**Doctoral (Ph.D.) thesis**

# Correlation between adverse reactions followed BNT162b2 vaccination against SARS-CoV-2 and the anti-spike protein antibody levels through a 6-month-long follow-up

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## 1. ABBREVIATIONS

ACE2: angiotensin-converting enzyme 2

AR: adverse reaction

CDC: Centers for Disease Control and Prevention

COVID-19: coronavirus disease 2019

2019-nCoV: novel coronavirus

DNA: deoxyribonucleic acid

EUA: Emergency Use Authorization

FDA: Food and Drug Administration

FKO: furin-cleavage site knocked out

HCoV-19 or hCoV-19: human coronavirus-19

HCWs: health care workers

IPV: inactivated polio vaccine

MERS-CoV: Middle Eastern respiratory syndrome

mRNA: messenger RNA

NIH: National Institutes of Health

NTD: N-terminal domain

OMV: outer membrane vesicle

OPV: oral polio vaccine

2P: diproline mutation

PRP: polyribosytol phosphate

RBD: receptor-binding domain

SARS-CoV-2: severe acute respiratory syndrome-coronavirus-2

SARSr-CoV: severe acute respiratory syndrome–related coronavirus

S-IgG: anti-spike immunoglobulin

S protein: spike protein

S-RBD: spike-receptor binding domain

VLP: virus-like particle

WHO: World Health Organization

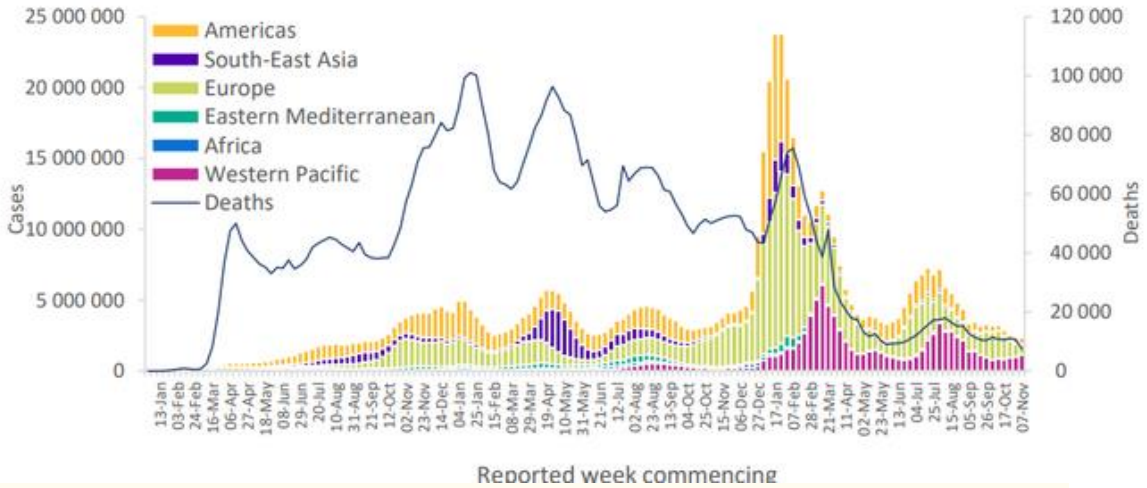
WT: wild type

## 2. INTRODUCTION

### 2.1 Definition and significance of SARS-CoV-2 and COVID-19

According to the WHO database[1], since 2019, there have been more than 600 million confirmed SARS-CoV-2 infections and over 6 million deaths all over the world (**Figure 1.**) , while in Hungary, by the first half of 2021, nearly 30,000 deaths related to SARS-CoV-2 infection were registered[2].

**Figure 1. COVID-19 cases reported weekly by WHO Region, and global deaths, as of 13 November 2022****




SOURCE: WORLD HEALTH ORGANIZATION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[3] is a strain of coronavirus which caused the respiratory illness responsible for the COVID-19 (coronavirus disease 2019) pandemic[4,5]. Its former names for 2019 novel coronavirus (2019-nCoV) also include the name 2019 human coronavirus (HCoV-19 or hCoV-19)[6–10].

The name was derived from June Almeida and David Tyrrell as the first observers of human coronaviruses [11,12] and it is inspired by the Latin word corona (meaning "crown" or "wreath"), which in turn comes from the Greek word κορώνη korṓnē, "garland, wreath"[13,14]. The word was first presented in 1968 by virologists in the journal *Nature* to describe a new family of viruses[15]. The infectious form of the virus is the virion with the typical electron microscopic image of reminiscent of the corona or halo of the sun at the

edge of large, spherical surface projections[11,12] (**Figure 3**.). The morphology is due to the proteins on the surface of the virus, the spiky peplomers[16]. The International Committee on the Taxonomy of Viruses approved the official name of "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2)[17,18]. It is a severe acute respiratory syndrome–related coronavirus (SARSr-CoV) species, which is related to the SARS-CoV-1 that caused the 2002–2004 SARS epidemic[3],[19]. Both belong to the family Coronaviridae in order Nidovirales[20].

This family contains two subfamilies, Coronavirinae and Torovirinae. Four genera of the latter are known: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. Wild birds are common reservoirs of Delta- and Gammacoronaviruses. One of the most dangerous characteristics of coronaviruses is their capability to break through the species barrier. In this regard, many reports have shown transmission to wild birds and some mammal species, including marine mammals[18,21,22].

Previously, lineages A, B, C and D of the genus Betacoronavirus existed. Now, these lineages have been categorized as subgenus of *Betacoronavirus*—as *Embecovirus* (lineage A), *Sarbecovirus* (lineage B), *Merbecovirus* (lineage C), and *Nobecovirus* (lineage D) (**Figure 2**.). SARS-CoV-2 belongs to a new evolutionary branch within the genus *Betacoronavirus* and subgenus *Sarbecovirus* and has 79% genetic similarity with SARS-CoV and nearly 50% similarity with MERS-CoV[23].

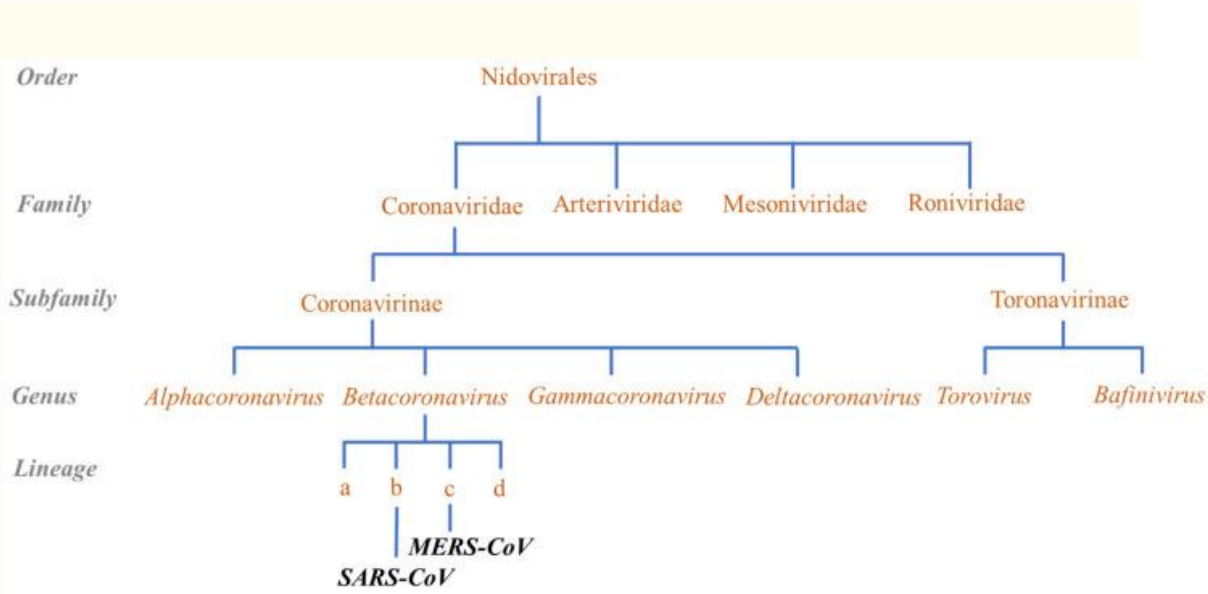



**Figure 2**. Classification scheme of coronaviruses. SOURCE: REFERENCES

Cases of human coronavirus infection date back to the 1960s. It was thought to be a cold and was only later recognized as the cause of respiratory diseases[5]. In 2002, the world perceived the first lethal coronavirus-induced disease which was named as severe acute respiratory syndrome (SARS-CoV)[5].

The first known case of atypical pneumonia associated with SARS-CoV was reported in Foshan, China, in November 2002[24]. Since then, the outbreak of the disease started to spread quickly across the globe, which spurred the World Health Organization (WHO) to declare the ailment "a worldwide health threat". Following the emergence of the disease in mainland China, within a few months, >300 cases were reported; the majority of them were healthcare workers. Consequently, the travelling of infected individuals further spread the disease to other countries as well[25].

A decade after the occurrence of SARS-CoV, in June 2012, a case of acute pneumonia and kidney disease was reported in Saudi Arabia. The death was linked to another new form of the coronavirus, MERS-CoV (Middle East respiratory syndrome coronavirus)[5]. It was isolated from the sputum of the patient[26]. Healthcare professionals and researchers were relatively more prepared when the MERS-CoV pandemic emerged due to advances in molecular diagnostic tools, such as the availability of advanced sequencing tools and next-generation sequencing technologies that facilitated full-length genome sequencing[5].

In 2019, the world was hit by another strain of the coronavirus, SARS-CoV-2. The mortality rate of SARS-CoV-2 is much lower, which increases sharply with age. However, it has a much higher transmission rate than SARS-CoV or MERS-CoV[27,28]. *Severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), was first announced in Wuhan, Hubei, China, in late December 2019[29]. In the early stages of the outbreak, the majority of patients reported contact with the Huanan seafood market in southern China, a live animal or "wet" market, suggesting a zoonotic origin of the virus[30–33]. A zoonotic origin has still not been confirmed yet. Some studies suggest that SARS-CoV-2 is a recombinant virus between a bat coronavirus and a coronavirus of unknown origin, with pangolins and minks being suggested as possible intermediate hosts. However, there is currently no evidence of a possible route from the bat reservoir to humans via one or more intermediary animal species[33,34].

Some further research is required to determine the origin of SARS-CoV-2. Since then, the epidemic has escalated and spread rapidly throughout the world, the WHO first declared it a public health emergency of international concern on January 30, 2020, and then officially declared it a pandemic on March 11, 2020[35]. The virus is previously unknown betacoronavirus that was discovered in bronchoalveolar lavage samples taken from clusters of patients who presented with pneumonia of unknown cause in Wuhan City, China, in December 2019[36]. It is the seventh known human infectious coronavirus after HCoV-229E, HCoV-OC43, HCoV NL63, HKU1, MERS-CoV and the original SARS-CoV[5,37].

The first four typically cause non-lethal mild upper respiratory diseases, while the last two, as well as SARS-CoV-2 (**Figure 3**.), can cause severe lethal respiratory illnesses[37].

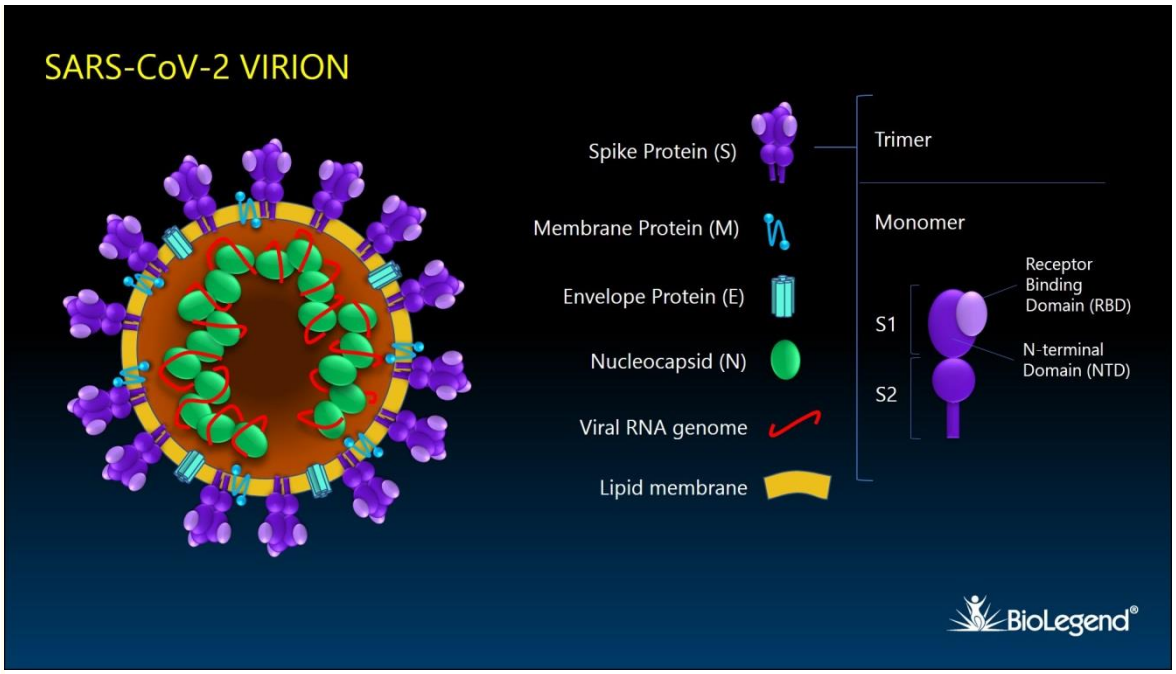



**Figure 3.** Schematic illustration of the SARS-CoV-2 virus particle and its structural proteins. SARS-CoV-2 has 4 major structural proteins: spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N). SOURCE: REFERENCES

Several variants of SARS-CoV-2 have been identified. The greater part of SARS-CoV-2 variants are now extinct, with the current circulating variant of concern being the Omicron variant (and its subvariants). The versions have replaced each other since the beginning of the pandemic, the most successful versions being Alpha, Delta and Omicron. Alpha and Delta were released in late 2020 and Omicron in late 2021[35,38].

Adjacent to the devastating health effects, the novel coronavirus disease 2019 (COVID-19) also had damaging economic and social consequences. Controlling the pandemic required joint and rapid action by science and pharmaceutical companies leading to the development of supposingly the most important vaccines in human medicine: the mRNA-based vaccines against SARS-CoV-2[39].

## 2.2. From the variolation to the mRNA vaccines

Diseases similar to the coronavirus were most recently caused by the Spanish flu, which claimed almost 50 million lives worldwide in 1918-1919. The H1N1 influenza virus that causes the Spanish flu and the SARS-CoV2 that causes COVID-19 to belong to different virus families and have different structures, genomic organization, and pathogenicity[40]. Nevertheless, the trajectory of the current COVID-19 outbreak shows a similar picture of the Spanish flu epidemic. In order to curb the spread of COVID-19 and prevent the situation that developed a century ago, it is essential to investigate and correlate these epidemics based on their origin, epidemiology, and clinical scenario[40].

Although advances in the prevention, control, and treatment of infectious diseases have improved our ability to respond to such outbreaks, globalization processes related to human behavior, demography, and mobility have increased the threat of pandemics and accelerated the spread of global diseases. Preparedness planning must continue to evolve to keep up with this increased risk[41].

The worldwide outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has highlighted the need for two major clinical interventions, such as the development of effective vaccines and acute therapeutic options for the moderate and severe stages of "coronavirus disease 2019" (COVID-19). They emphasized that effective vaccines, if successfully developed, would become the most effective strategy in the global fight against the COVID-19 pandemic[42].

The history of vaccination dating back to Edward Jenner's discovery in 1796, when the English physician Edward Jenner noticed signs of protection against smallpox in milkmaids with cowpox (**Figure 4**.)[43]. One method of protection was named after the virus that causes smallpox (variola virus). During **variolation**, people who had not previously suffered from smallpox had material from smallpox sores scratched into their arms or inhaled through their noses. After variolation, people usually noticed the symptoms associated with smallpox, however, fewer people died from variolation than if they had contracted smallpox naturally[43].

**Figure 4.** Vaccination: Dr Jenner Performing His First Vaccination, 1796, Ernest Board (1877-1934), Wellcome Collection, Art UK. SOURCE: REFERENCES

Jenner sensed a solution to prevent smallpox from exposure to cowpox. The name of the procedure comes from the Latin word for cow (vacca). Today, people can be vaccinated against many infectious diseases, but smallpox is not one of them. The popular global vaccination program freed the world from this life-threatening disease at the end of the 18th century[43,44].

Since then, a great number of vaccines have been developed to protect against infections and there have been continuous efforts to develop safer vaccine techniques (**Figure 5.,Table 1.**).

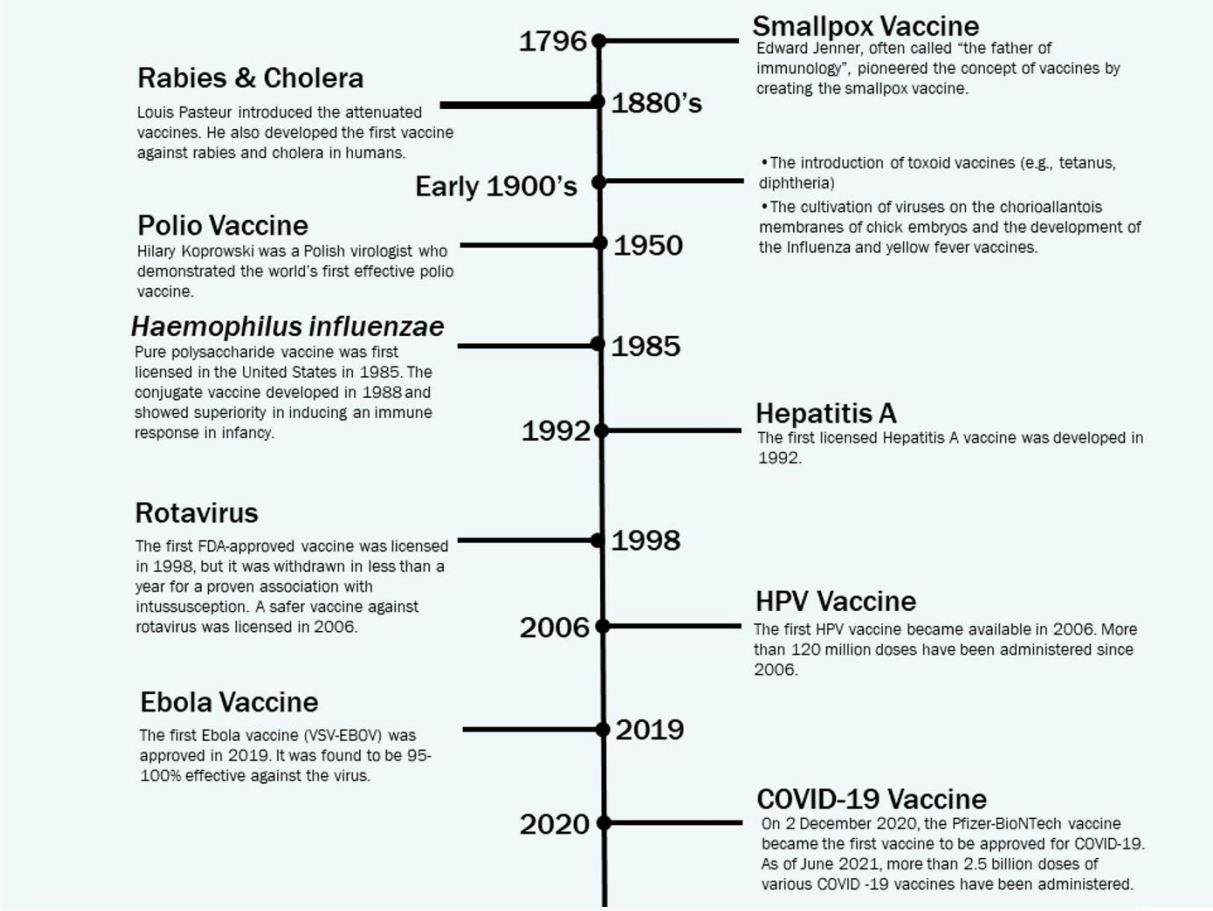



**Figure 5.** Vaccine history timeline. COVID-19, coronavirus disease 2019; FDA, U.S. Food and Drug Administration; HPV, human papillomavirus; SOURCE: REFERENCES

The French biochemist Louis Pasteur is credited with developing a **laboratory-developed vaccine** (the first in the world) against chicken cholera and later the first rabies vaccine using **attenuated or weakened** bacteria[45]. Pasteur was a pioneer in the development and success of the live, weakened vaccines[45,46] (**Table 1**.).

In the 19th century the world witnessed the evolution of germ theory through the discovery of numerous **microorganisms** by Koch[46]. The introduction of **attenuated toxins (toxoids)** paved the way for the development of the first generation of vaccines against diphtheria and tetanus. Significant advances in **laboratory techniques** made it possible to culture viruses enabled the development of vaccines against influenza and yellow fever in the 20th century[46].

