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Short communication

Evaluation of the immune response to COVID-19 vaccine mRNA BNT162b2 and correlation with previous COVID-19 infection

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ABSTRACT

Background: The kinetics of immune response after vaccination with mRNA-BNT162b2 (Comirnaty®) and the correlation with previous COVID-19 infection are still unclear.

Methods: Thirty-six subjects receiving mRNA-BNT162b2 were prospectively studied [10 days after the first dose (Time 1), 7 days and 16 weeks after the second dose (Time 2 and Time 3)] to determine antibody titers against nucleocapside, trimeric spike protein (TSP) and receptor-binding-domain (RBD) of the spike protein. Ten subjects had a previous COVID-19 infection not requiring hospitalization (Group 1) and 26 did not (Group 2).

Results: At Time 1 all subjects in Group 1 had IgG against TSP > 800 AU/mL compared to 11/26 (42.3%) in Group 2, whilst at Time 2 all subjects in both groups had > 800 AU/mL. The mean IgG against TSP titer at Time 3 was 711 AU/mL (95% CI 652–800) in Group 1 and 240 AU/mL (95% CI 112–375) in Group 2 (p < 0.0001). However, all subjects in both groups maintained antibody titers above the lower threshold limit at each time-point considered. These results were confirmed also using anti-RBD antibody tests. Antibodies against nucleocapside were reactive only in subjects in Group 1 and remained stable during the study period. No subject had a new onset of COVID-19 infection within 16 weeks of follow-up.

Conclusions: Subjects with previous COVID-19 infection have a more rapid immune response to mRNA-BNT162b2 than others and maintained higher antibody titers during 16 weeks of follow-up. However, no new COVID-19 infection also in subjects with lower antibody titers.

1. Introduction

Since the beginning of 2020 when the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection widely spread from China throughout the World [1-3] epochal changes have been observed in everyday life, leading to modification of interpersonal relationships and health habits; the innumerable number of deaths observed in the last year led to dramatic global economic and social upheavals [4-5], permanently putting the global health system in crisis. As a consequence, the introduction of large-scale vaccination against SARS-COVID-2 becomes mandatory to reduce the health and social impact of this infection. The first vaccine to be introduced in the clinical practice in Italy at the beginning of January 2021 was the mRNA-BNT162b2 (Comirnaty®, BioNTech/Pfizer, Mainz, Germany/-New York, United States). It works by injecting mRNA encoding the SARS-CoV-2 spike protein directly into the host. Although pure mRNA is rapidly downgraded, a number of technological advances in delivery methods and RNA carriers over the last decade allow efficient and safe uptake of mRNA into the cytosol, where ribosomes then translate the mRNA to produce a viable protein that can finally stimulate an immune response. The advantages of this technology over more conventional vaccine types are numerous, including better safety (as no infectious agents are involved in their production), low risk of developing mutations, lower risk of antigen degradation in vivo, and a very rapid mass production at lower cost, as in vitro reactions can rapidly generate high yields of therapeutic agent [6]. Safety and efficacy of mRNA-BNT162b2 vaccine have been studied in a large, randomized trial enrolling over 37,000 individuals [7]. In this study, the cumulative incidence of Covid-19 cases over time among individuals receiving placebo or vaccine begins to diverge by 12 days after the first dose of vaccine, demonstrating an

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early onset of an at least partially protective effect of immunization. Overall, 10 cases of severe Covid-19 were observed after the first dose, but only 1 occurred in the vaccine group, confirming protection against severe Covid-19 infections. Despite the demonstrated clinical efficacy of mRNA-BNT162b2 vaccine in the short term, very limited data are currently available on the modifications of the kinetics of antibody titers against the trimeric spike protein or against the receptor binding domain (RBD) of the spike protein after vaccination [8-10]. The aim of this study was to evaluate the immune response to mRNA-BNT162b2 vaccine in a cohort of subjects who received vaccination very early after the vaccine became available (first dose received from January 2nd to 5th); all the subjects received the second dose of vaccine 21 days after the first one. Finally, we correlated previous COVID-19 infection status with the kinetics of antibody titers using different antibody tests.

2. Methods

In this study, we included 36 subjects repeatedly tested to determine antibody titers at specific time-points after receiving vaccination with mRNA-BNT162b2: 10 days (±2 days) after the first dose (Time 1), 7 days (±2 days) and 16 weeks (±2 weeks) after the second dose (Time 2 and Time 3, respectively). Further, they were also tested to determine antibodies against nucleocapside immediately prior to vaccination, regardless of previous COVID-19 infection. Two groups of subjects were identified based on previous, documented COVID-19 infection: 10 had a previous COVID-19 infection and 26 had not. Three different antibody tests were performed at each time-point: Elecsys anti-SARS-CoV-2 (qualitative test determining antibodies against nucleocapside, cut-off index-COI: 1: reactive), Liaison SARS-CoV-2 TrimericS IgG (quantitative test determining IgG against the trimeric spike protein, protective titer > 13 AU/mL; upper threshold > 800 AU/mL) and Elecsys anti-SARS-CoV-2 (quantitative test to determine antibodies against the receptor binding domain –RBD- of the spike protein, protective titer > 0.8 UI/mL; upper threshold > 2500 UI/mL). The kinetics of the antibody titer between the two groups, that were not homogeneous for size, were compared using the Welch t-test for unpaired samples.

