42</td>
<td><strong>0.014</strong></td>
<td>-0.14</td>
</tr>
<tr>
<td>CD8 IL2</td>
<td>0.18</td>
<td>0.32</td>
<td>0.04</td>
</tr>
<tr>
<td>CD8 TFNα</td>
<td>0.39</td>
<td><strong>0.02</strong></td>
<td>-0.1</td>
</tr>
</tbody>
</table>

Spearman’s rank correlation coefficient, $r_s$
**Supplementary Figure 1.** Study design.
Immune response to vaccination was assessed in a sample of 34 NH residents six and twelve months after the first SARS-CoV-2 vaccination (T6 and T12). A booster SARS-CoV-2 vaccine dose was administered to NH residents after blood sampling at T6. Mean follow up time between booster dose and T12 assessment was 5 months.