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Comparison of Pfizer/BioNTech BNT162b2 COVID-19 (Comirnaty) Vaccination Effects in Aged Adults with Special Consideration for the Effectiveness of the Three-Color iSpot in Assessing Immune Response

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Abstract

Background: The roll-out of vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was certainly among the fastest in medical history. Vaccination campaigns around the world began a year after the outbreak in 2019. When Austria started vaccinating the population in 2020, we took the opportunity to collect data from the first sets of patients receiving the vaccine in our study region of East Tyrol. **Purpose:** Many studies have been conducted examining the immunogenicity of the new vaccines using classic serological test methods in combination with an IFN-γ ELISpot. Undeniable disadvantages of using IFN-γ to characterize the status of the cellular immunity are that 1) being an acute phase cytokine, IFN-γ loses signal strength in the long run and 2) IFN-γ does not provide information about the involvement of T helper 2 (Th2) cells in the immune process. This implies that it can affect false negative data about the cell-mediated immune status. **Method:** Therefore, in addition to a chemiluminescent immunoassay and the enzymatic IFN-γ ELISpot, this study included a fluorescent ELISpot assay using precoated human SARS-CoV-2-specific IFN-γ/IL-2/IL-5 ELISpot kits to show a more holistic overview on the involvement of T helper 1 (Th1) cells as signal senders of IL-2 and Th2 cells as senders of IL-5. **Results and Conclusion:** Our study confirms good immunogenicity of Pfizer/BioNTech BNT162b2 COVID-19 (Comirnaty) with strong Th1 and vanishingly small Th2 participation. The fluorescent three color iSpot can improve the diagnostic

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results' significance for the individual, especially when the infection has been longer in the past and the IFN-γ signal diminishes.

Keywords

Three-Color iSpot, Immune Response, Vaccination

1. Introduction

Since the first cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were reported in China in 2019, the international scientific community has been challenged to develop strategies to manage the impact of a global pandemic and to find ways to contain the spread of the disease and prevent severe cases of illness and death. Vaccine development began rapidly, with the goal of administering them quickly and immunizing a high percentage of the population.

The first vaccines approved and widely administered in Europe were BNT162b2 (Comirnaty, Pfizer-BioNTech) and mRNA-1273-SARS-CoV (Moderna) as mRNA vaccines and the Astra Zeneca/Oxford ChAdOx-SARS-CoV-2 as a vector-based vaccine. The efficacy of the developed vaccines has been intensively studied. In particular, their efficacy on the development of a humoral immune response in patients has been the subject of intensive investigation [1] [2] [3]. The cellular immune response, on the other hand, has been less extensively studied. This is due to the fact that several test systems are commercially available for the study of a patient's humoral immune response, whereas the study of the cellular immune response requires more work and training for the isolation and short-term cultivation of T cell lymphocytes. A common test for this is the enzymatic ELISpot. This test measures the release of a cytokine—usually interferon-γ (IFN-γ)—as an immune response of T cells to antigens administered as peptide pools.

IFN-γ promotes peptide-specific activation of CD4+ T cells. It is also a major product of T helper 1 (Th1) cells and directs the immune response toward Th1 phenotyping. The cytokine milieu present at the time of T cell receptor activation greatly influences the phenotype that a naive T cell adopts. At this time point, IFN-γ and IL-12 are the primary cytokines that control Th1 differentiation. IL-4, on the other hand, controls T helper 2 (Th2) cell differentiation. IFN-γ promotes IL-12 production in phagocytes and inhibits IL-4 secretion from Th2 populations, which may shift cell differentiation toward Th1 populations [4]. For a sound evaluation of the immunogenicity of a vaccine, consideration of IFN-γ alone is not sufficient. It is important to ensure that a vaccine not only triggers step 1 of the appropriate cascade but also initiates recruitment and proliferation of the desired type of T helper cells. It is also important to ensure that the proportion of Th2 helper cells in the recruited cells is not too high, since this could be the cause of an important complication of SARS-CoV-2—the cyto-

kine storm—with potentially fatal consequences [5] [6]. Only when this has been demonstrated, can the immunogenicity of the vaccine be reliably assessed.

