Maria Infantino*, Mariangela Manfredi, Lorenzo Stacchini, Claudia Cosma, Valentina Grossi, Barbara Lari, Edda Russo, Amedeo Amedei, Maurizio Benucci, Francesca Veneziani, Patrizia Casprini, Cateno Mario Catalano, Giuseppe Cirrincione, Guglielmo Bonaccorsi and Adolfo Pompetti

The role of neutralizing antibodies by sVNT after two doses of BNT162b2 mRNA vaccine in a cohort of Italian healthcare workers

Abstract

Objectives: Evaluating anti-SARS-CoV-2 antibody levels is a current priority to drive immunization, as well as to predict when a vaccine booster dose may be required and for which priority groups. The aim of our study was to investigate the kinetics of anti-SARS-CoV-2 Spike S1 protein IgG (anti-S1 IgG) antibodies and neutralizing antibodies (NAbs) in an Italian cohort of healthcare workers (HCWs), following the Pfizer/BNT162b2 mRNA vaccine, over a period of up to six months after the second dose.

Methods: We enrolled 57 HCWs, without clinical history of COVID-19 infection. Fluoroenzyme-immunoassay was used for the quantitative anti-S1 IgG antibodies at different time points T1 (one month), T3 (three months) and T6 (six months) following the second vaccine shot. Simultaneously, a commercial surrogate virus neutralization test (sVNT) was used for the determination of NAbs, expressed as inhibition percentage (% IH).

Results: Median values of anti-S1 IgG antibodies decreased from T1 (1,452 BAU/mL) to T6 (104 BAU/mL) with a percent variation of 92.8% while the sVNT showed a percent variation of 34.3% for the same time frame. The decline in anti-S1 IgG antibodies from T1 to T6 was not accompanied by a loss of the neutralizing capacity of antibodies. In fact at T6 a neutralization percentage <20% IH was observed only in 3.51% of HCWs.

Conclusions: Our findings reveal that the decrease of anti-S1 IgG levels do not correspond in parallel to a decrease of NAbs over time, which highlights the necessity of using both assays to assess vaccination effectiveness.

Keywords: antibody kinetics; anti-SARS-CoV-2 antibodies; health care workers; immune response; neutralizing antibodies; surrogate virus neutralization test; vaccine.

*Corresponding author: Dr. Maria Infantino, Immunology and Allergology Laboratory Unit, S. Giovanni di Dio Hospital, Via Torregalli, 3 50143 Florence, Italy, Phone: +390556932502, Fax: +390556932289, E-mail: maria2.infantino@uslcentro.toscana.it
Mariangela Manfredi, Valentina Grossi and Barbara Lari, Immunology and Allergology Laboratory Unit, S. Giovanni di Dio Hospital, Florence, Italy
Lorenzo Stacchini, Claudia Cosma and Guglielmo Bonaccorsi, Department of Health Science, University of Florence, Florence, Italy
Edda Russo and Amedeo Amedei, Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
Maurizio Benucci, Rheumatology Unit, S. Giovanni di Dio Hospital, Florence, Italy
Francesca Veneziani and Patrizia Casprini, Clinical Pathology Laboratory Unit, S. Giovanni di Dio Hospital, Florence, Italy
Cateno Mario Catalano and Giuseppe Cirrincione, Department of Technical Health Services, Preventive Medicine, S. Giovanni di Dio Hospital, Florence, Italy
Adolfo Pompetti, SOC Clinical Assistance Governance, SOS Preventive Medicine Unit, S. Giovanni di Dio Hospital, Florence, Italy

Introduction

Since the beginning of the coronavirus disease (COVID-19) pandemic [1], the public health measures implemented to prevent the spread of the Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) has led to social, political, and economic consequences [2, 3].

According to the global vaccination campaign, identifying the protection level generated by anti-SARS-CoV-2 vaccines is a worldwide priority to monitor immune tone and drive vaccinations, to determine when a booster dose might be necessary and especially for which priority groups.