The influenza (flu) was initially thought to be an infection caused by bacteria but was later identified as a viral infection[47,48]. The 1918 influenza pandemic, known as the

"Spanish flu", resulted in significant destruction, infecting millions of people and causing worldwide deaths[46,47]. Different strains of influenza viruses were discovered, which led to the introduction of a monovalent inactivated vaccine in 1938, and later the first bivalent vaccine[47,49]. Encapsulated and non-encapsulated forms of Haemophilus influenzae were targeted in the 1980s with **pure polysaccharide vaccines** and later with the development of **conjugate vaccines**, which proved to be more effective[46]. Antigenic shifts and drifts, as well as constant changes in viral composition, have required the establishment of a surveillance system for influenza strains coordinated by the World Health Organization (WHO), and have resulted in the development of vaccines that target specific circulating strains each year[47]. Advancements in **cell culture techniques**, **recombinant DNA**, and **whole genome sequencing** made it possible for scientists to be able to rapidly respond to the evolving Influenza pandemics like the "Avian flu" in 1997 and the "Swine flu" in 2009, by creating safe and effective vaccines within a few months' time[49].

The advance of cell culture led to the creation of the **polio vaccine**, and this marked the start of the golden age of vaccines[50]. By the mid-20th century, the poliovirus vaccine had been produced, and both the inactivated (IPV) and oral versions (OPV) proved successful in dramatically reducing polio cases (**Table 1**.)[50,51]. Hungary and Czechoslovakia were the first countries in the world to eradicate polio, after they were the first to use Sabin drops[52].

In 1971 the measles vaccine is combined with recently advanced vaccines against mumps and rubella into a single vaccination (MMR) by Dr Maurice Hilleman, resulted in an **attenuated measles vaccine**, which is still effective today[53]. His work resulted in the current vaccines used to prevent measles, mumps, hepatitis, chickenpox, meningitis, and pneumonia, saving millions of lives around the world[54].

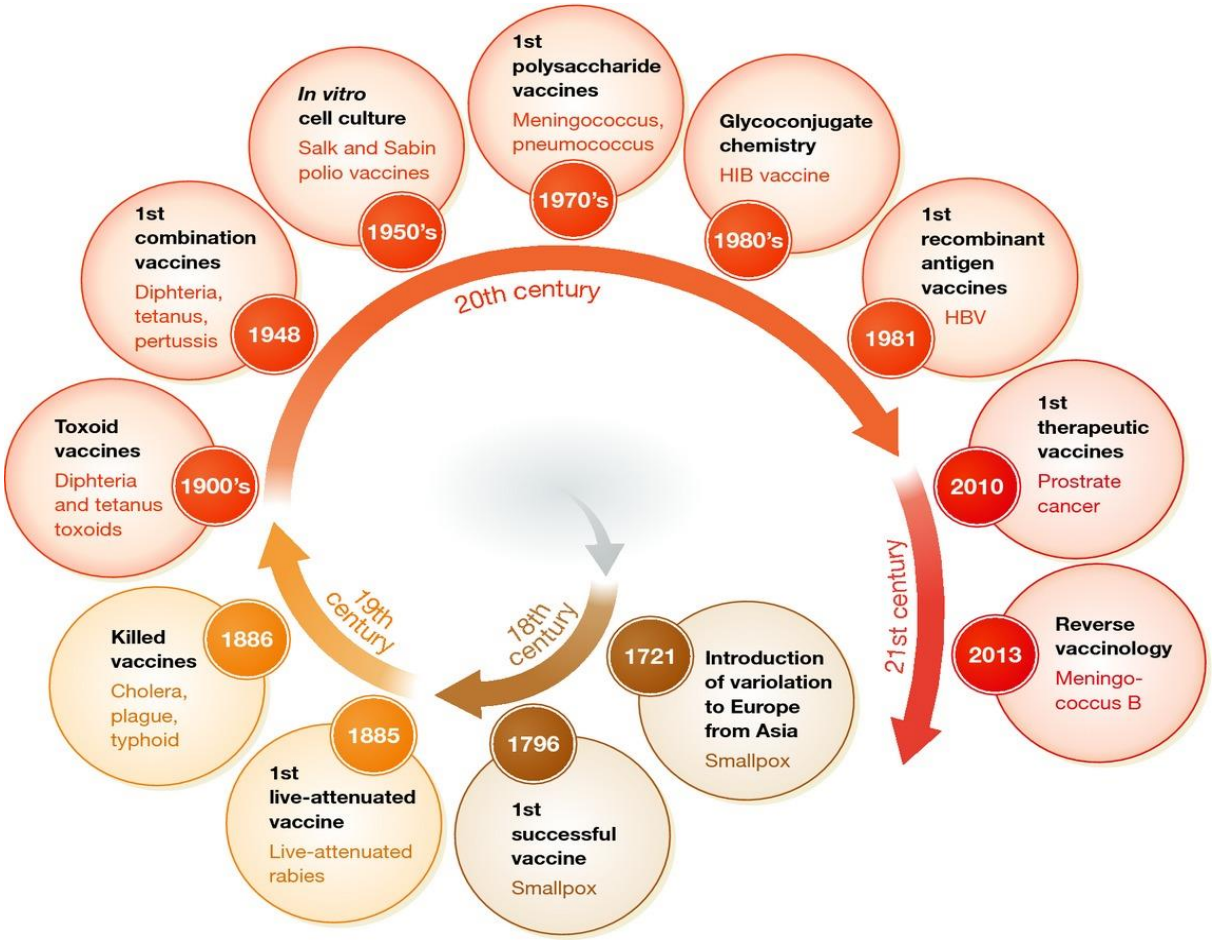



**Figure 6.** Major milestones in the historical path of the development of vaccinology and vaccine design. SOURCE: REFERENCES

Advances in science have always been the main driving force behind the development of effective vaccines (**Figure 6**.)[55]. **Table 1**.represents a list of different types of vaccine against pathogens. The first golden age of vaccines was born with the germ theory, with vaccines based on live, weakened or inactivated (killed) pathogens and inactivated toxins (**toxoids** (**Table 1**.)). These vaccines gave protection against rabies, diphtheria, pertussis, tetanus, and tuberculosis. The second golden age of vaccines resulted from innovations in cell culture technologies in the second half of the 20th century[55]. The 'cell culture revolution' made possible to gain effective inactivated vaccines to prevent polio (IPV) and **live-attenuated vaccines** against polio (OPV), mumps, rubella, measles (MMR) (**Table 1**.). Advances in microbiology have led to the emergence of polysaccharide vaccines against certain strains of pneumococcus and meningococcus[55].

To increase immunogenicity, the antigenic polysaccharides, which primarily lead to a B-cell-dependent immune answer, were covalently linked to carrier proteins, thereby providing helper T-cell activation. The resulting glycoconjugate vaccines brought about a better antibody response and were effective in all age groups. In our days, very effective **glycoconjugate vaccines** are available for Hemophilus influenzae, pneumococcus, and the meningococcus types A, C, W, and Y (**Table 1**.)[55]. For hepatitis B virus (HBV) and human papillomavirus (HPV), vaccine production is complicated because both are difficult to culture in vitro. The first-generation HBV vaccine was derived from the blood of infected donors and contained the surface antigen of purified HBV. Progress in molecular biology made possible the improvement of the vaccine against HBV and, more recently, the development of a new vaccine preventing HPV. Both vaccines are made of purified recombinant protein antigens that form a non-infectious viral-like particle (VLP)[55].

**Virus-like particles** (VLPs (**Table 1**.)) have made tremendous strides in vaccine science over the past three decades[56]. VLPs constitute versatile tools in vaccine development due to their favorable immunological characteristics such as their size, repetitive surface geometry, ability to induce both innate and adaptive immune answers[57]. The first HPV vaccine was authorized in 2006. HPV vaccination goes on to become a key part of the effort to eradicate cervical cancer[58]. The introduction of **recombinant DNA** and **whole-genome sequencing techniques** were major milestones in vaccine development. It gave researchers the opportunity and tools to develop new vaccines against pathogens[46].

Unlike other meningococci, Neisseria meningitidis type B (MenB) is enveloped by a capsular polysaccharide that is similar to that present in human tissues and is therefore weakly immunogenic[55]. As such, the MenB capsular polysaccharide cannot be used in a glycoconjugate vaccine, contrary to what has been done effectively for vaccines of type A, C, W and Y. Making a vaccine based on recombinant proteins was also quite demanding due to the extreme antigenic variation observed in the circulating MenB strains. The development of a universal type B meningococcal vaccine was achieved through **"reverse vaccination"** (the rational selection of candidate antigens based on genomic information), combining recombinant proteins (protective antigens specific to several MenB strains were expressed) and **outer membrane vesicles** (OMV (**Table 1**.)[55].

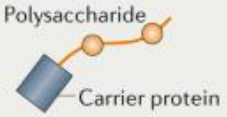
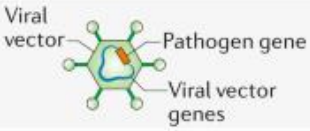
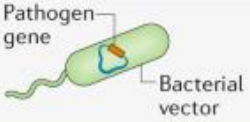
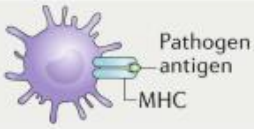
| Type of vaccine | | Licensed vaccines using this technology | First introduced |
|---|---|---|---|
| **Live attenuated (weakened or inactivated)** |  | Measles, mumps, rubella, yellow fever, influenza, oral polio, typhoid, Japanese encephalitis, rotavirus, BCG, varicella zoster | 1798 (smallpox) |
| **Killed whole organism** |  | Whole-cell pertussis, polio, influenza, Japanese encephalitis, hepatitis A, rabies | 1896 (typhoid) |
| **Toxoid** |  | Diphtheria, tetanus | 1923 (diphtheria) |
| **Subunit (purified protein, recombinant protein, polysaccharide, peptide)** |  | Pertussis, influenza, hepatitis B, meningococcal, pneumococcal, typhoid, hepatitis A | 1970 (anthrax) |
| **Virus-like particle** |  | Human papillomavirus | 1986 (hepatitis B) |
| **Outer membrane vesicle** |   | Group B meningococcal | 1987 (group B meningococcal) |
| **Protein–polysaccharide conjugate** |   | *Haemophilus influenzae* type B, pneumococcal, meningococcal, typhoid | 1987 (*H. influenzae* type b) |
| **Viral vectored** |   | Ebola | 2019 (Ebola) |
| **Nucleic acid vaccine** |   | SARS-CoV-2 | 2020 (SARS-CoV-2) |
| **Bacterial vectored** |   | Experimental | – |
| **Antigen-presenting cell** |   | Experimental | – |

**Table 1**.Schematic representation of different types of vaccine against pathogens; the text indicates against which pathogens certain vaccines are licensed and when each type of vaccine was first introduced. BCG, *Mycobacterium bovis* bacillus Calmette–Guérin. SOURCE: REFERENCES

Ebola vaccines have been developed using **viral vectors** (Table 1.)[59]. Ervebo (rVSV-EBOV), Zabdeno/Mvabea (Ad26-ZEBOV/MVA-BN-Filo) and cAd3-EBOZ are among the most advanced vaccines[60]. Their main advantage is that they can specifically deliver antigen to target cells and thereby induce robust, long-lived immunity[60,61].

The understanding of immunology and correlates of protection is crucial for developing vaccines against challenging pathogens, controlling outbreaks, and addressing age-related immune responses. Vaccines are made based on the ability of the developed human immune system to react to, and then remember, new encounters with pathogen antigens. To achieve this, the vaccine contains the antigen of pathogen as a component. These elements that make up most vaccines produce a protective immune response[62].

Correlates of protection are important for improving vaccines because they can be used to compare vaccines and predict if they will provide the same protection in different populations. Associates of protection can be measured in clinical trials, but large-scale serum collection is rarely done. Sero-epidemiological studies and human challenge studies are alternative ways to estimate correlates of protection, although the latter has limitations due to the dose and experimental circumstances not closely reflecting natural infection[62]. Vaccines are generally classified as live or non-live (sometimes loosely referred to as 'inactivated') to mark those vaccines that contain replicating strains of the relevant pathogenic organism. Beyond the 'traditional' **live and non-live vaccines**, numerous other platforms have been developed during the past few decades, including nucleic acid-based RNA and DNA vaccines (Table 1.).

The **mRNA** (messenger RNA) **technology** is a relatively new approach to vaccine development that has gained widespread attention in the context of the COVID-19 pandemic. This technology is based on the idea of using a small piece of genetic material, specifically mRNA, to instruct cells in the body to produce a protein that triggers an immune response. Messenger RNA was discovered in the early 1960s and took nearly 60 years to be approved as a COVID-19 mRNA vaccine[63]. In 1978 it was isolated for the first time in mammalian cells produced the coded protein. In vitro transcription introduced 6 years later using phage RNA polymerases from the coding plasmid became an effective tool for mRNA production. mRNA vaccines represent a promising alternative to conventional vaccine approaches due to their high efficacy, rapid development capability, and low-cost production and safe administration potential[64]. However, the development of technology has faced many challenges, including concerns about the safety and

efficacy of using mRNA in vaccines. Their use has been limited until recently by mRNA instability and inefficient in vivo delivery. Recent technological advances and the work of Katalin Karikó have now largely solved these problems[64]. Katalin Karikó, a Hungarian biochemist, played a key role in the development of mRNA technology since the 1990s[65]. Her research on modified mRNA laid the groundwork for the development of mRNA vaccines[65]. She focused on finding ways to overcome the natural response of immune system to foreign RNA, which can cause inflammation and other harmful effects. She discovered that modifying the RNA molecules by replacing uridine with pseudouridine could reduce the response of immune system while still allowing the RNA to function as intended[63,65,66]. Delivery of nucleoside-modified mRNA encoding viral antigens in the form of lipid nanoparticles has become a platform for an effective vaccine[63,66]. Its labile nature made it ideal for the temporary production of viral antigen and the creation of an effective antibody and cellular immune response[63].

Most recent clinical trials conducted by companies such as Moderna and Pfizer-BioNTech have shown promising results in terms of the safety and effectiveness of mRNA vaccines for COVID-19[67,68].

BNT162b2 (Pfizer-BioNTech) vaccine was one of the first mRNA vaccines to receive emergency use authorization in the United Kingdom in December 2020. The vaccine uses mRNA to instruct cells to produce the spike protein found on the surface of the SARS-CoV-2 virus, which triggers an immune response. The vaccine was shown to be highly effective in clinical trials in preventing COVID-19[67]. Similarly, mRNA-1273 vaccine (Moderna) is also using mRNA technology to instruct cells to produce the spike protein. It has been shown in clinical trials to be as effective as the Pfizer vaccine in preventing COVID-19[68].

The success of mRNA vaccines for COVID-19 has opened up new possibilities for the development of vaccines and therapies for a range of diseases. The technology is being explored for its potential use in cancer immunotherapy, gene therapy, and other applications.

People have been vaccinated against deadly diseases for more than 2 centuries since the world's first smallpox vaccine was developed[69]. History has shown that a full and effective global response to vaccine-preventable diseases takes time, financial support, and cooperation—and requires continued alertness. We have come a long way from the pioneering practices of the 1500s to the new technologies used in vaccines against

COVID-19. Vaccines now protect against more than 20 diseases, from pneumonia to cervical cancer and Ebola; and in the last 30 years, child mortality has fallen by more than 50%, largely due to vaccinations. However, more must be done[69].
In many parts of the world, countless children still remain unvaccinated. The coming decades will require global cooperation, funding, commitment, and vision to ensure that no child or adult suffers or dies from a vaccine-preventable disease[69].

Overall, mRNA technology represents an exciting new approach to vaccine development that has the potential to transform the field of medicine in the coming years[64].

### 2.2.1. *Types of vaccines against COVID-19*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the 2019 coronavirus disease (COVID-19), is the third novel beta-coronavirus that is among the severely pathogenic human coronaviruses that have caused a public health crisis during the last twenty years[70]. Compared it to its predecessors, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), we find that it spreads more efficiently among the population[71], this way the COVID-19 pandemic has intensified the development of vaccine platforms different from classical vaccines.
Understanding the structure and genomic construction of the virus is inevitable since git is the basis for the targets of different diagnostic tests and types of vaccines.The exact pathophysiology stays unknown, partly due to the shortage of postmortem studies[70]. The pathophysiology seems similar to other coronavirus infections. Even so, emerging evidence reveals that COVID-19 has specific pathophysiological characteristics that set it apart from respiratory failure of other origins[71]. SARS-CoV-2 joins to the angiotensin-converting enzyme-2 (ACE2) receptor on target host cells, followed by internalization and replication of the virus. ACE2 receptors are extraordinary manifested in the upper and lower respiratory tract cells, while are also expressed in myocardial cells, renal epithelial cells, enterocytes, and endothelial cells in multiple organs, which may serve as an explanation for the extrapulmonary marks associated with the disease[72](**Figure 7**.).Viral RNA has been identified in many organs in postmortem studies[70].
The SARS-CoV-2 virus is ellipsoidal in shape, with an average diameter of 60-140 nanometer[73]. It has single-stranded, non-segmented RNA of positive polarity (+ ssRNA).

RNA is able to act directly as mRNA in protein translation[42]. Coronaviruses are a huge family of enveloped RNA viruses and like other coronaviruses, SARS-CoV-2 has four configurational proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins[74] (**Figure 3.**).

The S protein is built as a homotrimer and is incorporated into the virion membrane in multiple copies, giving it a crown-like appearance[75]. Other structural proteins include the membrane (M) protein and envelope (E) protein, which form the ring-like structure, and the nucleocapsid (N) protein, which holds the RNA genome and plays a role in successful host cell entry (**Figure 8**.). In SARS-CoV-2, the spike protein (S), which has been imaged at the atomic level using cryogenic electron microscopy[76,77], performs two functional subunits[78,79]. The S protein is cleaved by proprotein convertases such as furin in the virus-producer cells[80,81] : S1 subunit containing the receptor-binding domain (RBD) that mediates binding to the host cell surface receptor angiotensin-converting enzyme-2 (ACE-2), and the S2 subunit which is fundamental to the following fusion between the viral and host cellular membranes[82].

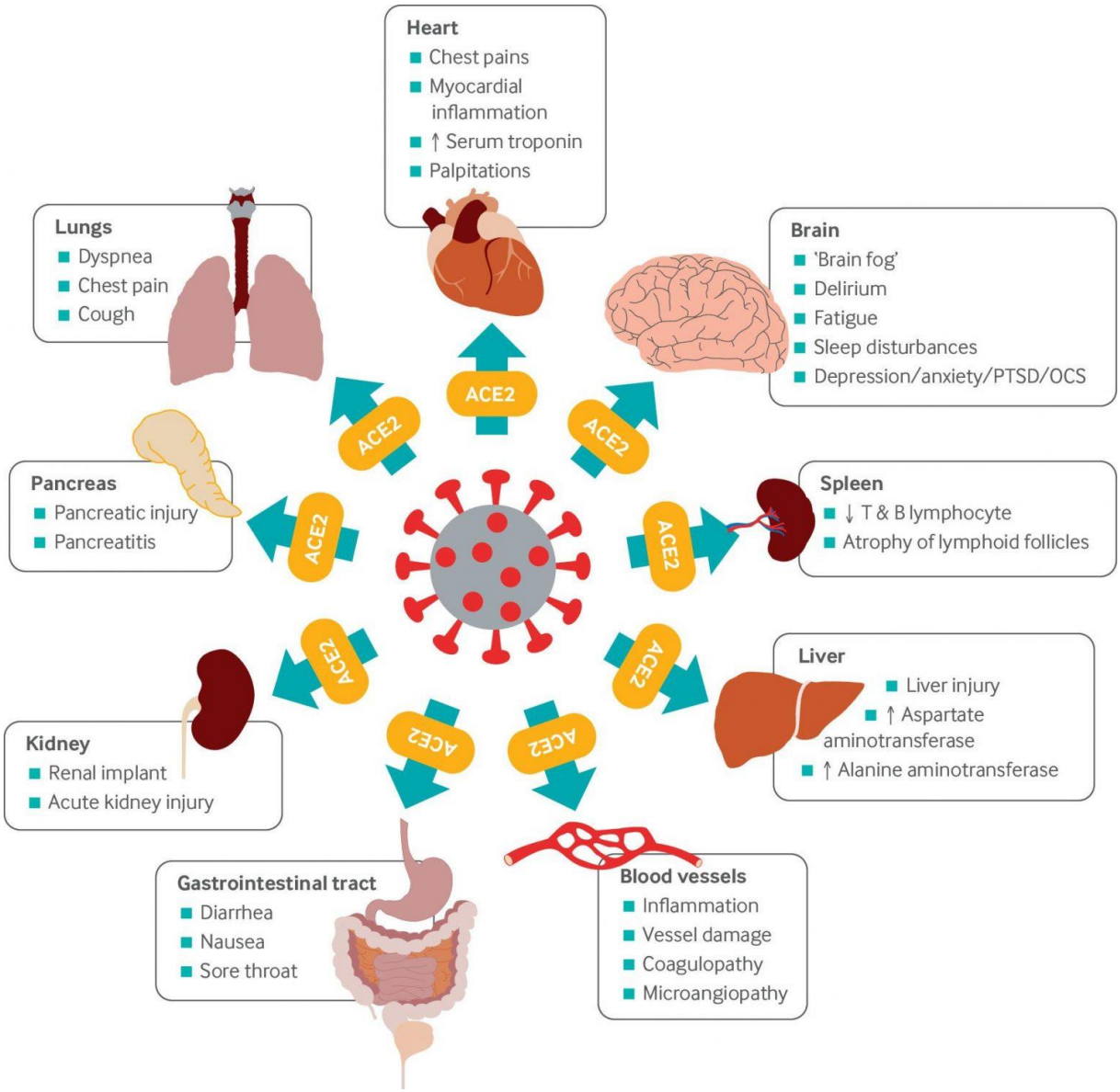



**Figure 7**. Multi-organ complications of COVID-19 and long COVID.

SOURCE: REFERENCES

A different structural feature of the spike glycoprotein receptor-binding domain (S-RBD) offers possibly higher binding affinity for ACE2 on host cells compared with SARS-CoV-1[31].This furin-like cleavage site does not seem to exist in other coronaviruses[83].The binding energy between the spike protein and ACE2 was highest for humans out of all species tested in one study, this way suggesting that the spike protein is especially evolved to bind to and infect human cells expressing ACE2[84].