Usually, a fluorescence-activated cell sorter (FACS) is used to reliably measure more than one cytokine in the specific T cell response, but this is not an option in routine diagnostics for economic reasons. In this study, a different assay method was chosen, which has potential for routine application. In addition to an enzymatic ELISpot, which measures one cytokine (IFN-γ), a three-color fluorescent immunospot assay (iSpot) was performed. This iSpot can simultaneously characterize 3 different cytokines (IFN-γ, interleukin-2 (IL-2), and interleukin-5 (IL-5) within the specific T-cell response without the use of a FACS.

IL-2 was first described in 1976 [7]. IL-2 promotes the expansion of antigen-specific clones and the proliferation of CD4+ and CD8+ T cells, but the most important role of IL-2 is to downregulate the immune response to prevent autoimmunity. IL-2 thus creates a negative feedback loop. Without new stimulation, IL-2 production is halted, and activated T cells die due to this deficiency [8]. IL-5 has been described as a growth factor for eosinophils and B cells. It is mainly produced by Th2 cells and activated eosinophils. IL-5 is also significantly involved in eosinophil activation, proliferation, differentiation, survival, and adhesion [9].

BNT162b2 is the most widely used COVID-19 vaccine in Austria and the EU to date [9]. BNT162b2 is a lipid nanoparticle-formulated, nucleoside-modified RNA vaccine that encodes a prefusion stabilized, membrane-anchored SARS-CoV-2 full-length spike protein, modified by two proline mutations to lock it in the prefusion conformation [1] [2] [10]. In the underlying technology, a transcript of interest encoding one or more immunogen(s) is delivered into the host cell cytoplasm for expression of the translated protein(s). The mRNA design offers several advantages over conventional systems, among others, mRNA vaccines are cell-free and scalable, the manufacturing process is timesaving, and it bears the possibility of expressing complex proteins that are difficult or impossible to generate with current expression systems [11] [12] [13].

In this study, the immune response of 175 volunteers, participating in the first vaccination campaign in the study region, to Pfizer/BioNTech's BNT162b2 COVID-19 vaccine was examined using diagnostic methods for humoral and cellular immune responses. As part of this investigation, the applicability of a three-color fluorescent immunospot assay (iSpot) to examine patients' multidimensional T-cell responses was tested. In the course of this, the aim was to find out whether the iSpot allows conclusions to be drawn about the different types of activated cells after vaccination.

2. Material and Methods

2.1. Characteristics of the Study Group

The study group consisted of 175 participants, of which slightly more were females than males (**Table 1**). The vast majority of participants were over 65 years

Table 1. Demographic data of study participants, n = 175.

Sex	**f**	**[%]**
Male	97	55.4
Female	78	44.6
Total	175	100
Age	**f**	**[%]**
≤65 years	19	10.9
>65 years	156	89.1
Total	175	100

of age. This is due to the fact that the study was conducted during the first vaccination roll out in February and March 2021, where primarily older individuals and high-risk patients received vaccination. The mean age was 82 years, with a standard deviation of 8.8 years. The study region was East Tyrol in Austria. Inclusion criteria for the study recruits were age of majority, no previous vaccinations against SARS-CoV-2 and consent to participate in the study.

For the study, blood was drawn from participants at three different time points. First, before receiving the first vaccine dose (T1); second, at the time of the second vaccination, *i.e.*, three to four weeks after the first blood draw (T2); and third, five to seven weeks after the second vaccination (T3). A graphical overview of the study design is given in **Figure 1**. After the patient gave explicit consent to participate in the study, 24 ml of lithium heparin and 8 ml of whole blood were drawn (Vacuette, Greiner bio-one, Austria). After centrifugation, the whole blood was used for humoral diagnostics and lithium heparin blood was used for the isolation of peripheral blood mononuclear cells (PBMCs).

2.2. Serological Diagnostics

Serological tests were performed using the LIAISON SARS-CoV-2 TrimericS IgG (DiaSorin S.p.A., Saluggia, Italy). The LIAISON SARS-CoV-2 TrimericS IgG is a Chemiluminescent Immunoassay (CLIA) which detects IgG antibodies reactive with the spike protein (S1/S2 domain). The assay was performed on the LIAISON XL Analyzer according to the manufacturer's instructions and gives the binding arbitrary units per mL (BAU/mL) according to the WHO International Standards for the Anti-SARS-CoV-2-immunoglobulin-binding activity (NIBSC 20-136).