After positively assessing the safety and efficacy of Pfizer/BioNT BNT162b2 mRNA vaccine [4], approved by the European Medicines Agency (EMA) {Agency, an Italian vaccination campaign started on 27 December 2020, targeting firstly healthcare workers (HCWs)}, to ensure their protection and safety worldwide [5].

Preliminary data showed that the number and the proportion of HCW cases reported started to drop significantly

30 days after the start of the COVID-19 vaccination campaign. Since then, a consistent decrease of cases has been observed, suggesting that vaccination is effective in reducing infections in this group [6]. As previously reported, different kinetics of cellular and humoral responses have been elicited by COVID-19 vaccines [7–9].

Currently, there are supporting data suggesting that neutralizing antibody (NAb) levels correlated with the protection threshold from infection [10–14]; however, specific correlates of protection from SARS-CoV-2 are not yet defined. Healthy individuals show high levels of the anti-SARS-CoV-2 Spike S1 protein IgG (anti-S1 IgG) antibodies and NAbs, as well as a persistent germinal center B-cell response following vaccination [15–22].

The BNT162b2 vaccine at peak immunity (2–4 weeks after the second dose) induces a high anti-virus Nabs titer and antibody titer against RBD antigen. This titer decreases six months after vaccination and further after eight months [8].

Despite one month after the primary vaccination, the kinetics of anti-SARS-CoV-2 NAbs and anti-S1 IgG showed a steep increase, on the contrary, in the following second and third month, the decline rate was lower, mostly for NAbs with a persistence of the antibody response above the positivity threshold against COVID-19 [23].

The aim of our study was to investigate the kinetics of anti-S1 IgG antibodies and NAbs by a surrogate virus neutralization test (sVNT) in an Italian cohort of HCWs following the BNT162b2 mRNA vaccine over a period of up to six months after the second dose. Finally, the variables of age and gender have been evaluated.

Materials and methods

Study population

Fifty-seven HCWs, without reported clinical history of COVID-19 infection and with persistently negative virus detection by RT-PCR on nasal swab monitoring, were enrolled in our study. Thirty-eight out of the total of 57 subjects studied (66.67%) were females and the median age was 50 years old (range 28–65 years). At T0 no patients had serological evidence of previous SARS-CoV-2 infection. All 57 HCWs had available serological data at prefixed time points according to the months distance from the second vaccine shot: T1 (one month), T3 (three months) and T6 (six months). The study was conducted in accordance with the ethical standards as formulated in the Helsinki Declaration and written informed consent was obtained from all the participants.

Methods

EliA SARS-CoV-2-Sp1 IgG: The EliA SARS-CoV-2-Sp1 IgG (Thermo Fisher S1 IgG) is a fluoroenzyme-immunoassay (FEIA) for the *in vitro* quantitative measurement of IgG antibodies in human serum and plasma directed to the S1 protein [24], performed on the Phadia 250 instrument (Thermo Fisher, Uppsala, Sweden). The EliA SARS-CoV-2-Sp1 IgG wells are coated with recombinant SARS-CoV-2 S1 protein. After washing non-bound antibodies, enzyme-labelled anti-human IgG are added to form antibody-conjugate complexes incubated with a development solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The cut-off value in AU/mL, the conversion factor to obtain BAU/mL, the cut-off value in BAU/mL and the linearity range in AU/mL are respectively: 7.0, 4.0, 28 and 0.7–204, as declared by the manufacturer. Samples with values above 204 AU/mL (816 BAU/mL) were diluted and measured 1:2 allowing the extension of the dynamic range up to 408 AU/mL (1,632 BAU/mL). According to the manufacturer, intra- and inter-assay precision data ranges between 2.1 and 3.1%; clinical specificity is 99.7% (95% CI: 98.4–100%) and clinical sensitivity is 100% (95% CI: 99.5–100%), calculated at eight days or more, after the first positive PCR.

