MULTIDISCIPLINARY COLLABORATION IN RESEARCH INTO THE DETERMINATION OF IGG AND IGM ANTIBODY LEVELS FOR COVID 19

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Summary

 COVID-19 has affected the whole world for the last 2 years. Residents were exposed to an unprecedented level of risk of SARS-CoV-2, which is still present around all of us, but through the   introduction of vaccination it was possible to partially bring it under Control.   As part of the study we designed, it was found that vaccination in combination with COVID-19 protects against severe course and possible death. The study involved a multidisciplinary team consisting of a general nurse, a statistician and an expert in immunology. The levels of specific IgG and IgM antibodies, which are a clear indicator of the state of the body's immune system, were monitored. The selected volunteers were   vaccinated by Pfizer BioNTech between December 2020 and November 2021.

Summary

COVID-19 has affected the entire globe in the last 2 years. Residents have been exposed to an unprecedented level of risk of the SARS-CoV-2 virus, which is still present around all of us, but with the introduction of vaccination, it has been partially brought under control. In a study we designed, vaccination in combination with past COVID-19 disease was found to protect against serious progression and possible death. A multisdisciplinary team of nurses, statisticians and immunologists participated in the study. The levels of specific IgG and IgM antibodies were monitored, which are a clear indicator of the state of the body's immune system. The selected volunteers were vaccinated by Pfizer BioNTech in the period December 2020 - November 2021.

Key words: SARS-CoV-2, COVID-19, Pfizer, vaccination, antibodies IgG and IgM.

Introduction

Infectious diseases are a major global healthcare problem. The current pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causing Coronavirus Disease 2019 (COVID-19) appeared in December 2019 in Wuhan, China and quickly has become a global pandemic. SARS-CoV-2 infection has a huge morbidity and mortality because it causes serious general illness with particular respiratory involvement . SARS-CoV-2 infection in susceptible patients leads to dysregulated immune responses triggered by the release of damage-associated molecular patterns (DAMP) that activate various

signaling pathways leading to dysregulation of immune responses, leading to the   secretion of a wide range of pro-inflammatory cytokines called cytokines. - so-called. cytokine storm correlating with severity of COVID-19 (Huang et al., 2020). So far (May 13, 2022), 517 million people have been infected, resulting   in approximately over 6 million deaths (World Health Organization (WHO), https:// www.who.int/data). The current pandemic is affecting not only the health sector, but also the economy. In particular, the Czech Republic was one of the most affected countries where the health sector reached the limit of its capacity. In 2020, new vaccines were introduced   to prevent the spread of SARS-CoV-2 and reduce the severity of clinical signs of possible development of COVID-19.   Unfortunately, new SARS-CoV-2 mutants are still emerging, which makes vaccination less effective.

The important thing is that it is a matter of time before a new mutant appears. It may   be necessary to adjust the current vaccine every year according to the future epidemiological situation, as is the case with the flu vaccine.   A better understanding of the mechanism of action of the SARS-CoV-2 vaccine   on the   host's immune system, together with knowledge of   the long-term persistence of antibodies and cellular immunity induced by vaccination, is therefore highly desirable   To consider vaccination schedules in healthy isdince and sick people. Recent studies report induction of specific antibodies against SARS-CoV-2, but the long-term persistence of these antibodies is unknown.   In addition, different methodological approaches to testing for the presence of specific SARS-CoV-2 antibodies make it difficult to draw conclusions. Despite the WHO's efforts to unify serological methods for the detection

of   SARS-CoV-2-specific antibodies   (Kristiansen et al, 2021), various research laboratories and diagnostic laboratories use Different

methods indicating the values of SARS-CoV-2 specific antibodies in different units or concentrations. Ranges based on the reagents used. In addition to the humoral immune responses represented by antibodies, cellular immunity plays a key role in the course of infection, limiting the number of cells infected with the virus and preventing further spread virus in the body. Some reports have already indicated induction of cellular immunity after vaccination with an mRNA vaccine, but again it remains to characterize the longevity of cellular immune responses.

Goals, hypothesis, methods

The main objective of the project was to characterize the longevity of both humoral and cellular immune responses after vaccination against SARS-CoV-2 in healthy individuals.

1) To test cellular immunity, the production of IFN-gamma whole blood of vaccinated individuals was stimulated by a spike protein that was determined by ELISA.

2) To identify the adaptive immune cell population producing IFN-gamma, IFN-gamma-positive CD4+ and CD8+ T cells were determined in whole blood after restimulation with spike protein by flow-through cytometry.

3) The effect of the spike protein on gene expression of cytokines (e.g. IFN-gamma, IL-2, TNF-alpha, IL-6, ILlbeta) in PBMCs isolated from peripheral blood was determined by real-time PCR.

4) The ability of the spike protein to induce proliferation of CD4+ and CD8+ T cells after restimulation with the PBMC spike protein was determined by dilution

carboxyfluorescein succinimidyl ester (CFSE) by flow cytometry.