2.3. SARS-CoV-2 Specific T Cell Response

2.3.1. Enzymatic IFN-γ ELISpot

The ELISpot assay was performed using precoated human SARS-CoV-2-specific IFN-γ ELISpot kits (AutoImmun Diagnostika, GmbH, Germany; Cat.no. ELSP 5500). PBMCs were separated from plasma and whole blood by gradient density (FicoLite-H, Linaris, Germany). After washing with phosphate-buffered saline (PBS), depleting erythrocytes (RBD-Lyse Buffer Life Technologies, 1xRBC Lysis

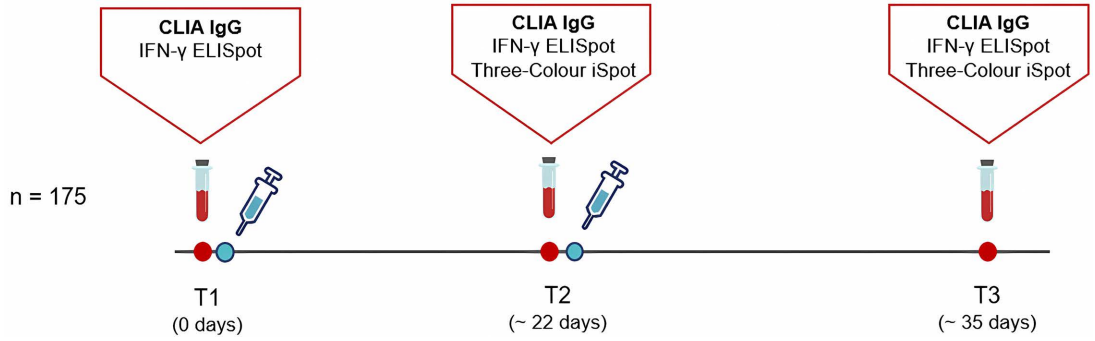

Figure 1. Study design. Blood samples were taken from recruited volunteers among the first vaccinees in the study area at time point 1 (T1) immediately before the first vaccination, time point 2 (T2), three weeks after T1 (mean = 22 days) and immediately before the second vaccination and at time point 3 (T3), on average 35 days after the second vaccination.

Buffer 200 mL; Invitrogen eBioscience, USA REF: 00-4333-57) and washing again with PBS, cells were counted and resuspended in x-vivo medium (x-vivo TM-10 Serum-free hematopoietic cell medium; BEBP02-055Q, Lonza, Switzerland). A total of 2×10^5 PBMCs was incubated in the precoated wells with x-vivo as a negative control, pokeweed mitogen (AutoImmun Diagnostika GmbH, Germany) as a positive control and 15 - 20 mer peptide pools for SARS-CoV-2 (AutoImmun Diagnostika GmbH, Germany). After incubation at 37˚C for 20 hours in a sterile and humidified atmosphere, plates were washed with washing buffer (AutoImmun Diagnostika GmbH, Germany) and stained with the kit-specific reagents according to the manufacturer's protocol. Plates were then washed several times under running water and dried overnight. Spot forming units (SFU)/100,000 cells were counted using an automated AID ELISpot reader system (AutoImmun Diagnostika GmbH, Germany).

The assessment criteria for the ELISpots were a minimum of 50 SFU in the positive control and a maximum of 10 SFU in the negative control. When those criteria were fulfilled, the stimulation index (SI) was calculated by dividing the mean SFU numbers in the antigen-specific wells with the mean SFU numbers of the negative control. A test was assessed negative with an SI < 2 according to previous determination of the cut-off by well-defined negative samples and clearly positive with an SI of at least 3.

2.3.2. Fluorescent IFN-γ/IL-2/IL-5 ELISpot

The fluorescent ELISpot was performed using an iSpot Assay-Kit (AutoImmun Diagnostika, GmbH, Germany; Cat.no. ELSP 6010). PBMCs were obtained as described above and mixed with anti-human CD 28 in a ratio of 1:1000. 2×10^5 PBMCs in 100 µl x-vivo medium were transferred to a 96-well plate precoated with anti-human IFN-γ, anti-human IL-2 and anti-human IL-5. PBMCs were incubated with 100 µl per well of either x-vivo medium as negative control, pokeweed mitogen as positive control or 15 - 20 mer peptide pools for SARS-CoV-2 (AutoImmun Diagnostika GmbH, Germany), for 24 hours at 37˚C. After incubation, plates were emptied and washed five times with washing buffer and

stained with the kit-specific reagents according to the manufacturer's protocol. Plates were dried for at least three hours before evaluated with the AID iSpot reader system. The assessment criteria for the iSpot were the same as for the ELISpot. Because the criteria for validity for each cytokine were calculated separately per patient, the number of valid tests per cytokine and test time point differs from the total number of study participants.

