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Immunantwort auf SARS-CoV-2-Vakzine Immune Response to Vaccines against SARS-CoV-2

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Bareiß, Alan

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Dekan: Professor Dr. B. Pichler

1. Berichterstatter: Prof. Dr. T. Bakchoul
2. Berichterstatter: Prof. Dr. N. P. Malek

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Für Laura und Theo

Auch wenn dieses akademische Pendant zum Unterarm-Namenstattoo den Monaten eures Verzichts und bedingungsloser Unterstützung nicht gerecht werden kann.

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Abbreviations

COVID-19	coronavirus disease
SARS-CoV-2	severe acute respiratory syndrome coronavirus
MERS-CoV	middle east respiratory syndrome coronavirus
RNA	ribonucleic acid
ACE2	angiotensin-converting enzyme 2
TMPRSS2	transmembrane protease, serine 2
ORF	open reading frame
mRNA	messenger ribonucleic acid
NSP	non-structural protein
rER	rough endoplasmic reticulum
ERGIC	endoplasmic-reticulum–golgi intermediate compartment
CFR	case fatality rate
UK	United Kingdom of Great Britain and northern Ireland
LDH	lactate dehydrogenase
ESR	erythrocyte sedimentation rate
CRP	c-reactive protein
ICU	intensive care unit
ARDS	acute respiratory distress syndrome
IL	interleukin
TNF-α	tumor necrosis factor alpha
PE	pulmonary embolism
MODS	multiple organ dysfunction syndrome
HPV	human papilloma virus
VLP	virus-like particle
DNA	desoxyribonucleic acid
CNS	central nervous system
NK	natural killer cells
MHC	major histocompatibility complex
DC	dendritic cell
LN	lymph node
TCR	t-cell receptor
CD	cluster of differentiation
MAC	membrane attack complex
WHO	world health organization
(S)AEFI	(severe) adverse event following immunization
VITT	vaccine-induced immune thrombotic thrombocytopenia
RBD	receptor-binging domain
UKT	university hospital of Tübingen
ZKT	institute for clinical and experimental transfusion medicine
NMI	natural and medical sciences institute
(N)HS	(non-)hospital staff
BNT	Biontech/Pfizer BNT162b2 vaccine “Comirnaty”
AZE	AstraZeneca ChAdOx1 nCov19 vaccine “Vaxzervria”
MOD	Moderna mRNA-1273 vaccine
JAN	Janssen Johnson & Johnson vaccine

ANOVA	analysis of variance
CI	confidence interval
SD	standard deviation
STIKO	Ger: „Ständige Impfkommission“: Engl: Standing Committee on Vaccination at the Robert Koch Institute

Introduction

COVID-19

Global public health has been put at risk by the rapid spread of coronavirus disease 2019 (COVID-19). The first infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was discovered in December 2019 in Wuhan City, Hubei Province, China. Although quickly a narrative around a Wuhan seafood market emerged, cluster analysis raises doubts about it. SARS-CoV-2 is suspected of infecting humans from other species initially and is now disseminated predominantly by human-to-human transmission. The case fatality rate in June 2022 in Europe is about 0.9% after an initial surge of up to almost 10% in April 2020. With a high transmission rate, a volatile epidemiological scenario unfolded, leading to significant differences in transmission and fatality rates across continents and even neighboring counties. Compared to SARS-CoV-2, SARS-CoV, and Middle East respiratory syndrome coronavirus (MERS- CoV), each also induces severe pneumonia with a lower transmission rate but a mortality rate of 9.6%[1], and 34.0%[2], respectively.

Pathogen (SARS-CoV-2)

Coronaviridae belong to the order Nidovirales, and the now-existing forms are divided into the genera *alpha, beta, gamma and delta*[3]. The distinction between the genera is made according to their genome structure and history of development.[4] Multiple approaches to dating back the emergence of the first coronaviridae have been performed years before the outbreak of SARS-CoV-2. Conventional analysis by calculating the age of a genome from the individual nucleotide replacement rate, as well as cross-species evolution, estimates the most common ancestor to 8100 BC.[5] Another study that considers the varying influence of natural selection to model the development of coronaviridae more accurately concludes that this might be a vast underestimation and states that the time of the most common recent ancestor (tMCRA) could date back 193

million years.[6] However, both extensive dissertations, among others, state an origin of alpha- and betacoronaviridae, a coronavirus of bat origin and the emergence of gamma- and deltacoronaviridae as avian-related. A fascinating feature of the entire family of Coronaviridae is the size of their genome, which is considered the largest of all RNA viruses. Their genomic structure is known to be single-stranded and positive-sense, meaning that the ribonucleic acid exists in a simple form without the congruent counterpart such as double-stranded RNA and that the RNA codes for the proteins directly so it can be translated without previous transcription.[7] SARS-CoV-2 is a betacoronavirus.

The SARS-CoV-2 virion has a lipid double layer with three structural proteins, spike (S), envelope (E), and membrane (M), on the surface. A fourth structural protein, nucleocapsid, is found inside the virion, wrapped around the RNA. Upon inhalation of virus particles, the receptor binding domain (RBD) of the spike protein attaches to ciliated respiratory epithelial cells as well as respiratory macrophages, specifically to the angiotensin-converting enzyme II (ACE2) on their surface, which is present on the surface of cells throughout the body, mainly in cells of the small intestinal lining, and renal cells in the proximal tubules.[8,9] In the respiratory tract, ACE2 is primarily expressed in ciliated epithelial cells, limited to the upper and transitional respiratory tract. Type II alveolar cells express very little ACE2 proteins, yet some that do and are infected undergo upregulation of ACE2 expression, facilitating further viral entry.[10] The spike protein is then cleaved by a protease on the target cell, namely transmembrane protease serine subtype 2, TMPRSS2 in short, exposing the S2-subunit of the S-Protein which now acts as a fusogenic protein. This protein attaches to the host cell's lipid membrane. It undergoes structural changes that pull the membranes of the virus and target cell towards each other, followed by endocytosis of the virion genome. Virus RNA is exposed to the hosts cytoplasm.[9]

All coronaviruses demonstrate a similar order of genes on their RNA. An initial sequence of amino acids that are not translated. This is followed by two open reading frames (ORF) with a programmed -1 ribosomal frameshift. As the viral RNA is present in a positive sense host, ribosomes start translating these regions into two replicase polyproteins that act as replicase-transcriptase complex after proteolysis. An ORF with nucleotide sequences for the aforementioned structural proteins S, E, M and N follows at the 3’ end

of the genome.[11] Once the positive sense is transcribed, the pathway temporarily splits into two: In the transcription process, the original RNA in the form it has been initially injected can be synthesized. In a second process, the discontinuous transcription, subgenomic RNAs that act as messenger RNAs (mRNAs) of different length coding for several non-structural proteins (NSP) as well as the structural proteins are transcribed from different parts of the same negative-sense replicate.[11]

All mRNAs now move to the rough endoplasmic reticulum (rER) in the perinuclear region of the cell, the term *rough* in this case describing the microscopic appearance of host ribosomes located on the surface. Once translated, because of their hydrophobic characteristic, structural virus surface proteins are embedded into the lipid double layer of the ER. Vesicles then transport the S-proteins to the Golgi apparatus for maturation and the M-proteins and E-proteins to the ER-Golgi intermediate compartment (ERGIC). After the maturation of S-proteins, they are moved to the ERGIC. The replicates of the complete virus genome, now transcribed into a positive sense, are inside the ER packed in a lipid double layer. Once the RNA is ready, it leaves the ER through pores. In the cytoplasm, it coils up with the nucleocapsid.[12] In a process similar to endocytosis, this complex is then covered with the lipid double layer containing the surface proteins at the ERGIC. The round morphology of the virion is facilitated by coulomb forces between the positively charged M-protein and the negatively charged E-protein. The virion is now in its final form, moves through the Golgi apparatus to be packed into the vesicle and is now ready for exocytosis and repetition of the cycle.[11,12]

Epidemiology of (COVID-19)

The SARS-CoV-2, initially known as the 2019 novel coronavirus (2019-nCov), was reported by an unknown source in late December 2019. On March 11th, 2020, WHO declared COVID-19 the sixth global public health emergency.[13] As with most human viruses, a zoonotic event is the most plausible theory of how SARS-CoV-2 came to be. Viruses strongly linked to SARS-CoV-2 have been found in pangolins and bats in various South and East Asia regions, including Cambodia, China, Japan, and Thailand. Serological proof confirms the supposition.[14] Wuhan seafood market has been broadly discussed as the location of the first transmission. As of today, it remains unclear whether the spillover of the zoonotic virus occurred before or after it became pathogenic to

humans as well as easily transmittable in human-to-human fashion or if it has been pathogenic before spilling over.[15] The first patient that was recognized as a COVID-19 case and was linked to the Wuhan seafood market was dated December 1st 2019.[16] Upon analysis of genomic data of different patients early on, evidence arose that a first common precursor of the new pathogen could have been circulating already since October 1st 2019.[17] Cluster analysis of the first COVID-19-Patients revealed that the Wuhan market accounted for some of the infections, while other were not linked to it geographically. It may therefore be questioned that – contrary to media portraits – the Wuhan seafood marked the origin of the pandemic or even acted as a setting for the first spillover event.[18] The highly contagious virus started spreading throughout China at first. However, it soon reached France and, therefore, the European Union on February 21st 2020, by confirming three cases of travellers who recently returned from China. In a highly globalized society, the spread was soon omnipresent throughout Europe and the globe.[19]

As mortality depends on many factors, from preventive measurements to individual risk factors, the case fatality ratio (CFR) in continental Europe, the United States of America and the world average peaked in early May 2020 with 9.93%, 6.12% and 7.34% respectively. In early July of 2022, the rates dropped to 0.9% for Europe and 1.16% for the US and the world. [20] Although CFR has several limitations and distortions, it provides a rough estimate of the changing impact of the pandemic over time.[21,22]

Factors contributing to this development include the adaption of prevention from monitoring and screening to rules of isolation, constantly improving the immunity status of the population, whether by vaccination or infection and potentially decreasing virulence during pathogen evolution and mutation.[23]

Clinical picture in COVID-19 patient

After contraction the incubation period initially, i.e. wild type infection, was estimated to be of a mean of 5.8 days.[24] Through mutations, the incubation period has shortened drastically, showing the capacity of pathogen evolution. As it dominated the UK, the Alpha variant had a mean incubation period of 4.3 days.[25] Omicron has shortened the time to development of symptoms to 3 days. SARS-CoV-2 can cause mild to severe symptoms, but asymptomatic carriers are estimated at 30 - 40 % and, therefore quite common.[26,27] Fever and dry cough being the most prominent symptom while other

common symptoms include headache, pharyngalgia, fatigue and dyspnea.[28–31] With later variants such as Delta and Omicron, flu-like symptoms, i.e. besides the aforementioned sneezing and rhinorrhea, became the most prevalent presentation. The milder and upper respiratory tract-focused clinical appearance of the latter is explained by the decreased capacity of Omicron to spread from cell to cell by fusion of membranes which causes more severe tissue damage in lower respiratory tract spread but instead rather relying on endosomal uptake, leaving the cell structure intact.[32] Gastrointestinal symptoms which include diarrhea, abdominal pain and vomiting are less frequent but still account for a significant part in the clinical picture.[33] For some COVID-19 cases, gastrointestinal manifestations may be the only cluster of symptoms and should not be overlooked as such.34 Neurological manifestations in a wide range associated with COVID-19 are reported, although only affecting a small fraction of patients.[34]

Besides the meanwhile broadly available methods for pathogen detection, e.g. via rapid antigen testing or genome detection through a polymerase chain reaction, there are typical findings upon hospital admissions. A differential blood test will likely show lymphocytosis, neutrophilia, lymphopenia, and thrombocytopenia. Each parameter plays its own role in the pathology and prognosis of COVID-19.[35] LDH, ESR and CRP are frequently elevated.[36]

Age plays a vital factor in as good as every facet of COVID-19. Severity of disease, admission to ICU and ultimately lethality are significantly and negatively influenced by progressing age of patients.[35]

A potential outcome of contracting SARS-CoV-2 is the development of acute respiratory distress syndrome (ARDS). This potential, more commonly seen in earlier variants, gave rise to the name of this pathogen and others from the same family, such as MERS-CoV and SARS-CoV, with potentially severe pulmonary manifestations. ARDS is defined by the Berlin criteria that include a state of respiratory failure in an acute setting, a triggering event such as an infection and the absence of cardiogenic etiology.[37] Pathophysiologically, ARDS results from the host's immune response, starting with inflammatory signals and procoagulant mediators primarily released by granulocytes. Capillaries are damaged because of endothelial disturbance. The same leads to increased capillary permeability and consequentially to ‘flooding’ of alveoli with protein-rich fluid, giving rise to the name of the first phase of three distinct phases of ARDS, the ‘exudative

phase'. On the third day, the so-called 'proliferative phase' starts and is marked by microthrombi in the pulmonary capillaries, further diminishing gas exchange capacity. After seven days, the 'fibrotic phase' begins, characterized by consolidation of the fibrin-containing exudate. ARDS clinically leads to hypercapnic acute respiratory insufficiency and often makes intubated ventilation necessary.[38,39]

More than just the local pulmonary inflammation and locally induced hypercoagulability, the systemic cytokine release, including cytokines like tumor necrosis factor-α (TNF-α) and interleukin-1 (IL1) and IL-6, leads to increased thrombin generation by macrophages and monocytes.[40] Laboratory markers such as a markedly increased D-dimer count, prolonged coagulation times and thrombocytopenia indicate that. Post-mortem analysis showed fibrin microthrombi in blood vessels and muscle tissue of the heart, capillaries and venules of peritubular renal tissue, hepatic sinusoids as well as neutrophilic plugs in brain tissue.[41,42] Macrothrombi, i.e. thrombotic events in larger vessels such as deep vein thrombosis (DVT) of the lower limbs and pulmonary embolism (PE) have been reported with an incidence of 3 % and 8% respectively in pooled analysis, being even higher when only looking at ICU patients where the incidence is 8% and 17% respectively.[43]

The criteria of disseminated intravascular coagulation (DIC) being roughly defined as "widespread hypercoagulable state that can lead to both microvascular and macrovascular clotting and compromised blood flow, ultimately resulting in multiple organ dysfunction syndrome […] (MODS)"[44] are therefore met in many cases. With the progress of research COVID-19 became ever more recognized as a multi-organ disease rather than being limited to the respiratory tract.