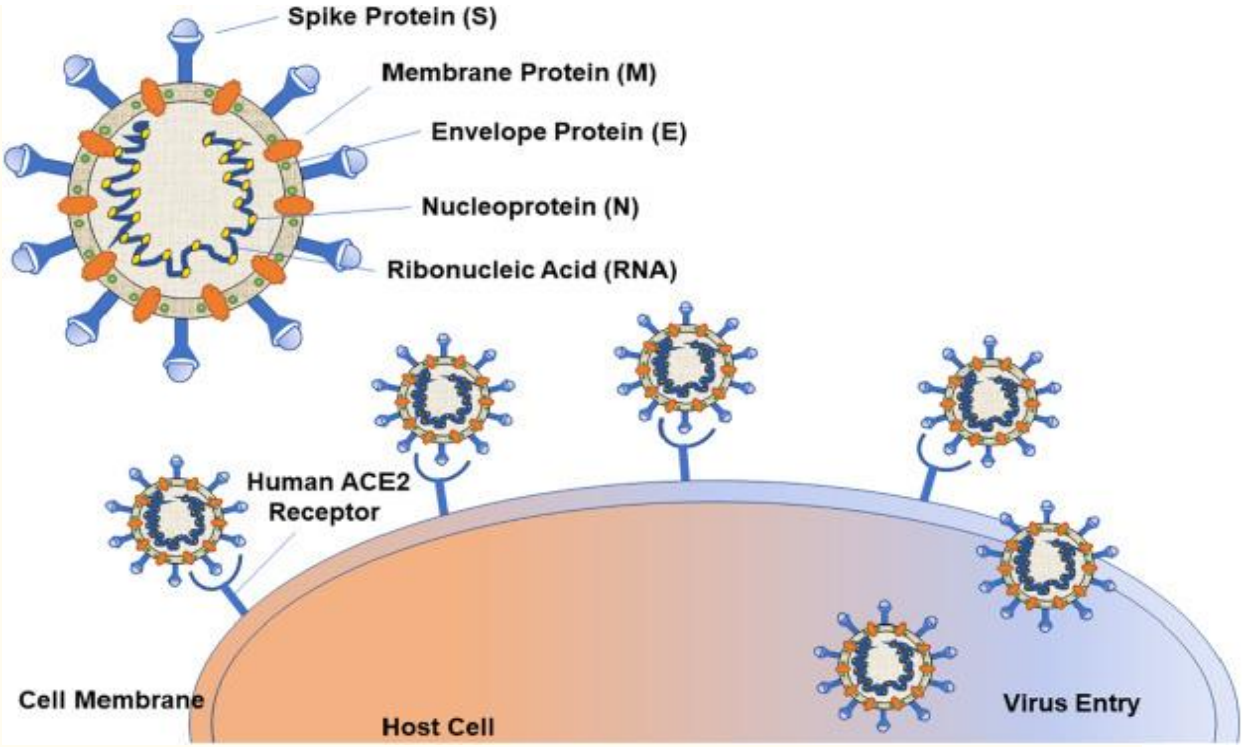



**Figure 8**. Typical scheme of human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entering the cell. SOURCE: REFERENCES

**Vaccine products**

The majority of the candidate vaccines for COVID-19 that employ administration of viral antigens or viral gene sequences aspire to induce neutralizing antibodies against the viral spike protein (S), holding back uptake through the human ACE2 receptor and, this way, blocking infection[85,86].

Since the publication of the genome sequence of SARS-CoV-2, on January 11th, 2020, efforts of unparalleled speed and magnitude set out to develop a vaccine against the disease. Early scientific opinions predicted that it would take at least a year to a year and a half to get a SARS-CoV-2 vaccine permitted for use in the United States. Still, recent advances on the field have made possible the issuing of emergency use authorizations (EUAs) by a lot of national and international drug regulation agencies for various vaccine candidates against SARS-CoV-2 in less than a year since the release of the viral genome sequence[86]. A perfect SARS-CoV-2 vaccine should meet the following requirements: give protection not only from severe disease but also thwart infection in all vaccinated populations, covering less immunocompromised individuals, elicit long term memory immune answers after a minimal number of immunizations or booster doses, the

manufacturing company should be capable to increase production very fast to produce billions of doses per year and has the power to make it readily available for vaccination campaigns worldwide at an affordable price and for a limited time[86,87].

Understandably, candidates first entering Phase 3 clinical trials use rapid entry strategies, namely nucleic acid platforms, non-replicating viral vector platforms, inactivated viruses, or recombinant subunit vaccines[86] (**Table 2**.). Other traditional vaccine development programs, such as **attenuated virus vaccines**, even though historically leading to very successful vaccines against viral diseases[88,89] need long cell culturing processes to get attenuated strains[86]. Not surprisingly, no SARS-CoV-2 vaccine candidate has entered clinical trials using this strategy. It is quite possible that the second-generation SARS-CoV-2 vaccines will show the capacity to elicit more powerful and longer memory responses with just one immunization[86,90].

Since May 14th of 1796, the day when Edward Jenner executed the emblematic experimental inoculation of an 8-year-old boy with pus obtained from a milkmaid infected with cowpox that gave him immunization against smallpox, vaccination has been proven to be a successful story in Medicine. Traditional vaccine development strategies, though proven to be efficient for a number of pathogens, are slowly giving space to more sophisticated techniques involving recombinant DNA technology, adding new options in vaccine composing strategies[86,91].

| Product | Developers | Platform | Antigen | First deployment | Ref. |
|---|---|---|---|---|---|
| CanSino Ad5-nCoV | CanSino Biologics | Non-replicating adenoviral vector rAd5 | WT S protein[a] | Limited use for 1 year in Chinese military personnel/ 25 June 2020 | 92 |
| CoronaVac | Sinovac Research and Development Co. | Inactivated virus (alum adjuvant) | Whole virion, including WT S protein[a] | 29 Aug 2020 (China) | 93 |
| BNT162b2 (Comirnaty) | Pfizer, BioNTech | mRNA–lipid nanoparticle | S protein (2P)[b] | 2 Dec 2020 (United Kingdom) | 67 |
| Gam-COVID-Vac (Sputnik V) | Gamaleya Research Institute, Health Ministry of the Russian Federation | Non-replicating adenoviral vectors rAd26 and rAd5 | WT S protein[a] | 5 Dec 2020 (Russia) | 94 |
| Covilo BBIBP-CorV /BIBP vaccine | Sinopharm's Beijing Institute of Biological Products | Inactivated virus (alum adjuvant) | Whole virion, including WT S protein[a] | 9 Dec 2020 United Arab Emirates | 95 |
| mRNA-1273 | Moderna, US National Institute of Allergy and Infectious Diseases | mRNA–lipid nanoparticle | S protein (2P)[b] | 18 Dec 2020 (United States) | 96 |
| AZD1222 Covishield) | AstraZeneca, University of Oxford | Non-replicating adenoviral vector ChAdOx1 | WT S protein[a] | 30 Dec 2020 (United Kingdom) | 97 |

| Product | Developers | Platform | Antigen | First deployment | Ref. |
|---|---|---|---|---|---|
| BBV152 (Covaxin) | Bharat Biotech International | Inactivated virus (Algel-IMDG adjuvant) | Whole virion, including WT S protein[a] | 2 Jan 2021 (India) | 98 |
| Ad26.COV2.S | Janssen Pharmaceutical (Johnson & Johnson) | Non-replicating adenoviral vector rAd26 | S protein (FKO + 2P)[c] | 17 Feb 2021 (South Africa) | 99 |
| NVX-CoV2373 | Novavax | Protein subunit–nanoparticle (Matrix M adjuvant) | S protein (FKO + 2P)[c] | Pending[d] | 100 |

**Table 2**. Selected products used in global vaccination campaigns: The vaccines that had received or are close to receiving emergency use authorization at the time of writing are included. S protein, spike protein; 2P, diproline mutation (K986P and V987P); FKO, furin-cleavage site knocked out; WT, wild type. [a]Full-length WT S protein. [b]Full-length S protein with 2P mutation in the S2 subunit to stabilize prefusion conformation. [c]Full-length S protein with FKO and 2P mutation added to stabilize prefusion conformation. [d]Application for emergency use authorization had been submitted in numerous countries as of August 2021 but have not been received at the time of writing.

There are always distinctive advantages and challenges behind the strategies[86]. However, any vaccine strategy must have two main features: one is the safety of the vaccine, while the other is related to the activation of adaptive immune responses to provide long-term protection against multiple strains of the pathogen – ideally – with a single dose of vaccine[86].

The vast majority of licensed vaccines have traditionally been aimed at inducing strong protective and neutralizing antibodies against the target pathogen, thus aiming at sterilizing immunity in vaccinated people[86]. Sterilizing immunity describes the immune status whereby virus infection of the host is completely inhibited and, as a result, disease and further transmission of the virus prevented. It differs from innate trained, or T-cell mediated immunity that allows for infection, but efficiently controls and subsequently

eliminates the pathogen[86]. Sterilization of immunity is quite rare, especially against viruses that infect the lower respiratory tract, such as the influenza virus or various coronaviruses[86,101]. However, there is growing evidence that T-cell mediated responses against SARS-CoV-2 are outstandingly important and more long-lasting than B-cell immunity[102,103]. Therefore, vaccine strategies that bring about strong cellular responses apart from humoral immunity present a significant advantage in the present pandemic[86]. Vaccine products against SARS-CoV-2 based on several technology platforms (**Figure 9**.) have been advanced to clinical tests and emergency use worldwide (**Table 2**.).

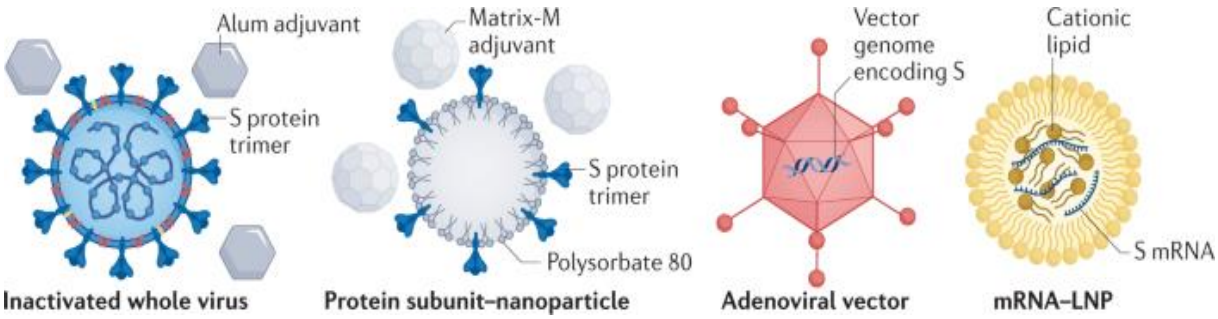



**Figure 9**. Vaccines against SARS-CoV-2. SOURCE: REFERENCES

**Inactivated pathogen vaccines** contain complete pathogen that has been submitted to heat, radiation or chemical (i.e., formalin, β-propiolactone) treatment inactivation, this way to guarantee a better safety profile than live attenuated vaccines. Still, pathogens, sometimes lose their immunogenicity causing this strategy less efficacious than live attenuated pathogen immunization. Consequently, inactivated pathogen vaccines often fail to produce cellular adaptive responses unless and thus require the addition of adjuvants, specific compounds that act as stimulants of immune cells and amplifiers of immune answers, is required[86].

In a traditional approach, **inactivated whole-virus vaccine** (far left in the **Figure 9**.) is generated by inactivating purified SARS-CoV-2 with formaldehyde or β-propiolactone (i.e. **CoronaVac**, **Covilo**) and mixing it with an adjuvant such as alum (CoronaVac) or Algel-IMDG (**BBV152**)[93,95]. On the other hand, the schemes using whole virus -either attenuated or inactivated- aspire to induce a broader, more heterologous polyclonal answer against several viral antigens[86].

**Subunit vaccines** the basis underlying the development of subunit vaccines was based upon the observation that do not need to rule the entire pathogen to elicit strong immune responses, but simply an immunogenic fragment. **Protein subunit vaccines,**

**polysaccharide** and **conjugate vaccines**, and **virus-like particle vaccines** can all be considered different forms of subunit delivery strategies that differ in the chemical nature of the antigen administered, the platform used to deliver the antigen, and the need for adjuvant use to strongly activate the immune system[86]. Protein subunit vaccines are produced by recombinant technology or protein isolation and purification methods after culturing large quantities of pathogens[104]. This strategy excludes the possibility of serious adverse effects, but frequently raises the necessity to increase booster doses and optimize the adjuvant added to reach stronger and more durable immunization. The administered antigen is taken up by adjuvant-activated antigen-presenting cells (APCs) and presented to adaptive immune cells[86]. The burst of genetic engineering observed in the last two decades of the 20th century caused the capacity to clone and ramp up antigen production in vitro[86]. Such techniques enabled the production of large quantities of hepatitis B surface antigen in yeast cells, leading to a breakthrough in the production of the Hepatitis B vaccine[86,105].

In human clinical tests against SARS-CoV-2 each one of the candidates mentioned above, is using different immunogens, basically different forms of the entire Spike protein or its receptor binding domain (RBD), the region of the S protein that mediates viral binding to the ACE2 receptor of target host cells[86]. Upon binding to the host cell ACE2 receptor the prefusion conformation of the S protein is subjected to an extended conformational change to a highly stable post fusion conformation that makes possible the fusion between the viral particle and host cell membranes[106]. Generally, prefusion-stabilized viral glycoproteins are usually more immunogenic, thus being more attractive vaccine targets[86,107–109].

The **protein subunit–nanoparticle vaccine** (middle left in the **Figure 9**.) is produced by incorporation of purified recombinant S protein into polysorbate 80 micelles with the addition of the saponin-based adjuvant Matrix-M (**NVX-CoV2373**)[86,100,110] . Novavax COVID-19 vaccine, also known as Nuvaxovid (Biocelect Pty Ltd/Novavax Inc) has been transitionally allowed by the Therapeutic Goods Administration (TGA) for use in a primary course of vaccination in people aged 18 years and older. This vaccine has been demonstrated to be highly efficient in preventing symptomatic COVID-19 in adults in a primary schedule, based on phase II-III clinical tests involving over 45,000 participants. Novavax COVID-19 vaccine is not currently registered by the TGA for use as a COVID-19 booster vaccine[111,112].

Two vaccine platforms using **gene therapy technologies** to elicit production of the S protein antigen in host cells have also been proved effective and have exceptional intrinsic immunogenicity. These are **non-replicating recombinant adenoviral vector systems** (middle right in the **Figure 9**.), which carry the gene encoding the S protein (Gam-COVID-Vac, AZD1222, Ad5-nCoV and Ad26.COV2.S)[9799,113] and **mRNA–LNP (lipid nanoparticle) systems** (far right in the **Figure 9**.), wherein chemically modified mRNA encoding the S protein bound to ionizable lipids is encapsulated inside a layer of mixed lipids (BNT162b2 and mRNA-1273)[67.]

The efficacies of the vaccines are Pfizer—95%, Moderna—94.1%, AstraZeneca—70.4% and Janssen—66.9%, proving that these vaccines are efficient at reducing the incidence and seriousness of SARS-CoV-2 infection among a study population[114].

Adenoviral vector platform was explored by the Oxford/AstraZeneca vaccine and the Janssen Pharmaceuticals vaccine by Johnson & Johnson. Both these vaccines encode the S protein of the SARS-CoV-2 virus[115].After vaccination, it is trusted that the surface spike protein is produced, encouraging the immune system to attack when it encounters the SARS-CoV-2 virus. ChAdOx1-S-(AZD1222) uses a chimpanzee adenovirus vector while Ad26.COV2.S is based on a recombinant human adenovirus vector[115]. Still, the Janssen vaccine is advantageous over the other candidate, as it is administered in only one dose, which reduces manufacturing costs[115]. Antibody directed against the S protein avoids invasion of the SARS-CoV-2 virus in type 2 alveolar cells of the lungs, this way reducing the seriousness and morbidity of the infection[116].

Advantages of adenoviral vectors are adjuvant qualities, scalability, and their broad tissue tropism[117].On the downside, there is these laboratories need to have biosafety level 2. In addition, there is the possibility of pre-existing immunity to viral vectors, decreasing the efficiency of the vaccine. The Oxford/AstraZeneca was able to overcome this disadvantage by using the Chimpanzee adenovirus (ChAdOx1) which means an alternative to the human Ad vector and lacks preexisting immunity in humans[118,119].

The response to the COVID-19 pandemic has called attention to distinct advantages of the mRNA–LNP technology in rapid prototyping and manufacturing on a large scale[120].

**BNT162b2/ Pfizer**

This is a lipid nanoparticle–formulated, nucleoside-modified RNA vaccine that works against the S protein of the SARS-CoV-2 virus[67].This vaccine lets for the body to create an antibodies respond for S protein-dependent virus neutralization via the ACE2 receptor into type 2 alveolar cells[121]. Pfizer efficacy indicates a 95% effectiveness at defending from COVID-19 infections[68,114].

**mRNA-1273/ Moderna**

This is a lipid nanoparticle–encapsulated nucleoside-modified messenger RNA (mRNA)–based inoculation. It encodes the prefusion stabilised full-length spike protein of SARS-CoV-2. This spike glycoprotein moderates host cell attachments. Therefore, it is important for viral entry and thus the primary vaccine target. The vaccine gives rise to an intense binding and neutralising antibody response[122]. Besides it, this contains $CD4^+$ T-cell and $CD8^+$ cytotoxic T-cell response to get rid of the virus[117]. Moderna efficacy was 94.1% at preventing COVID-19. Negative events appeared more frequently in the Moderna group following the first and second doses[114].

All current vaccines are based on the S protein sequence of the Wuhan-Hu-1 strain predating the D614G mutation, which stabilizes the S protein[123–126] and is present in all currently circulating variants. To stabilize the S protein, some vaccine designs included furin-site knockout and/or diproline mutations in the S protein. 'Diproline mutation' refers to the mutation of two consecutive residues in the S2 subunit (Lys986 and Val987) to proline and used to stabilize prefusion conformation of the S protein[127]. Other inoculations , such as AZD1222 and the inactivated whole-virus vaccines, use the unmodified S protein[97,98,128] (**Table 2**.). This way, differences in vaccine efficacy may be due to not only their differing vectors and formulations but also the particular S protein construct employed.

### 2.2.2. *Adverse reactions and side effects of vaccines*

Vaccines are one of the greatest success stories in public health. To ensure the continued success of vaccines, it is vital to make it absolutely sure that vaccines are safe, but in reality, no vaccine is 100% safe or effective for everyone, as each body of person reacts to vaccines differently[129–132].

Licensing a vaccine is a lengthy process that can take 10 years or more[132,133]. In public health emergencies, such as a pandemic, the development strategy may differ from the usual, as the case of the COVID-19 pandemic showed. However, the fast-track process for vaccines developed to treat the COVID-19 pandemic did not compromise scientific standards, the integrity of the vaccine review process, or safety[134]. The FDA quickly recognized the severity of the current public health emergency and the need for an emergency use authorization (EUA) to protect the public as soon as possible with reliable vaccines[134].

Vaccines are generally safe and well-tolerated, but like any medical intervention, they can cause adverse events and side effects. Quite often vaccine adverse events are identified during clinical trials[135].

**Adverse Reaction**: An adverse reaction refers to an unintended, undesired occurrence that is a consequence of taking the drug correctly or a harmful response that occurs after exposure to a medical treatment or intervention, while a side effect is a secondary unwanted effect that occurs because of drug therapy. An adverse event can be a type A or B reaction. The former adverse event is predictable and usually dose dependent[136]. Type B reactions can occur unexpectedly regardless of dose. An adverse event may occur because neither the healthcare provider nor the individual knows the drug and its underlying mechanism. The event surprises both the doctor and the patient, but the effects can be mitigated by reducing or omitting the dose. Individual sensitivity factors, such as allergies and intolerances, may be influencing factors[136].

It is a common misconception that adverse events and side effects are identical, although they have different meanings[136]. Adverse reaction can include any unexpected or unwanted effects that may occur in addition to the intended therapeutic effect. It can range from mild to severe and can affect various systems or organs in the body[136,137].

