Aspects of Humoral Immunity after Vaccination with Bnt162b2: A Small Study

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ABSTRACT: Starting from December 2020, vaccination against COVID-19 became available in Romania. There are a lot of uncertainties regarding the kinetics of immunity and its persistence over time. This is a small prospective study developed between January-September 2021 in the Infectious Diseases Clinic from Craiova and comprising 61 subjects immunised with BNT162b2 (Comirnaty). We have found that after two doses of vaccine there is a strong humoral response, but the immunity lowers six months later. Subjects with a diagnosis of COVID-19, previously or in between the two doses, have had the most significant immunological response, but, also, the sharpest decline in antibody titer. The immune response seems to be the same, regardless the gender of the subjects. There are a variety of responses at the individual level, but overall vaccine effectiveness is 96.72% two weeks following immunisation and 88.52% after six months; however for those who have been in contact with the virus, they all had an antibody titer well above the laboratory limit.

KEYWORDS: SARS-CoV-2, COVID-19, BNT162b2.

Introduction

Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) is an emerging pathogen detected at the end of December 2019 and responsible for the Coronavirus Infectious Disease 2019 (COVID-19).

It rapidly swept the world and the World Health Organization (WHO) declared the pandemic on March 11, 2020.

Starting from December 2020 the first vaccine against SARS-CoV-2 became available.

For the European Union (EU) space there are five vaccines approved (as for January 1, 2022): BNT162b2 (Comirnaty, Pfizer BioNTech), mRNA 1273 (Moderna, Moderna Tx), ChAdOx-1 S (Vaxzevria, Astra Zeneca), JNJ-78436735 (Johnson&Johnson, Janssen Pharmaceuticals), and NVX-CoV2373 (Novaxvovid, Novavax Inc).

Under investigations there are two more vaccines produced by Sanofi GlaxoSmithKline and Valneva companies.

According to WHO, until April 8, 2022, a number of 11.250.782.214 doses of vaccine has been used around the world; 5.063.588.105 persons received at least one dose, while 4.437.098.395 are fully vaccinated [1].

United Arab Emirates has 99% of its population with at least a dose of vaccine received, while Nigeria has 4.9% [2].

In the EU/EEA countries (data from April 7, 2022) there were 1.204.679.528 vaccines administered, 75.2% of the population has received at least one dose, while 52.8% completed immunization with a booster/additional dose [3].

Up to 4.2 billion doses have been secured through COVID-19 Vaccine Global Access Facility (COVAX) [4].

In Romania vaccination started on December 27, 2020; as for March 13, 2022 a number of 16.737.321 vaccines have been used; 8.116.026 persons received one dose and 2.548.948 three doses [5].

There are a lot of aspects that need to be clarified, mainly regarding the kinetics of the early humoral response and its persistence over time.

Materials and Method

The present study is a prospective one and it was developed between January-September 2021 in the Infectious Diseases Clinic from Craiova.

Subjects were all recruited using electronic media. They agreed to participate to the present study and signed an informed consent; also they had to complete an electronic survey regarding personal data and medical history.

Copies of the original laboratory results were sent using electronic media.

The persons from the study group were immunised with two doses of BNT162b2 (21 days apart between doses) and the humoral immunity was assessed by determination of the level of antibodies directed against the S (spike) protein of the SARS-CoV-2; there was two...
determination, the first at 14 days from the last vaccine dose and the second assessment was implemented six months apart.

Antibody detection (against S1/S2 epitopes) was performed by the chemoluminescence (CLIA), using the Liaison® SARS-CoV-2 S1/S2 IgG kit (DiaSorin Inc); the lower limit for positivity was 50 arbitrary units per milliliter (au/ml).

In selected cases, to confirm previous asymptomatic infection, the anti nucleocapsid antibodies were determined.

A Microsoft Excel database was created to help the analysis. For the statistical analysis we have used the Chi² tests (two tails, with Yates correction) and Mann Whitney U test.

The limit for statistical significance is p<0.05.

The study has been approved by the Ethics Committee of the “Victor Babes” Hospital from Craiova.

**Results**

Initially, during January-March 2021, we have recruited 78 subjects.

The period for data collecting has ended in September 2021, when the last person sent his laboratory result regarding anti S (SARS-CoV-2) antibodies level.