SARS-CoV-2 NeutraLISA: The "SARS-CoV-2 NeutraLISA" enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Lübeck, Germany) is a commercial sVNT for the semiquantitative *in vitro* determination of neutralizing anti-SARS-CoV-2 antibodies [25]. The assay is based on the competition between neutralizing antibodies and S-RBD antigens for the binding to ACE2. Briefly, during the first incubation, samples and controls are diluted with a sample buffer containing biotinylated ACE2. If neutralizing antibodies are present in the sample, they compete with the receptor ACE2 for the binding sites of the SARS-CoV-2 S/RBD proteins. A second incubation step is performed to detect the bound ACE2 by the addition of peroxidase-labelled streptavidin, which catalyzes a color reaction. The intensity of the product absorbance is inversely proportional to the concentration of neutralizing antibodies in the sample. The inhibition percentage (% IH) is calculated with the following formula: % IH=100% – (extinction of patient sample $\times$ 100%/extinction of blank). Results below 20% IH are considered negative; ≥20% IH to <35% IH as borderline and ≥35% IH as positive. Sensitivity and specificity are 95.9 and 99.7%, respectively, as declared by manufacturer.

Statistical analysis

Descriptive statistics were used to analyze main characteristics of the variables. Once the assumption of normal distribution for the quantitative measures was rejected by the Kolmogorov-Smirnov test, non-parametric tests were chosen to verify the research hypothesis. The associations between continuous variable were evaluated by ANOVA, Student's t test or corresponding non parametric tests. The Spearman's coefficient of rank correlation was used to compare sVNT and anti-S1 IgG assay. A p-value less than 0.05 was considered statistically significant. p-Values inferior to 0.001 were referred as <0.001. Statistical analysis was performed by using R 4.1.1 (R Foundation for Statistical Computing) software.

Results

Median values of anti-S1 IgG antibodies significantly decreased from T1 (1,452 BAU/mL; IQR: 980–1,632) to T6 (104 BAU/mL; IQR: 64–184) with a percent variation of
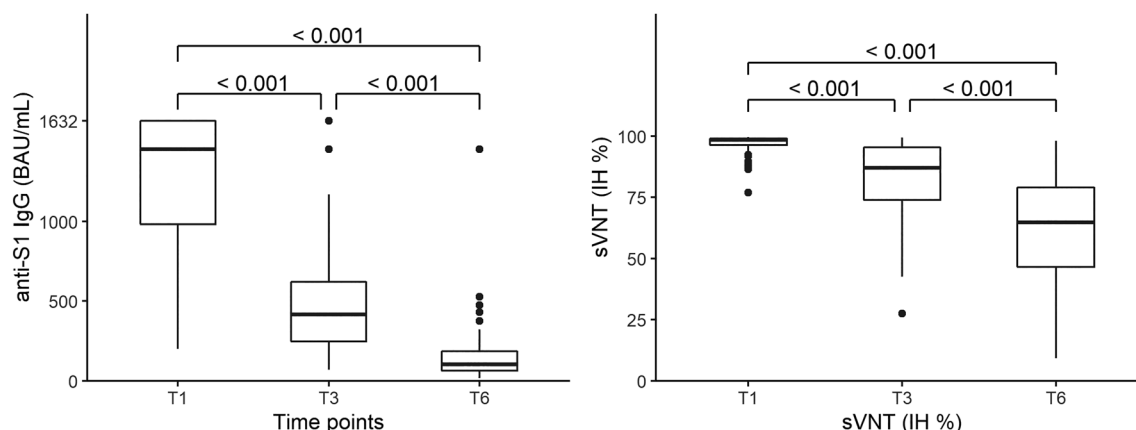
Figure 1: Boxplots of anti-S1 IgG levels and sVNT (IH %) at different time points.

92.8%. Moreover, sVNT showed a significant decrease of the values from T1 (98.60% HI; IQR: 96.50–99.20) to T6 (64.80% HI; IQR: 46.60–79.10) with a percent variation of 34.3%. Notably, all time points (T1 and T2; T1 and T3; T2 and T3) significantly differed for both anti-S1 IgG antibodies and sVNT (% IH) (Figure 1).

The individual trajectories of the two different tests at each time point and the exponential regression performed to predict the concentration decrease during time from the second dose of BNT162b2 vaccine are shown in Figure 2.

Comparing age and gender with antibody levels, no significant correlation (Mann-Whitney U test show a p-value<0.001 for both of variables) was found (data not shown).

