Vaccination strategy and anti-SARS-CoV-2 S titers in healthcare workers of the INT – IRCCS “Fondazione Pascale” Cancer Center (Naples, Italy)

Ernesta Cavalcanti (e.cavalcanti@istitutotumori.na.it)
Istituto Nazionale per lo Studio e la Cura dei Tumori: Fondazione IRCCS Istituto Nazionale dei Tumori
https://orcid.org/0000-0001-6302-1579

Maria Antonietta Isgrò
Istituto Nazionale Tumori IRCCS Fondazione Pascale

Domenica Rea
Istituto Nazionale Tumori IRCCS Fondazione Pascale

Lucia Di Capua
Istituto Nazionale Tumori IRCCS Fondazione Pascale

Giusy Trillò
Università degli Studi di Napoli Federico II

Luigi Russo
Istituto Nazionale Tumori IRCCS Fondazione Pascale

Gerardo Botti
Istituto Nazionale Tumori IRCCS Fondazione Pascale

Leonardo Miscio
Istituto Nazionale Tumori IRCCS Fondazione Pascale

Franco Maria Buonaguro
Istituto Nazionale Tumori IRCCS Fondazione Pascale

Attilio Antonio Montano Bianchi
Istituto Nazionale Tumori IRCCS Fondazione Pascale

Research Article

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Abstract

**Background:** Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection and the resulting disease, coronavirus disease 2019 (COVID-19), have spread to millions of people globally, requiring the development of billions of different vaccine doses. The SARS-CoV-2 spike mRNA vaccine (named BNT162b2/Pfizer), authorized by the FDA, has shown high efficacy in preventing SARS-CoV-2 infection after administration of two doses in individuals 16 years of age and older.

In the present study, we retrospectively evaluated the differences in the SARS-CoV-2 humoral immune response after vaccine administration in the two different cohorts of workers at the INT - IRCCS “Fondazione Pascale” Cancer Center (Naples, Italy): previously exposed to SARS-CoV-2 subjects and not exposed to SARS-CoV-2 subjects.

**Methods:** We determined specific anti-RBD (receptor-binding domain) titers against trimeric spike glycoprotein (S) of SARS-CoV-2 by Roche Elecsys Anti-SARS-CoV-2 S immunoassay in serum samples of 35 healthcare workers with a previous documented history of SARS-CoV-2 infection and 158 healthcare workers without, after 1 and 2 doses of vaccine, respectively. Moreover, geometric mean titers and relative fold changes (FC) were calculated.

**Results:** Both previously exposed and not exposed to SARS-CoV-2 subjects developed significant immune responses to SARS-CoV-2 after the administration of 1 and 2 doses of vaccine, respectively. Anti-S antibody responses to the first dose of vaccine were significantly higher in previously SARS-CoV-2-exposed subjects in comparison to titers of not exposed subjects after the first as well as the second dose of vaccine. Fold changes for subjects previously exposed to SARS-CoV-2 was very modest, given the high basal antibody titer, as well as the upper limit of 2500.0 BAU/mL imposed by the Roche methods. Conversely, for naïve subjects, mean fold change following the first dose was low (≈1.6), reaching 3.8 FC in 72 subjects (45.6%) following the second dose.

**Conclusions:** The results showed that, as early as the first dose, SARS-CoV-2-exposed individuals developed a remarkable and statistically significant immune response in comparison to those who did not contract the virus previously, suggesting the possibility of administering only one dose in previously SARS-CoV-2-exposed subjects. FC for previously exposed subjects should not be taken into account for the generally high pre-vaccination values. Conversely, FC for not exposed subjects, after the second dose, were ≈3.8 in >45.0% of vaccinees, and ≤3.1 in 19.0%, the latter showing a potential susceptibility to further SARS-CoV-2 infection.

**Background**

Coronavirus disease 2019 (COVID-19) is a severe acute respiratory syndrome caused by Coronavirus 2 (SARS-CoV-2), a positive-sense single-stranded RNA virus belonging to the Coronaviridae family. In 20% of patients the disease evolves to severe pneumonia, respiratory and multi-visceral failure and is responsible for death in patients who present comorbidity, such as diabetes, hypertension, cardiovascular...
disease and chronic lung disease [1]. The immune system represents an important component against the viral infection through neutralising antibodies production. The trimeric spike glycoprotein (S) of SARS-CoV-2 is a key target for virus neutralising antibodies and the prime candidate for vaccine development [2]. The protein S binds its cellular receptor on the host cells, human angiotensin converting enzyme 2 (ACE), through a receptor-binding domain (RBD). One of the first vaccines approved by the European Medicines Agency (EMA) was BNT162b2 produced by Pfizer and BioNTech, a vaccine containing the messenger RNA that encodes the SARS-CoV-2 S, in small lipid particles. On 22nd December 2020, AIFA (Agenzia Italiana del Farmaco) authorized in Italy the use of BNT162b2/Pfizer vaccine, in 2 doses with an interval of 21 days between the doses [3].