While some symptoms in mild to moderate courses of the clinical complex disappear earlier such as fever (5.8[45] – 10 days[46]). Other symptoms like disruption of smell and taste have a quite high percentage of persistence for a year and longer after onset especially with wild type and alpha variants.[47]

Special attention in clinical medicine and neuroscience was given to a complex of symptoms persisting after a covid infection, leading to potentially drastic lifestyle changes in patients. "Long COVID" describes a long-lasting manifestation of any COVID-related symptoms but typically involves headache, fatigue, and dyspnea with a persistence of 4 weeks and more.49 As research continues, some authors differentiate

long-COVID etiologically between chronified inflammation and actual organ damage such as pulmonary fibrosis or irreversible kidney damage.[48]

Vaccination against SARS-CoV-2

Since the first successful attempt of vaccination performed by Edward Jenner by inoculating cowpox in human skin to prevent the infection with smallpox in the 18th century a wide variety of diseases has been successfully eradicated or is routinely prevented by induction of immunity before acquiring the corresponding pathogen.[49] The term vaccine stems from the Latin word *vaccinus*, meaning "derived from a cow". While with today's safety regulations, the development of vaccines is a yearlong process, potentially taking decades from the idea to development, testing and governmental approval, the COVID-19 pandemic made it necessary to shorten the process. The unprecedented global effort, attention and resources have been dedicated to speeding up the process and facilitating the launch of a safe and efficient vaccine. Governments worldwide have approved the rolling review process, i.e. the preliminary authorization of a vaccine while continuously monitoring its effects. A primary differentiation in the vaccines nowadays available is being made between active and passive immunization, the latter not strictly counting as such because the procedure involves administering immune globulins intended to support the recipient's immune system without generating lasting immunity. Vaccines of active immunization are grouped based on their mechanisms of action and type.

Live attenuated vaccines are genetically modified pathogens with a reduced capacity to replicate and therefore do not induce the corresponding disease. Examples that have been in use for a long time are vaccines against viral diseases like measles, mumps and rubella. Also, vaccines aiming to prevent bacterial infections such as tuberculosis or typhoid fever are available. In an attempt to establish a SARS-CoV-2-vaccine of this type, COVI-VAC entered first-phase clinical trials without any available results.[50]

Inactivated, whole-particle vaccines are well established in the prevention of for example, hepatitis A (viral) and pertussis (bacterial). An example of the COVID-19 vaccines of this type is CoronaVac®, developed by *Sinovac biotech*, a Chinese company. It is currently, except for central Europe, used worldwide.[51]

Virus-like particles (VLP) resemble the actual virus, containing no genetic information but one or more of the aforementioned surface proteins that act as antigens and stimulate the hosts immune system.[52] The common human papilloma virus (HPV) vaccine is an example of this type. Canada has authorized CoVLP®, a VLP produced in a tobacco-like plant.[53] No SARS-CoV-2 vaccine of this kind, as of today, is in use in central Europe.

Another type of vaccine, the subunit vaccine, works by presenting antigenic structural proteins (subunits) to the patient's immune system. Established examples are vaccines against hepatitis B and influence. The most recent in Germany authorized example of this type is Novavax®. It consists of a recombinant S-glycoprotein attached to a nanoparticle. Some sources therefore classify the agent as VLP.

A newly emerged vaccine technology, the messenger ribonucleic acid (mRNA) vaccine, has built the backbone of central European efforts in the vaccine campaign. It contains messenger RNA, coding for the S-antigen of SARS-CoV-2. The RNA needs to reach the 'hosts' cytoplasm. To do so it is packed into a lipid vesicle that can enter immune cells by phagocytosis. The cells own ribosome then translates the genome and starts to generate the desired antigen, S in this case. After some time, the mRNA degrades and the immune system has successfully processed the S-protein making it much less susceptible to the actual pathogen. Two examples from the realm of COVID vaccines are Comirnaty® (BioNTech/Pfizer) and Spikevax® (Moderna).

Vector based vaccines work in a very similar fashion. Yet there are two main differences. One lays in the fact, that the carrier particle is an attenuated adenovirus. The other is that the genome that is transmitted is in the form of a desoxyribonucleic acid (DNA). Thus once in the 'host's cytoplasm it has to undergo transcription in the nucleus to be transcribed into mRNA. Vaxzevria® (AstraZeneca) is one example that was temporarily used in Germany. Janssen® (Johnson & Johnson) is another.[54]

Immune System

In order to protect the organism from foreign and therefore potential malicious agents the immune system is equipped with an arsenal of physical, chemical and physiological-cellular protection mechanisms. Mainly the immune system can be divided into an innate and an acquired part as well as into specific and unspecific. Often these terms are used interchangeably.

Innate and Unspecific Immunity

Unfolding its action already from birth, a series of defence mechanisms provide protection against pathogens. The thick keratinized layer of the outer skin, the cutis, acts as a physical barrier to prevent pathogen entry into the organism. Additionally, substances like fatty acids excreted on the skin form an unfavorable habitat for some pathogens. Mucosal surfaces are in some way more susceptible to pathogen entry than skin by expressing surface proteins that can be used for cell entry for specific pathogens. To counteract that weakness, a glycoprotein-rich mucus layer shields the cells from the outside. Some mucosal epithelial cells form cilia on the surface to eliminate pathogens by transporting them out of the organism's system. An example of a liquid and chemical barrier is the lacrimal fluid containing an antibacterial enzyme or the low pH of hydrochloric acid-rich gastric acid. Besides these liquid factors, the surfactant in the alveoli fulfils a slightly more sophisticated task. It is capable of coating parts of some bacteria by interacting with lipopolysaccharides to stimulate and lead the way for macrophages in a process named opsonization.[55]

When it comes to the actual "active" immune response to pathogens, once a pathogen got beyond the aforementioned barrier systems, the unspecific immune system acts with cellular and humoral components. Macrophages, through a series of receptors, initially bind to a pathogen, then ingest it by phagocytosis and lyze the pathogen intracellularly. This not just eliminates the pathogen but also initiates the release of cytokines, a series of proteins that upregulate local inflammation to attract other cells of the unspecific immune system but also regulate the acquired immune response later on. Cytokines are also referred to as interleukins (IL), a hybridism stemming from the Latin word *inter* and the Greek word *λευκός* [leukos]. The most important representatives of cytokines released by active macrophages are IL-1, -6, -8 and -12 as well as tumor necrosis factor alpha (TNF-α). Whilst different cytokines have their own scope of action, they are considered functionally redundant, meaning in that several cytokines share the same or a similar effect and being pleiotropic they are able to exert their effects on multiple cell types.[56] Collectively their effect induces both local and systemic reactions. On local level, vascular permeability is increased, allowing extravasation of further immune components such as granulocytes around the site of pathogen entry. Neutrophilic granulocytes have a

strong phagocytic capacity and release of their granules, hence the name, has an antibacterial effect. Granules released by eosinophilic granulocytes comprise various enzymes, each targeting another aspect of the intruding pathogen. Macroscopically painful local erythema and edema formation result from these local immune mechanisms. Circulating in the infected organism's cardiovascular system, cytokines unfold their systemic effects as well. Both directly and indirectly affecting the central nervous system (CNS) by stimulation of hepatocytic production of acute phase proteins (as part of the unspecific immune system), causing a rise in body temperature, clinically resulting in fever. Another key player in humoral immunity is the complement system, a group of peptides freely contained in the blood plasma. Upon encounter with a pathogen, the main task of the complement is to attach to its surface, i.e. either to opsonize it, priming it for phagocytosis or directly disrupting the pathogen's cell membrane. The complement system works in an activation cascade, meaning that lacking a factor in the activation chain may disrupt this system. Moving towards a transition to the specific part of the immune system, natural killer (NK) cells, still part of the unspecific response, constantly scan cells of the own system for expression of a healthy quantity of major histocompatibility complex (MHC) class I molecules. If through cellular stress, either by intracellular infection or by genome damage in malignant transformation, the amount of MCH class I molecules decreases, NK cells release granules to induce apoptosis of the affected cell.[57] NK cells though as well as cytokines produced by phagocytic cells do interfere with the specific immune system through both stimulation of lymphocytes as well as refining the maturation process of for example dendritic cells (DC).[58]

DC, much like the unspecific macrophages, are capable of ingesting, i.e. phagocytosis and lysis of pathogens.[59,60] The one very important difference is that parts of the surface structure are presented on the surface of DCs, which then migrate to the closest lymph node (LN) and settle in the paracortical aspect. In this location, naïve T cells, i.e. fully matured but not yet activated T cells (and therefore not specialized yet), frequently pass and are exposed to the antigenic surface proteins brought in by dendritic cells. Chemokines released by the DC aid this process by attracting T cells. T cells interact with DCs repeatedly in countless fashions until a T cell receptor (TCR) matches the presented antigen on the DC’s MHC-class-I molecule. If also a co-receptor between the two cells matches, the priming of the T cell is now complete.[61–63] Depending on the level and kind

of interleukin that predominantly surrounds this interaction, the T cell is able to differentiate into one of the following effector T cells: T-helper cell, T-regulatory cell and T-memory cell. While T helper cells' main task is to stimulate B lymphocytes, regulatory T cells help downregulate the immune response to avoid an overshoot. Memory T cells remain in the system for years, increasing the speed of immune response upon further encounters with the same antigen. T cells that are activated through this pathway express a glycoprotein called cluster of differentiation (CD) 4, which acts as a coreceptor to the TCR.[64–67]

Naïve T cells with glycoprotein CD8 (CD8+) need their antigen presented on another kind of MCH, namely class II. Once a naïve CD8+ T cell is activated, it differentiates into a cytotoxic t-cell with the capability of inducing apoptosis in infected cells.[68,69]

T cells account for the cellular part of the specific and acquired immune system. B cells are responsible for the highly specific humoral immune response. While T cells mature in the **t**hymus, B cells originate and mature in the **b**one marrow.[70,71]

Similarly to T lymphocytes, B cells start to circulate freely as in a naïve, dormant state. Their activation requires a two-step process: First, a matching antigen has to be presented to the B cell on an MHC-II molecule (e.g. on a DC), engulfed and presented on the surface by the B cell itself. If now a T helper cell that has already specified to this particular antigen binds to it and reacts by releasing large amounts of cytokines, the B cell becomes activated and differentiates either into a plasma cell that produces antibodies or into a B memory cell.[72]

In the process of activation, B lymphocytes undergo somatic hypermutation, a series of point mutations in the part of the genome that codes for the variable antibody portion leaving only lymphocytes with high antigen affinity for differentiation and emigration from the lymph node.[73,74]

Once differentiated into plasma cells, multiple kinds of antibodies are produced. Ultimately the action of these immune globulins is threefold:

- By attaching to the surface of pathogens, often forming conglomerates of multiple pathogenic organisms, it becomes neutralized and can no longer harm the host
- Pathogens that have antibodies attached to their antigenic surface portions are marked and therefore prone for phagocytosis

- Antibodies play a role in one of the activation pathways of the complement system. Ultimately different proteins of the complement system form a pore complex on the surface of pathogens and, in the case of antibody activation on the surface of infected cells, called the membrane attack complex (MAC). Once several MACs are embedded into the cell wall, free diffusion of electrolytes is enabled, making it impossible for the cell to uphold the vital membrane potential.[75]

Vaccination after induction of unspecific and specific immune responses leads to the formation of memory cells. Whilst both T and B memory cells are "stored" in secondary lymphatic organs such as LN, B cells reside in the spleen and T cells also circulate freely. Once formed, they need less stimulation and co-stimulation to proliferate and demonstrate a faster and more efficient immune response upon a second encounter with the same antigen.