- Local adverse reactions, including local pain, swelling and redness at the injection site, are among the most common topical reactions. They usually appear within a few hours, do not harm the body, and disappear on their own. Depending on the type of vaccine, they can appear in up to 80% of vaccine doses. It can rarely become severe. These events, called Arthus reactions, are most often seen with diphtheria and tetanus toxoids. Arthus reactions are not allergic reactions. It is thought to result from high antibody titers through an excessive toxoid dose.[135].

- Systemic adverse reactions, include fever, fatigue, myalgia, arthralgia, skin rash, headache, chills, nausea, lymph node swelling, or other[39] may occur following vaccination. Adverse reactions following the administration of live, attenuated vaccines mimic the mild form of the natural disease, such as fever and skin rash. Systemic reactions are mostly mild and can occur from 3 days to 3 weeks after the vaccine is administered. In the case of live attenuated vaccines, systemic effects may occur at longer intervals because they are caused by the replication of the vaccine virus in the body, which occurs after several days[135].

An adverse case is an undocumented therapeutic/pharmacological event that is either unforeseen or a harmful reaction to the medication. These types of cases are not typically studied during drug development because they are unique to the dose, patient, and possible interaction. Adverse events are unpredictable and occur much less often than side effects. Immunization Safety Office of CDC, along with FDA and other federal government partners, leads research on adverse events that occur after vaccination[135]. Adverse events (serious health problems), including severe allergic reactions, after COVID-19 vaccination are rare but can cause long-term health problems. That is why it is important for the vaccination provider to observe those receiving the vaccine against COVID-19 for at least 15 minutes. They usually occur within six weeks of getting a vaccine[138]. Unfortunately, there is no way to know whether a patient is going to experience an adverse event. Keeping track of the event and reporting it as soon as possible are the best ways to decrease the chances of it getting worse[139].
An adverse event can be illustrated with an example: Out of two patients taking 5 mg of Warfarin, patient A shows a normal response. The INR value increases as expected. No one expects a blood clot to form after taking a blood thinner; however, one patient may

differ physiologically from another, since differences in weight may also occur, may genetically contain more vitamin K, and an adverse event already occurs[139].

**Side Effect**: A side effect, on the other hand, is a specific type of adverse reaction that occurs when the drug is used regardless of dosage. Side effects are secondary, often unwanted effects of the primary therapeutic action of a medication. They are usually known and anticipated based on the pharmacological properties of the drug. Unlike adverse events, the doctor usually foresees the side effects and clarifies them with the patient, thus he is aware of the effects occurring during the therapy[136]. Some drugs are even used because of their side effects, such as mirtazapine, used in anorexic patients, because the drugs can cause weight gain. Common examples of side effects include drowsiness, nausea, headache, or dry mouth. Side effects are typically mentioned in the product information or medication package inserts[136,137]. Side effects are carefully monitored and investigated in clinical trials before entering the market[136].

A two-dose regimen of BNT162b2 and mRNA-1273 were found to be safe and more than 90% effective against COVID-19[67,68].

*Dighriri et al.* noted that adverse effects regarding to the **Pfizer-BioNTech COVID-19** vaccine are common, usually mild, and self-limiting[140]. Local adverse effects occur more times than systematic ones. The most common local reaction was pain and swelling at the injection site[140]. Examining the safety of mRNA vaccines, *G. Alicandro et al.* observed that most adverse effects had a mild course, while those of moderate severity were more common after the second dose, as in our case[39,141]. The most common ARs were fatigue (59–65%), headache (52–58%), fever (16%), and chills (44%)[67,68]. Fortunately, cases of anaphylaxis to mRNA COVID-19 vaccines have been very rarely reported , mainly in individuals with a history of sensitivity and allergy[142]. It is important to detect it in time and treat it immediately! A severe allergic reaction contraindicates another repeated vaccination[143]. In summary, adverse reactions encompass a broader range of unintended and harmful responses that can occur due to any medical treatment or intervention, whereas side effects specifically refer to the known and anticipated secondary effects of medications or treatments. It is important to note that adverse events and side effects can vary depending on the individual and other factors, such as age and health status, but the benefits of vaccination usually outweigh the risks!

### 2.2.3. *Relationship of adverse reactions and subsequent antibody titers in the literature*

Vaccines induce provisional inflammation that forms the desired immune response while also causing short-lived local and systemic reactions called reactogenicity. The hypothesis that the extent of local reactions (pain, redness, or swelling at the injection site) or systemic reactions (e.g., headache or fever) following vaccination is predictive of immunogenicity and efficacy (so to speak "no pain, no gain") remains controversial[144].

The sudden rapidity of vaccine development and the uncertainty of possible unpredictable adverse effects have created hesitancy towards mRNA vaccines in the global community[145]. By 21 January 2022, 60.3% of the world's population had got at least one COVID-19 vaccine[146]. In the context of the world's largest ever vaccination campaign, the uncertain relationship between vaccine-related reactogenicity and immunogenicity has attracted the interest of numerous studies, but the results are incongruent[147,148].

- A weak, but statistically significant correlation was found between reactogenicity (intensity of pain after vaccination) and immunogenicity after herpes zoster vaccine[144].
- In a 2009 study clear correlation was found between systemic adverse event (including fever) and antibody titer following H1N1 vaccination[39]. Hemagglutination-inhibition titers were 60% higher in children with fever ≥38 °C after vaccination, suggesting a more effective immune response[149].

Several studies have also examined the relationship between reactogenicity and immunogenicity in recipients of mRNA vaccines against COVID-19 and have found incongruent results. The main objective of the present thesis was to clarify the long-term effect of adverse reactions following vaccination on antibody production in healthcare workers with and without prior COVID-19 infection.

- In two independent studies, *Koike and Kobashi* found a significant correlation between immunogenicity, as reflected by the titer of anti-SARS-CoV-2 S protein IgG antibodies, and reactogenicity after the second dose of the vaccine[150]. In these studies, the correlation was found to exist only with some side effects. In the first

study[151], a significant positive correlation was found between higher body temperature and higher antibody titer 3 months, but not 6 months after vaccination, in the second study[152] significance existed between muscle-joint pain and anti-S1 protein IgG antibody titer- neutralizing activity. [150–152].

- *Otani et al.* showed that flushing at the injection site after the first dose, induration, heat, swelling at the injection site, and systemic symptoms (fatigue, fever, and headache) after the second dose were associated with a higher anti-SARS-CoV-2 IgG antibody level[153].
- The results of *Bruna Lo Sasso et al.* showed that anti S-RBD IgG levels were lower in subjects with previous SARS-CoV-2 infection than vaccinated subjects with or without prior infection ($p < 0.001$)[154]. No difference was observed between vaccinated participants, regardless of previous COVID-19 positivity. In fact, the anti-RBD IgG level was increased in women compared to men (2110 vs. 1341 BAU/ml; $p < 0.001$) as well as in symptomatic subjects compared to asymptomatic members (2085 vs. 1332 BAU /ml; $p = 0.001$).) and lower in olders than in youngers. Their conclusions are remarkable given their results. They reported an effective antibody response after vaccination, with age-, time-, and gender-dependent differences[154].
- However, in another study, *Zhang et al.*[155] looked for a correlation between neutralizing activity against SARS-CoV-2 with BNT162b2 or the CoronaVac vaccine, a whole inactivated virus COVID-19 vaccine, and noticed only a low correlation between adverse reaction and the BNT162b2 vaccine.
- *Takeuchi et al.* indicated no correlation between reactogenicity and antibody production in a study of 67 HCWs[156], while *Held et al.* determined that adverse events were weakly but persistently correlated with spike protein antibody levels after vaccination with the BNT162b2 vaccine in a study of 80 HCWs. In a recent study to determine whether humoral immune responses after BNT162b2 vaccine administration were associated with local and systemic side effects, a prospective observational cohort study was performed at a single tertiary referral center[157]. Healthcare workers who received the first dose of BNT162b2 vaccine were studied. SARS-CoV-2 anti-spike IgG antibody titers were measured three weeks after the second dose and information on post-vaccination side effects was collected. 72.3% of the participants were women with a median age of 38 (22-

74) years[157]. All but one had anti-spike IgG levels well above the cut-off. Essentially, 92.2% of participants reported some reaction after the first dose and 96.3% after the second dose. Significantly more participants reported a systemic reaction after the second dose, as seen in our study, than after the first dose ($P < 0.01$), and 73.6% of subjects reported that reactions were more severe after the second dose[157]. Factors positively associated with increased anti-spike IgG levels were history of asthma (24% higher if present, $P = 0.01$) and more severe reactions after the second dose (19% higher if experienced, $P = 0.02$). Most of the participants had good humoral responses and reported few side effects after vaccination. Anti-spike IgG levels were significantly higher when adverse events after the second dose were more serious than after the first dose[157].

- *Naaber et al.* detected that fever was significantly associated with spike-receptor binding domain (S-RBD) IgG levels at 1, 6, and 12 weeks after the second dose of the COVID-19 mRNA vaccine Comirnaty (Pfizer-BioNTech)[158]. The presence and score of adverse effects were correlated with S-RBD IgG responses[158]. It has recently been shown that the occurrence of systemic effects is more frequent in vaccinated people who already have immunity compared to those who do not have immunity[158]. The mRNA vaccine-induced antibody levels were higher in subjects with more systemic side effects and the seriousness of the side effects of vaccination was proposed to be a surrogate indicator of short-term antibody responses[159]. Lower antibody levels have been reported in asymptomatic individuals infected with SARS-CoV-2, suggesting that more severe symptoms correlate with stronger antibody responses[158,160–162].
- *Levy et al.* found a significant correlation between reactogenicity and immunogenicity after adjusting for age and sex in a larger study of 831 health care workers vaccinated with the BNT162b2 mRNA vaccine[150]. Systemic adverse reactions were more common after the second dose of the vaccine, which is consistent with our results.

Similar to other mRNA vaccine trials, adverse reactions[67,68,163,164] and reactogenicity[165] were less common in older participants, as observed in our study. Anti-RBD IgG levels were higher in younger participants after the second dose[166,167]. Immunoescence and aging may explain these results[168]. Immunosenescence reduces the ability of both CD4+ and CD8+ cells to function properly, reduces the frequency of naïve T cells, expands

memory T cells, and reduces T cell diversity[169]. Aging alters the microenvironment and developmental checkpoint regulation, resulting in quantitative and qualitative changes in B-cell generation[170], as well as impaired peripheral B-cell recruitment, reduced regenerative B-cell capacity, and ultimately humoral responses. After vaccination against influenza[171,172], vaccine efficacy was reduced in the elderly.

In this study also found that **women reported more side effects** than men, even after adjusting for age and professional sector. Both registration studies of mRNA vaccines and real-life studies have shown that women have higher rates of side effects. Other vaccination studies, such as influenza and diphtheria, tetanus, and pertussis (DTP), have also shown higher reactogenicity in women[173,174].

Several studies have shown that individuals who have **recovered from COVID-19 have increased reactogenicity** following vaccination and higher titers of RBD-IgG compared to those who were vaccinated and uninfected[175,176] or infected but with mild disease[177]. Local ADRs usually result from locally immune cell activation (e.g., macrophages, dendritic and mast cells) by adjuvants or lipid nanoparticles in mRNA vaccines used to stabilize mRNA. Systemic reactogenicity is the result of the release of inflammatory mediators or products into the circulation or immune system activation by the protein used as an antigen (e.g., protein S)[177]. The latter may be deeper after the provocation of the second dose. This may explain why a correlation between immunogenicity and reactogenicity is found after the second dose with only systemic side effects, but less so with the first dose[177].

It is important to note that while these studies suggest a correlation between adverse reactions and subsequent antibody titers, causation cannot be established based on these results alone. The relationship between adverse events and antibody titers is may not always be straightforward, multifactorial, and further research is needed to fully understand the mechanisms involved.

### 2.2.4. *Vaccine campaign against COVID-19 in Hungary*

The first registered case of the Covid-19 coronavirus epidemic in Hungary was announced on March 4, 2020, by Prime Minister Viktor Orbán as the head of the Operational Group Responsible for the Protection Against the Corona Virus Epidemic, and the first patient who died was reported on March 15. On March 18, the national chief medical officer, epidemiologist Cecília Müller, already gave information that the infectious virus can be present anywhere in Hungary[178,179]. The epidemic has hit the country in three waves so far. During the first wave, which began in March 2020, the number of active cases increased until the beginning of May, exceeding 2,000, and then began to decrease continuously. This decrease lasted until the second half of July 2020, but from then on, the number first started slowly, and then started to rise rapidly from August, with the arrival of the second wave[178]. During the second wave, many more patients were identified, but this time a greater proportion of young people, for whom the disease is less dangerous, so the death rate in the second wave was much lower than in the first. The second wave began to weaken in December 2020, but when it had not completely disappeared, in mid-February 2021, the number of cases began to rise again due to the appearance of a British mutant that was much more infectious than the original virus. In this third wave, the number of people treated in the hospital broke all previous records[178] The country was one of the first European countries to start its COVID-19 vaccination campaign, with the first doses of the Pfizer-BioNTech vaccine administered on December 26, 2020[178]. The Hungarian government has prioritized vaccination as a key strategy for controlling the pandemic, and as of 19 March 2023, over 16 million vaccine doses have been administered in the country, with 63.5% of the population fully vaccinated and 69.2% having received at least one dose[180]. The Hungarian vaccination campaign initially focused on healthcare workers and the elderly, with vaccination centers established throughout the country. In addition to the Pfizer-BioNTech vaccine, Hungary has also authorized the use of vaccines from Moderna, AstraZeneca, Johnson & Johnson, and Sinopharm. The country was also one of the first in Europe to approve the use Sputnik V vaccine. The Hungarian government has taken a number of measures to encourage vaccination, including offering incentives such as discounts on certain products and services for those who get vaccinated[178]. The government has also launched a nationwide information campaign to address vaccine hesitancy and misinformation and has worked to make vaccination as convenient as possible by allowing walk-in appointments and

establishing mobile vaccination units. Despite these efforts, Hungary has experienced a surge in COVID-19 cases and hospitalizations in the fall of 2021, with a peak in January 2022[178]. However, the number of cases has declined since then, with a daily average of around 500 cases reported in April 2023[180].

## 3. AIMS

Our goal is to observe the adverse reactions following the SARS-CoV-2 mRNA vaccine, as well as to look for a correlation between the spike antibody level and the number and type of adverse reactions. Another goal of our study series is to follow the reactions observed after the homologous and heterologous booster vaccinations, as well as any correlation between the antibody levels measured after the vaccinations.

The main steps and assumptions of our investigation:

1. Correlation between SARS-CoV-2 spike protein antibody (SARS-CoV-2-S-Ig) levels in peripheral blood samples obtained by vein puncture and adverse reaction detected after the second vaccination is investigated in a half-year follow-up period. We assume that there is a correlation between the detected antibody levels and the intensity and frequency of adverse reaction.

2. Investigation of the possible relationship between the dynamics of the SARS-CoV-2-S-Ig level obtained during the analysis of peripheral blood samples and the COVID-19 infection status. Immunological changes caused by previous COVID-19 may also affect adverse reaction and detected antibody levels.

3. The SARS-CoV-2 S-Ig level of people being infected previously and of those who were not infected but vaccinated and showed adverse reaction, suggest some presence of similar phenomena in both groups, , according to which the previous COVID-19 infection and the symptomatic status after vaccination are similar can result in a humoral immune reaction.

4. Adverse reaction observed after the mRNA-based two-dose base vaccination predict the frequency and intensity of the adverse reactions noted even after the booster vaccination.

5. An adverse reaction detected after the second vaccination may affect the probability of a COVID-19 infection after the booster vaccination.

## 4. MATERIALS AND METHODS

### 4.1. Study Design and Population

Health care workers in Szigetvár Hospital were recruited for the present study. Participants were scheduled to initiate BNT162b2 mRNA (Pfizer/BioNTech, Comirnaty, Reinbek, Germany) vaccination according to the original protocol of 2 doses, which they received at the prescribed three-week interval between January 27 and May 9, 2021. In our study, both participants with SARS-CoV-2 infection and infection-free volunteers confirmed 3-5 months prior to the study by RT-PCR (reverse transcriptase-polymerase chain reaction) were taken part.

The study protocol included collection of venous blood samples for anti-SARS-CoV-2 spike protein immunoglobulin (SARS-CoV-2-S-Ig) determination at seven different time points (namely 12, 30, 60, 90, 120, 150 and 180 days following the second vaccine dose; designated Day 12, Day 30, Day 60, Day 90, Day 120, Day 150, and Day 180, respectively). Before administration of the first dose, registration of anamnestic data (high blood pressure, diabetes, hypothyroidism, autoimmune diseases, malignancy, smoking, recent flu vaccination and an inquiry allergies, age, sex, height, body weight, use of medications, including non-steroid anti-inflammatory drugs (NSAIDs), statins, antihypertensives, ACE inhibitors, beta blockers, calcium channel blockers, immunosuppressants, platelet inhibitors, steroids was performed using a questionnaire) were recorded.

Based on the presence of vaccination induced adverse reactions; (i) symptomatic (detection of an adverse reaction within 7 days after each vaccination dose) vs. (ii)

asymptomatic (no adverse reaction occurred after any dose), and according to the prior COVID-19 infection status, the following subgroups were created:

Group 1: previously COVID-19 negative and asymptomatic individuals (--)
Group 2: previously COVID-19 negative and symptomatic individuals (-+)
Group 3: previously COVID-19 positive, but asymptomatic individuals (+-)
Group 4: previously COVID-19 positive and symptomatic individuals (++)

### 4.2. Adverse Reaction Assessment

Adverse reactions were recorded immediately after the first vaccination before the administration of second dose and adverse reactions after the second vaccination session simultaneously with the first blood sampling on Day 12 follow-up visit. Adverse reactions were investigated in a questionnaire where the volunteer was required to clearly indicate if they experienced an adverse reaction within 1 week after vaccination. Volunteers had to select the symptoms they experienced within 1 week after vaccination from the following list: local pain, fatigue, fever, myalgia, arthralgia, headache, chills, nausea, lymph node swelling or other (free description).

After the booster vaccination plan became available, in the other part of our study, we again contacted the volunteer group of our study, the health care workers of the Szigetvári Hospital, to participate in our follow-up study. In accordance with the methodology used in our previous study, we collected venous blood samples before and after the third vaccination dose for anti-SARS-CoV-2 spike-immunoglobulin determination (14, 60 and 120 days after the administration of the third vaccine dose) and our volunteers recorded the adverse reactions after local and systemic booster vaccination (asymptomatic and symptomatic group) similar to what happened after primary immunization. After the administration, the type of vaccination (heterologous or homologous vaccination scheme) and the SARS-CoV-2 infection status were also recorded.

## 4.3. Measurement of Antibody Titers

For the measurements, the blood samples of the volunteers were drawn via venipuncture with a 21-gauge needle into a closed system anticoagulant-free serum separator tube (Vacuette®, Greiner Hungary LTD, Mosonmagyaróvár, Hungary). Peripheral blood samples were tested for IgG antibodies against SARS-CoV-2 spike proteins on a fully automated benchtop Access2 analyzer according to the manufacturer's instructions (Beckman Coulter Hungary LTD, Budapest, Hungary). We used the Beckman-Coulter Access SARS-CoV-2 IgG II assay (Beckman Coulter Hungary LTD) for the determination of antibodies against the SARS-CoV-2 spike protein. The test measures IgG antibodies directed to the receptor-binding domain (RBD) of the spike protein of the coronavirus.

The two-step enzyme assay is a chemiluminescent immunoassay consisting of paramagnetic particles, which is based on the semiquantitative determination of IgG antibodies against the SARS-CoV-2 virus in human serum. Briefly about the test: the sample is prepared in a reaction vessel filled with buffer and paramagnetic particles coated with recombinant SARS-CoV-2 protein. After incubation in the reaction vessel, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. Next, a monoclonal anti-human IgG alkaline phosphatase conjugate is added to the mixture, and the conjugate binds to the IgG antibodies captured on the particles. A second separation and washing step removes any unbound conjugates. A chemiluminescent substrate is added to the vessel and the light generated by the reaction is measured with a luminometer. Light production is directly proportional to the concentration of SARS-CoV-2 IgG antibody in the sample.

The number of antibodies in the sample is determined based on a multipoint calibration curve. The results are given in IU/mL, that are correlated with the First WHO International Standard Anti-SARS-CoV-2 Immunoglobulin (Human), NIBSC code, 20/136, in BAU/mL (BAU: Binding Antibody Units). The conversion of IU/mL concentrations to BAU/mL, can be done by multiplying IU/mL by multiplication factor 1. The results can be interpreted as follows: cut-off index <10 AU/mL as non-reactive and reactive >/=10 AU/mL.

## 4.4. Ethics Statement

The methodology of the tests listed above corresponds to that described in the literature and was prepared with the approval of the National Public Health Center (40576-8/2021/EÜIG). The research plan was compiled in accordance with the current legislation and the ethical guidelines of the 1975 Declaration of Helsinki. All participants provided written informed consent prior to inclusion in the present study.