2.4. Statistics

Dichotomous data was tested with a chi-squared test or, in the case of a small group size ($n < 60$), with Fisher's exacta (SPSS V.15.0; SPSS Inc., Chicago, IL, USA). A two-sided significance level of $\alpha = 0.05$ was set. Results were considered statistically significant if $p < \alpha$. After testing for distribution (Kolmogorov-Smirnov-test), non-parametric continuous independent variables were compared using the Mann-Whitney-U test for each time point. Dependent non-parametric variables were compared with the Wilcoxon rank test (SPSS V.15.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. The Humoral Immune Response

At the time of the first blood drawing, before receiving the first vaccination dose, 10.6% of all patients tested positive for IgG antibodies against SARS-CoV-2. After the first vaccination dose, already 88% formed IgG antibodies, and after the second dose, the value increased to 99.4%. At this time, 5 to 7 weeks after the second vaccination dose, only one patient did not test positive for SARS-CoV-2 specific IgG antibodies (Table 2). The amount of IgG antibodies formed, indicated

Table 2. Evolution of IgG antibodies against SARS-CoV 2 spike protein 1/2 within the study group over different testing time points.

CLIA T1	n	positive	[%]	borderline	[%]	negative	[%]
Male	78	5	[6.4]	2	[2.6]	71	[91.0]
Female	92	13	[14.1]	3	[3.3]	76	[82.6]
Total	170	18	[10.6]	5	[2.9]	147	[86.5]
CLIA T2	**n**	**positive**	**[%]**	**borderline**	**[%]**	**negative**	**[%]**
Male	78	66	[84.6]	0	[0.0]	12	[15.4]
Female	97	88	[90.7]	1	[1.0]	8	[8.2]
Total	175	154	[88.0]	1	[0.6]	20	[11.4]
CLIA T3	**n**	**positive**	**[%]**	**borderline**	**[%]**	**negative**	**[%]**
Male	77	76	[98.7]	0	[0]	1	[1.3]
Female	94	94	[100.0]	0	[0]	0	[0.0]
Total	171	170	[99.4]	0	[0]	1	[0.6]

T1—time point 1, before vaccination; T2—time point 2, 3 - 4 weeks after the first vaccination dose; T3—time point 3, 5 - 7 weeks after the second vaccination dose. There were no significant differences between sexes in the change in specific IgG antibodies.

in BAU/ml, changed significantly from T1 to T2, and also from T2 to T3 (2). At T2 the median BAU/ml was 151.5 BAU/ml and increased to 1330 BAU/ml at T3. No significant differences were found between the sexes at any time point, as shown in Figure 2.

3.2. The Cellular Immune Response

INF-γ ELISpot

The specific cellular immune response of the patients was assessed with a SARS-CoV-2 specific IFN-γ ELISpot. The aggregated results are shown in Table 3. Even before the first dose, 13% of patients showed a positive result, and another 13% showed a borderline result with an SI between 2 and 3.

After the first dose, this value hardly changed, and only after the second dose (T3) did the number of positive results increase significantly (Figure 3). At this time, 60% of the patients showed a positive result, and another 8.6% showed a borderline result. No significant difference between sexes was detected ($p > 0.05$). In almost one third of the patients, IFN-γ could not be detected even after the second dose.

3.3. iSpot

The results of the SARS-CoV-2 specific three-color iSpot are shown in Figure 4. With respect to IFN-γ, 29.3% of patients were positive after the first vaccination dose (T2). Broken down by sex, 22.9% of male patients and 35% of female patients tested were positive at this time point. However, these differences were not found to be significant. At 5 - 7 weeks after the second vaccination dose (T3),