	Humoral	**Cellular**
Unspecific	Complement System Cytokines	Granulocytes Macrophages NK-Cells
Specific	Antibodies (produced by plasma cells and B-Lymphocytes)	T-Lymphocytes (cytotoxic and regulatory)

Table 1: Rough classification of key players of the immune response[76]

Vaccine side effects

Various terms are used for unwanted symptoms or clinical events directly caused by the administered agent, be it a drug or a vaccine. The World Health Organization (WHO), which plays a leading role in monitoring vaccine safety, uses the term side effect.[77] In general, the term *side effect* seems to be associated with less harmful and predictable symptoms, while for more severe and potentially life-threatening occurrences, the term *adverse reaction* is used. Some authors argue that the terms are used interchangeably, and the latter is to be preferred.[78] In contrary the term *adverse event* is not to be confused as the definition lacks the necessary causative link of the administered agent and the undesired outcome.[79] Actually in the context of publications evaluating unwanted effects of COVID-19 vaccines the terms vary. Discussably, even the term

adverse event following immunization (AEFI) and severe AEFI (SAEFI) is used.[80] To align the terminology with the most commonly used, we chose the simple term *side effect*. The capacity to elicit side effects is referred to as reactogenicity. Local side effects around the injection site can be explained through the direct activation of nociceptors through tissue damage. Local inflammatory reaction facilitates this and may cause local side effects other than pain. The systemic side effects are the result of antigen-induced immune response involving increased transcription and release of pyrogenic cytokines
.[81] It remains unanswered whether a higher reactogenicity leads to higher immunogenicity of SARS-Cov-2 vaccines although there is evidence that heterologous vaccination schemes rank higher on both scales without stating reactogenicity as the causative link.[82]

Vaccinations against SARS-CoV-2 are reported with modest to moderate side effects for a majority of recipients lasting 1-3 days while the most common complication is a fever with chills, muscle and joint soreness, fatigue, and headache. Local injection-site side effects include discomfort, pain, redness and swelling.[83,84] Reports about having at least one local or one systemic side effect vary from 70-80% and seem to significantly depend on the vaccine type.[85,86] The proportion of population reporting side effects also depends on age, comorbidities and whether a pre-vaccine SARS-CoV-2 infection had taken place.[87]
However more severe side effects have been reported but they are rare.[88–92] One of the most discussed is cerebral sinus vein, splanchnic vein and even azygos and hemiazygos vein thrombosis.[93,94] The underlying mechanism of the so called vaccine-induced immune thrombotic thrombocytopenia (VITT) is etiologically linked to antibody-mediated platelet activation.[95] VITT is linked to the vector based ChAdOx1 vaccine.
Another rare occurrence that is associated with vaccination using mRNA-based vaccines BNT162b2 and mRNA-1273 is that of myocarditis and pericarditis.[96] This particular side effect as seen so far primarily affects young, male adolescents and adults.[97,98]

Heterogeneous vaccination is reported to cause more or more severe side effects than homologous regimens, especially when combining a mRNA-based vaccine with a vector based vaccine.[99] Also in terms of immunogenicity heterologous vaccination schemes appear to be superior to homologous schemes.[100]

Breakthrough infections after vaccination

Although it might be a compelling narrative in the campaign to get the world population covered with vaccines against the novel coronavirus, no vaccine that has been developed today has 100% efficacy. A plethora of circumstances inside and outside of our control and knowledge contribute to this. These range from incorrect handling to individual anamnestic and medical factors. The same is true for COVID-Vaccines. A breakthrough infection is basically defined as an infection that occurs after what is considered a full vaccination. Because of the inherent dynamics of the immune system, most studies add a couple of days after the completion dose to allow for immunogenicity to unfold. For COVID-19 in Germany, the official statement by the RKI defines it as a "PCR confirmed SARS-CoV-2 Infection" 14 days after the second dose.101 International studies often set their own time frame.[101]

To get to the core of the topic, the question at hand must be specified and split up: How effective are vaccines in preventing an infection? What influences the efficacy? And last but not least: How do we measure efficacy in a practical approach in a highly volatile pandemic context?

Fueled by the heated argument around vaccination in a time of vast internet accessibility, anti-vaccine campaigners are using the fact of vaccine efficacy of less than 100% to promote vaccine hesitancy (among other factors).[102] However in a more systemic outlook equally important should be the question whether a vaccination is capable of reducing the risk of a severe, potentially life-threatening manifestation after contraction and whether a broad population immunity may lead to a "return to normal" in terms of medical service capacities. [103]

In literature three factors and their connection to vaccine efficacy are mainly discussed: Vaccination scheme, time after vaccination and pathogen mutation. Generally the capability of a vaccination declines after time, SARS-CoV-2 tends to bring forth ever more infections variants and a booster dose increases protection against or against severe infection.[104] Exemplarily a study published in the New England Journal of Medicine concludes that two doses of Vaxzevria (AstraZeneca) are 89% effective against symptomatic delta infection 2-4 weeks after the second dose but only 49 % against the

Omicron variant. After 25 weeks the effectiveness declines to 44 % even for Delta and no effectiveness is shown against Omicron. A booster dose of Comirnaty fixes the effectiveness back to 95 % and 62 %.[105]

Methods

Aims

1) To investigate the incidence and severity of vaccine side effects on the background of a longitudinal data set with a broad base of anamnestic data.
2) To determine the correlation between vaccine side effects and antibody levels in vaccinated individuals.
3) To identify factors influencing the strength or likelihood of side effects upon vaccination

Objectives

1) To recruit a representative cohort of each control group (healthcare workers versus non-healthcare workers).
2) To collect data on vaccine side effects and anamnestic data through self-reported surveys.
3) To measure antibody levels in participants' blood samples at multiple time points following vaccination.
4) To analyze the data to identify any patterns or correlations between vaccine side effects and antibody levels.
5) To determine factors that correlate with or influence the time point of vaccine breakthrough.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the institutional review board of the University of Tübingen (556/2021BO1) on September 8, 2021 as well as registered in the German Clinical Trials Register, https://drks.de, registration number: DRKS00029013.

Recruitment

The study was announced through the central email service of the university hospital of Tübingen, which distributes the invitation to all employees. The announcement was repeated weekly over a one-month period. Similarly, “Zentrum für klinische und

experimentelle Transfusionsmedizin" (ZKT; engl.: Center for Clinical and Experimental Transfusion Medicine) and "Naturwissenschaftliches und Medizinisches Institut Reutlingen" (NMI; engl.: Natural and Medical Sciences Institute at the University of Tübingen, in Reutlingen, Germany) employees were invited to participate in the study through their unfiltered distributor.

Volunteers could make an appointment through an online calendar. All participants gave informed consent to participate in the study.

Study Design

Aiming to better understand the immune response, the study is designed to collect longitudinal data from study participants over a year. Anamnestic data was collected from study participants through several questionnaires in an online platform. Each participant received their own profile, accessible through a QR code that was handed to them after entering the study. In the initial questionnaire, the study participants answered questions about their medical records and demographic data as well as the date and kind of vaccine they had received. Probands then gave information of ordinal quality on the side effects experienced after the first dose of the COVID-19 vaccine. In separate questionnaires, side effects for each consecutive dose were gathered.

Demographic Data	**Comorbidities**	**Medication**
▪ Age ▪ Gender ▪ Date of each Blood Sample	▪ Cardiovascular ▪ Neurologic ▪ Dermatologic ▪ Hematologic ▪ Pulmonary ▪ Hepatic / Renal ▪ Gastrointestinal ▪ Chronic Diseases such as Allergies or Diabetes Mellitus ▪ Tumor (benign and malignant) ▪ Have you had a COVID-19 infection?	Do you take any medication for: ▪ Hypertension ▪ Hyperlipidemia ▪ Immune Suppression ▪ Anticoagulation ▪ Diabetes mellitus ▪ Pain ▪ Thyroid dysfunction ▪ Cancer

Table 2: Initial Questionnaire. Participants hat to fill it only once.

Local Side Effects	Systemic Side Effects
▪ Pain on Injection Site ▪ Skin Sensitivity ▪ Swelling ▪ Redness / Local Erythema	▪ Headache ▪ Fever ▪ Shivers ▪ Muscle Pain ▪ Joint Pain ▪ Fatigue ▪ Nausea and Vomiting ▪ Diarrhea
▪ Date and Name of the Vaccine:	
▪ Did you take analgesics because of the vaccination?	

Table 3: Side Effects Questionnaire – The answers to each symptom was ranked as none, mild, moderate and severe.

Determination of Humoral Response

Besides the anamnestic and subjective data blood samples were collected on three occasions throughout one year to be tested for antibody levels against SARS-CoV-2 spike trimer, S1 and S2 subunits and RBD using the *MultiCoV-Ab* assay that allows the analysis of these parameters simultaneously and ranks high in specificity and sensitivity. The test was developed by a team from NMI and has been validated against commercially available kits by known and established companies. Quality control samples are processed in parallel within every assay run to ensure stability and comparability. A signal-to-cutoff ratio is calculated and used for comparison.[106]

To assess the performance of the antibodies *NeutrobodyPlex,* an inhibitory ACE-II binding assay measuring RBD binding capacity, was used. It utilizes nanobodies and can be evaluated by its fluorescence (mean fluorescence index) in a multiplex system. Nanobodies are derived from single-domain antibodies like those found in alpacas or dromedaries.[107] In principle, nanobodies behave similarly to conventional antibodies, but they have the advantage of being significantly smaller and more stable than usual antibodies. Their production is cheap and relatively easy.[108] For the development of the NeutroboyPlex assay, nanobodies were initially obtained from B lymphocytes of immunized alpacas. After transferring the nanobody DNA into bacteria, the molecules can be produced quickly and efficiently in the laboratory.[107]

For the *NeutrobodyPlex* assay, nanobodies were developed that recognize distinct regions within the receptor binding domain (RBD) of SARS-CoV-2. As discussed in the *Pathogen* section, RBD composes a portion of the spike protein on the surface of the SARS-CoV-2 virion. It plays a unique role as the virus uses the RBD to dock with and consecutively enter human cells. The protective, neutralizing antibodies, such as those formed after infection or vaccination, are also directed against RBD as one of the spike domains.

The analysis allows for simultaneous analysis of antibody performance against RBDs of different variants. Wild type (WT), beta, delta and two omicron strains (BA2, BA5) RBDs were tested for each serum. This allows detecting what variant was responsible for a potential previous infection and how well the individuum is protected against each variant from a humoral viewpoint.[107]

Proband Selection and Exclusion Criteria

To be enrolled in the TüSeRe:exact study, probands had to be of an age of 18 and above, be employed at the University Hospital of Tubingen (UKT), the Institute for Clinical and Experimental Transfusion Medicine (ZKT) or the Natural and Medical Sciences Institute (NMI) of Reutlingen. Probands from the latter primarily serve the purpose of comparison between those exposed to SARS-CoV-2 through their work at the hospital and those whose at-work-exposure is equal to the broad German population. Therefore the latter will be referred to as non-hospital staff (NHS), and the first two groups will be referred to as hospital staff (HS).

Pregnant women and probands who changed workplaces during data acquisition were excluded from enrollment or further participation.

Statistical Analysis

Data for the analysis was extracted from the studies' SQL Server in September 2022 and includes measurements performed at ZKT. Publications that use later datasets or repetition of antibody measurements at the NMI can therefore vary in outcome.

For statistical analysis the software *DataTab* was used.[109] For each analysis only those probands were included who filled the corresponding questionnaire. To clean the data, implausible cases were eliminated (e.g. contradictory information about vaccination dates; invalid dates, etc..).

Vaccine side effects were given in ordinal format. In an approach to broaden the spectrum of analytic measures, the data was copied and dichotomized as some statistical techniques, such as chi-squared tests and logistic regression, are designed for categorical data and are not appropriate for continuous data. Accepting a certain degree of loss in data depth, the interpretation of the result may also become more explicit. A dual approach was therefore chosen (i.e., *frequency* and *severity* in separate analyses).

A Chi-square test was used to determine significant differences in the *frequency* of side effects, which were in dichotomous format. The Dunn-Bonferroni test was used to compare side effect data in respect to *severity* among three vaccine types, where the data was in ordinal format (*i.e., none, mild, moderate, severe*). The Mann-Whitney U test was performed to compare side effect severity between the vaccines for the third vaccination, as for the latter there were only two considered.

Depending on the nature and distribution of the data as well as each individual research question a suitable statistical test was used. The assumptions for each respective analysis were tested before (e.g., Gaussian distribution, min(n) of observed frequencies). What test was used is indicated in the legend of each table and figure.

For the analysis of vaccine breakthroughs, the Kaplan-Maier survival analysis was used. Kaplan-Meier survival analysis is a commonly used method for analyzing time-to-event data, such as time-to-disease progression or time-to-death. This method is particularly useful for studying vaccine breakthroughs because it allows for the estimation of the probability of an event (e.g., days to infection after vaccination) over time while also allowing for comparison of the survival curves between different groups (e.g. mRNA-based vaccines vs vector-based) to determine if there are significant differences in the probability of breakthroughs between the groups.