## 4.5. Statistical Analysis

During the analysis of our results, the patient data were anonymized, and, after coding, the data were stored in a database accessible only to the research participants. The summary statistics of the participants were constructed based on the frequency and proportions of the categorical data, and the mean and standard deviation (SD) of the continuous variables. The statistical analysis of the collected data was performed by software version SPSS 23.0 (version 26; IBM Corporation, Armonk, NY, USA). Conformity of data to normal distribution was determined by histogram and Kolmogorov–Smirnov test. The between-group difference was calculated with $\chi 2$, Fisher's exact, Mann–Whitney U, and Kruskal–Wallis tests in line with suitability. To explore the independent predictors of S-Ig level and SARS-CoV-2 positivity after booster dose, a binary logistic regression was used. The odds ratio (OR) and 95% confidence interval 112 (95% CI) were calculated. The significance level was considered as $p < 0.05$. Data with nonparametric distribution were presented as median and interquartile range (IQR). Correlations of Ig levels with adverse reactions were tested by linear regression using Spearman correlation coefficient (R).

## 5. RESULTS

### 5.1. Study Participants

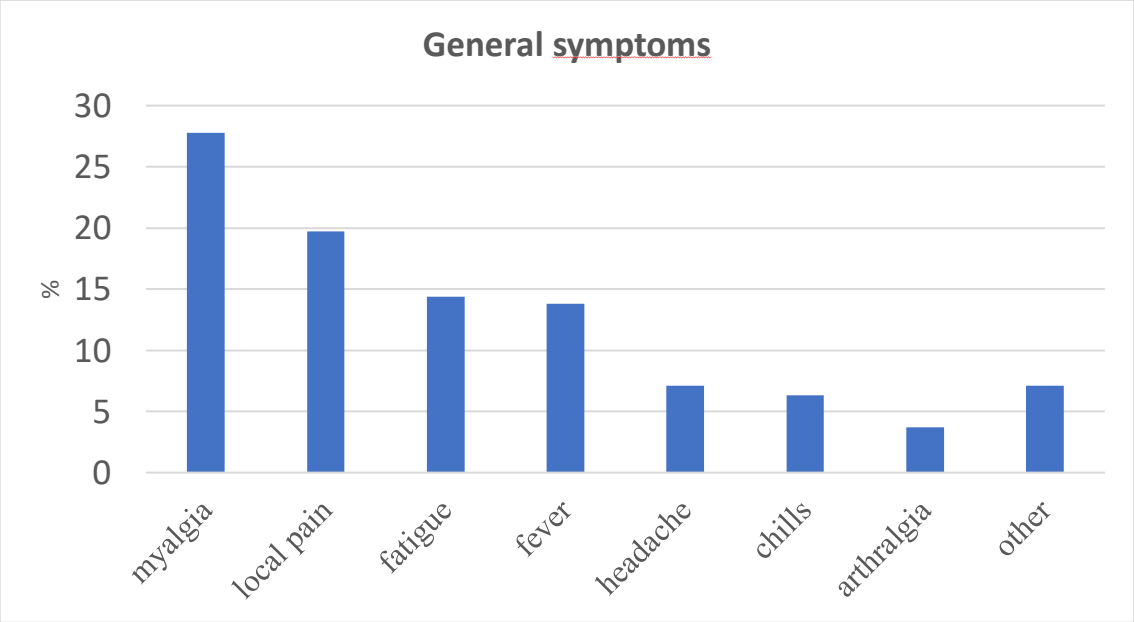
Between 10th of February and 13th of June 2021, a total of 395 people received the second dose of Pfizer-BioNTech vaccine (BNT162b2) and provided informed consent for study enrollment. From these, 383 individuals completed the questionnaire on post-vaccination ARs and gave post-vaccination blood samples at Day 12, 323 at Day 30, 320 at Day 60, 303 at Day 90, 268 at Day 120, 220 at Day 150 and 279 at Day 180. The age of the vaccinated volunteers ranged from 20 to 77 years (median 47 years; IQR 39-55). 76.7% were females and 34.7% were current smokers. A total of 169 (44.1%) subjects had at least one AR within 7 days of any vaccination (symptomatic group), and 214 (55.9%) reported no vaccine related ARs (asymptomatic group). There were significantly more patients with history of allergy in the symptomatic group. The characteristics of the participants are shown in **Table 3**.

**Table 3**. Baseline characteristics of study participants based on post-vaccination adverse event status. Data are presented as means with standard deviation or median with interquartile range as appropriate. Proportions are expressed both as numbers and percentages. A *p*-value less than 0.05 was considered statistically significant. BMI, body mass index; ACE-inhibitors, Angiotensin-converting enzyme-inhibitors; COVID-19, Coronavirus disease; NS, non-significant.

| | **Total population (N=383)** | **Asymptomatic group (N=214)** | **Symptomatic group (N=169)** | **p-value** |
|---|---|---|---|---|
| Age, (mean±SD) | 46.5±12 | 47.6±12 | 45.3±12 | NS |
| Female, (N, %) | 303 (76.7) | 159 (74.3) | 139 (82.2) | NS |
| BMI, (mean±SD) | 27.6±6 | 28.1±7 | 26.9±5 | NS |
| Smoking, (N, %) | 123 (34.7) | 73 (37.2) | 50 (31.8) | NS |
| Flu vaccination, (N, %) | 67 (17.6) | 37 (17.5) | 30 (17.9) | NS |
| Hypertension, (N, %) | 95 (26) | 55 (28.2) | 40 (23.7) | NS |
| Diabetes, (N, %) | 22 (6) | 14 (7.2) | 8 (4.7) | NS |
| Hypothyreosis, (N, %) | 25 (6.9) | 15 (7.7) | 10 (5.9) | NS |
| Autoimmune disease, (N, %) | 20 (5.5) | 10 (5.2) | 10 (5.9) | NS |
| Allergy, (N, %) | 96 (26.2) | 36 (18.3) | 60 (35.5) | <0.001 |
| ACE inhibitors, (N, %) | 63 (17.4) | 34 (17.6) | 29 (17.2) | NS |
| Beta blockers, (N, %) | 60 (16.5) | 30 (15.5) | 30 (17.8) | NS |
| Calcium channel blocker, (N, %) | 25 (6.9) | 9 (4.7) | 16 (9.5) | NS |
| Prior COVID-19 infection, (N, %) | 85 (23.2) | 47 (24) | 38 (22.5) | NS |

5.2. Adverse Reactions

ARs occurred in 125 patients after the first dose and in 131 after the second dose. The total number of ARs within 7 days after the first vaccination was 314, while 365 ARs occured within 7 days after the second dose. In 87 participants (22.7%) at least one AR occurred after both vaccinations and in 214 cases (55.9%) no ARs occurred after either dose. The most common ARs during vaccinations were myalgia (27.8%) and local pain (19.7%). A detailed description of adverse reactions is shown in **Table S1**.




| | Number of adverse reactions after 1st dose (N=314) | Number of adverse reactions after 2nd dose (N=365) | Sum of adverse reactions after each dose (N=679) |
|---|---|---|---|
| Myalgia | 77 | 112 | 189 |
| Local pain | 74 | 60 | 134 |
| Fatigue | 38 | 60 | 98 |
| Fever | 57 | 37 | 94 |
| Headache | 18 | 30 | 48 |
| Chills | 19 | 24 | 43 |
| Arthralgia | 8 | 17 | 25 |
| Other | 23 | 25 | 48 |

**Table S1.Frequency of adverse reactions after 1st and 2nd vaccination. Only symptoms that occurred immediately after vaccination and for 7 days thereafter were considered vaccination adverse reactions.**

5.3. Relationship between antibody levels, demographics and clinical variables

Age showed a negative correlation with serum antibody levels at all time points in this follow-up study (**Figure 10**.);data of Day 30, 60, 120 and 150 are not displayed). Significantly lower serum S-IgG antibody levels were observed in smoking individuals over the entire 6-month study period when compared to non-smokers (**Table S3**). Neither female gender nor BMI showed a significant association with antibody production during follow-up. A mild negative correlation was observed between antibody production and ACE inhibitor and statin use respectively, while oral contraceptive treatment was associated with higher antibody levels in the first month.

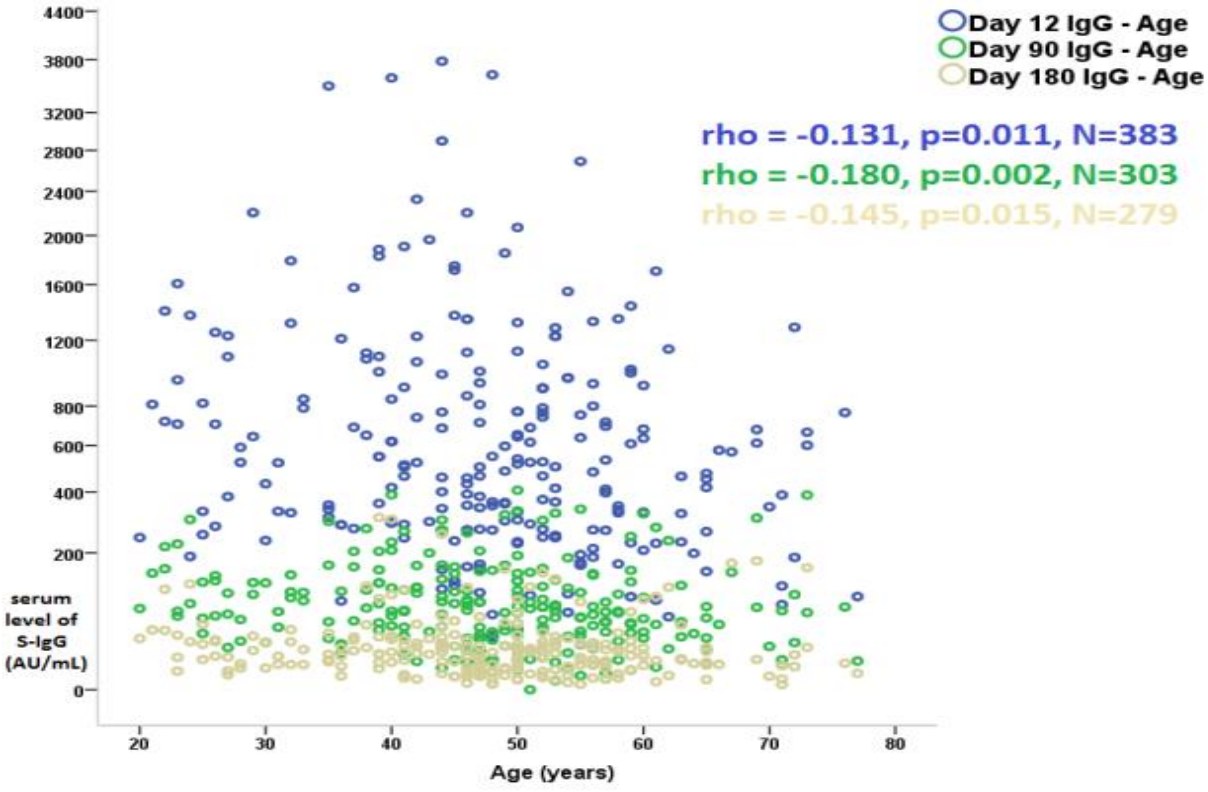



**Figure 10**. Correlation of serum level of S-IgG and age at Day 12, Day 90, and Day 180 follow-up visit after the 2nd dose of BNT162b2 mRNA (Pfizer/BioNTech, Comirnaty) vaccine. Values are Spearman correlation coefficients (rho). S-IgG; anti-spike immunoglobulin G, mRNA; messenger ribonucleic acid.

| | Day 12 IgG | Day 30 IgG | Day 60 IgG | Day 90 IgG | Day 120 IgG | Day 150 IgG | Day 180 IgG |
|---|---|---|---|---|---|---|---|
| Gender | 0.088 | 0.050 | 0.041 | 0.044 | 0.002 | 0.055 | 0.040 |
| Smoking | -0.107* | -0.134* | -0.177* | -0.142* | -0.164* | -0.091 | -0.187** |
| BMI | -0.006 | 0.016 | -0.024 | -0.092 | -0.057 | -0.018 | -0.001 |
| Autoimmunity | -0.121* | -0.119* | -0.167** | -0.138* | -0.153* | -0.122 | -0.054 |
| Allergy | 0.056 | 0.111* | 0.118* | 0.154** | 0.106 | 0.088 | 0.080 |
| ACE inhibitors | -0.126* | -0.117* | -0.112* | -0.105 | -0.087 | -0.047 | -0.065 |
| Contraceptives | 0.121* | 0.117* | 0.101 | 0.096 | 0.114 | 0.128 | 0.120 |
| Statins | -0.084 | -0.096 | -0.095 | -0.121* | -0.121* | -0.153* | -0.181** |
| Hyperlipidaemia | -0.074 | -0.085 | -0.073 | -0.092 | -0.124* | -0.151* | -0.081 |

**Table S3. Correlation of S-Ig antibody levels with demographic and clinical factors after 2nd dose of BNT162b2 vaccine manufactured by Pfizer/BioNTech, during the 6-month follow-up period. Values are Spearman correlation coefficients. *p<0.05, **p<0.01**

5.4. Relationship between antibody levels and Adverse Reactions

After the first dose fever, chills, and muscle pain showed a strong positive correlation with antibody levels during the 6-month follow-up period. However, after the 2nd dose the strongest positive correlation with antibody titer was observed for fever and chills (**Table S2**). Significantly higher serum anti-SARS-CoV-2 spike IgG antibody levels were observed at all time points of the six-month follow-up period in the symptomatic group (**Figure 11**A,B).

| | Day 12 IgM | Day 12 IgG | Day 30 IgG | Day 60 IgG | Day 90 IgG | Day 120 IgG | Day 150 IgG | Day 180 IgG |
|---|---|---|---|---|---|---|---|---|
| After 1st dose | | | | | | | | |
| Myalgia | -0.044 | 0.027 | 0.113* | 0.092 | 0.120* | 0.204** | 0.153* | 0.141* |
| Local pain | 0.030 | 0.103 | 0.105 | 0.063 | 0.102 | 0.101 | 0.091 | 0.094 |
| Fatigue | -0.061 | 0.050 | 0.027 | 0.004 | 0.041 | 0.063 | 0.058 | 0.097 |
| Fever | -0.061 | 0.047 | 0.127* | 0.091 | 0.131* | 0.216** | 0.212** | 0.185** |
| Headache | 0.028 | 0.077 | 0.113* | 0.089 | 0.115* | 0.081 | 0.080 | 0.080 |
| Chills | -0.026 | 0.124* | 0.167** | 0.178** | 0.185** | 0.215** | 0.195** | 0.232** |
| Arthralgia | -0.074 | -0.002 | -0.021 | 0.017 | -0.029 | -0.007 | -0.091 | -0.059 |
| After 2nd dose | | | | | | | | |
| Myalgia | 0.138** | 0.181** | 0.186** | 0.129* | 0.134* | 0.110 | 0.149* | 0.156* |
| Local pain | 0.023 | 0.038 | 0.041 | 0.038 | 0.067 | 0.055 | 0.026 | 0.060 |
| Fatigue | 0.076 | 0.151* | 0.130* | 0.093 | 0.077 | 0.065 | 0.060 | 0.074 |
| Fever | 0.036 | 0.196** | 0.246** | 0.236** | 0.296** | 0.305** | 0.362** | 0.311** |
| Headache | 0.073 | 0.129* | 0.174** | 0.163** | 0.187** | 0.155* | 0.165* | 0.180** |
| Chills | 0.126* | 0.217** | 0.232** | 0.236** | 0.220** | 0.201** | 0.217** | 0.184** |
| Arthralgia | -0.039 | 0.049 | 0.055 | 0.038 | -0.033 | -0.031 | -0.011 | -0.049 |

**Table S2. Correlation of S-Ig antibody levels with adverse reactions after 1st and 2nd dose of BNT162b2 vaccine manufactured by Pfizer/BioNTech, during the 6-month follow-up period. Values are Spearman correlation coefficients. *p<0.05, **p<0.01**

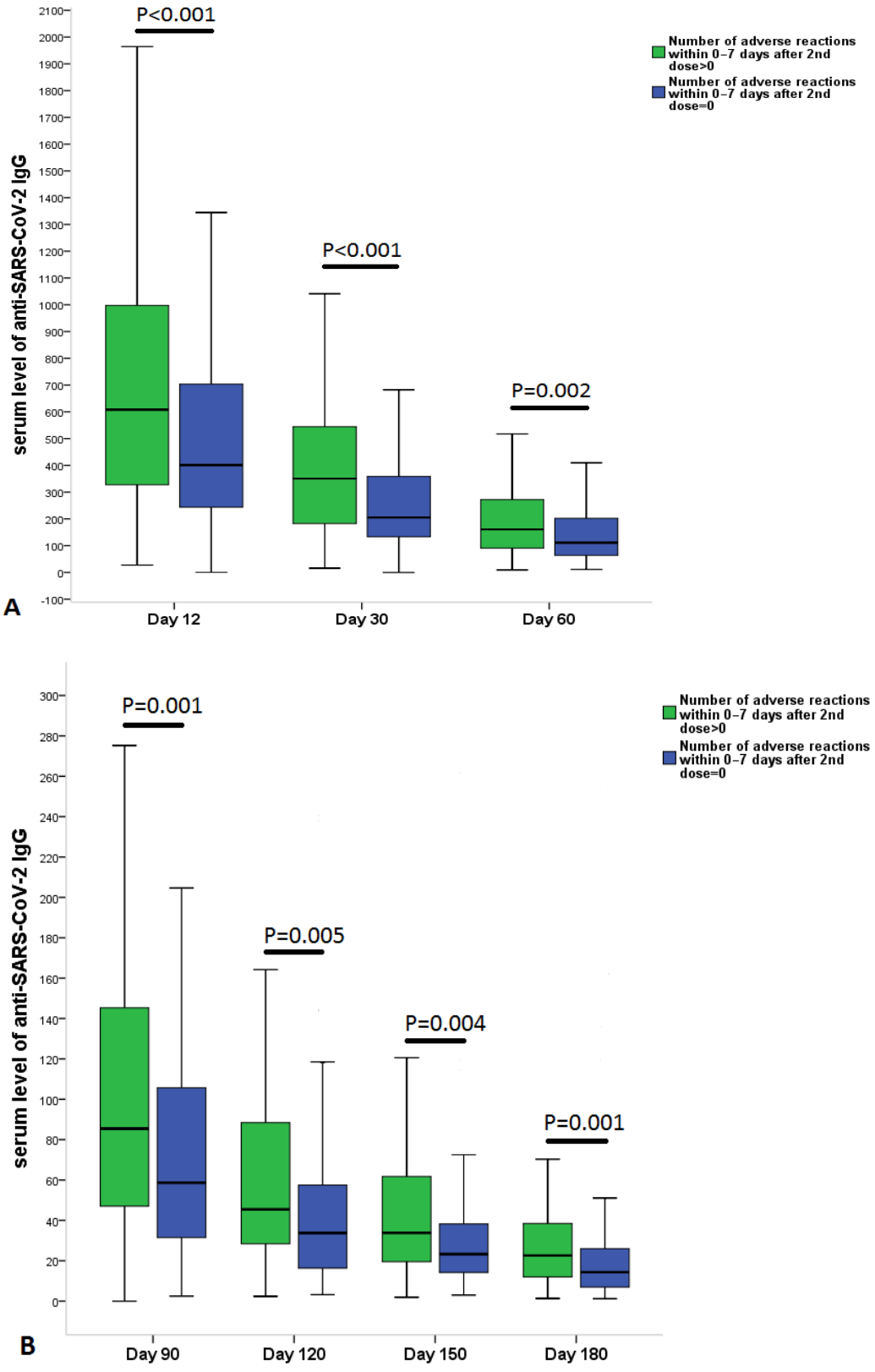
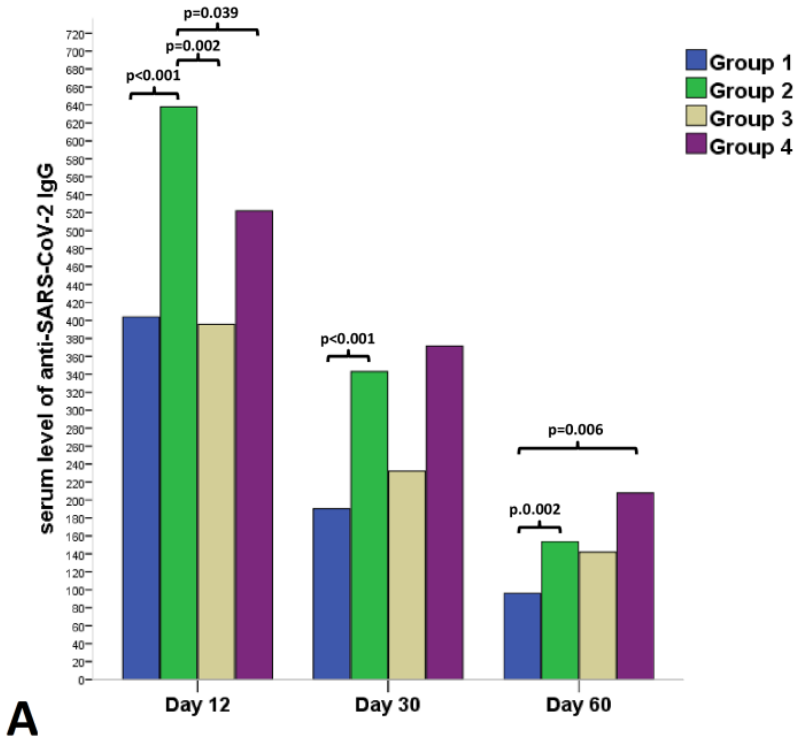



**Figure 11**. Comparison of serum level of anti-SARS-CoV-2 IgG at (A) 12, 30, 60 and (B) 90, 120, 150, 180 days after the second dose of vaccination (BNT162b2 mRNA) in patients without or with at least one adverse reaction after each vaccine dose. The data are provided as median and interquartile range. The between-group differences were calculated by the Kruskal-Wallis test.