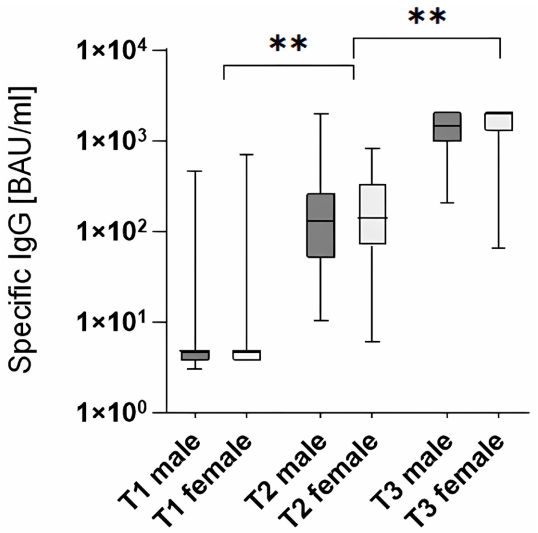

Figure 2. SARS-CoV-2 specific IgG antibodies reactive with spike protein 1/2 at three time points: T1—before vaccination; T2—after first vaccination dose (3 - 4 weeks after T1); T3—after second vaccination dose (5 - 7 weeks after T2). Wilcoxon rank test showed no significant differences in IgG responses between sexes. Wilcoxon rank test showed a significant increase in IgG antibodies, both from T1 to T2 (**, $p < 0.000$) and from T2 to T3 (**, $p < 0.000$); n (T1) = 170; n (T2) = 175; n (T3) = 171.

Table 3. Summary of the examination of the patients' T cell-mediated immunity against SARS-CoV-2 using ELISpot IFN-γ kits.

ELISpot T1	n	positive	[%]	borderline	[%]	negative	[%]
Male	54	5	[9.3]	9	[17]	40	[74]
Female	61	10	[16.4]	6	[9.8]	45	[73.8]
Total	115	15	[13]	15	[13]	85	[74]
ELISpot T2	**n**	**positive**	**[%]**	**borderline**	**[%[**	**negative**	**[%]**
Male	70	9	[1.9]	6	[8.6]	55	[78.6]
Female	75	16	[21.3]	6	[8.0]	53	[70.7]
Total	145	25	[17.2]	12	[8.3]	108	[74.5]
ELISpot T3	**n**	**positive**	**[%]**	**borderline**	**[%]**	**negative**	**[%]**
Male	29	16	[55.2]	2	[6.9]	11	[37.9]
Female	41	26	[63.4]	4	[9.8]	11	[26.8]
Total	70	42	[60]	6	[8.6]	22	[31.4]

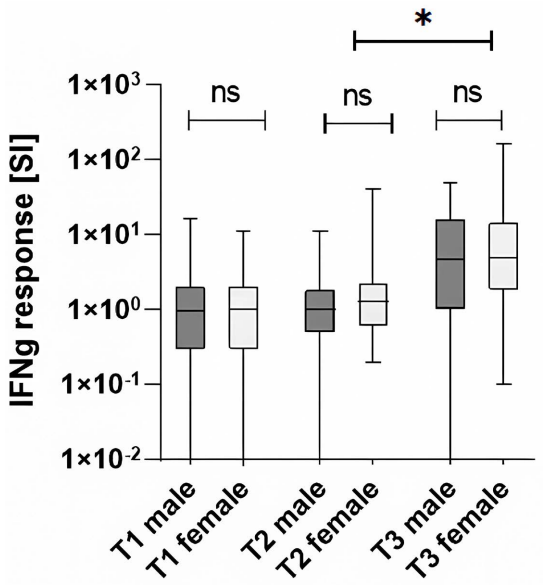

Figure 3. SARS-CoV-2 specific IFN-γ response of isolated T-cells against peptide pools at three time points: T1—before vaccination; T2—after first vaccination dose (3 - 4 weeks after T1); T3—after second vaccination dose (5 - 7 weeks after T2). Wilcoxon rank test showed no significant differences in IFN-γ responses between sexes. Wilcoxon rank test showed a significant increase in IFN-γ response from T2 to T3 (*, $p < 0.05$); n (T1) = 115; n (T2) = 145; n (T3) = 70.

only 17% tested positive for IFN-γ. Although both, the enzymatic ELISpot and the iSpot measured the release of IFN-γ, there were different results for this value in the two assays. Whereas in the ELISpot there was a significant increase in patients testing positive between T2 and T3, this significance could not be demonstrated in the iSpot. In both cases, the differences between male and female patients at both time points proved to be non-significant.