Results

Cohort Demographics

	NMI	ZKT/UKT	Total
female	55 (88)	82 (736)	824
male	45 (72)	18 (159)	231
diverse	1 (1)	0	1
total	100 (161)	100 (895)	1056

Table 4: Number of participants according to institution and gender. The percentage in groups is displayed and the absolute numbers are in brackets.

The overall included number of participants is 1056 (Tab. 4). Out of these, 84% (n=895) were hospital staff and 15% (n=161) from non-hospital staff. Most of the participants were female, with a predominance of 82% in the hospital workers group and 55% in the control group (Tab. 4).

The hospital staff had a slightly higher mean age of 45.3 (±12.5) than the non-hospital group averaging 39.2 (±13), with this difference being statistically significant ($p = 0.03$).

Only complete data sets were included in the analysis.

Vaccine Side Effects

Vaccination Schemes

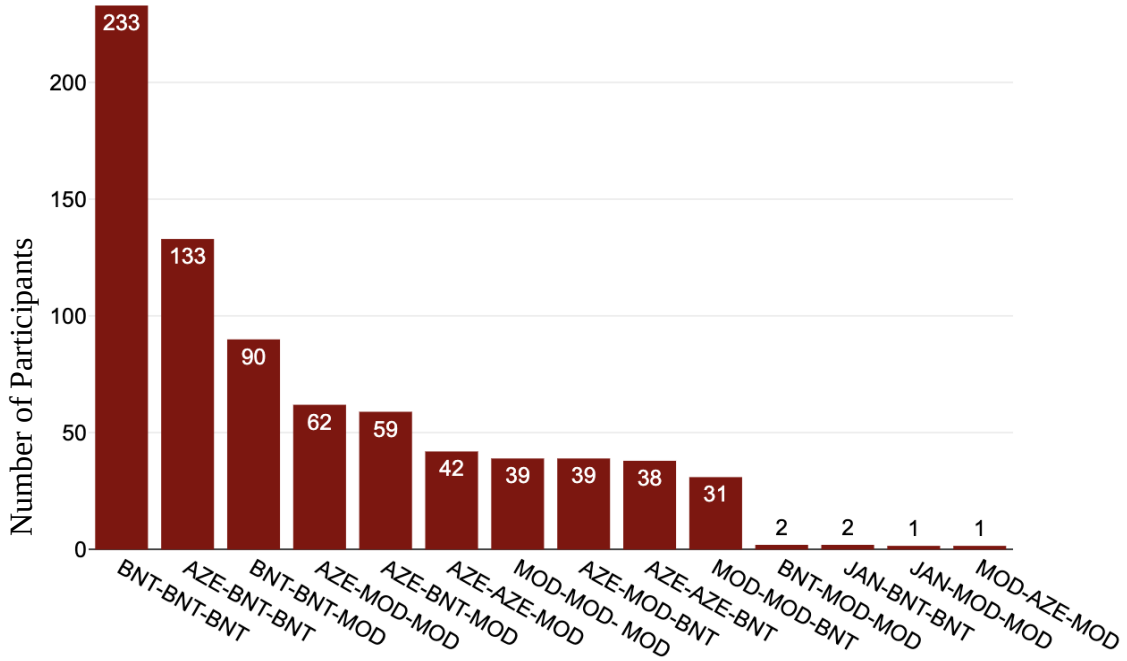

Figure 1: Number of participants according to their vaccination scheme.

By far most participants (n=233, Fig. 1) received a homologous regimen of BNT followed by AZE-BNT-BNT (n=133). Only three participants reported that they have received JAN. The latter were excluded from further analysis because of the small sample size.

First Dose

	Total (n)	BNT	MOD	AZE
n	1046	460	103	483
Age (± SD)	44 (±12.9)	43 (±13)	40 (±14)	46 (±12)
Gender, % (n)				
male	22 (231)	25 (114)	24 (25)	19 (92)
female	78 (815)	75 (346)	74 (78)	81 (391)
local side effects, % (n)	78 (821)	79 (362)	86 (89)	77 (370)
pain on injection site	73 (762)	73 (338)	80 (83)	71 (341)
skin sensitivity	52 (547)	47 (215)	64 (66)	55 (266)
swelling	29 (307)	27 (124)	46 (47)	28 (136)
local erythema	19 (195)	15 (71)	31 (195)	19 (92)
systemic side effects % (n)	72 (761)	60 (277)	57 (59)	88 (425)
headache	50 (527)	33 (154)	34 (35)	70 (338)
fever	30 (319)	12 (54)	17 (17)	51 (248)
shivers	32 (331)	12 (53)	17 (17)	54 (261)
general muscle pain	44 (457)	29 (132)	31 (32)	61 (293)
joint pain	44 (461)	25 (116)	25 (26)	66 (319)
fatigue	62 (646)	47 (215)	48 (49)	79 (382)
nausea	7 (71)	5 (21)	3 (3)	10 (47)
diarrhea	3 (30)	3 (12)	1 (1)	4 (17)

Table 5: Age, gender, 4 local and 8 systemic side effects are shown for the first vaccination. For the side effects the numbers indicate the percentage of participants reporting each symptom respectively and the number in parenthesis display the absolute number.[110]

	AZE- BNT	BNT-MOD	MOD-AZE
local side effects			
pain on injection site	1	.798	.24
skin sensitivity	.06	.006*	.564
swelling	1	.006*	.006*
local erythema	.852	.006*	.042*
systemic side effects			
headache	.006*	1	.006*
fever	.006*	1	.006*
shivers	.006*	.996	.006*
general muscle pain	.006*	1	.006*
joint pain	.006*	1	.006*
fatigue	.006*	1	.006*
nausea	.012*	1	.15
diarrhea	1	1	1

Table 6: P-values for the comparison of different vaccines in terms of side effect frequency (n = 1046). A Chi2 test has been performed for the administered vaccine on first vaccination and the proportion of each reported side effect. P-values were adjusted with Bonferroni correction.[110]

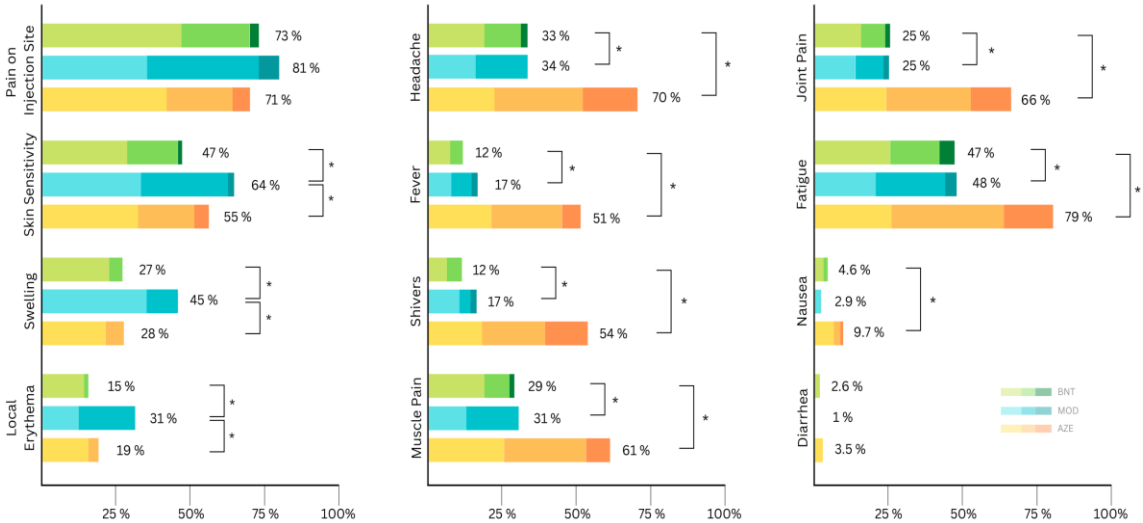

Figure 2: Distribution of self-reported local (left) and systemic (middle, right) side effects according to symptom severity (mild, moderate, severe) after the first vaccination (AZE:AstraZeneca (ChAdOx1 nCov19); BNT: Biontech/Pfizer (BNT162b2); MOD: Moderna (mRNA-1273); n=1046). Asterisks indicate significant differences in severity.[110]

The distribution of local side effects according to symptom severity is presented in Fig 2. Compared to AZE, the severity of local side effects, except for skin sensitivity and diarrhea, was significantly higher after vaccination with MOD (Fig. 2). However, the severity of most local side effects was similar after receiving AZE and BNT vaccines after the first vaccination (Fig. 2, Tab. 11). On the other hand, the severity of most of the local side effects was higher after MOD compared to BNT (Fig. 2, Tab. 11). [110]

At least one systemic side effect was reported by 72% of the study participants (Tab. 5). The most frequent side effect was fatigue, which was reported by 62% of the participants. Systemic side effects were reported by 88% of those participants receiving AZE as the first vaccine. In contrast, the percentage of participants with systemic side effects was 60% and 57% after receiving BNT and MOD vaccines, respectively. All systemic side effects, except nausea and diarrhea, differed significantly between AZE and both mRNA-based vaccines (Tab. 5 and 6). The severity and frequency of self-reported systemic side effects are presented in Fig. 2 and Tab. 11. In terms of the severity of systemic side effects, BNT and MOD vaccines were not significantly different (Tab. 5, 6 and 11). [110]

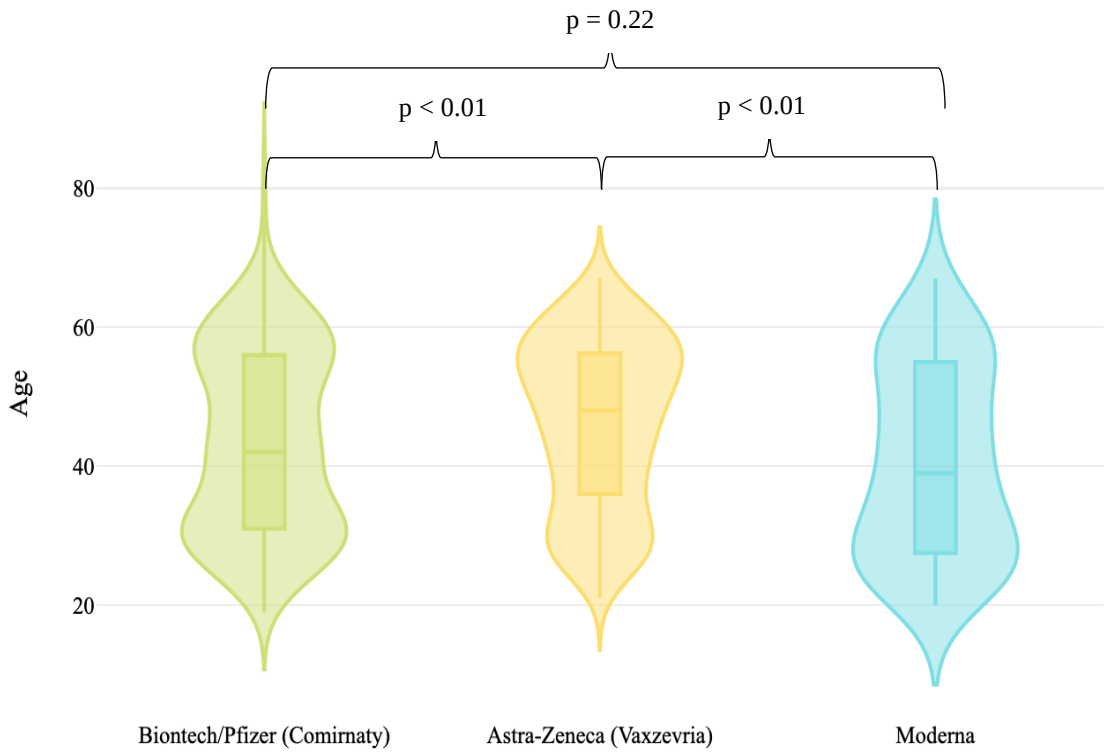

Figure 3: Violin boxplots of proband age according to vaccine type for the first dose.

The visual representation (Fig. 3) shows that the data is distributed bimodally rather than normally. The Kolmogorov-Smirnov test ($p < 0.01$) confirms that the distribution is not Gaussian. The same is true for each administered vaccine. Hence for the comparison, a non-parametric test is indicated.

For the representation of the data (Fig. 3), violin plots were selected to visualize the modality. A potentially confusing point that needs clarification is that each violin's pointy end extends beyond the actual data points. The reason is that violin plotting incorporates a kernel density estimation to smoothen out the borders. The overall range of age was 19 - 79 years upon entering the study.

A Kruskal-Wallis test showed a significant difference between the categories of the independent variable first vaccine with respect to the dependent variable *Age*, p=<.001.

A Bonferroni Post hoc test was used to compare the groups in pairs to determine which was significantly different. It revealed that participants receiving *Astra-Zeneca* were

significantly older than those receiving *BioNTech* or *Moderna* ($p < 0.01$). However, the age of participants receiving Biontech or Moderna were similar ($p = 0.22$).