After grouping patients according to previous COVID-19 infection and adverse reactions after vaccinations, the following results were observed in antibody levels (**Figure 12**A,B): (i) At the earliest time point at follow-up (Day 12) symptomatic COVID-19 negative patients (Group 2) had the highest antibody levels among the groups; (ii) COVID-19 negative and symptomatic patients (Group 2) had higher antibody levels during the entire 6-month follow-up period than COVID-19 negative and asymptomatic patients (Group 1) (**Figure 12**A,B); (iii) in the first 60 days (Day 12, Day 30 and Day 60) COVID-19 positive status has not led to significantly higher antibody levels in the asymptomatic group compared to COVID-19 negative individuals. This trend was reversed from Day 90, because prior COVID-19 positivity resulted in significantly higher antibody levels at 90-, 120-, 150-, and 180-day follow-up visits in the asymptomatic group. Interestingly, COVID-19 positive but asymptomatic subjects (Group 3) and COVID-19 negative but symptomatic individuals (Group 2) produced similar antibody levels over the 6-month follow-up period, except initial levels at Day 12.



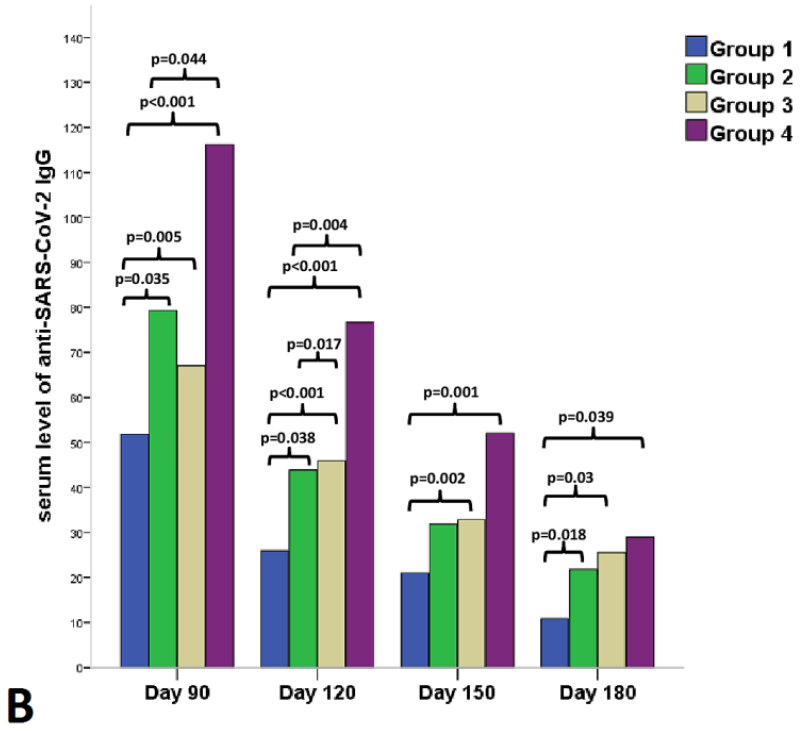



**Figure 12**. Comparison of serum levels of anti-SARS-CoV-2 IgG at (A) 12, 30, 60 and (B) 90, 120, 150, 180 days after the 2nd dose of vaccination (BNT162b2 mRNA). Healthcare workers were divided into four study groups: Group 1=individuals without prior SARS-CoV-2 infection and with no adverse reaction after vaccination; Group 2=individuals without prior SARS-CoV-2 infection and with at least one adverse reaction after vaccination; Group 3=individuals with prior SARS-CoV-2 infection and with no adverse reaction after vaccination; Group 4=those who had prior SARS-CoV-2 infection and at least one adverse reaction after vaccination. Sample size at each follow-up time point:

| | Day 12 | Day 30 | Day 60 | Day 90 | Day 120 | Day 150 | Day 180 |
|---|---|---|---|---|---|---|---|
| Group 1 | 167 | 129 | 136 | 129 | 108 | 90 | 110 |
| Group 2 | 131 | 119 | 115 | 115 | 109 | 84 | 107 |
| Group 3 | 47 | 42 | 36 | 47 | 26 | 21 | 27 |
| Group 4 | 38 | 33 | 33 | 28 | 24 | 24 | 25 |

Data are presented as medians and IQR.

**Table S4.** Results of the binary logistic regression analysis examining associations between level of S-Ig, adverse events after vaccination and demographic-clinical variables at seven time points, namely, 12 and 30, 60, 90, 120, 150 and 180 days following second vaccine doses (designated Day 12, Day 30, Day 60, Day 90, Day 120, Day 150 and Day 180, respectively). § In these binary logistic regression models, serum S- Ig levels were converted to a binary dependent variable, based on the median value of the sample (0: ≤median, 1: >median).

A. Day 12, value of S-Ig (AU/mL, median as the cutoff) §

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Gender | -0.078 | 0.264 | 0.087 | 1 | 0.768 | 0.925 | 0.551 | 1.553 |
| Chills | -0.915 | 0.610 | 2.254 | 1 | 0.133 | 0.400 | 0.121 | 1.323 |
| Myalgia, 2nd | -0.282 | 0.372 | 0.574 | 1 | 0.449 | 0.754 | 0.364 | 1.565 |
| Headache, 2nd | -0.520 | 0.503 | 1.071 | 1 | 0.301 | 0.594 | 0.222 | 1.592 |
| Age | -0.013 | 0.010 | 1.787 | 1 | 0.181 | 0.987 | 0.969 | 1.006 |
| Prior COVID + | 0.450 | 0.265 | 2.868 | 1 | 0.090 | 1.568 | 0.932 | 2.637 |
| Fever, 2nd | -1.264 | 0.496 | 6.491 | 1 | 0.011 | 0.283 | 0.107 | 0.747 |

B. Day 30, value of S-Ig (AU/mL, median as the cutoff) §

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Fever, 2nd | -1.349 | 0.535 | 6.349 | 1 | 0.012 | 0.260 | 0.091 | 0.741 |
| Smoking | 0.491 | 0.256 | 3.681 | 1 | 0.055 | 1.634 | 0.989 | 2.700 |
| Headache, 2nd | -0.372 | 0.516 | 0.520 | 1 | 0.471 | 0.689 | 0.251 | 1.895 |
| Myalgia, 2nd | -0.339 | 0.403 | 0.706 | 1 | 0.401 | 0.712 | 0.323 | 1.571 |
| Chills, 2nd | -0.954 | 0.693 | 1.896 | 1 | 0.169 | 0.385 | 0.099 | 1.498 |
| Age | -0.013 | 0.011 | 1.396 | 1 | 0.237 | 0.987 | 0.966 | 1.008 |
| Prior COVID+ | 0.073 | 0.288 | 0.064 | 1 | 0.801 | 1.075 | 0.612 | 1.890 |
| Gender | 0.179 | 0.298 | 0.362 | 1 | 0.547 | 1.196 | 0.667 | 2.145 |

C. Day 60, value of S-Ig (AU/mL, median as the cutoff) §

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Gender | 0.269 | 0.297 | 0.820 | 1 | 0.365 | 1.309 | 0.731 | 2.345 |
| Chills, 2nd | -0.268 | 0.664 | 0.162 | 1 | 0.687 | 0.765 | 0.208 | 2.812 |
| Chills, 1st | -1.029 | .618 | 2.777 | 1 | 0.096 | 0.357 | 0.106 | 1.199 |
| Myalgia, 2nd | -0.067 | 0.399 | 0.028 | 1 | 0.868 | 0.936 | 0.428 | 2.047 |
| Headache, 2nd | -0.475 | 0.528 | 0.808 | 1 | 0.369 | 0.622 | 0.221 | 1.752 |
| Age | -0.021 | 0.011 | 3.747 | 1 | 0.053 | 0.979 | 0.958 | 1.000 |
| Smoking | 0.651 | 0.258 | 6.375 | 1 | 0.012 | 1.917 | 1.157 | 3.176 |
| Prior COVID+ | -0.191 | 0.302 | 0.399 | 1 | 0.527 | 0.826 | 0.457 | 1.493 |
| Fever, 2nd | -1.372 | 0.551 | 6.188 | 1 | 0.013 | 0.254 | 0.086 | 0.748 |

D. Day 90, value of S-Ig (AU/mL, median as the cutoff) §

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Gender | 0.301 | 0.313 | 0.929 | 1 | 0.335 | 1.352 | .732 | 2.494 |
| Chills, 2nd | -0.397 | 0.758 | 0.274 | 1 | 0.601 | 0.673 | 0.152 | 2.971 |
| Chills, 1st | -1.672 | 0.820 | 4.158 | 1 | 0.041 | 0.188 | 0.038 | 0.937 |
| Headache, 2nd | -1.145 | 0.622 | 3.393 | 1 | 0.065 | 0.318 | 0.094 | 1.076 |
| Age | -0.019 | 0.012 | 2.409 | 1 | 0.121 | .981 | 0.958 | 1.005 |
| ACE-inhibitor | 0.552 | 0.371 | 2.212 | 1 | 0.137 | 1.736 | 0.839 | 3.592 |
| Smoking | 0.510 | 0.273 | 3.483 | 1 | 0.062 | 1.666 | 0.975 | 2.846 |
| Prior COVID+ | -0.352 | 0.335 | 1.109 | 1 | 0.292 | 0.703 | 0.365 | 1.354 |
| Fever, 2nd | -2.482 | 0.784 | 10.020 | 1 | 0.002 | 0.084 | 0.018 | 0.389 |

E. Day 120, value of S-Ig (AU/mL, median as the cutoff) §

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Gender | 0.234 | 0.348 | 0.451 | 1 | 0.502 | 1.263 | 0.639 | 2.499 |
| Chills, 2nd | -0.656 | 0.744 | 0.776 | 1 | 0.378 | 0.519 | 0.121 | 2.232 |
| Age | -0.037 | 0.013 | 8.557 | 1 | 0.003 | 0.964 | 0.941 | 0.988 |
| Smoking | 0.780 | 0.294 | 7.054 | 1 | 0.008 | 2.181 | 1.227 | 3.878 |
| Prior COVID+ | -1.159 | 0.378 | 9.380 | 1 | 0.002 | 0.314 | 0.150 | 0.659 |
| Fever, 2nd | -2.518 | 0.795 | 10.046 | 1 | 0.002 | 0.081 | 0.017 | 0.382 |

F. Day 150, value of S-Ig (AU/mL, median as the cutoff) §

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Gender | -0.314 | 0.392 | 0.642 | 1 | 0.423 | 0.730 | 0.339 | 1.575 |
| Chills, 2nd | -0.403 | 0.792 | 0.259 | 1 | 0.611 | 0.669 | 0.142 | 3.155 |
| Chills, 1st | -0.835 | 0.739 | 1.274 | 1 | 0.259 | 0.434 | 0.102 | 1.849 |
| Age | -0.016 | 0.013 | 1.366 | 1 | 0.243 | 0.984 | 0.959 | 1.011 |
| Headache, 2nd | -0.872 | 0.643 | 1.839 | 1 | 0.175 | 0.418 | 0.119 | 1.474 |
| Smoking | 0.318 | 0.321 | 0.985 | 1 | 0.321 | 10.375 | 0.733 | 2.577 |
| Prior COVID+ | -0.781 | 0.384 | 4.133 | 1 | 0.042 | 0.458 | 0.216 | 0.972 |
| Fever, 2nd | -2.414 | 0.781 | 9.554 | 1 | 0.002 | 0.089 | 0.019 | 0.413 |

G. Day 180, value of S-Ig (AU/mL, median as the cutoff) §

| | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Gender | 0.046 | 0.348 | 0.018 | 1 | 0.894 | 1.048 | 0.530 | 2.070 |
| Chills, 1st | -1.552 | 0.809 | 3.676 | 1 | 0.055 | 0.212 | 0.043 | 1.035 |
| Age | -0.015 | 0.012 | 1.424 | 1 | 0.233 | 0.985 | 0.962 | 1.009 |
| Myalgia, 2nd | -0.298 | 0.411 | 0.527 | 1 | 0.468 | 0.742 | 0.332 | 1.660 |
| Smoking | 0.651 | 0.284 | 5.273 | 1 | 0.022 | 1.918 | 1.100 | 3.345 |
| Priod COVID+ | -0.683 | 0.355 | 3.700 | 1 | 0.054 | 0.505 | 0.252 | 1.013 |
| Fever, 2nd | -1.632 | 0.582 | 7.852 | 1 | 0.005 | 0.196 | 0.062 | 0.612 |

A statistical analysis was run at all follow-up time point with median value of S-IgG as the outcome of interest. Based on binary logistic regression analysis, fever after 2nd dose proved to be independent predictor of median S-IgG level at all follow-up time points (**Table 4**., **Table S4**.).

**Table 4**. Serial associations among level of S-IgG, adverse events after vaccination and demographic-clinical variables at seven time points (Day 12, Day 30, Day 60, Day 90, Day 120, Day 150 and Day 180 following second vaccine dose, respectively). § In this binary logistic regression model, serum S-IgG levels were converted to a binary dependent variable based on the median value of the sample (0: ≤median, 1: >median).

| Variable | B | Odds ratio | 95% C.I. | | p-value |
|---|---|---|---|---|---|
| **Day 12, value of S-IgG (AU/mL, median as the cutoff) §** | | | | | |
| **Fever, 2nd** | -1.264 | 0.283 | 0.107 | 0.747 | 0.011 |
| **Day 30, value of S-IgG (AU/mL, median as the cutoff) §** | | | | | |
| **Fever, 2nd** | -1.349 | 0.260 | 0.091 | 0.741 | 0.012 |
| **Day 60, value of S-IgG (AU/mL, median as the cutoff) §** | | | | | |
| **Smoking** | 0.651 | 1.917 | 1.157 | 3.176 | 0.012 |
| **Fever,2nd** | -1.372 | 0.254 | 0.086 | 0.748 | 0.013 |
| **Day 90, value of S-IgG (AU/mL, median as the cutoff) §** | | | | | |
| **Chills, 1st** | -1.672 | 0.188 | 0.038 | 0.937 | 0.041 |
| **Fever,2nd** | -2.482 | 0.084 | 0.018 | 0.389 | 0.002 |
| **Day 120, value of S-IgG (AU/mL, median as the cutoff) §** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| **Age** | -0.037 | 0.964 | 0.941 | 0.988 | 0.003 |
| **Smoking** | 0.780 | 2.181 | 1.227 | 3.878 | 0.008 |
| **Prior COVID+** | -1.159 | 0.314 | 0.150 | 0.659 | 0.002 |
| **Fever, 2nd** | -2.518 | 0.081 | 0.017 | 0.382 | 0.002 |
| **Day 150, value of S-IgG (AU/mL, median as the cutoff) §** | | | | | |
| **Prior COVID+** | -0.781 | 0.458 | 0.216 | 0.972 | 0.042 |
| **Fever,2nd** | -2.414 | 0.089 | 0.019 | 0.413 | 0.002 |
| **Day 180, value of S-IgG (AU/mL, median as the cutoff) §** | | | | | |
| **Smoking** | 0.651 | 1.918 | 1.100 | 3.345 | 0.022 |
| **Fever,2nd** | -1.632 | 0.196 | 0.062 | 0.612 | 0.005 |

B, B coefficient; Odds ratio, the exponentiation of the B coefficient EXP(B); 95%CI, 95% confident interval; S-IgG, anti-spike immunoglobulin; AU, arbitrary unit; COVID-19, confirmed corona virus disease-19.

**Table S4. Results of the binary logistic regression analysis examining associations between level of S-Ig, adverse events after vaccination and demographic-clinical variables at seven time points, namely, 12 and 30, 60, 90, 120, 150 and 180 days following second vaccine doses (designated Day 12, Day 30, Day 60, Day 90, Day 120, Day 150 and Day 180, respectively). § In these binary logistic regression models, serum S- Ig levels were converted to a binary dependent variable, based on the median value of the sample (0: ≤median, 1: >median).**

5.5. Correlation between Adverse Reactions, Antibody Levels after booster vaccination

5.5.1. *Participants Characteristics*

Collectively, 218 patients were enrolled and underwent blood sampling before and after (Day 14, 60, 120) the 3rd dose of SARS-CoV-2 vaccination. The initial cohort consisted of 383 volunteers, 218 volunteers from the previous cohort were included in the present study. Reasons for dropout: discontinued study (n=101), loss to follow-up (n=25), withdrawal of consent (n=14), physician decision (n=3), dead (n=2), other (n=20). The mean age was 47.6 years, with a prevalence of females (79%). The time difference between the 2nd and 3rd vaccine doses was 249±44 days. 35% (N=77) of participants experienced adverse reactions following the 3rd vaccination. 28% (N=62) of the participants in the study experienced SARS-CoV-2 infection before the 3rd dose, 25% (N=54) of them became positive after the 3rd dose. The median serum SARS CoV-2 spike

Ig level was 72 AU/mL (IQR: 36-135) before the 3rd dose, 639 (424-1100) at 14 days, 413 (215-742) at 60 days, and 268 (128-594) at 120 days after the third dose. In the symptomatic group, the proportion of volunteers receiving the heterologous booster vaccine was significantly higher (16% vs. 3%, p=0.002). The serum SARS-CoV-2 spike Ig level decreases rapidly after a homologous booster dose, after a 14-day peak, while a gradual increase in the antibody level can be seen after a heterologous booster, **Figure 13**. Patient characteristics are outlined in **Table 5**.

**Table 5**. Characteristics of study population. The patients considered symptomatic if experienced adverse reactions within 7 days after vaccination vs. asymptomatic if no adverse reaction occurred after vaccination.

| | Asymptomatic (N=141) | Symptomatic (N=77) | p-value |
|---|---|---|---|
| Age, (mean±SD) | 49.6±11 | 44±11 | 0.001 |
| Female, (N, %) | 116 (82%) | 57 (74%) | 0.151 |
| BMI, (mean±SD) | 28±7 | 27±5 | 0.474 |
| Smoking, (N, %) | 45 (33%) | 23 (32%) | 0.947 |
| Time lag between 2nd and 3rd vaccine dose, days, (mean±SD) | 250±45 | 247±41 | 0.827 |
| Pfizer BioNTech vaccine, 3rd dose, (N, %) | 136 (97%) | 65 (84%) | 0.002 |
| Hypertension, (N, %) | 40 (29%) | 17 (23%) | 0.359 |
| Diabetes, type II, (N,%) | 10 (7%) | 5 (7%) | 0.904 |
| Allergy, (N, %) | 32 (23%) | 28 (38%) | 0.022 |
| Autoimmune disease, (N, %) | 10 (7%) | 3 (4%) | 0.361 |
| Symptomatic after 1st dose, (N, %) | 43 (31%) | 43 (56%) | <0.001 |
| Symptomatic after 2nd dose, (N, %) | 40 (28%) | 45 (58%) | <0.001 |
| SARS-CoV-2 positivity before 3rd dose, (N, %) | 45 (32%) | 17 (22%) | 0.124 |
| SARS-CoV-2 positivity after 3rd dose, (N, %) | 31 (22%) | 23 (30%) | 0.197 |

Abbreviations: BMI, body mass index; SD, standard deviation; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; N, number.