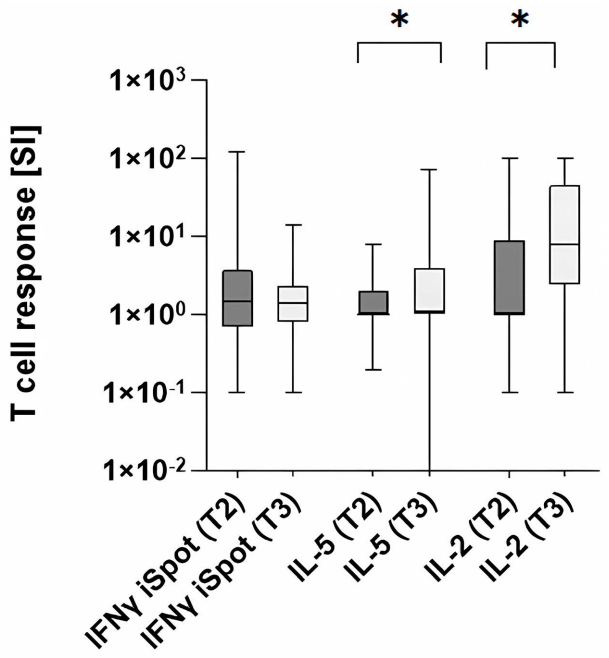

Figure 4. SARS-CoV-2 specific cytokine release of isolated T-cells against peptide pools at three time points: T1—before vaccination; T2—after first vaccination dose (3 - 4 weeks after T1); T3—after second vaccination dose (5 - 7 weeks after T2). Wilcoxon rank test showed a significant increase in IL-5 and IL-2 response from T2 to T3 (*, $p < 0.05$). IFN-γ: n (T2) = 75; n (T3) = 56; IL2: n (T2) = 100, n (T3) = 75; IL-5: n (T2) = 39, n (T3) = 84.

Regarding the second cytokine investigated, IL-2, 38.7% of all patients showed a positive result 3 - 4 weeks after the first vaccination (T2). 5 - 7 weeks after the second vaccination (T3), 70% of all tested patients showed a positive result in the SI for IL-2. Again, as at T2, the percentage of positive results was lower in male (65.1%) than in (73.7%) patients. However, this difference did not prove to be significant.

The third cytokine measured in the iSpot was IL-5. Three to four weeks after the first dose, 10.3% of all patients tested positive for IL-5. Of these, one was male and three were female. However, the difference between the sexes did not prove significant. Five to seven weeks after the second dose, 32.1% showed a positive SI for IL-5. Although again more females (36.2%) than males (27%) tested positive, this difference was not significant. Both the increase in positive results for IL-2, and IL-5 between T2 and T3 proved to be significant.

The fluorescent iSpot with its cytokine-specific coating provided the opportunity to also assess the ratio of IL-2 and IL-5 secreting T cells. To this end, we compared data from 26 patients whose iSpot assay showed results for IL-2 as well as for IL-5 at two time points (T2: 3 - 4 weeks after the first vaccine dose, T3: 5 - 7 weeks after the second vaccine dose). Of these, 46.2% were male and 53.8% were female. After the first vaccination dose, the average stimulation index (SI) for all 26 patients was 26.6 for IL-2 and 2.0 for IL-5, corresponding to a ratio of 13.3:1. After the second vaccination dose, the average SI for IL-2 de-

creased to 23.0, while the average SI for IL-5 increased to 2.6, changing the ratio to 8.7:1.

4. Discussion

This study compared three different test methods, all of which can be used to evaluate a patient's immune response to SARS-CoV-2. Vaccination with Pfizer/BioNTech BNT162b2 COVID-19 (Comirnaty) was found to have high immunogenicity in elderly patients. In the humoral immune response, seroconversion occurred in 90% of patients tested after the first dose, and in the T-cell-mediated immune response, as measured by the IFN-γ ELISpot, in 30%. There was a significant increase in specific IgG antibodies 3 - 4 weeks after the first vaccination. In contrast, the cell-mediated immune response tested with an enzymatic ELISpot assay showed a significant increase in positive test results only after 8 weeks, *i.e.*, only after the second dose.

The study of Tauzin *et al.* (2022), showed a similar development [14]. While no direct comparison was made between the cellular and humoral responses, it showed a clear rise in total immunoglobulins even after the first vaccination dose. For example, the anti-RBD total IgG jumped from a mean of 1.2 to a mean of 45.4 after the first vaccination (given in relative light units normalized to CR3022). Similarly, the anti-RBD IgG jumped from a mean of 3.5 to a mean of 56.7 after the first dose (also given in RLU normalized to CR3022). Both sets of numbers are taken from the study's naïve cohort.