Recent studies have found that subjects infected with COVID-19 present protective immunity for at least 6 months [4], but the impact of previous exposure to SARS-CoV-2 on immune response elicited by the vaccines needs to be verified in a large trial study. Preliminary data reported by Krammer et al., have shown that the immune response to the vaccine after the first dose is substantially more pronounced in individuals with pre-existing immunity and it is similar to the immune response developed after the second dose in individuals not previously infected [5]. However, no data are available for people with a history of COVID-19 regarding the booster responses or the ideal dosage, for which reason there is no definitive indication about the administration of the vaccine: whether they should be vaccinated and/or should receive one or two doses [6].

In the present study, we retrospectively analyse antibody responses induced by vaccination in two different cohorts of workers at the INT - IRCCS “Fondazione Pascale” Cancer Center (Naples, Italy): previously exposed to SARS-CoV-2 subjects and not exposed to SARS-CoV-2 subjects.

**Materials And Methods**

**Sample size:** According to our internal health surveillance program, healthcare workers underwent BNT162b2/Pfizer vaccine: 1 dose was administered to subjects previously exposed to SARS-CoV-2 (seropositive for anti-N immunoglobulins), and 2 doses (with an interval of 21 days) to subjects not exposed. The program contemplated the evaluation of antibody responses by determining anti-RBD titers at three times: basal, 20 days after the first dose and 8 days after the second dose. Data regarding 193 healthcare workers of INT - IRCCS “Fondazione Pascale” Cancer Centre (35 and 158 with history/no history of COVID-19 infection, defined as exposed and not exposed to virus subjects, respectively) were collected retrospectively.

**Assay**

Roche Elecsys Anti-SARS-CoV-2 S electrochemiluminescence immunoassay (ECLIA) for the in vitro quantitative determination of antibodies (including IgG) against spike RBD of SARS-CoV-2 in human serum was performed on Roche Cobas e 601 module. According to the manufacturer, the correlation test between Roche Elecsys Anti - SARS-CoV-2 S units per mL and WHO International Standards for anti-
SARS-CoV-2 immunoglobulins showed an excellent correlation ($r^2 = 0.9992$, slope $= 0.972$, intercept $= 0.0072$), thus allowing to consider specific Roche Elecsys Anti-SARS-CoV-2 S U/mL units equivalent to WHO International Standard BAU/mL (Binding Arbitrary Units per mL). Measuring range spanned from 0.4 BAU/mL to 2500.0 BAU/mL; values higher than 0.8 BAU/mL were considered positive.

### Statistical analysis

Statistical analysis was performed by using the Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA), version 27.0. Distribution of variables was evaluated by Shapiro-Wilk test; parametric data were represented as mean ± standard deviation (SD), whilst non-parametric variables were expressed as median (IR - Interquartile Range). Two-tailed Mann-Whitney (for independent variables) and Wilcoxon (for paired variables) tests were used to compare groups. Values lower than 0.4 BAU/mL were assumed as 0.4 and values higher than 2500.0 BAU/mL were reported as 2500.0; p values < 0.05 were considered statistically significant.

To overcome the novelty of general bead-based linear models, used to evaluate post-to-pre vaccination antibody titer increase, the used statistic methods were extrapolated from Zaccaro et al. (2013) [7]:

$$
\log_{10}(FC) = \log_{10}\left(\frac{z_{28,i}}{z_{0,i}}\right) = \log_{10}(z_{28,i}) - \log_{10}(z_{0,i})
$$

defining $z_{0,i}$ and $z_{28,i}$ to be the Day 0 (pre-vaccination) and Day 28 (post-vaccination) assay results for the $i^{th}$ participant, respectively.