In the end only 61 participants have had all the data required by the study protocol.

From the 61 subjects, 37 were females (60,65%) and 24 were males (39,35%).

The median age was 44 years, the youngest person had 26 years old, while the oldest had 79.

Most subjects were from urban area (60, 98.36%), and from Dolj county (52, 85.24%).

Most participants had university degree (53, 86.88%) and were part of the medical staff (40 physicians-65.57%-and 8 nurses-13%).

From all the recruited subjects, a number of 9 (14.75%) had a medical condition that may impair the vaccination result by lowering the immune response to the biological product administered to them (4 persons with autoimmune disorders, 2 with malignancies and one each with either immunosuppressive therapy, diabetes mellitus or cirrhosis).

A number of 14 persons (9 females and 4 males) have been diagnosed with COVID-19, previously or in between the two vaccine doses.

Eleven cases were mild forms of disease and other three were moderate forms; no severe cases of COVID-19 were reported prior or during vaccination process.

Following the immunisation, the median level of anti spike-protein (SARS-CoV-2) antibodies was 1490au/ml, with a minimum level of 16.76 and a maximum of 80000au/ml.

After six months the median level drops to 517.7au/ml, with a minimum of 6.25au/ml and a maximum of 11398.7au/ml.

The difference has statistical significance (p=0.001581, Mann Whitney U test)

Figure 1 demonstrates the level of specific antibodies for each individual subject.

**Figure 1. Antibodies level for each individual subject.**

Legend: blue diamond=subjects with no diagnosis of COVID-19; red dots=subjects with a previous or in between vaccination diagnosis of COVID-19; yellow triangle=subjects with no known previous diagnosis of COVID-19, but having anti SARS-CoV-2 nucleocapsid antibodies (retrospective diagnosis of COVID-19).
We have separated the subjects in two groups: group A (n=42), those with no previous contact with a case or diagnosis of COVID-19 and group B (n=19), those with a diagnosis of COVID-19, previously or in between the two doses of vaccine (n=14) or retrospective diagnosis of asymptomatic infection (n=5) proven by the presence of anti SARS-CoV-2 nucleocapsid antibodies.

Differences of the median levels of anti S (SARS-CoV-2) antibodies between the two groups (initially and six months later, respectively) are shown in Figure 2.

There is a 69.98% loss in the antibodies level for the group A and 92.94% for group B (p<0.0001, Chi² with Yates correction).

Initially there was two persons (one female and one male) who failed to reach an antibodies level above the threshold considered for immunisation (>50au/ml), but, six months apart, there was seven (4 females and 3 males) and three more (2 females and 1 male) with less than 5au/ml above the limit.

However, all subjects from the B group had an antibody level above the laboratory threshold. Table 1 demonstrates the differences regarding the level of immunisation in the two considered groups.

Table 1. Differences in immunisation level for the two groups considered.

| Anti S antibodies level (au/ml) | Initially | 6 months later |
|:-----------------------------|:---------|:-------------|
| >20000                      | No. Px. group A | No. Px. group B | No. Px. group A | No. Px. group B |
| 10000-19999                 | 0        | 1           | 0            | 1       |
| 1000-9999                   | 14       | 6           | 5            | 12      |
| 100-999                     | 22       | 0           | 17           | 6       |
| 50-99                       | 3        | 0           | 13           | 0       |
| <50                         | 2        | 0           | 7            | 0       |

Legend: No=number; Px=participant(s)

When considering the gender of the subjects, the present study has not identified a statistical difference of the immunisation level, initially and, respectively, 6 months later, in either group A or group B (see Table 2).

Table 2. Differences in immunisation level for the two groups and subjects’ gender

| Group | Gender | Antibodies level (au/ml) | Statistical significance |
|-------|--------|-------------------------|-------------------------|
|       |        | Initially | After 6 months | p (Chi²) |
| A     | Female | 420.95    | 154           | NS       |
|       | Male   | 436.5     | 153.65        |          |
| B     | Female | 19124.95  | 1365          | NS       |
|       | Male   | 26049.6   | 1605          |          |
For the subjects known with medical conditions that may impair the immunisation, the median level of anti S (SARS-CoV-2) antibodies was 334.7 au/ml initially and 102.2 after six months.

Two females from this subgroup failed to have an antibody level above the laboratory limit for positivity, six months after immunisation.