3. Results

Ten subjects (27.8%, Group 1) had a previous, documented COVID-19 infection (7 got infected between March-April 2020 and 3 between October-December 2020) whilst 26 never had COVID-19 infection (Group 2). Antibodies against nucleocapside were detected only in subjects who had previous COVID-19 infection. All subjects in Group 1 had a mild COVID-19 disease not requiring hospitalization. Table 1 summarizes clinical and demographic characteristics of the two groups of individuals. No differences were found according to gender, age and chronic co-morbidities (co-morbidities considered were: diabetes, renal, hepatic or cardiac failure, hypertension, cancer, auto-immune diseases, chronic hepatitis); finally, no subject in either group had known immune-depression at the time of first dose of vaccine. All recipients in Group 1 had reactive antibodies against nucleocapside, and their levels remained substantially stable after the two doses of vaccine at each time-point considered (mean values: 49.2 vs 51.3 vs 50.8 at Time 1, 2 and 3, respectively); as expected, anti nucleocapside antibodies remained not reactive in all individuals in Group 2 (COI < 1) at each time-point. Using Liaison SARS-CoV-2 TrimericS IgG test, at Time 1 all the recipients included in Group 1 had antibody titers > 800 AU/mL compared to 11/26 (42.3%) in Group 2. At Time 2, all subjects in both Group 1 and Group 2 had antibody titers > 800 AU/mL. At Time 3, only 2 individuals, both in Group 1, had antibody titers > 800 AU/mL. Further, the mean antibody titers at Time 3 was 711 AU/mL (95% CI 652–800) in Group 1 and 240 AU/mL (95% CI 112–375) in Group 2 (p<0.0001), with a difference in decline of 471 AU/mL (95% CI: 359.4–564.6). Nevertheless, at every time-point all the recipients in both groups maintained antibody titers > 13 AU/mL. These results were confirmed also when using anti-RBD antibody tests. In particular, all Group 1 subjects had antibody titers > 2500 UI/mL at Time 1 and Time 2 and had mean titers of 2407 at Time 3 (95% CI 1789–2500). In contrast, at Time 1, 10/26 subjects (38.5%) in Group 2 had antibody titers > 2500 UI/mL; all the individuals had titers > 2500 UI/mL at Time 2, whilst no individual maintained these levels at Time 3. Mean antibody titers at Time 3 were 503 UI/mL (95% CI 178–1195). In both groups, antibody titers were >0.8 UI/mL during all the time-points considered, ensuring an immune-protection. During follow-up, no subject was diagnosed with a new onset COVID-19 infection.

4. Discussion

This study, though including only a small number of individuals, provides information on the kinetics of antibody titers after the vaccination with two doses of mRNA-BNT162b2. Firstly, we confirmed that antibodies against nucleocapside are present only in case of natural infection by COVID-19 and that their level is not significantly affected by vaccine administration. This finding was easily predictable as mRNA-BNT162b2 vaccine produces a spike antigen only and, consequently, spike antibodies only. Further, subjects with previous COVID-19 infection have a more rapid immune response to mRNA-BNT162b2 than others, achieving very high titers soon after the first dose. This specific finding could have been biased in our study by the lack of baseline antibodies against the RBD of the spike protein and of the Trimeric test. Immediately after the second dose, all the recipients had antibody titers above the upper threshold limit. However, the most important finding of our study is that antibody titers decline significantly more rapidly in subjects without previous COVID-19 infection, although all the subjects in both groups still maintained protective antibody titers towards SARS-CoV-2 infection and no subject developed COVID-19 infection within the 16-weeks follow-up period. These observations were confirmed using two different antibody tests, against the trimeric spike protein and the RBD of the spike protein. A prolonged period of follow-up and larger confirmatory studies are mandatory to better assess these results, in order to eventually choose the optimal timing for

| Table 1 Clinical and demographic characteristics of the two groups of subjects and kinetics of immune response. Group 1: subjects with previous COVID-19 infection; Group 2: patients without previous COVID-19 infection. |
|----------------------------------|---------------------------------|----------------|-----------|
|                                | Group 1 (n = 10) | Group 2 (n = 26) | p         |
| Males, n (%)                    | 3 (30)            | 7 (26.9)         | NS        |
| Mean age, years (95% CI)        | 46.1 (34–60)      | 47.5 (37–59)     | NS        |
| Number of co-morbidities ≥ 4 (%)| 8 (80)            | 19 (73.1)        | NS        |
| IgG against RBD protein, mean, AU/mL (95% CI) | > 800 (46.1–50.8) | 611 (47.4–52.9) | <0.0001 |
| Cut-off index > 13 AU/mL        | 711 (652–800)     | > 800 (375–503)  |           |
| Time 1                          | 240               |                 |           |
| Time 2                          | (112–375)         |                 |           |
| Time 3                          | Antibodies against RBD, mean, UI/mL (95% CI) | > 2500 | 1601 | <0.0001 |
| Cut-off index > 0.8 UI/mL       | > 2500 (978–2500) |                 |           |
| Time 1                          | 2407              | > 2500 (978–2500) |     |
| Time 2                          | (1789–2500)       | 503              |           |
| Time 3                          | (178–1195)        |                 |           |
| Antibodies against nucleocapside, mean, UI/mL (95% CI) | 49.2 | 0 | <0.0001 |
| Cut-off index > 1               | 51.3              |                 |           |
| Time 1                          | (45.8–56.1)       |                 |           |
| Time 2                          | 50.8              |                 |           |
| Time 3                          | (46.6–55.1)       |                 |           |
administration of the third dose of vaccine.

Declaration of Competing Interest

No conflict of interest is present for the authors

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