Other studies show a different development—where the cellular immune response was shown to set on immediately [15] [16]. For instance, in their study of vaccinated subjects, Almendro-Vá Zquez *et al.* (2021) [16] show a significant increase in S1 IFN-γ even after the first dose. In this study, the measurement was expressed in spot-forming units (SFU)/106 peripheral blood mononuclear cells (PBMCs) and jumps from 0 to 100 to 200 after the first vaccination and subsequently to almost 1000 after the second.

Romero-Olmedo *et al.* (2022) [15] examined the differences in responses to vaccine between older adults and a younger control group. Compared with the data in the present study, in that study the jump in the median for IFN-γ after the first vaccination in older adults is much more pronounced.

The fact that not much information is available on the intricacies of both arms of the immune response shows that more research is needed in this area. This study provides a first insight into this area and allows more nuanced questions to be asked in further research.

According to this study, the CLIA still appears to be the test of choice for providing a clear and precise answer to a patient seeking a simple answer to their immune status. It provides a higher amplitude of results, making it easier for laboratory personnel to interpret and communicate to the client. At the outset of the study, one goal was to determine whether the IFN-γ-ELISpot could be used in conjunction with or even replace the CLIA test. It became clear quite quickly

that looking at only one cytokine raises more questions than it answers. Considering IFN-γ as a Th1 signaling agent immediately led to questions about the nature of the underlying Th2 response which a test measuring only one cytokine cannot answer. Working with the IFN-γ-ELISpots, a question arose: How does the ratio of Th1 to Th2 cells behave after vaccination? The enzymatic ELISpot proved to be helpful neither from the customer's point of view—due to the lower amplitude of the results according to our interpretation—nor on the research side, as it left questions unanswered. This prompted us to investigate the iSpot designed for simultaneous detection of multiple cytokines. It has been shown that a preponderance of Th2 helper cells may be responsible for the major complication of SARS-CoV-2—the cytokine storm—making the study of the ratio of Th1 to Th2 helper cells in the immune response of essential interest [6] [17]. This study shows no evidence that the vaccine studied may be in any way responsible for Th2 overhang. Even over time, Th1 cells (as measured by IFN-γ and IL-2 response) clearly predominated over Th2 cells (as measured by IL-5 response). For this objective, the fluorescent iSpot has proven to be the assay of choice. Its ability to test multiple cytokines simultaneously is critical for answering deeper questions about an individual's immune response.

One aim of the study was a detailed investigation of the immune development of the test groups over the period from before vaccination to after the second doses. The results clearly indicate the desired immune response, which keeps the intricate balance of Th1 and Th2 cells appropriate and safe. Because previous studies provided evidence that uncontrolled proliferation of Th2 cells may be responsible for the dangerous and potentially fatal side effect of cytokine storm, the concern about the effect of vaccination on this development seemed very valid. This study clearly shows that vaccination triggers the desired development of naïve T cells in favor of Th1. On the contrary, the development into Th2 cells seems to be rather suppressed, which seems beneficial since a higher proportion of Th2 cells has been shown to pose a risk [18].

The limitations of our study include, on the one hand, the high average age of the recruited patients, which does not correspond to the population average. On the other hand, the difference in the measured IFN-γ concentration between the enzymatic and fluorescence assays shows certain technical difficulties we had in performing the iSpot. The overall level of signal in the iSpot was lower than in the ELISpot, but the data within each assay were comparable and reliable, and still gave us the response we obtained in the involvement of Th1 and Th2 cells that we were primarily aiming for.

To conclude, the study showed high immunogenicity of Pfizer/BioNTech BNT162b2 COVID-19 (Comirnaty) in the elderly. The humoral immune response occurred promptly and in nearly 100% of patients, while the cellular immune response, as measured by IFN-γ ELISpot, was slightly later in onset and was detectable in only about two-thirds of patients. This study was one of the few to consider the crucial balance between Th1 and Th2 cells when examining

immunogenicity. Although the handling of the iSpot requires more laboratory experience, the simultaneous detection of IFN-γ, IL-2, and IL-5 in the fluorescent iSpot is a suitable tool for an initial assessment of the effect of new vaccines.

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Conflicts of Interest

The authors have no conflict of interest to declare.

Ethics Statement

All participants were informed about the study in the course of the medical consultation before vaccination, provided written informed consent, and the study was performed according to the principles of the declaration of Helsinki 2013. The Ethics Committee of the Medical University of Innsbruck approved the use of vaccinees (ECS1166/2020) for scientific purposes.

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