No cases of COVID-19 were noted previous of immunisation or in between the two vaccine doses given of these individuals.

Table 3 shows the changes in antibody titers (percentages from the initial value), after six months following immunization.

For all the subjects with an increases of the level, there was a positive test for the anti nucleocapsid antibodies.

Table 3. Changes in antibodies level after six months following immunisation as compared with the initial level.

| % antibodies level after 6 months as compared with initial level | No. Px. |
|---------------------------------------------------------------|---------|
| 4 times increases                                             | 1       |
| 3 times increases                                             | 1       |
| 2 times increases                                             | 1       |
| same level                                                    | 3       |
| 50-75%                                                       | 13      |
| 25-50%                                                       | 10      |
| 10-25%                                                       | 12      |
| 5-10%                                                        | 8       |
| <5%                                                          | 12      |

Legend: No=number; Px=participant(s)

Discussion

In Romania vaccination against SARS-CoV-2 started in December 2020.

The first vaccine available was BNT162b2 (and, also, the most purchased and used type of vaccine [6]), while the second available was mRNA1273 starting from March 2021.

The present study recruited subjects during January-March and all them were immunised with BNT162b2.

The initial strategy of immunisation in Romania was to offer the vaccine to the medical personnel and other essential staffs that is why most of the subjects are physicians or nurses.

There are studies showing that, shortly after the second dose of BNT162b2 vaccine, there is a robust immunological response in both humoral and cellular component, but the immunity protection has the tendency to lower over time [7,8,9].

A recent paper assessing the immunity against SARS-CoV-2 after COVID-19 vaccination and previous infection proved that there are differences regarding the length of seroprotection in non-infected versus previously infected subjects.

Those vaccinated with two doses of BNT162b2 had a high short-term protection that waned significantly after six months while the combination of previous infection and vaccination resulted in high level of protection for more than one year [10].

Our study shows a decline of the antibody level, with a 69.98% loss in the antibodies level for the group A and 92.94% for group B.

The exposure to SARS-CoV-2 leads to a higher antibody level, but the decline is also sharper.

However, considering the laboratory limit of 50 au/ml, the absolute antibody level is more than 27 times higher for group B and only 2.6 times for group A.

Differences in antibody titers between the two groups are to be expected because, for the group B, it is a secondary immune response and other study also highlighted it [11,12].

After the initial two doses of BNT162b2 COVID-19 vaccine, overall there was two persons who have failed to reach the laboratory threshold of 50 au/ml, so the effectiveness of vaccine (considering this point of view) was 96.72%, but the parameter lowers to 88.52% after six months (seven persons with an antibody level below the threshold limit).

However all of the subjects from the group B had an antibody level well above laboratory threshold, both after the initial immunisation and six months apart.

At least two recent studies (including a meta-analysis of 267 articles) found that the anti S antibody level are lower in men than in women following COVID-19 vaccination [8,13]; it seems that, due to biological sex differences, vaccines uptake and immunisation results against other pathogens (influenza virus, Clostridium tetani, Bordetella pertussis, varicella zoster virus or Streptococcus pneumoniae) are also lower for males than for females, mainly after 65 years of age [14].

A previous study of our infectious diseases department showed similar results after immunisation against hepatitis B virus in adults [15].

However, the present study has failed to find similar data for COVID-19 vaccination, but this is only a small research and there are necessary a larger number of participants to clarify the divergence.
At the individual level there are various responses after immunisation. At least in three cases there was a proven contact with the SARS-CoV-2 which heightened the protection level six months after the first dose of vaccine.

Conclusions
Vaccination with BNT162b2 lead to a strong specific humoral response two weeks after the second dose; the antibody titers trend lower six months after immunisation, but the overall humoral response remains above the laboratory limit in most cases.

Subjects with a diagnosis of COVID-19, previously or in between the two doses, have had the most significant immunological response.

Limitation of the Study
The present study is a small one regarding the number of participants. It was not able to evaluate the cellular immunity response following vaccination against SARS-CoV-2.

Also, the detection of viral anti nucleocapsid antibodies was performed only in selected cases.

Authors’ contribution
LIG developed the study design and performed the analysis of the data. LIG, FD, DL, SAC, IC, SI were responsible for data collection. LIG, FD, IC, SI wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Conflict of interests
None to declare.

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