### Results

Data regarding 193 (35 previously exposed and 158 not exposed healthcare workers) were collected retrospectively: the 35 seropositive cases included 25 female subjects and 10 male subjects with an overall mean age of 48.1 years (SD ± 9.7, range 31 – 69); the 158 seronegative cases included 74 female subjects and 84 male subjects with an overall mean age of 47.6 years (SD ± 10.0, range 22 - 70) (Table 1).

| Male (age) | Female (age) | Total (mean ± SD) |
|------------|--------------|-------------------|
| n (mean ± SD) |
| **Previsouly exposed subjects** | 10 (55.2 ± 9.5) | 25 (45.2 ± 8.4) | 35 (48.1 ± 9.7) |
| **Not exposed subjects** | 84 (49.7 ± 9.7) | 74 (45.2 ± 10.0) | 158 (47.6 ± 10.0) |
| **Total** | 94 | 99 | 193 |

Previously exposed subjects = subjects previously exposed to SARS-CoV-2
In previously exposed to SARS-CoV-2, antibody response 20 days after the first dose of vaccine was statistically higher in comparison to pre-vaccination: median >2500.0 BAU/mL vs. 36.6 BAU/mL (IR 14.5 - 99.0) (Wilcoxon test, p <0.001) (Table 2, Figure 1).

In not exposed to SARS-CoV-2 subjects, overall antibody response 20 days after the first dose of vaccine was statistically significant in respect to pre-vaccination values: 18.9 BAU/mL (IR 4.3 - 58.2) vs. <0.4 BAU/mL (Wilcoxon test, p <0.001) (Table 2, Figure 1). Also the titers determined 8 days after the second dose revealed a statistically increasing trend in respect to the first dose: 2111.0 BAU/mL (IR 713.8 - >2500.0) vs. 18.9 BAU/mL (IR 4.3 - 58.2) (Wilcoxon test, p <0.001) (Table 2, Figure 1).

|                      | Pre     | Post I   | Post II  |
|----------------------|---------|----------|----------|
| **Previously exposed subjects** | 36.6 (14.5-99.0) | >2500.0 | ---      |
| **Not exposed subjects** | <0.4    | 18.9 (4.3-58.2) | 2111.0 (713.8 - >2500.0) |

Comparing basal values and titers after the first dose of vaccine in the 2 groups, it was evident that previously exposed subjects presented significantly higher basal titers of antibodies in comparison to those not exposed (36.6 BAU/mL [IR 14.5 - 99.0] vs. <0.4 BAU/mL) and, as early as the first dose, previously exposed workers developed a significantly higher antibody response to SARS-CoV-2 respect to values of not exposed subjects (>2500.0 BAU/mL vs. 18.9 BAU/mL [IR 4.3 - 58.2]), Mann-Whitney test, p <0.001) (Table 2, Figure 1).

Further interesting information derived from analysis which compared antibody titers after the first dose of vaccine in the previously exposed cohort, in comparison to antibody titers after the second dose in the not exposed cohort, revealing values significantly higher (>2500.0 BAU/mL vs. 2111.0 BAU/mL [IR 713.8 - >2500.0], Mann-Whitney test, p <0.001) (Table 2, Figure 1).
Fold changes for subjects previously exposed to SARS-CoV-2 was very modest (1.8) (Table 3), given the high basal antibody titer, as well as the upper limit of 2500.0 BAU/mL imposed by the Roche methods. Conversely, for not exposed to SARS-CoV-2 subjects, mean fold change following the first dose was low (1.6), reaching 3.8 FC in 72 subjects (45.6%) following the second dose (Table 3). In the average, following the second dose, the FC of not exposed subjects was 3.5 in comparison to pre-vaccination and 1.9 in comparison to the titer obtained after the first dose of vaccine. In this cohort, given the high variability of FC, prompted us to divide it into 3 different groups, using two arbitrary antibody titers of 500.0 and 800.0 BAU/mL, whose FC values were 3.1 and 3.3, respectively (Table 3). The validation of such arbitrary values will be obtained in the following monitoring of those groups in order to verify and evaluate their relative susceptibility to further SARS-CoV-2 infection.

**Table 3. Antibody titers’ fold changes (FC) in previously exposed and not exposed cohorts of healthcare providers to monitor vaccine immune responses.**

| Analyzed cohorts | Mean FCmaxFC n (%) |
|------------------|--------------------|
| **Previously exposed subjects** | |
| Post I / Pre | 1.8 | 0.08 (100.0%) |
| **Not exposed subjects** | |
| Post I / Pre | 1.6 | 0.06 (100.0%) |
| Post II / Post I | 1.9 | 0.08 (100.0%) |
| Post II / Pre | 3.5 | 0.05 (100.0%) |
| Post II/Pre ≤ 3.1 | 2.6 | 1.03 (19.0%) |
| Post II/Pre 3.1< ≤ 3.3 | 3.2 | 3.01 (7.6%) |
| Post II/Pre 3.3< ≤ 3.8 | 3.7 | 3.01 (73.4%) |
| Post II/Pre = 3.8 | 3.8 | 72 (45.6%) |