5) The potential of PBMCs in vaccinated individuals to specifically respond to spike protein stimulation by IFN-gamma secretion   was investigated by the Enzyme Linked Immuno Spot Assay (ELISPOT). The use of various methodological approaches to monitor the cellular immunity capacity of vaccinated patients to produce IFN-gamma will lead to the design of the most appropriate methods documenting cellular immunity after mRNA vaccination   Vaccine. The humoral immunity represented by the production of SARS-CoV-2 specific antibodies was determined using ELISA.

Methodology

First, it was necessary to submit a   proposal with all   relevant documentation, including the methodology of the study, to the Ethics Committee, which voted positively that the study could be started.

As part of the   cooperation with the   General University Hospital in Prague, potential volunteers were addressed in the form of advertising on the hospital's website and 1. medical schools. These were employees of the General University Hospital and 1. medical schools. An online questionnaire was created,   which contained essential and relevant information about the health status of the potential volunteer (age, height, weight, male/female, date of vaccination, date of recovery from COVID   19,   concomitant diseases,   chronic medication, workplace). According to clearly defined criteria, candidates for the study were selected. A total of 173 volunteers. The aim was to recruit volunteers who are between the ages of 18. up to 80. year of life, with a previous

illness, after vaccination appropriate

Comirnaty (Pfizer BioNTech) vaccine with and without chronic medication. It was necessary to monitor the possibility of influences that may act on the human immune system.

All selected volunteers were asked when and how peripheral venous blood samples needed to determine their own IgG and IgM antibodies would take place. These two types of antibodies were selected for a reason that clearly shows the immune response, whether to vaccination or to the actual reaction after COVID-19.

All of them were informed about the course of the study and at the same time signed Informed Consents, concerning not only the study, but the possible sending of results from the given samples.

Samples were taken 2-4 months after vaccination or illness. A general nurse with many years of experience in intensive care, but also with experience with " covid" patients, planned visits of volunteers to the collection point, where she also collected them herself. The laboratory examination was performed by an immunologist in the laboratory of Immunology of the Institute of Immunology and Microbiology 1. Faculty of Medicine, Charles University. All statistical processing was carried out by a statistician from the Institute of BiofYzika and Informatics 1. Faculty of Medicine, Charles University.

Outcomes

From our pilot study involving vaccinated healthy individuals, we were able to detect strong antibody production persisting for 19 weeks after the second dose of the PfizerBioNTech mRNA vaccine, (Image 1). The IgM antibody titer is surprisingly relatively stable over the study period.

The concentration of antibodies is exactly the same with a similar interindividual

variability from 4. week after vaccination until 19. week after vaccination, (Figure 2).

In the case of   SARS-CoV-2 IgG-specific,   levels of specific IgG antibodies are fairly stable at the upper limit of detection within 10 weeks, followed by a slow gradual decrease up to 19.   week after vaccination, (Figure 3). These pilot data are influenced by the varying number of individuals attending regular time intervals of blood collections.   Expansion of the monitored cohort would be highly desirable. However, the ability of the PfizerBioNech mRNA vaccine to induce specific IgM and IgG is self-evident.
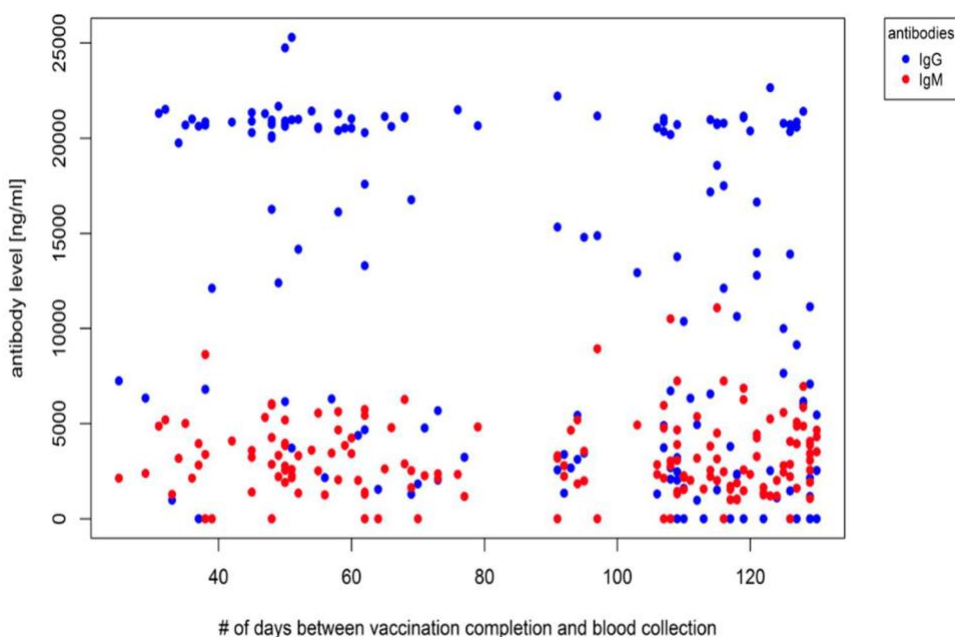