This finding can be explained by an adjustment early in the vaccination campaign regulations by the Standing Committee on Vaccination (German: "Ständige Impfkommission", STIKO). In a press release from March 2021, Astra-Zeneca was recommended to be administered only for persons above 60 years of age on the background of multiple reported cases of thromboembolic events in younger patients.

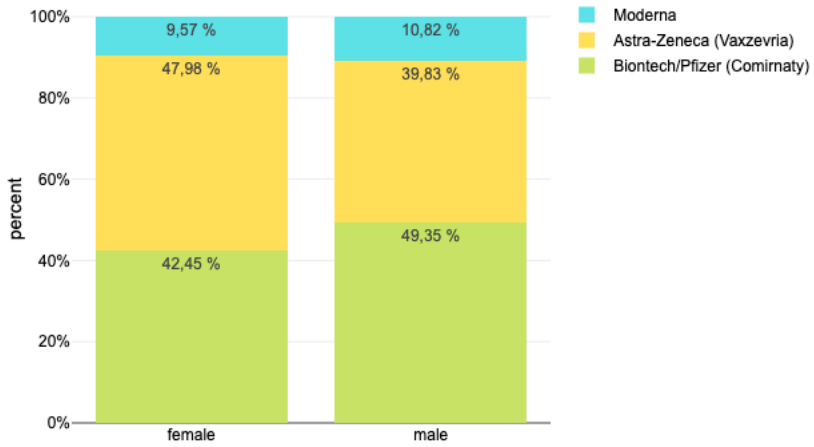

Figure 4: Vaccine distribution across Genders in the 1st dose (n=1046).

With 48 % most of female participants received Astra-Zeneca (Fig. 4). It is also the greater proportion when compared to male participants (40 %). To male participants mostly BioNTech has been administered as a 1st dose. Also, more males received Moderna. However, the differences do not pass a Chi2 test ($p = 0.09$) and are therefore not statistically significant.

Second Dose

	Total (n)	BNT	MOD	AZE
n	1046	706	238	102
Age (± SD)	43 (±13)	44 (±12)	42 (±12)	54 (±10)
Gender, % (n)				
male	22 (231)	23 (164)	19 (46)	21 (21)
female	78 (815)	77 (542)	81 (192)	79 (81)
local side effects, % (n)	75 (782)	75 (528)	85 (200)	53 (54)
pain on injection site	87 (718)	70 (492)	80 (187)	38 (39)
skin sensitivity	52 (546)	49 (342)	70 (165)	38 (39)
swelling	29 (303)	26 (182)	42 (99)	22 (22)
local erythema	16 (163)	13 (95)	24 (57)	11 (11)
systemic side effects % (n)	73 (771)	71 (497)	92 (216)	57 (58)
headache	54 (563)	49 (342)	79 (185)	35 (36)
fever	32 (338)	27 (189)	56 (132)	17 (17)
shivers	30 (317)	26 (180)	52 (122)	15 (15)
general muscle pain	46 (482)	42 (293)	71 (166)	23 (23)
joint pain	47 (493)	42 (294)	70 (165)	33 (34)
fatigue	64 (670)	61 (427)	83 (196)	46 (47)
nausea	6 (65)	5 (37)	11 (26)	2 (2)
diarrhea	2 (23)	2 (16)	3 (6)	1 (1)

Table 7: Age, gender, 4 local and 8 systemic side effects for the second vaccination are shown. For the side effects the numbers indicate the percentage of participants reporting each symptom respectively and the number in parenthesis display the absolute number.[110]

	AZE- BNT	BNT-MOD	MOD-AZE
local side effects			
pain on injection site	.006*	.018*	.018*
skin sensitivity	.33	.006*	.006*
swelling	1	.006*	.024*
local erythema	1	.006*	.03*
systemic side effects			
headache	.072	.006*	.006*
fever	.174	.006*	.006*
shivers	.108	.006*	.006*
general muscle pain	.006*	.006*	.006*
joint pain	.678	.006*	.006*
fatigue	.036	.006*	.006*
nausea	.894	.012*	.03*
diarrhea	1	1	1

Table 8: P-values for the comparison of different vaccines in terms of side effect frequency. A Chi2 test has been performed for the administered vaccine on second vaccination and the proportion of each reported side effect. P-values were adjusted with Bonferroni correction.[110]

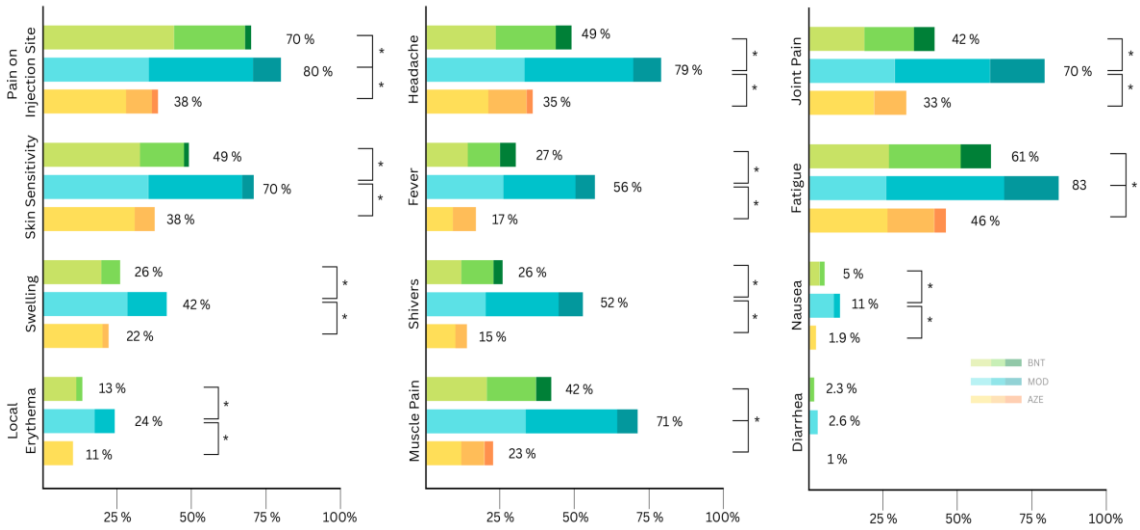

Figure 5: Distribution of self-reported local (left) and systemic (middle, right) side effects according to symptom severity (mild, moderate, severe) after the second vaccination (AZE:AstraZeneca (ChAdOx1 nCov19); BNT: Biontech/Pfizer (BNT162b2); MOD: Moderna (mRNA-1273), n=1042). Asterisks indicate significant differences in severity.[110]

The overall percentage of study participants that reported any local and systemic side effects were similar after the second vaccination, being 75% and 73% (Tab. 7), respectively. The most reported local side effect was pain at the injection site, reported by 87%, and the most common systemic side effect was fatigue, with 64%. Recipients of the MOD vaccine as the second dose not only reported the highest proportion of local side effects, but also systemic ones, where the difference is most significant (Tab. 8). All local side effects were more frequent after MOD compared to AZE and BNT (Tab. 8). Pain at the injection site was more common after BNT compared to AZE (Tab. 8). Although the frequency of skin sensitivity was similar after receiving AZE and BNT, the severity of the symptom was significantly higher after receiving BNT (Fig. 5, Tab. 7 and 8). [110]

All side effects apart from diarrhea were reported with a significantly (statistically) higher frequency and severity after receiving MOD compared to after receiving AZE or BNT (Tab. 7 and Fig. 5). AZE had the lowest proportion of reported adverse events compared with the first dose, with 53% of the participants experiencing local side effects and 54% experiencing systemic side effects (Tab. 7). General muscle pain and fatigue were significantly more common after BNT compared to AZE (Tab. 7 and 8). The vast majority of all reported side effects after the second dose were mild to moderate (Fig. 5). [110]

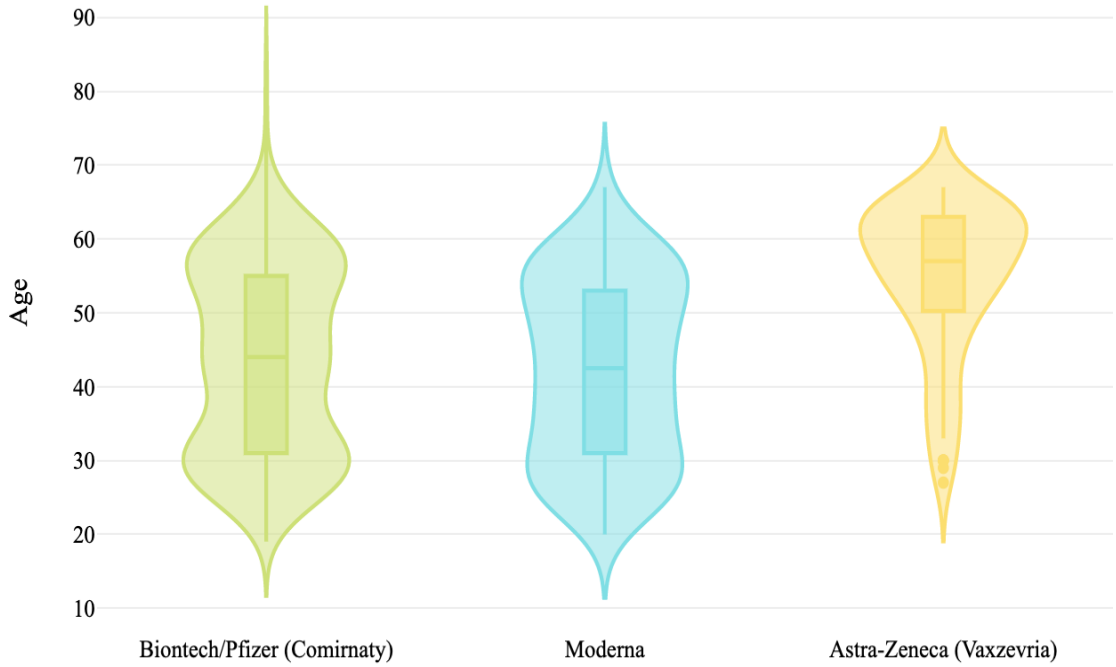

Figure 6: Violin boxplots of proband age according to vaccine type for the second dose.

When sketching out the age distribution after the second dose (Fig. 6) and performing the same statistical analysis as in the section before it becomes quite apparent that AZE-vaccinees are now not just statistically significantly older but now outrank the mRNA-based vaccinees by far averaging at 55 years (versus 44 years for BNT-vaccinees and 42 years for MOD-vaccinees).

Two effects come into play that are able to explain this progressive pattern. One being that the aforementioned change in regulation is now broadly applied (quite a part of vaccinees have received AstraZeneca as a first dose before the regulation was adjusted). Additionally, we only examined working age participants. As on average in Germany the retirement age is 64 years[111] the sample size of participants receiving AstraZeneca decreased drastically from 483 in the first to 102 in the second dose.

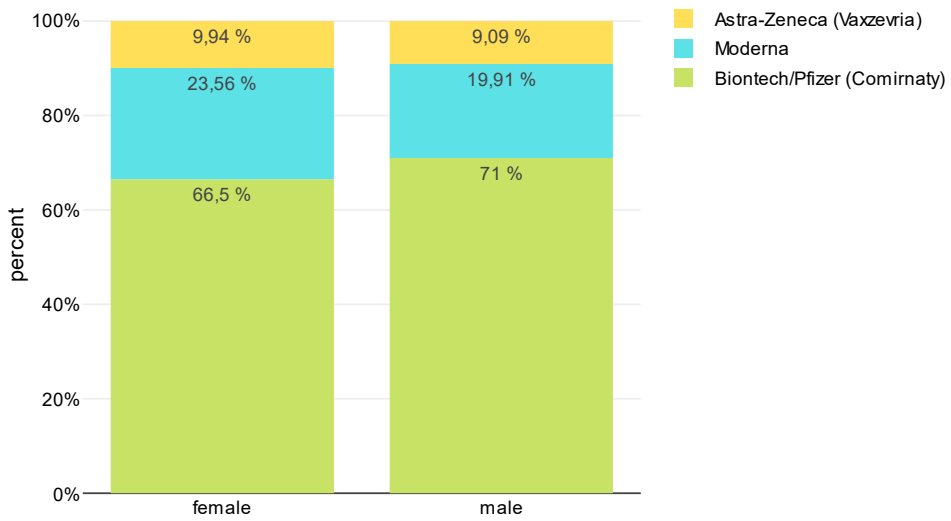

Figure 7: Gender distribution across the vaccines in the 2nd dose (n=1042).

Figure 7 shows the distribution of vaccine manufacturers across the genders upon the 2nd dose. Compared to the 1st dose the proportion of AZE shrinks. The largest proportion belongs to BNT for both male and female.