Previously exposed subjects = subjects previously exposed to SARS-CoV-2
Not exposed subjects = subjects not previously exposed to SARS-CoV-2
Pre = anti-SARS-CoV-2 S titers before the first dose
Post I = anti-SARS-CoV-2 S titers 20 days after the first dose
Post II = anti-SARS-CoV-2 S titers 8 days after the second dose
mean FC = mean fold changes of anti-SARS-CoV-2 S mean geometric titers
n (%) = number of subjects (percentage)

**Discussion**

Our preliminary data suggest that, differently from behaviour of not previously exposed to SARS-CoV-2 subjects, who require 2 doses of vaccine in order to develop a substantial immune response to SARS-CoV-2, antibody titers in previously exposed cases present a significant increase since the first dose, as reported for the SARS-CoV-2 infection following the first dose of vaccine. Indeed, the infection would induce a short-lived immunity in some case even “silent” along with a good immune memory, able to
trigger a powerful antibody production after an induced stimulus, such as a single vaccine dose. Moreover, although a second dose of vaccine in previously exposed subjects will not significantly contribute to their immunization level, it will increase the vaccine reactogenicity [5].

Moreover, these current data suggest that the second dose in not exposed subjects reaches the maximal induction in < 50.0% of the vaccinated subjects, with a large variability in the rest of the vaccinees. For such reason, two further subgroups were identified in order to evaluate prospectively their susceptibility to further SARS-CoV-2 infection. It should always be kept in mind that, although we are currently evaluating preferentially the Th2 humoral immune response, most (if not all) anti-SARS-CoV-2 vaccines have been targeted to Th1 immune response [8], and that subjects with low antibody titers could be protected by a strong cellular immunity, which will need to be evaluated in further studies.

**Conclusions**

In accordance with first data reported in literature [5-6], our findings argue that administration of a single dose to previously exposed subjects is necessary and that it would be sufficient to elicit an adequate immune response. The vaccine dose serving as booster in naturally infected individuals provides a rationale for updating vaccine recommendations to consider a single vaccine dose to reach protective immunity and to use quantitative anti-RBD serological standardized assays to screen individuals prior to vaccination if the infection history is unknown or uncertain [9-10].

Nevertheless, further studies are mandatory to confirm these data in larger cohorts of subjects (including patients and immunocompromised individuals) and to monitor antibodies’ titers over time. Moreover, more efforts are needed to overcome the use of non-standardized antibodies’ units, the lack of specific cut-offs defining the protective antibody titer levels and the large number of not equivalent diagnostic platforms already commercially available.

**List Of Abbreviations**

ACE: human angiotensin converting enzyme 2

AIFA: Agenzia Italiana del Farmaco

BAU/mL: Binding Arbitrary Units per mL

COVID-19: Coronavirus disease 2019

ECLIA: electrochemiluminescence immunoassay

EMA: European Medicines Agency

FC: Fold Change
RBD: receptor-binding domain

S: trimeric spike glycoprotein

SARS-CoV-2: Severe Acute Respiratory Syndrome caused by Coronavirus 2

Declarations

**Ethics approval and consent to participate** The study was conducted under the statements of Declaration of Helsinki.

**Consent for publication** Not applicable.

**Availability of data and materials** All relevant data and their evaluation are reported in the manuscript.

**Competing interests** All authors declared to have no conflict of interest.

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**Authors' contributions** EC and FMB conceived, designed and supervised the project. GB, LM and AAMB supported the internal health surveillance program. DR, LDC and GT assisted with data collection. LR performed the tests. MAI performed the statistical analysis. EC analysed and interpreted the data. EC, MAI, LR and FMB wrote the manuscript draft. All authors reviewed and edited the manuscript.

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Figures

Figure 1

Vaccine immune response monitoring in previously exposed and not exposed cohorts of healthcare providers. Previously exposed subjects = subjects previously exposed to SARS-CoV-2 Not exposed subjects = subjects not previously exposed to SARS-CoV-2 Pre = anti - SARS-CoV-2 S titers (expressed in
BAU/mL) before the first dose Post I = anti-SARS-CoV-2 S titers (expressed in BAU/mL) 20 days after the first dose Post II = anti-SARS-CoV-2 S titers (expressed in BAU/mL) 8 days after the second dose