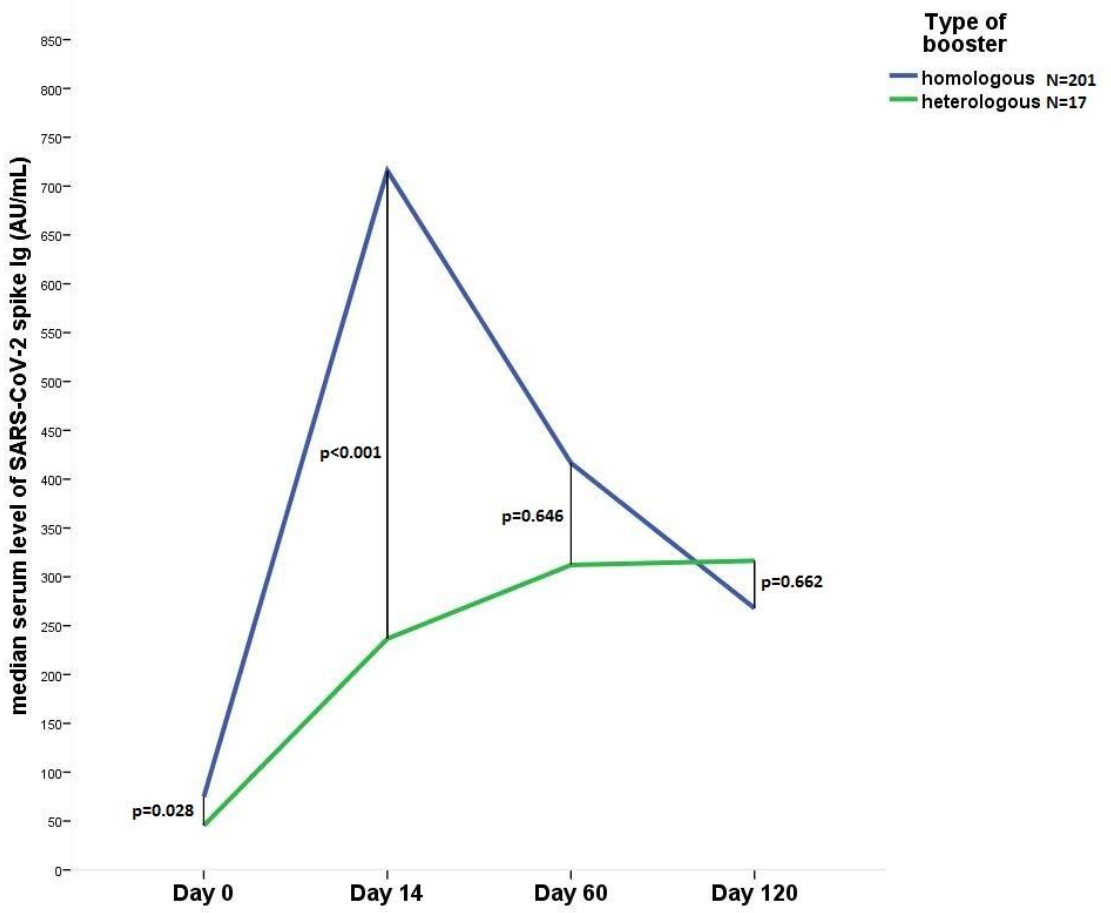



**Figure 13**. Line diagram shows the change in the median anti SARS-CoV-2 IgG level 14, 60 and 120 days after heterologous (N=17) or homologous (N=201) booster vaccination. Day 0, immediately before 3rd dose.

5.5.2. *Frequency of Adverse Reactions after 1st, 2nd and 3rd Dose of Vaccination*

Adverse reactions occurred in 88/218 patients after the first vaccination, 87/218 after the 2nd vaccination, and 77/218 patients after the 3rd vaccination within 7 days after the dose. The total number of adverse reactions was 234 (1st dose), 252 (2nd dose) and 284 (3rd dose) after each vaccination. The frequency of fever (N, %) was 27 (13%), 22 (11%) and 36 (17%) after the 1st, 2nd, and 3rd vaccinations respectively.

The most common adverse reaction following the 2nd vaccination was local pain (47%), limb pain (47%), myalgia (36%) and fever (25%) while after the 3rd vaccination local pain was observed in 47%, limb pain in 47%, fever in 46% and chills in 34%. Fever was significantly more frequent after the 3rd vaccination than after the 2nd dose, $p<0.05$.

5.5.3. *Correlation of antibody titers with adverse reactions after the vaccinations*

The serum median SARS-CoV-2 Spike IgG level was significantly higher in the symptomatic group than in the asymptomatic group at all three time points after the second vaccination (Day 14, 60 and 120) **Figure 14A**.. After the 3rd vaccination, this correlation disappeared and we did not detect any significant difference between the serum SARS-CoV-2 spike Ig levels of the two groups **Figure 14B**.. If only the serum levels of the volunteers receiving the homologous booster vaccine (N=201) are examined, slightly significantly higher serum levels were observed in the symptomatic patients for the levels measured on days 14, 60 and 120 ($p=0.035$, $p=0.049$ and $p=0.170$, respectively). In the case of volunteers who received a heterologous booster dose (N=17), there was no difference in serum antibody levels between the symptomatic and the asymptomatic group during the studied period. In the case of fever appearing within 7 days after vaccination, significantly higher serum levels were found in the group with fever after both vaccinations, although the correlation is weaker after the 3rd vaccination, **Table 6**.. We examined the correlations of serum S-IgG levels measured at 4 time points (Day 0, 14, 60 and 120) with demographic and clinical parameters. The Spearman r coefficient of correlation between all these parameters is presented in **Table 7**..

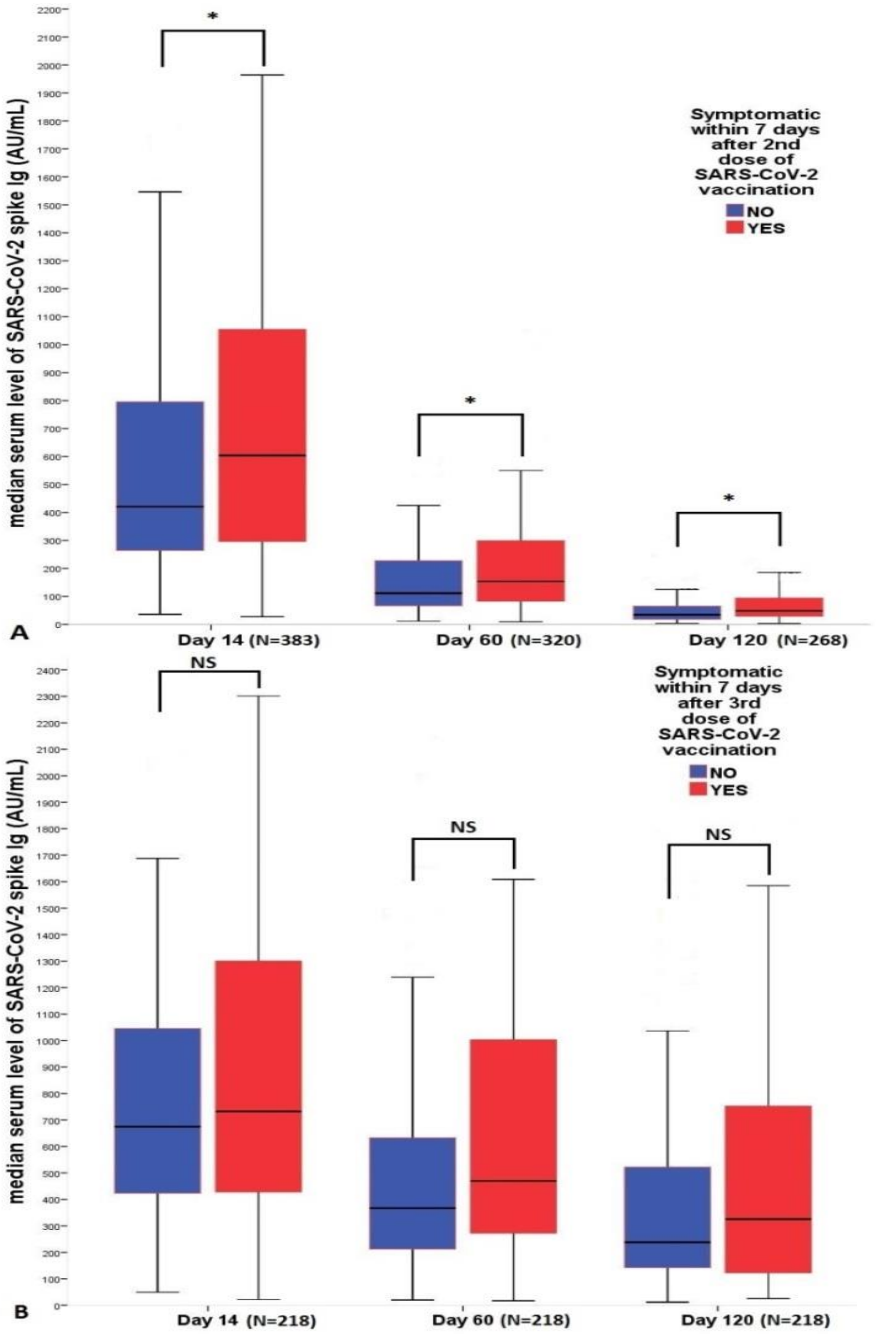



**Figure 14**. Correlation of antibody titers with symptomatic status after 2nd and 3rd vaccinations. (A) Antibody response of symptomatic versus non-symptomatic patients at 14 (N=383), 60 (n=320) and 120 (N=268) days after the 2nd vaccination, (B) after the 3rd dose (N=218) at all time points. Definition of a symptomatic individual: a local or systematic reaction occurring within 7 days after vaccination. Statistical analysis was performed using Mann-Whitney-U test in each group

respectively. NS, non-significant; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. * Indicates p<0.05.

| Fever | Minimum | 25% | Median | 75% | Maximum | *p*-Value |
|---|---|---|---|---|---|---|
| After 2nd dose | | | | | | |
| Day 14 (N=383) | | | | | | |
| **+ (N=56)** | 266 | 689 | 986 | 1402 | 7785 | <0.001 |
| **- (N=327)** | 27 | 262 | 442 | 810 | 655 | |
| Day 60 (N=320) | | | | | | |
| **+ (N=49)** | 96 | 164 | 274 | 457 | 998 | <0.001 |
| **- (N=271)** | 9 | 70 | 123 | 237 | 655 | |
| Day 120 (N=268) | | | | | | |
| **+ (N=45)** | 28 | 49 | 76 | 148 | 251 | <0.001 |
| **- (N=223)** | 2 | 19 | 36 | 68 | 379 | |
| After 3rd dose | | | | | | |
| Day 14 (N=218) | | | | | | |
| **+ (N=41)** | 47 | 388 | 955 | 1570 | 3209 | 0.045 |
| **- (N=177)** | 22 | 425 | 663 | 1014 | 5948 | |
| Day 60 (N=218) | | | | | | |
| **+ (N=41)** | 107 | 331 | 790 | 1190 | 4117 | 0.002 |
| **- (N=177)** | 17 | 208 | 379 | 670 | 7101 | |
| Day 120 (N=218) | | | | | | |
| **+ (N=41)** | 56 | 260 | 494 | 815 | 3005 | 0.014 |
| **- (N=177)** | 12 | 124 | 240 | 541 | 2706 | |

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ig, immunoglobulin.

**Table 6**. Changes in serum SARS-CoV-2 spike Ig levels on Days 14, 60 and 120 after the 2nd and 3rd vaccination, depending on whether fever occurred within 7 days after the vaccination. Number of patients with fever (N,%) after 3rd dose: 41/218 (19%).

| Variable | Day 0 S-IgG | Day 14 S-IgG | Day 60 S-IgG | Day 120 S-IgG |
|---|---|---|---|---|
| **Age** | -0.190** | -0.030 | -0.028 | -0.089 |
| **Smoking** | -0.163* | -0.050 | -0.128 | -0.080 |
| **Gender** | 0.013 | 0.158* | 0.134* | 0.149* |
| **mRNS type vaccine** | -0.149* | -0.317** | -0.032 | 0.031 |
| **COVID+ before 1st dose** | 0.293** | 0.004 | -0.024 | -0.042 |
| **COVID+between 2nd and 3rd dose** | 0.144* | 0.174* | 0.204** | 0.116 |
| **NSAID** | -0.163* | -0.140* | -0.115 | -0.145* |
| **Hyperlipidaemia** | -0.138* | -0.166* | -0.157* | -0.082 |
| **Chills after 2nd dose** | 0.209** | 0.189** | 0.138 | 0.091 |
| **Fever after 2nd dose** | 0.281** | 0.261** | 0.226** | 0.172** |
| **Chills after 3rd dose** | N/A | 0.138* | 0.203** | 0.143 |
| **Fever after 3rd dose** | N/A | 0.145* | 0.262** | 0.189** |
| **Use of beta blocker** | -0.169* | -0.054 | -0.123 | -0.019 |
| **Time lag between 2nd and 3rd dose** | 0.097 | 0.012 | 0.118 | 0.216** |

**Table 7**. Variables associated with levels of S-IgG (AU/mL) in cross-sectional analysis. Day 0, S-IgG measurements immediately before 3rd dose; Day 14, Day 60, and Day 120 S-IgG, S-IgG measurements on 14, 60 and 120 days after 3rd dose. Values are Spearman correlation coefficients. ∗P<0.05, ∗∗P<0.001. S-IgG, anti-spike immunoglobulin; AU, arbitrary unit; COVID+, confirmed corona virus disease-19, mRNS, messenger ribonucleic acid.

5.5.4. *Factors related to SARS-CoV-2 positivity after the 3rd vaccination*

SARS-CoV-2 serum spike Ig levels measured before the 3rd dose and 14 days after the 3rd dose were significantly lower in patients who became COVID-19 positive within 120 days after the 3rd dose (before 3rd dose, COVID+: 64 [26-94] vs. COVID-: 79 [38-143], p=0.036, 14 days after 3rd dose, COVID+: 527 [392-778] vs. 734 [461-1203], p=0.015). COVID-19 positivity after 2nd dose and before 3rd dose (OR=2.65; 95%CI=1.05-6.69, p=0.039) was independently associated with COVID-19 positivity within 120 days after booster vaccination, while fever after the 2nd dose (OR=0.14; 95%CI 0.02-0.82; p=0.028) was found to be independently associated with a reduction in the likelihood of COVID-19 positivity within 120 days after the booster dose. The SARS-CoV-2 spike Ig level immediately before the 3rd dose or 14 days after the 3rd vaccination did not prove to be an independent predictor of subsequent COVID positivity.

## 6. DISCUSSION

In this prospective, single-center follow-up study serum anti-SARS-CoV-2 spike Ig antibody levels were serially recorded in healthcare workers at 12, 30, 60, 90, 120, 150, and 180 days after the 2nd dose of BNT162b2 vaccine. The key results of this study are the following: (i) on day 12 after administration of the 2nd dose, volunteers with at least one vaccine related adverse reaction (symptomatic group) had the highest S-IgG antibody levels, regardless of prior COVID-19 status; (ii) significantly higher S-IgG levels were observed in the symptomatic group of subjects without prior COVID-19 infection when compared to the asymptomatic group throughout the entire follow-up period; (iii) in the asymptomatic groups prior COVID-19 positivity (Group 3) resulted in higher S-IgG levels from only Day 90 of the follow-up period compared to Group 1; (iv) prior COVID-19 disease with asymptomatic status (Group 3) and symptomatic status without prior COVID-19 (Group 2) infection resulted in nearly identical, not significantly different S-IgG antibody levels; (v) fever after the 2nd dose was independently associated with higher median S-IgG level at all follow-up time points.

In our study, we observed significantly lower serum S-IgG antibody titers in older individuals, which is consistent with results previously reported in the literature[148,181]. A previous study demonstrating that aging decreased antibody response among COVID-19 patients and the fact that aged people demonstrated weaker immunologic responses[148].

Coordination of SARS-CoV-2 antigen-specific responses was disrupted in individuals ≥ 65 years old, resulting in an uncontrolled response between CD4 + and CD8 + cells and antibody production that can lead to failure of disease control[181]. Significantly lower serum antibody levels were observed in smoking subjects over the entire 6-month study period when compared to non-smokers. In addition, smoking status was an independent predictor of the median S-IgG level at Day 60, 120 and 180 follow-up visits. However, we have no information on the proportion of seroconversion among smoking and non-smoking volunteers. There is more evidence that smoking lowers serum IgG levels. Smoking was associated with a decrease in serum IgG levels in a small case-control

study[182]. In a larger study of 1,787 patients, it was found that cigarette smoking was associated with reduced IgG median concentrations[183]. There are several explanations for the effect of smoking on the humoral immune response. These might include direct effects on B cells and indirect effects on T cells and antigen-presenting cells, which could affect Ig class switching and/or differential survival of naive B cells or memory B cells[184]. Activity of nicotinic acetylcholine receptors can suppress B-cell activation in response to antigenic challenge[185]. In smokers, we observed significant negative correlation with antibody response to vaccination for a minimum of six months, suggesting that smoking affects the immunogenicity of vaccines in our cohort.

In this study we did not observe a significant difference between genders in terms of antibody response however, the majority of participants were female. Several mechanisms can cause a different antibody response between males and females such as hormonal, genetic, and microbiota differences[186]. Growing body of data provide evidence that sex-specific effects may lead to different outcomes of vaccine safety and efficacy[187]. Therefore, it would be important that sex-based differences were to be considered and investigated in pre-clinical and clinical trials.

In our study, systemic events such as chills and fever showed a strong correlation with subsequent antibody response against SARS-CoV-2 spike protein. Besides, fever after the 2nd dose proved to be an independent predictor of median S-IgG level at all follow-up time points. *Naaber et al.* found that fever was significantly associated with the spike-receptor binding domain (S-RBD) IgG levels at 1, 6 and 12 weeks after second dose of COVID-19 mRNA Comirnaty (Pfizer-BioNTech) vaccine[158]. The importance of body temperature elevation in an adequate immune response was previously highlighted. Physiological temperature change like fever acts to regulate the emergence of new immune responses but does not restrict the activity of existing effector mechanisms once they have been formed[188]. There is a growing body of evidence suggesting that febrile temperatures boost the effectiveness of the immune response during infections by stimulating both the innate and adaptive arms of the immune system[189]. This previous evidence and our results both confirm that the attenuation and elimination of fever in any

form (such as the use of NSAIDs) at the beginning of the immune response may adversely affect the immune process, even in the long run.

*Coggins et al.* found no correlation between symptom severity following the first or second vaccine doses and IgG reactivity with spike protein, but at the same time a significant correlation was observed with duration of symptoms after the second shot of vaccination and anti-spike IgG titers[147]. *Müller et al.* found that there was not any general correlation between vaccination-induced SARS-CoV-2 spike-specific IgG or neutralizing antibody production and the presence or absence of individual post-vaccination reaction reports[190]. In contrast, a study with the H1N1 vaccine found that titers were 60% higher in children with fever ≥38°C after vaccination, suggesting an enhanced immune response in those who had side effects after vaccination[149]. During the examination of hospital workers who received a prime-boost vaccination with BNT162b2, only a weak but existing correlation was found between the ARs and SARS-CoV-2 antibody levels[191]. In contrast, *Hwang et al.* concluded after vaccination of 135 healthy individuals with either AZD1222 (AstraZeneca) or BNT162b2 (Pfizer/BioNTech) that the local and systemic reactogenicity may not be associated with humoral immunogenicity[192]. However, in two recent studies a clear correlation was found between systemic adverse events including fever and antibody titer following COVID-19 vaccination, which is also consistent with our results[148,153]. The literature on the relationship between reactogenicity and immunogenicity of vaccines is limited and contradictory. The inconsistent results shown in the studies are difficult to explain. One possible explanation is that there is no information about the medications taken before and after vaccination, especially regarding the use of NSAIDs. Two (consecutive, randomised controlled, open-label) vaccination studies provided evidence that after vaccination of infants with a ten-valent pneumococcal non-typeable Haemophilus influenzae protein D-conjugate vaccine (PHiD-CV) co-administered with the hexavalent diphtheria-tetanus-3- component acellular pertussis-hepatitis B-inactivated poliovirus types 1, 2, and 3-H influenzae type b (DTPa-HBVIPV/ Hib) and oral human rotavirus vaccines, antibody concentration was significantly lower in the group receiving prophylactic paracetamol than in the group not receiving it[193]. Thus, it is hypothesized that the use of regular or occasional analgesic

NSAIDs (e.g., paracetamol) in the peri-vaccination period may affect the production of antibodies. This assumption is supported by several previous evidence. *Bancos et al* reported that a panel of widely used NSAIDs blunts antibody synthesis in human peripheral blood mononuclear cells and in purified B cells[194], and it reduces antibody synthesis which may negatively affect the post-vaccination immune response. NSAIDs suppress T-cell activation by inhibiting p38 MAPK induction, thus the immunosuppressant activity of NSAID on T-cells underlines the role of COX activity in the normal process of lymphocyte activation[195]. *Ryan at al.* found evidence that NSAIDs and the new Cox-2-selective drugs negatively affect B-cell function and attenuate antibody production in humans[196]. NSAIDs are one of the most commonly used drugs; they are recommended for all age categories, are prescribed for relieving transient pain, therefore, their uncontrolled use might affect post-vaccination side effects and may alter the humoral immune response to antigen stimuli. However, the mechanism by which antipyretic analgesics reduce antibody response remains unclear and not fully explained by COX enzyme inhibition, and the involvement of nuclear and subcellular signaling pathways also arises[197]. More detailed immunological studies are needed to accurately determine the effect of NSAIDs or other antipyretic or analgesic drugs on the vaccine-induced immune response.

Overall, this evidence may explain the contradictory results in the literature between post-vaccination adverse effects and antibody production.

*Morales et al.* provided evidence that a single dose of the BNT162b2 vaccine could be sufficient to confer a similar immunization in those patients with previous history of COVID-19 (individuals vaccinated at least 3–5 months after SARS-CoV-2 infection)[198]. This hypothesis was supported by other's work[199]. In a cohort of 1,025 individuals, a steeper slope of decline for IgG and neutralizing antibodies was found in vaccinated individuals without previous COVID-19 infection compared to those with previous COVID-19 infection[200]. IgG antibodies in most patients with COVID-19 can last for at least 12 months after discharge and the IgG titers decreased significantly in the first 6 months and remained stable in the following 6 months[201]. These results support the findings of our study that previous COVID-19 infection compensates for the decrease in

antibody levels following vaccination, an effect that occurs primarily in the late phase beyond 90 days.