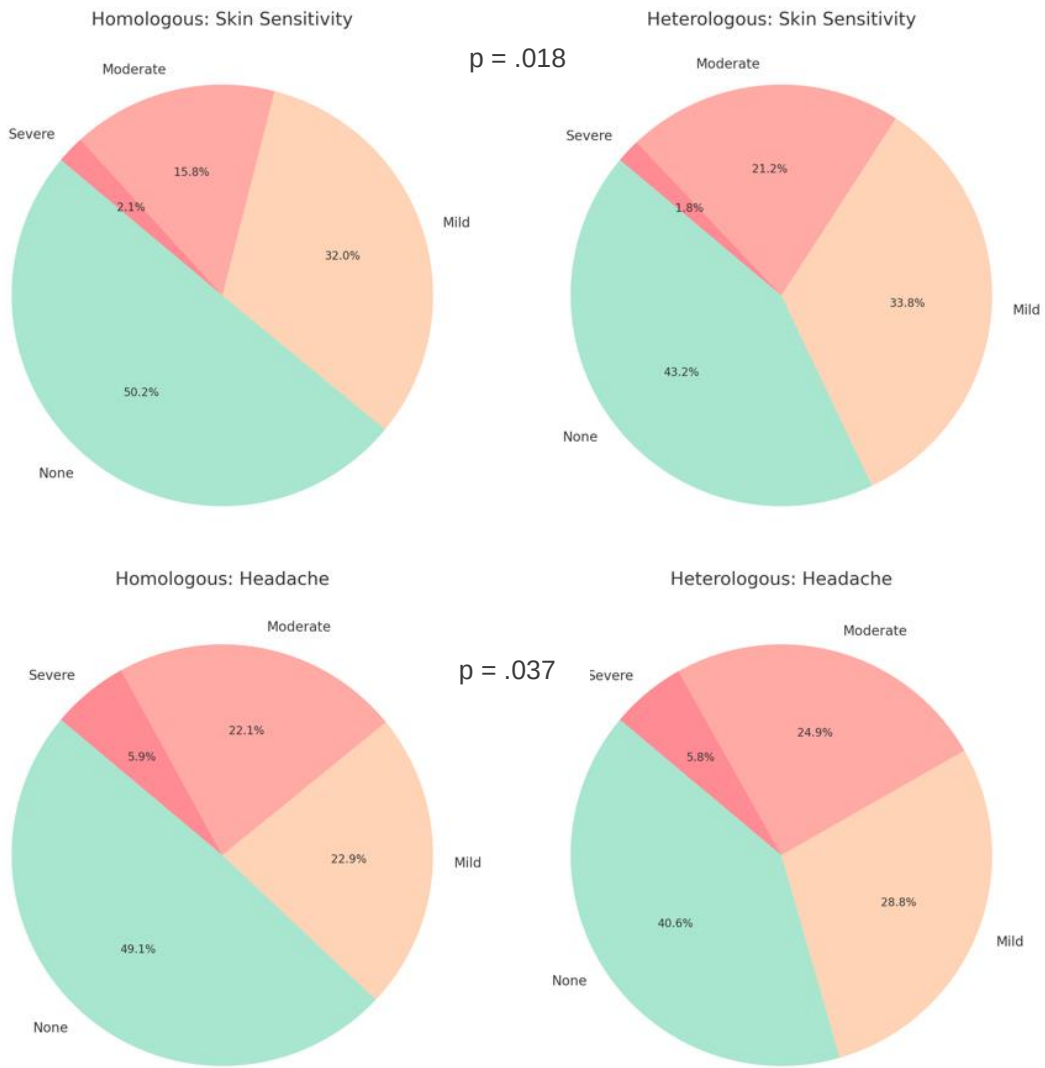

Figure 8: Severity of side effects (green- "none" to red - "severe") after the second vaccination according to the vaccination scheme. The values on top indicate the asymptotic significance (2-tailed) of a Mann-Whitney U-Test.

Local Side Effects	**p-value**
Pain on Injection Site	0.707
Skin Sensitivity	0.018*
Swelling	0.662
Local Erythema	0.944
Systemic Side Effects	
Headache	0.037*
Fever	0.047*
Shivers	0.050*
General Muscle Pain	0.074
Joint Pain	0.544
Fatigue	0.285
Nausea	0.438
Diarrhea	0.268

Table 9 – The values in this table indicate the asymptotic significance (2-tailed) of a Mann-Whitney U-Test. Asterisks show significance.

Four out of twelve side effects showed a statistically significantly higher severity for the group of heterogeneously vaccinated participants than those who received the same vaccine twice. Those were skin sensitivity, headache, fever, and shivers as portrayed in Figure 8.

Third Dose

	Total (n)	BNT	MOD
n	766	473	293
Age (± SD)	43 (±13)	42 (±13)	49 (±10)
Gender, % (n)			
male	21 (168)	23 (107)	21 (61)
female	79 (598)	77 (366)	79 (232)
local side effects, % (n)	76 (579)	73 (344)	80 (235)
pain on injection site	70 (537)	66 (312)	77 (225)
skin sensitivity	57 (439)	56 (267)	59 (172)
swelling	31 (241)	29 (136)	36 (105)
local erythema	17 (133)	15 (73)	20 (60)
systemic side effects % (n)	66 (502)	62 (292)	72 (210)
headache	44 (339)	41 (195)	49 (144)
fever	22 (171)	21 (101)	24 (70)
shivers	22 (170)	21 (100)	24 (70)
general muscle pain	41 (313)	36 (170)	49 (143)
joint pain	38 (289)	34 (162)	43 (127)
fatigue	58 (446)	55 (262)	63 (184)
nausea	7 (52)	7 (31)	7 (21)
diarrhea	4 (31)	4 (19)	4 (12)

Table 10: Age, gender, 4 local and 8 systemic side effects for the third (i.e., booster) vaccination are shown. For the side effects the numbers indicate the percentage of participants reporting each symptom respectively and the number in parenthesis display the absolute number.[110]

A total of 772 participants received a booster vaccine. Of these, 720 participants, who filled out the online questionnaires about side effects were included for analysis. In accordance with current local recommendations, no participants received AZE as a third dose (Tab. 10). The proportion of recipients reporting at least one local side effect is 76% and for systemic side effects is 66%, which is comparable to the previous two administrations. Pain at the injection site was reported by 70% of participants and fatigue by 58% (Fig. 9). A statistically significant difference in frequency between MOD and BNT was found in seven of the twelve included side effects (Tab. 11) and five out of the twelve in terms of severity (Tab. 12). [110]

	BNT-MOD
local side effects	
pain on injection site	.001*
skin sensitivity	.626
swelling	.046*
local erythema	.081
systemic side effects	
headache	.037*
fever	.488
shivers	.407
general muscle pain	.001*
joint pain	.013*
fatigue	.05*
nausea	.769
diarrhea	.978

Table 11: P-values for the comparison of different vaccines in terms of side effect frequency. A Chi2 test has been performed for the administered vaccine on the third vaccination and the proportion of each reported side effect.[110]

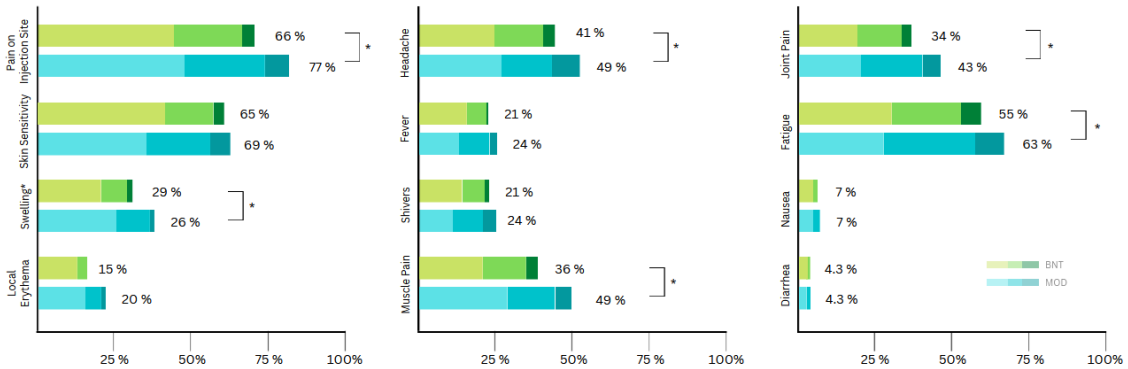

Figure 9: Distribution of self-reported local (left) and systemic (middle, right) side effects according to symptom severity (mild, moderate, severe) after the third vaccination; BNT: BioNTech/Pfizer (BNT162b2); MOD: Moderna (mRNA-1273); n=720). Asterisks indicate significant differences in severity.[110]

	AZE vs BNT		AZE vs MOD		BNT vs MOD		
	V1	V2	V1	V2	V1	V2	V3
local side effects							
pain on injection site	1	.001*	.01*	.001*	.005	.001*	.001*
skin sensitivity	.037*	.116	.094	.001*	.001*	.001*	.131
swelling	1	.918	.03*	.001*	.006*	.001*	.111
redness	.586	1	.035	.32	.003*	.001*	.22
systemic side effects							
headache	.001*	.1	.001*	.001*	1	.001*	.029*
fever	.001*	.164	.001*	.001*	.618	.001*	.438
shivers	.001*	.076	.001*	.001*	.481	.001*	.39
general muscle pain	.001*	.035*	.001*	.001*	.664	.001*	.003*
joint pain	.001*	.419	.001*	.001*	1	.001*	.015*
fatigue	.001*	.019*	.001*	.001*	1	.001*	.012*
nausea	.07*	1	.105	.005*	1	.001*	.892
diarrhea	1	1	.222	.877	.654	.877	.989

Table 12: P-values for comparison between the three vaccines (V) in terms of severity according to each dose. The values for the first and second vaccination (V1, V2) were corrected using Bonferroni correction, as multiple comparisons were made. V3 needed no correction as such because the only two different vaccines have been administered upon this occasion.[110]

Figure 10: Violin boxplots of proband age according to vaccine type for the third dose (n=720).

		t	df	p (2-tailed)
Age	Equal variances	7.14	767	<.001

Table 13: T-Test for independent samples

The results of the descriptive statistics show that the Moderna group has higher values for the dependent variable *Age* (M = 49.23, SD = 10.03) than the Biontech/Pfizer (Comirnaty) group (M = 42.7, SD = 13.57).

The Levene test of equality of variance yields a p-value of <.001, which is below the 5% significance level. The Levene test is therefore significant and the null hypothesis that all variances of the groups are equal is rejected. Thus, there is no variance equality in the samples.

A two-tailed t-test for independent samples (equal variances not assumed) showed that the difference between Moderna and Biontech/Pfizer (Comirnaty) with respect to the dependent variable *Age* was statistically significant, t(744.67) = 7.65, p = <.001, 95% confidence interval [4.85, 8.22] as portrayed in Table 13. Thus, the null hypothesis is rejected. Recipients of BNT are significantly younger than MOD-recipients.

Use of Analgesics

General

	V1	V2	V3
Analgesic	28 (291)	28 (294)	23 (164)
No Analgesic	72 (755)	72 (750)	77 (562)
Total	100 (1046)	100 (1044)	100 (726)

Table 14: percentage of participants reporting the use of analgesics according to the number of consecutive dose (V1, V2, V3). The numbers are expressed in percentage and the brackets show the absolute numbers.

A Chi2 test was performed between the group of participants who used analgesics on the second and on the third dose. No expected cell frequencies were less than 5. There was a statistically significant relationship between these two variables, $\chi^2(1) = 135.21$, $p = <.001$, Cramér's $V = 0.43$. The Chi2 test is therefore significant, and the null hypothesis is rejected. Therefore, it becomes evident that whether participants use analgesics is influenced moderately by whether they used it before. The comparison of the use of analgesics of the first and second dose as well results in $p = <.001$, therefore the same must be true.

According to Vaccine

		Analgesic Use		
		0	**1**	**Total**
Vaccine V1	Biontech/Pfizer (Comirnaty)	88 (404)	12 (56)	460
	Astra-Zeneca (Vaxzevria)*	54 (261)	46 (222)	483
	Moderna	87 (90)	13 (13)	103
	Total	72 (755)	28 (291)	1046
		0	**1**	**Total**
Vaccine V2	Biontech/Pfizer (Comirnaty)	76 (540)	24 (167)	707
	Astra-Zeneca (Vaxzevria)	78 (80)	22 (22)	102
	Moderna*	55 (130)	45 (105)	235
	Total	750	294	1044
		0	**1**	**Total**
Vaccine V3	Biontech/Pfizer (Comirnaty)	80 (354)	20 (91)	445
	Moderna	74 (206)	26 (72)	278
	Total	560	163	723

Table 15: Observed frequencies (% (n)) of analgesia (1) and no analgesia (0) according to the vaccine administered. Asterisks indicate that the *observed* frequency of "1" is **higher** than the *expected* frequency with p = <.001 by performing a Chi2 test of independence.

Put into more comprehensive terms in specific constellations, we can observe a potential positive relationship between the vaccine choice and the tendency to use analgesia. Participants who received AZE as a first dose showed a statistically significant higher analgesia use than expected under entirely unrelated conditions (Tab. 15). The same is true for MOD recipients for the second dose. No relevant effect was observed for the third dose (Tab. 15).

This finding does align with the reported differences in frequency and severity of local and primarily systemic side effects. While AZE outranked the other vaccine types in the 1st, MOD took the lead in the second dose. The finding is, therefore, internally coherently explained by obvious and almost intuitive cofounders.