The highest correlation coefficient (r=0.76) was obtained at T1 and the lowest at T6 (r=0.59), decreasing at each time point, corresponding to a decrease of correlation between the two tests (Figure 3).

Stratifying the cohort according the sVNT (% IH) in three categories (<20, 20–59 and ≥60%), we observed that 100% of the HCWs had a percentage ≥60% at T1, of which 84.21% had anti-S1 IgG antibodies ≥800 BAU/mL and 15.79% between 40 and 799 BAU/mL. At T3 89.47% of all HCWs had a sVNT percentage ≥60%, but the remaining 10.53% of all HCWs had a sVNT percentage between 20 and 59%, all with a correspondent anti-S1 IgG antibody concentration between 40 and 799 BAU/mL. At T6 we observed a lower percentage (56.14%) of HCWs compared to T1 and T2 points with a sVNT percentage ≥60%, and 40.35% had a sVNT percentage between 20 and 59%, both mainly with an anti-S1 IgG antibody concentration between 40 and 799 BAU/mL. A neutralization percentage
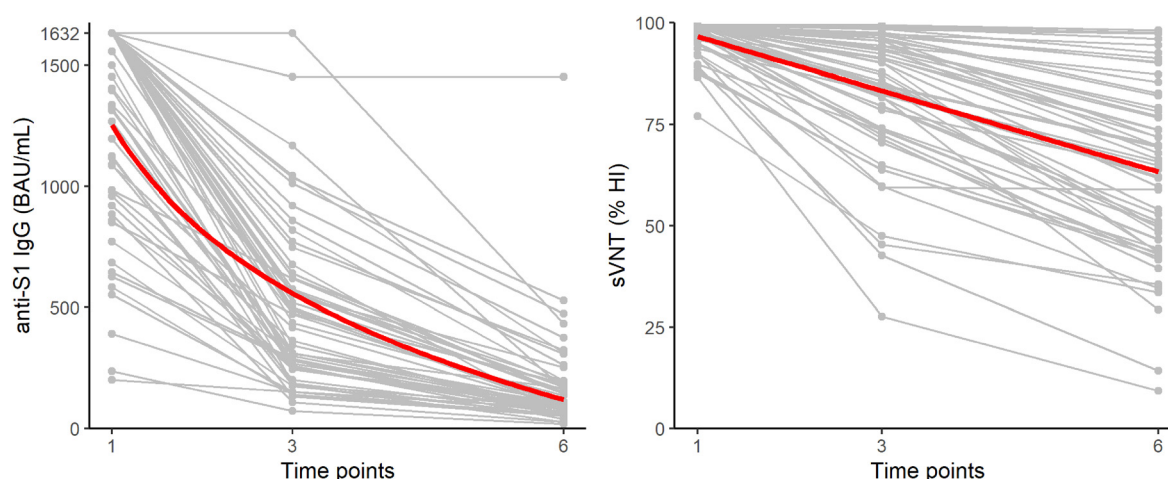