In a recent systematic review and meta-analysis, significantly more ARs were reported in vaccine groups compared with placebo groups after COVID-19 vaccination trials, but the rates of reported ARs in the placebo arms were still substantial[202]. The result of this work is remarkable; however, the study did not examine the rate of seroconversion or subsequent antibody response in the placebo group and the vaccine group. In addition, the correlations observed in our study (correlation between post-vaccination fever and antibody titer) showed a robust association for 6 months.

The strength of our results is given by the relatively large number of volunteers and the long follow-up period. A significant limitation of our study is that the fact and frequency of NSAID use after vaccination were not recorded in our study questionnaire. Thus, their potential effect on the association between antibody production and adverse events after vaccination cannot be established. Furthermore, the male population was underrepresented and conclusions on gender differences in vaccine response is limited. Some volunteers missed follow-up dates, which reduced the number of participants at consecutive follow-up times, but the statistical power of our results remained strong. To accurately report adverse reactions, volunteers kept a diary. Using this will help reduce the possibility of recall bias, but it cannot be completely ruled out.

-- Our previous study showed that adverse reactions after the second vaccination against SARS-CoV-2 are associated with a significantly higher serum SARS-CoV-2 spike Ig level for up to 6 months[39]. Other studies have also shown that specific IgG titers after basic BNT162b2 vaccination (2 doses) showed a positive correlation with the occurrence of adverse reactions[150,203]. In contrast, in the present study we demonstrated that (i), the booster dose in the same cohort no longer shows any correlation between the appearance of adverse reactions and the subsequent antibody level, except a positive correlation with fever. However, this correlation is significantly weaker than observed after the second dose.

Other important findings of our study are the following: (ii) Volunteers in the symptomatic group were younger, (iii) there were more symptomatic patients in the group

receiving the heterologous booster vaccine, (iv) those who were symptomatic after the 1st and 2nd dose were more likely to be symptomatic after the 3rd vaccination as well, and (v) fever after 2nd dose was independently associated with a reduction in the likelihood of COVID-19 positivity after booster dose.

The adverse reactions after the booster vaccination were similar to those after the second dose according to recently published studies[204,205]. In our study, there was no difference between the adverse reactions observed after the 2nd and 3rd vaccination, although fever occurred more often after the 3rd dose. Next, the volunteers were grouped based on the booster vaccination into heterologous and homologous groups. After analysis, we did not find correlation between subsequent antibody levels and adverse reactions, although there were more symptomatic volunteers in the heterologous group. Previous studies have found a clear association between adverse events and subsequent antibody levels when using the basic homologous vaccination regime containing 2 doses[39,150,203]. After the 2nd COVID-19 vaccination, the number of memory B cells increased, and the number of memory T cells gradually decreased. However, with the third dose of the vaccine, the level of memory B cells continued to increase, while the levels of neutralizing antibodies and memory T cells returned to the level after the second vaccine administration[206]. mRNA COVID-19 vaccines generated functional memory B cells that increased from 3 to 6 months post-vaccination and further induced antigen-specific $CD4^+$ and $CD8^+$ T cells, and early $CD4^+$ T cell responses[207]. Booster vaccinations could lead to a rebound in immune response against SARS-CoV-2 variants compared to a two-dose vaccination schedule. The broad responses may be due to the co-evolution of B cells in response to different variants, including SHM and memory B cell clonal turnover[208]. In a large cohort, heterologous boosters showed higher vaccine effectiveness than a homologous booster for laboratory-confirmed COVID-19 cases, hospitalization, admission to the ICU, and death, although there are no data on the distribution of adverse reactions[209]. A heterologous booster immunization strategy, primarily due to the differences in T-cell response, provides an immune response that may prove beneficial for long-term prevention[210]. Based on our results, our assumption is that the heterologous booster vaccine induces also induces additional immune mechanisms in the individual, which do

not result in adverse reactions of the same strength and severity as in the case of the homologous vaccine. In contrast to the basic vaccination scheme, heterologous booster vaccines were also used in our study. Although the 3rd vaccine was heterologous in only a few patients in the present study, it could have still affected the positive correlation between antibody levels and side effects, which we also described earlier in the case of the basic vaccination scheme[39].

Although the difference in the increase of immunoglobulin levels between patients with and without fever is smaller after the third vaccination than after the second vaccination in our study, the booster effect of the vaccine remains evident: it triggered better sustained and higher levels of immunoglobulin compared to those observed following the second vaccination dose. While the second vaccination is part of the basic immunization, where the goal is to form immune memory and teach the immune system to respond to the spike protein, the booster dose is to strengthen the process, making these mechanisms permanent. Overall, it is likely that the different immune activation caused by the heterologous immunization is also associated with different adverse reactions, this could be one of the likely mechanisms why the adverse reactions after the 3rd vaccination did not show a correlation with the later antibody response. At the same time, the low number of cases might also explain why we were unable to demonstrate the clear correlation between adverse events and antibody levels that we observed in our previously examined population[39]. In addition, in contrast to the basic homologous vaccination strategy the booster vaccination also featured a heterologous version, which despite the low number of cases, may have influenced the number and quality of adverse reactions, given the different immunological mechanisms. A further study involving a higher number of volunteers would help to correctly interpret the differences between the quality and quantity of side effects and their correlation with subsequent antibody levels following the primary vaccination schedule and the booster vaccine that we observed in our current cohort of patients. Similar to a previous study[67], we also observed that systemic adverse reactions were reported more often by the younger volunteers than the older ones. This can be explained by the reduced strength of the inflammatory response in the elderly exposed to immune stress[211]. Another potential mechanism of this phenomenon is that

older people show a greater tolerance for pain and disease symptoms acquired through life[212]. We found that after the heterologous booster vaccination, there were more symptomatic patients than after the homologous 3rd dose, which corresponds to what is described in the literature[166,213]. According to a recent study, spike-specific CD8+ T-cell levels after heterologous vaccination were significantly higher than after homologous regimens[214]. Peripheral blood CD8+ T cells can be useful predictive markers of adverse events associated with the immune system during lung cancer therapy. The incidence of AEs was higher in the high CD8+ T cells group[215]. Another study also confirms that the severity of adverse reactions is associated with CD4+ and CD8+ T-cell response in mRNA-1273 vaccinated health care workers[216]. Based on these results, it is reasonable to assume that the immune processes triggered by the heterologous vaccine, which are different compared to those elicited by the homologous version, may be responsible for the more frequent adverse reactions.

Based on our data, it is evident that among the volunteers who experienced adverse reactions after the 3rd vaccination, there were significantly more patients who were also symptomatic after the 1st or 2nd dose. Our results are consistent with a recent study which found that adverse events after the second dose of the COVID-19 vaccine were predictors of more intense systemic adverse events notified within 7 days after booster dose[217]. Finally, we found that fever after the 2nd dose was independently associated with a reduction in the likelihood of COVID-19 positivity after a booster dose. This can presumably be explained by the fact that fever following the second vaccination induces a stronger humoral immune response and the protective effect of the consequent higher antibody level prevails even after the booster vaccination.

Our study has some limitations. The number of patients receiving a heterologous booster dose is much lower than the number of patients receiving homologous vaccination, which may affect the strength of the statistical results. The follow-up period was only 3 months; a longer observation period would have allowed a better follow-up of the change in antibody levels of the respective groups. We only observed the humoral immune response and not the cellular immune response, so we can only explain the immunological changes that took place after the 3rd vaccination to a limited extent. At the same time, the strength

of our study is that it points out that the type of booster vaccination (heterologous vs. homologous) probably causes different immune activation, which can also be inferred from the different vaccination reactions.

## 7. FURTHER AIMS AND PERSPECTIVES

It is theoretically possible to observe the antibody levels in the case of post-vaccination fever even with the use of non-steroidal anti-inflammatory drugs, thereby studying their effect on the immune response. Large, prospective studies are needed to fully explore the effect of post-vaccination fever on the developing immune response.

## 8. CONCLUSION

The overall aim of this thesis was to draw general conclusions that can essentially influence the assessment of adverse effects following the administration of vaccines, especially vaccines against SARS-CoV-2. According to our current knowledge, adverse reactions are the adequate reaction of the immune system to a foreign antigenic stimulus, the strength and pattern of which can also affect the humoral immune response.

As a novelty, we investigated the correlation of fever with antibody levels during prime and boost vaccination. The major findings were the following:

(i) After primary immunization against SARS-CoV-2, serum median S-IgG level measured at all follow-up time points during the examined period (6 months) was significantly higher in the symptomatic group (fever!) compared with asymptomatic.

(ii) Lower S-IgG levels were detected in older individuals, which correlates with the results reported in the literature. Several studies have demonstrated that aged people produce a weaker immunological response.

(iii) Significantly lower serum antibody levels were observed in smoking subjects over the entire 6-month study period compared with non-smokers. There are several evidence for the negativ effect of smoking on the humoral immune response.

(iv) In our study we did not detect a significant difference between genders in the antibody response, which could be caused by several mechanisms.

(v) In this study, systemic events such as chills and fever were strongly correlated with the subsequent antibody response to SARS-CoV-2 spike protein. In addition,

fever occurring after the second dose proved to be an independent predictor of median S-IgG level at the follow-up times.

We were also able to show that:

(vi) The correlation between the appearance of adverse events and the subsequent antibody level disappeared in the same cohort following the booster vaccination, except in the case of fever.

(vii) The volunteers in the symptomatic groups were younger.

(viii) There were more symptomatic individuals in the heterologous booster group.

(ix) Those who reported some kind of adverse reaction after the first and second dose were more likely to be symptomatic after the 3rd vaccination as well. The type of booster vaccination (heterologous or homologous) theoretically causes different immune activation, which can also be supposed from the different vaccination reactions.

(x) Fever after the second dose was independently associated with a reduced likelihood of COVID-19 positivity after the booster dose.

Several factors have an impact on antibody levels after SARS-CoV-2 vaccination including age, smoking status, prior COVID-19 positivity, and adverse reactions after each dose of vaccines. Fever was associated with higher median S-IgG level during a 6-month follow-up period. These results may convince those who refuse vaccination due to fear of vaccination reactions. In addition, an individual approach that takes all factors influencing antibody levels into account might be useful when developing a vaccination strategy. Large, prospective studies are needed to fully explore the effect of post-vaccination fever on the developing immune response.

## 9. LIST OF PUBLICATIONS

### Publications related to the thesis:

**Kanizsai A**, Molnar T, Varnai R, Zavori L, Tőkés-Füzesi M, Szalai Z, Berecz J, Csecsei P. Fever after Vaccination against SARS-CoV-2 with mRNA-Based Vaccine Associated with Higher Antibody Levels during 6 Months Follow-Up. Vaccines (Basel). 2022 Mar 14;10(3):447. doi: 10.3390/vaccines10030447. PMID: 35335080; PMCID: PMC8950492.

**IF: 4,961**

**Kanizsai A**, Zavori L, Molnar T, Tőkés-Füzesi M, Szalai Z, Berecz J, Varnai R, Peterfi Z, Schwarcz A, Csecsei P. Adverse Reactions after Booster SARS-CoV-2 Vaccination Have Less Impact on Antibody Response than after Basic Vaccination Scheme. Vaccines (Basel). 2023 Jan 15;11(1):182. doi: 10.3390/vaccines11010182. PMID: 36680026; PMCID: PMC9864401.

**IF: 4,961**

Cumulative impact factor related to the thesis: **9,922**

### Other publications:

Varnai R, Molnar T, Zavori L, Tőkés-Füzesi M, Illes Z**, Kanizsai A**, Csecsei P. Serum Level of Anti-Nucleocapsid, but Not Anti-Spike Antibody, Is Associated with Improvement of Long COVID Symptoms. Vaccines (Basel). 2022 Jan 21;10(2):165. doi: 10.3390/vaccines10020165. PMID: 35214624; PMCID: PMC8924883.

Zavori L, Molnar T, Varnai R, **Kanizsai A**, Nagy L, Vadkerti B, Szirmay B, Schwarcz A, Csecsei P. Cystatin-c May Indicate Subclinical Renal Involvement, While Orosomucoid Is Associated with Fatigue in Patients with Long-COVID Syndrome. J Pers Med. 2023 Feb 19;13(2):371. doi: 10.3390/jpm13020371. PMID: 36836605; PMCID: PMC9958557.

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## IMAGES

*Figure 1.* Number of COVID-19 cases reported weekly by WHO Region, and global deaths, as of 4 December 2022 World Health Organization.

*Figure 2.* Classification scheme of coronaviruses. Mujeeb Khan [1], Syed F Adil [1], Hamad Z Alkhathlan [1], Muhammad N Tahir [2], Sadia Saif [3], Merajuddin Khan [1], Shams T Khan [4] COVID-19: A Global Challenge with Old History, Epidemiology and Progress So Far Molecules. 2020 Dec 23;26(1):39. doi: 10.3390/molecules26010039.

*Figure 3.* This image is a work of BioLegend (SARS-CoV-2 Proteins) with title of, Schematic illustration of SARS-CoV-2 viral particle and its structural proteins". Available on the following page: https://www.biolegend.com/en-us/sars-cov-2-proteins

*Figure 4.* Vaccination: Dr Jenner Performing His First Vaccination, 1796, Ernest Board (1877-1934), Wellcome Collection, Art UK.

*Figure 5.* Vaccine history timeline. Saleh A, Qamar S, Tekin A, et al. (July 26, 2021) Vaccine Development Throughout History. Cureus 13(7): e16635. doi:10.7759/cureus.16635

*Figure 6.* Major milestones in the historical path of the development of vaccinology and vaccine design. Review 6 May 2014. Vaccines for the 21st century. Isabel

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*Figure 7.* Multi-organ complications of COVID-19 and long COVID. The SARS-CoV-2 virus gains entry into the cells of multiple organs via the ACE2 receptor BMJ. 2021;374: n1648

*Figure 8.* Typical scheme of human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entering the cell. Naqvi AAT, Fatima K, Mohammad T, Fatima U et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. Biochim Biophys Acta Mol Basis Dis. 2020 Oct 1;1866(10):165878. doi: 10.1016/j.bbadis.2020.165878. Epub 2020 Jun 13. PMID: 32544429; PMCID: PMC7293463.

*Figure 9.* Vaccines against SARS-CoV-2. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol. 2022 Jan;23(1):3-20. doi: 10.1038/s41580-021-00418-x. Epub 2021 Oct 5. PMID: 34611326

*Figure 10.* Correlation of serum level of S-IgG and age at Day 12, Day 90, and Day 180 follow-up visit after the 2nd dose of BNT162b2 mRNA (Pfizer/BioNTech, Comirnaty) vaccine.

*Figure 11.* Comparison of serum level of anti-SARS-CoV-2 IgG at (A) 12, 30, 60 and (B) 90, 120, 150, 180 days after the 2nd dose of vaccination (BNT162b2 mRNA) in patients without or with at least one adverse reaction after each vaccine dose.

*Figure 12.* Comparison of serum levels of anti-SARS-CoV-2 IgG at (A) 12, 30, 60 and (B) 90, 120, 150, 180 days after the 2nd dose of vaccination (BNT162b2 mRNA).

*Figure 13.* Line diagram shows the change in the median anti SARS-CoV-2 IgG level 14, 60 and 120 days after heterologous (N=17) or homologous (N=201) booster vaccination.

*Figure 14.* Correlation of antibody titers with symptomatic status after 2nd and 3rd vaccinations. (A) Antibody response of symptomatic versus non-symptomatic patients at 14 (N=383), 60 (n=320) and 120 (N=268) days after the 2nd vaccination, (B) after the 3rd dose (N=218) at all time points. Definition of a symptomatic individual: a local or systematic reaction occurring within 7 days after vaccination.

**TABLES**

*Table 1.* Pollard, A.J., Bijker, E.M. A guide to vaccinology: from basic principles to new developments. *Nat. Rev. Immunol* **21**, 83–100 (2021). https://doi.org/10.1038/s41577-020-00479-7.

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*Table 3.* Baseline characteristics of study participants based on post-vaccination adverse event status. Kanizsai A, Molnar T, Varnai R, Zavori L, Tőkés-Füzesi M, Szalai Z, Berecz J, Csecsei P. Fever after Vaccination against SARS-CoV-2 with mRNA-Based Vaccine Associated with Higher Antibody Levels during 6 Months Follow-Up. Vaccines (Basel). 2022 Mar 14;10(3):447. doi: 10.3390/vaccines10030447. PMID: 35335080; PMCID: PMC8950492.

*Table 4.* Serial associations among level of S-IgG, adverse events after vaccination and demographic-clinical variables at seven time points (Day 12, Day 30, Day 60, Day 90, Day 120, Day 150 and Day 180 following second vaccine dose, respectively). Kanizsai A, Molnar T, Varnai R, Zavori L, Tőkés-Füzesi M, Szalai Z, Berecz J, Csecsei P. Fever after Vaccination against SARS-CoV-2 with mRNA-Based Vaccine Associated with Higher Antibody Levels during 6 Months Follow-Up. Vaccines (Basel). 2022 Mar 14;10(3):447. doi: 10.3390/vaccines10030447. PMID: 35335080; PMCID: PMC8950492.

*Table 5.* Characteristics of study population. Kanizsai A, Zavori L, Molnar T, Tőkés-Füzesi M, Szalai Z, Berecz J, Varnai R, Peterfi Z, Schwarcz A, Csecsei P. Adverse Reactions after Booster SARS-CoV-2 Vaccination Have Less Impact on Antibody Response than after Basic Vaccination Scheme. Vaccines (Basel). 2023 Jan 15;11(1):182. doi: 10.3390/vaccines11010182. PMID: 36680026; PMCID: PMC9864400.

*Table 6.* Changes in serum SARS-CoV-2 spike Ig levels on Days 14, 60 and 120 after the 2nd and 3rd vaccination, depending on whether fever occurred within 7 days after the vaccination. Kanizsai A, Zavori L, Molnar T, Tőkés-Füzesi M, Szalai Z, Berecz J, Varnai R, Peterfi Z, Schwarcz A, Csecsei P. Adverse Reactions after Booster SARS-CoV-2 Vaccination Have Less Impact on Antibody Response than after Basic Vaccination Scheme. Vaccines (Basel). 2023 Jan 15;11(1):182. doi: 10.3390/vaccines11010182. PMID: 36680026; PMCID: PMC9864400.

*Table 7.* Variables associated with levels of S-IgG (AU/mL) in cross-sectional analysis. Kanizsai A, Zavori L, Molnar T, Tőkés-Füzesi M, Szalai Z, Berecz J, Varnai R, Peterfi Z, Schwarcz A, Csecsei P. Adverse Reactions after Booster SARS-CoV-2 Vaccination Have Less Impact on Antibody Response than after Basic Vaccination Scheme. Vaccines (Basel). 2023 Jan 15;11(1):182. doi: 10.3390/vaccines11010182. PMID: 36680026; PMCID: PMC9864400.

*Table S1.* Frequency of adverse reactions after 1st and 2nd vaccination. Kanizsai A, Molnar T, Varnai R, Zavori L, Tőkés-Füzesi M, Szalai Z, Berecz J, Csecsei P. Fever after Vaccination against SARS-CoV-2 with mRNA-Based Vaccine Associated with Higher Antibody Levels during 6 Months Follow-Up. Vaccines (Basel). 2022 Mar 14;10(3):447. doi: 10.3390/vaccines10030447. PMID: 35335080; PMCID: PMC8950492.

*Table S2*: Correlation of S-IgG antibody levels with adverse reactions after 1st and 2nd dose of BNT162b2 vaccine manufactured by Pfizer/BioNTech, during the 6-month follow-up period. Kanizsai A, Molnar T, Varnai R, Zavori L, Tőkés-Füzesi M, Szalai Z, Berecz J, Csecsei P. Fever after Vaccination against SARS-CoV-2 with mRNA-Based Vaccine Associated with Higher Antibody Levels during 6 Months Follow-Up. Vaccines (Basel). 2022 Mar 14;10(3):447. doi: 10.3390/vaccines10030447. PMID: 35335080; PMCID: PMC8950492.

*Table S3*: Correlation of S-IgG antibody levels with demographic and clinical factors after 2nd dose of BNT162b2 vaccine manufactured by Pfizer/BioNTech, during the 6-month follow-up period. Kanizsai A, Molnar T, Varnai R, Zavori L, Tőkés-Füzesi M, Szalai Z, Berecz J, Csecsei P. Fever after Vaccination against SARS-CoV-2 with mRNA-Based Vaccine Associated with Higher Antibody Levels during 6 Months Follow-Up. Vaccines (Basel). 2022 Mar 14;10(3):447. doi: 10.3390/vaccines10030447. PMID: 35335080; PMCID: PMC8950492.

*Table S4:* Results of the binary logistic regression analysis examining associations between level of S-Ig, adverse events after vaccination and demographic-clinical variables at seven time points. Kanizsai A, Molnar T, Varnai R, Zavori L, Tőkés-Füzesi M, Szalai Z, Berecz J, Csecsei P. Fever after Vaccination against SARS-CoV-2 with mRNA-Based Vaccine Associated with Higher Antibody Levels during 6 Months Follow-Up. Vaccines (Basel). 2022 Mar 14;10(3):447. doi: 10.3390/vaccines10030447. PMID: 35335080; PMCID: PMC8950492.