Figure 1.   Dynamics of SARS-CoV-2 specific IgM and IgG antibodies in vaccinated healthy individuals detected by commercially available ELISA kit from Matriks Biotek.
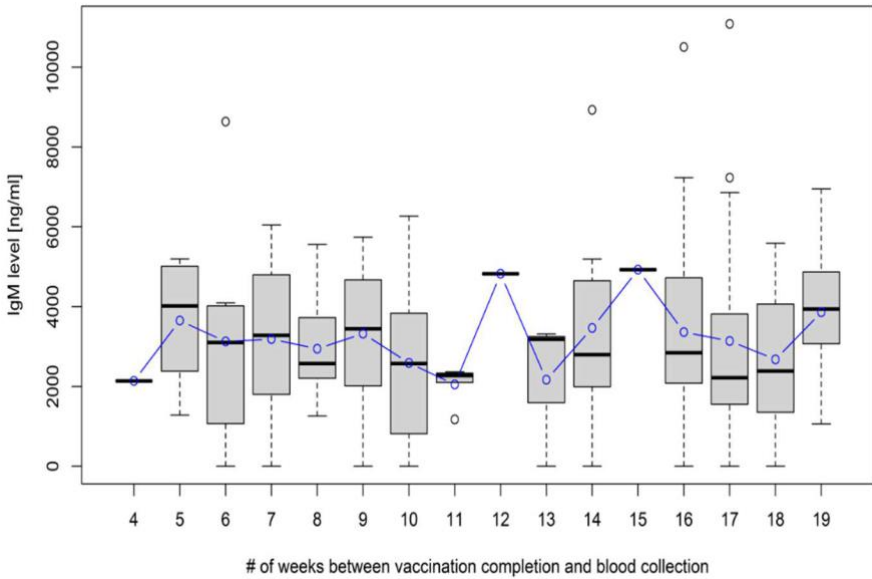
Figure 2. Detection of SARS-CoV-2 specific IgM in vaccinated healthy individuals in peripheral blood serum using ELISA. A total of 173 individuals were enrolled.
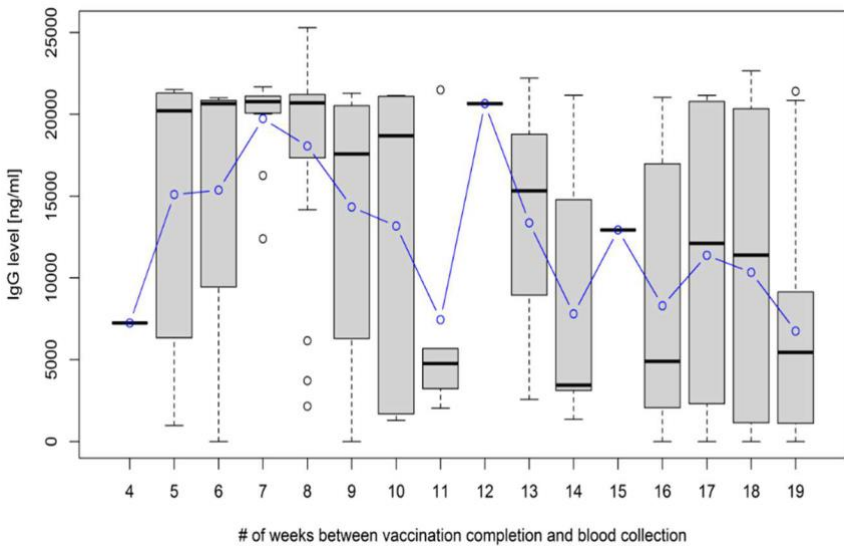
Figure 3. Detection of SARS-CoV-2 specific IgG in vaccinated healthy individuals in peripheral blood serum using ELISA. A total of 173 individuals were enrolled.

Conclusion

We concluded the whole study as very important and successful. It has been proven that vaccination alone protects a person from Covid -19 to some extent. It should be emphasized that it is very clear that people should get vaccinated, but also that the specialists in the development centers for vaccine development are constantly developing new, more responsive ones vaccines for the various types of mutants that are present and very likely will occur. Experiencing COVID-19 alone is not sufficient protection against reinfection. The combination of vaccination and undergoing the disease proved to be very effective according to the results of antibodies, because their levels were many times to immeasurable as they were high.

It turned out that the cooperation of the teams is extremely important, without it it would not be possible not only to conduct studies, but also to come up with new facts regarding diseases, treatment options, etc.

I would like to thank my team with whom we worked on the study, but also, and above all, our volunteers, because without them nothing could ever have happened.

Resources

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