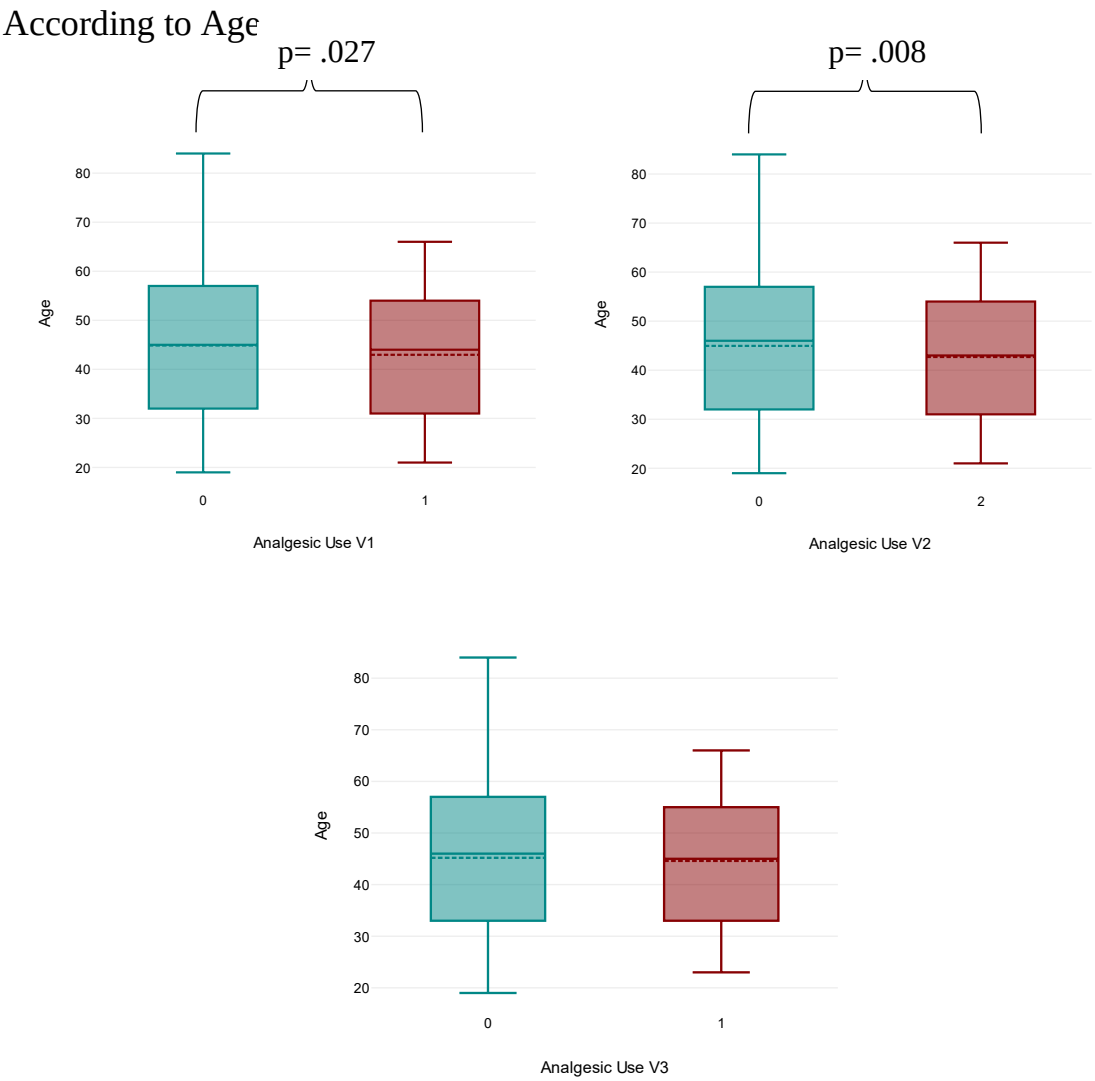

Figure 11: Boxplots of participant's age grouped into whether analgesics have been used (1) or not (0) for each vaccination dose (n=1046, 1042, 720 respectively).

The mean age of participants at the first vaccination who did not use analgesics is 44.86 ± 13.21 (Fig. 11). For those who did the mean age is 42.97 ± 11.97. The Levene test of equality of variance showed there is no variance equality in the samples. A two-tailed t-test for independent samples (equal variances not assumed) showed that the difference between 0 and 1 with respect to the variable *Age* was statistically significant, t (576.87) = 2.22, p =.027, 95% confidence interval [0.21, 3.57]. Thus, the difference is statistically significant.

For the second vaccination mean age of those who reported not having used analgesics is 44.96, ±13.18, the mean age of analgesic users is 42.69 ± 12.06 (Fig. 11) and therefore

lower as well. A two-tailed t-test returned with statistical significance (t (581.79) = 2.66, p=.008, 95% confidence interval [0.59, 3.95].

There is also a difference in age when looking at the third dose between those who have not used analgesics 45.19 ± 12.98 and the group who has 44.59 ± 11.98 (Fig. 11). The result of the t-test returns insignificant though.

According to Side Effects

	Cramér's V		
local side effects	**V1**	**V2**	**V3**
pain on injection site	.2	.27	.27
skin sensitivity	.22	.23	.25
swelling	.15	.17	.24
local erythema	.15	.16	.23
systemic side effects			
headache	.48	.42	.52
fever	.46	.44	.4
shivers	.41	.39	.36
general muscle pain	.41	.38	.38
joint pain	.46	.42	.38
fatigue	.41	.34	.39
nausea	.14	n/a	.11
diarrhea	n/a	n/a	n/a

Table 16: A Chi2 test was performed between each dichotomous side effect and whether analgesics have been used. Cramér's V is displayed if the assumptions for the Chi2 test were met (all cell frequencies > 4) and if $p < .05$.

According to the Chi2 test, there are distinct statistical associations between the occurrence of headaches and the use of analgesics following the first vaccination, fever after the second, and headaches again after the third (Tab. 16). Additionally, systemic side effects are more closely associated with the use of analgesics than local side effects throughout the vaccination series (Tab. 16). However, these findings should not be interpreted as causative for analgesic use decisions, primarily due to the lack of specific data on the timing of analgesic administration in relation to the onset of side effects.

According to Gender

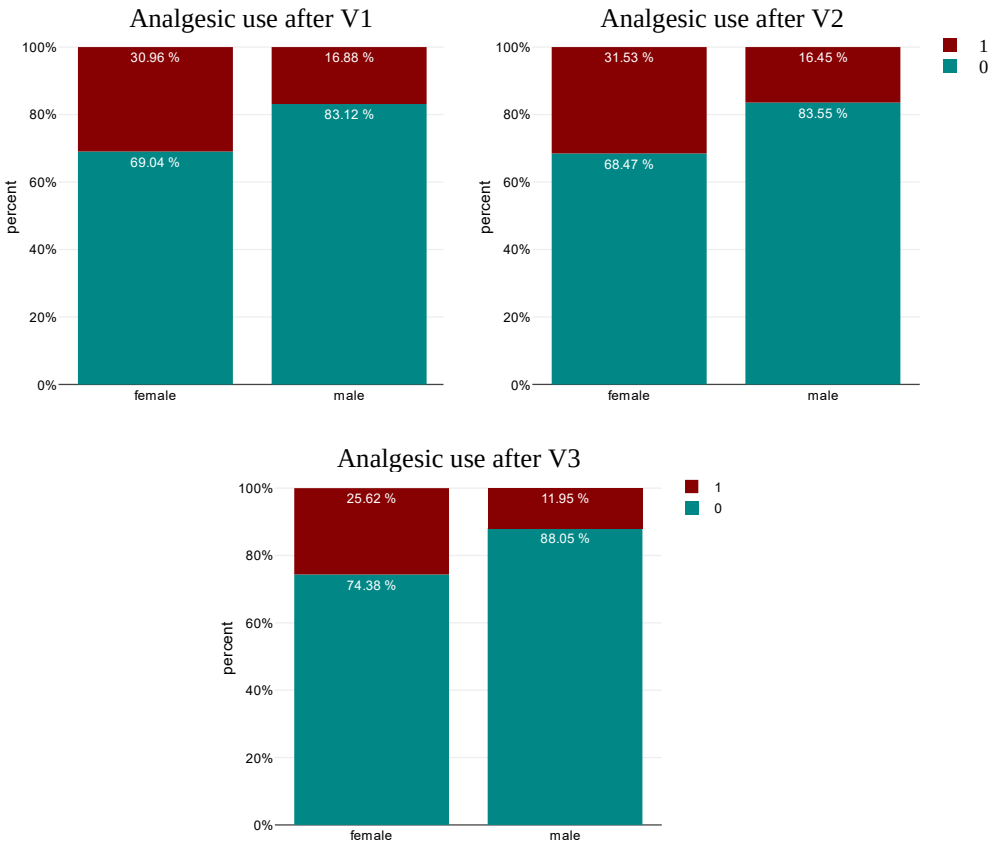

Figure 12: percentage of female and male participants in regard of whether analgesics have been used (red) or not (green).

In the first vaccination 31% of female participants reported the use of analgesics compared to 17% of the male participants. Also, in the second and third vaccination more female participants reported using analgesics with 32% and 25% respectively compared to their male counterparts (16%, 12%) (Fig. 12).

A Chi2 test conducted for each vaccination separately resulted in $p < .001$ each. The differences therefore are statistically significant for each administration.

Vaccine Breakthroughs

	After V2	After V3	Total
Vaccine Breaktroughs % (n)	11 (77)	29 (298)	37 (375)
No Breakthrough % (n)	89 (644)	71 (644)	63 (644)

Table 17: Total breakthroughs recorded.

By the end of data collection, a total of 37% of participants reported a vaccine breakthrough infection. After the second dose (V2) which was initially considered a full vaccination with the included regimens and before receiving a booster dose 11 % of participants reported a breakthrough infection. After the booster dose (V3) 29% of participants reported an infection as depicted in Table 17. Fourth infections were not considered.

To assure the quality of the data a comparison to the reported infections in Baden-Württemberg from 16 October 2022 can be done.

$$\frac{infections\ reported\ in\ the\ federal\ state^{112}}{inhabitants\ of\ the\ federal\ state}$$

$$\frac{4.440.148}{11.124.642} = 39.9\%$$

It is important to note that 39 infections in our data accounting for 3.8% of the total as shown in Table 17 have occurred before V2, as well as the fact that double or until today rare third infections are not counted. In conclusion the sample represents the background infection dynamics quite well. The rate in our study is even slightly higher reflecting the vast proportion of health workers working in direct contact with COVID-19 patients.

From further analysis those who reported two SARS-CoV-2 Infections were excluded. When looking at the vaccine breakthrough infections after the second vaccination but before the third, 6 participants were excluded accounting for 8% of the recorded breakthrough infections as listed in Table 17.
The same was done for vaccine breakthrough infections after the third dose, resulting in an elimination of 15 participants, accounting for a reduction in case numbers of 4% compared to the total record (Table 17).

After the 2nd Vaccination

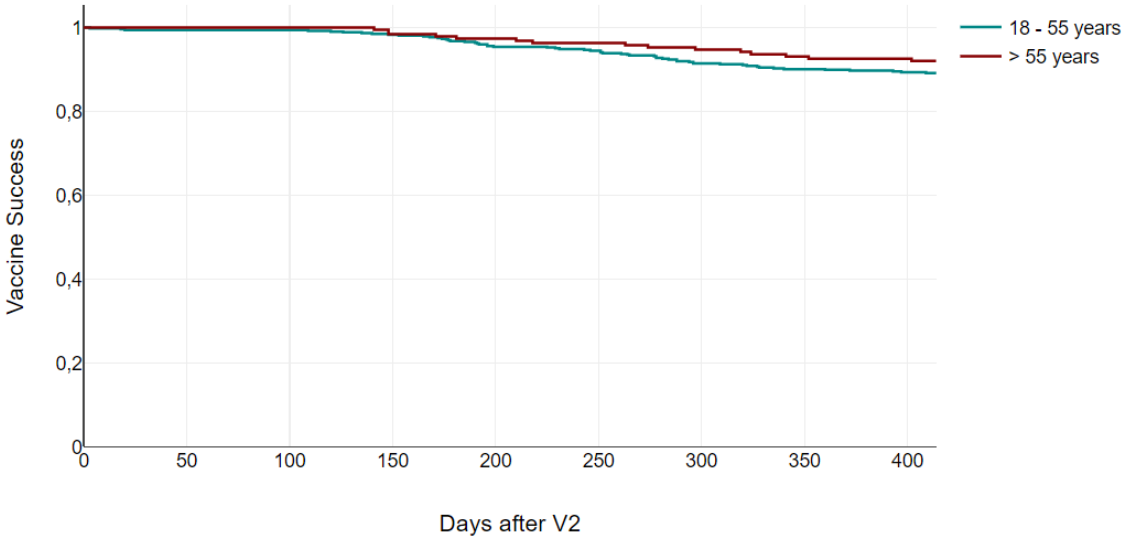

Figure 13: Kaplan-Meier survival function for the age groups 18-55 years and > 56 years after "full vaccination". Vaccine breakthrough infection serves as measured event.