Figure 2: Anti-S1 IgG antibody concentrations (BAU/mL) for each HCW at each time point (on the left), and neutralizing capacity of sVNT (IH %) for each HCW at each time point (on the right).
The red line indicates, respectively, the log fit and the linear fit between time points (independent variables) and y axis.
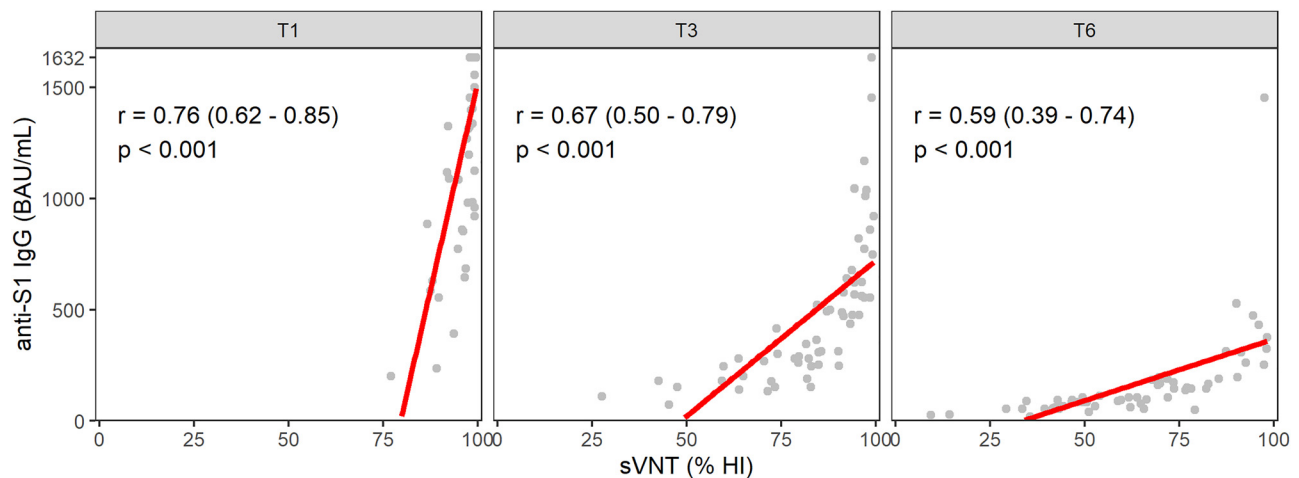
Figure 3: Correlation between anti-S1 IgG antibody concentrations (BAU/mL) and neutralization capacity of sVNT (IH %) at three different time points (months from the second vaccine shot).
T1 (one month); T3 (three months); T6 (six months).

<20% was observed only at T6 in 3.51% of HCWs (Figure 4).

Discussion

As of 22 February 2022, around 78% of Italian population had been given at least two doses of a COVID-19 vaccination (https://ourworldindata.org/covid-vaccinations). Considering the overall vaccinated population, the effectiveness of protection against symptomatic and asymptomatic infection remains high (89%), up to seven months following the second dose, as well as six months against hospitalization and death (96 and 99%) [26].

WHO suggested that efficacy can be assessed against "disease, severe disease, and/or shedding/transmission" endpoints [27] and studies on a vaccine's efficacy against COVID-19 have been based on the capability of decreasing the number of COVID-19 cases [4, 28–34], without considering the NAbs as a complementary tool to support it [35].

Studies evaluating the decrease of anti-SARS-CoV-2 antibodies and NAbs have been widely reported [36–40] with different results due to the poor harmonization among assays [41–45]. The influence of sex and age is still unclear [46].
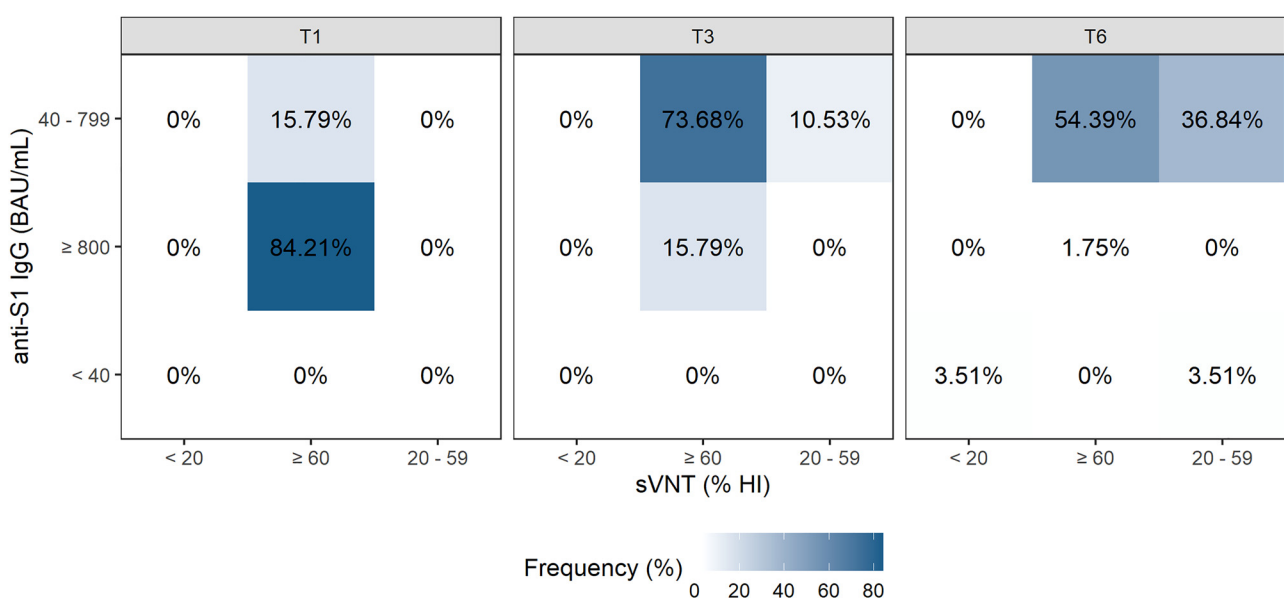

Figure 4: Frequency (%) of HCWs according to each sVNT (<20; 20–59; ≥60) and anti-S1 IgG antibodies (<40; 40–799; ≥800) categories at three different time points (months from the second vaccine shot).
T1 (one month); T3 (three months); T6 (six months).

However great efforts have been made to provide reliable serological assays (both for anti-SARS-CoV-2 antibody assays and for ACE2-RBD competitive assays) and to implement diagnostic platforms, in order to achieve an ever-better correlation with the virus neutralization test (VNT) gold standard and to improve the harmonization process [47, 48].

The high correlation between serological assays and VNT gold standard allows us to evaluate the potential role of serological tests [34] to provide information on: 1) the effectiveness of anti-COVID-19 vaccines across population (mass testing); 2) the duration of immune protection from infection and how long this protection lasts after natural infection or vaccination [13, 14, 49]; 3) vaccine clinical trials; 4) disease control in health and non-healthcare settings; 5) research studies.

In this study both anti-S1 IgG antibodies and NAb declined in parallel at three months after the second dose of BNT162b2 mRNA vaccine, compared to the peak response at one month. Nevertheless, the humoral response remained robust in almost all HCWs tested, according to the literature studies [21, 36, 50].

This documented kinetics reflects a dynamic that sees the decrease in the titer of anti-S1 IgG antibodies after natural infection or vaccination without a decrease in the immune activity.

Indeed, when an infection starts, the acute antibody response commonly determines a high antibody concentration that decreases over time as most acute response plasma cells are short-lived, but about 10–20% of these will become long-lived memory plasma cells. Over time, a phenomenon known as maturation of the antibody affinity of memory B cells occurs, as well as a constant maintenance of the neutralizing capacity of long-lived memory plasma cells [51].

Effectively, memory plasma cells are able to respond to subsequent exposures to the pathogen [52] and can be stored for decades, if not for life, in the bone marrow [53]. Immunological memory against many viruses and vaccines is stable over decades, if not for a lifetime [54].

Our data, showing the correlation between anti-S1 IgG levels and NAbs that progressively decrease longitudinally (T1>T3>T6), stress the importance of both tests to monitor the efficacy of vaccine. In particular, on one side high levels of anti-S1 IgG antibodies are always associated with high rates of NAbs and on the other side, low and medium levels may be associated with different rates of NAbs.

Therefore, it would be important to stratify HCWs with low and medium levels of anti-S1 IgG antibodies on the rate basis of the NAbs. We believe that this notion is crucial to better define the potential role of both tests in prioritizing the use of booster doses regardless of setting of fragility.

The main limitations of our study are the small number of patients investigated and the non-inclusion of past-COVID-19 patients. However, the strength of our work is the diagnostic approach suggested for health surveillance on HCWs through commercially available assays that are easy to use in the daily clinical practice. We did not test NAbs using the Plaque Reduction Neutralization Test (PRNT) reference method, since it requires Biosafety Level 3 (BSL-3) containment. Even if the data comparing sVNT and PRNT are still not very robust and sometimes controversial [37, 47, 48], the accessibility and user-friendliness of sVNT make it an intriguing opportunity.

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