For the present data, the log-rank test showed that there is no difference between the groups in terms of the distribution of time until the event occurs, p=.311. The null hypothesis is thus not rejected. There is no significant difference between the age groups (Fig. 13) although in a merely visual approach younger participants' vaccine efficacy seems to decline faster than that of the older age group.

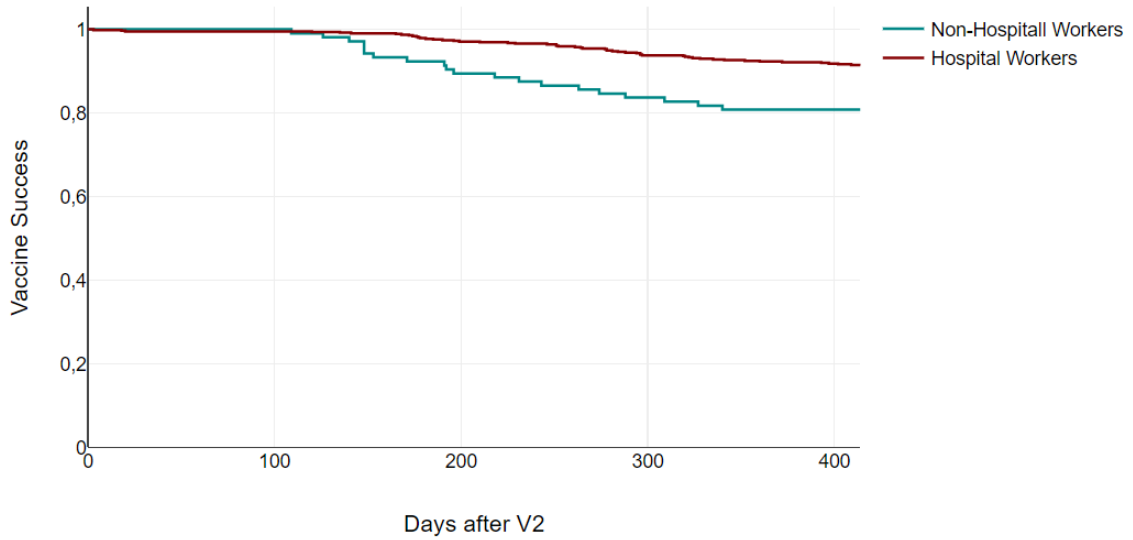

Figure 14: Kaplan-Meier survival function for the hospital workers group compared to the general population control group after "full vaccination". Vaccine breakthrough infection serves as measured event.

For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the breakthrough occurs, p=.001. The null hypothesis is thus rejected and delivers a significant result in the comparison of healthcare workers and non-healthcare workers for breakthrough infections after the "full vaccination" (2nd dose, Fig. 14).

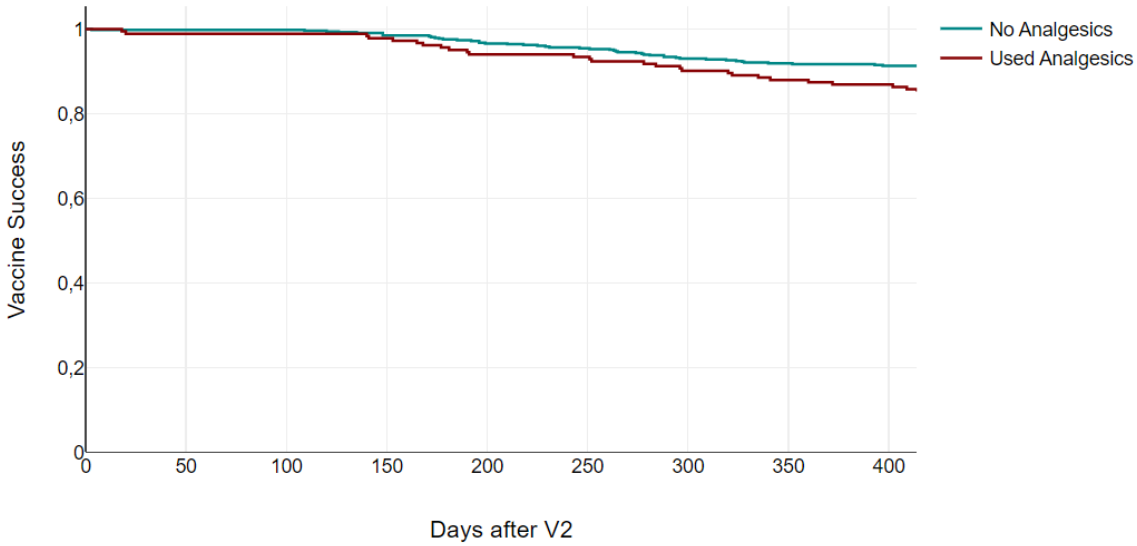

Figure 15: Kaplan-Meier survival function for the comparison of participants who used analgesics after the *second* vaccination and those who did not. Vaccine breakthrough infection serves as measured event.

For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the breakthrough infection occurs, p=.035. The null hypothesis is thus rejected. Therefore, the vaccine upholding rate is factually preserved for a longer time when analgesics have not been used around V2 (Fig. 15). To increase the informative value the analysis has been repeated to check if the difference in breakthrough after a *full vaccination* becomes significant when looking at whether analgesics were used after the *1st dose*. The p-value returned as .31 and is therefore above the significance threshold.

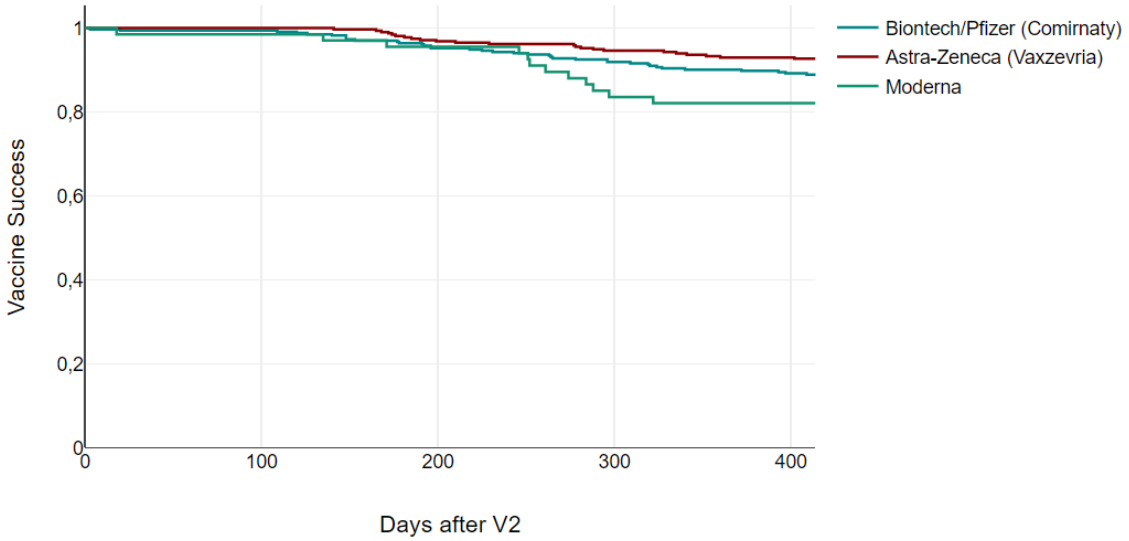

Figure 16: Kaplan-Meier survival function for the comparison of the vaccine that was administered **first**. Vaccine breakthrough infection serves as measured event.

For the present data, the log-rank test showed that there is a difference between the vaccine type groups in terms of the distribution of time until the event occurs, p=.015 (Fig. 16). There is an inter-group difference with statistical significance. The effect is strongly influenced by the difference between AZE and MOD (pairwise p = .005). BNT-MOD and BNT-AZE showed no significant difference (p = .138 and .074 respectively).

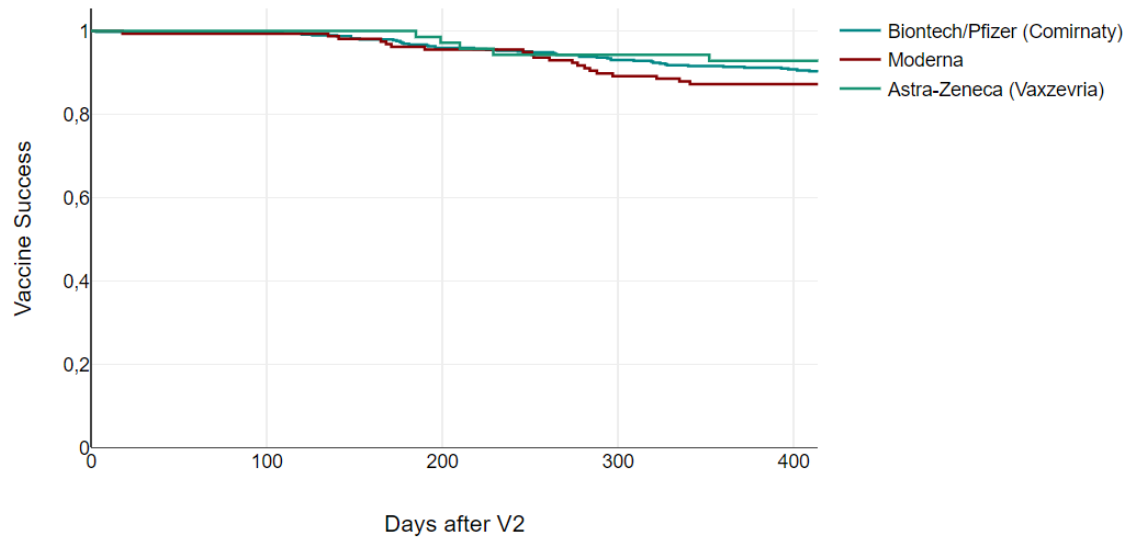

Figure 17: Kaplan-Meier survival function for the comparison of the vaccine that was administered **second**. Vaccine breakthrough infection serves as measured event.

For the second comparison the log-rank test showed that there is no difference between the groups in terms of the distribution of time until the breakthrough occurs, p = .7 (Fig.

17). The null hypothesis is thus retained and the difference therefore not significant. A pairwise analysis yields no significance either.

When comparing the Kaplan-Meier curves of the first and second vaccine according to manufacturer (Fig. 16, Fig. 17) although only significant in the log-rank test for the first analysis, the outcome looks somewhat similar. While having received AZE seems to result in a longer period until a breakthrough occurs and receiving MOD tends to result in a shorter time to contracting a vaccine breakthrough infection. A noteworthy finding is that the decline vaccine success starts shortly after 100 days and seems to have a divergence at around 220 days between the different vaccines. Hence one might speculate that when expanding the observation time also the p-value of a log-rank test for the second analysis (Fig. 17) might move in the direction of statistical significance.

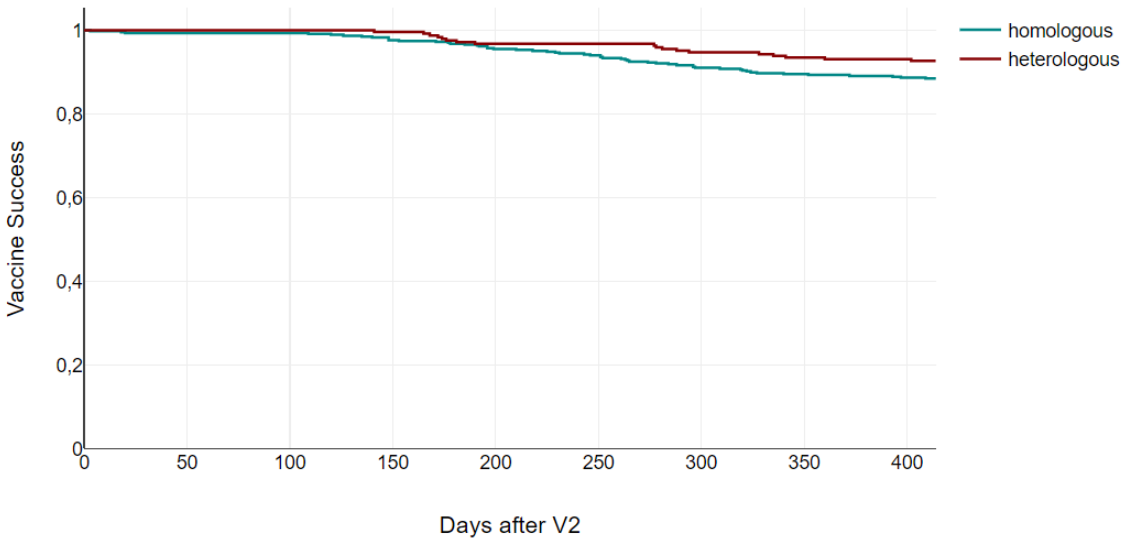

Figure 18: Kaplan-Meier survival function for the homologous vaccine regimen compared to the heterologous one considering V1 and V2. Vaccine breakthrough infection serves as measured event. For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the event occurs, p=.015.

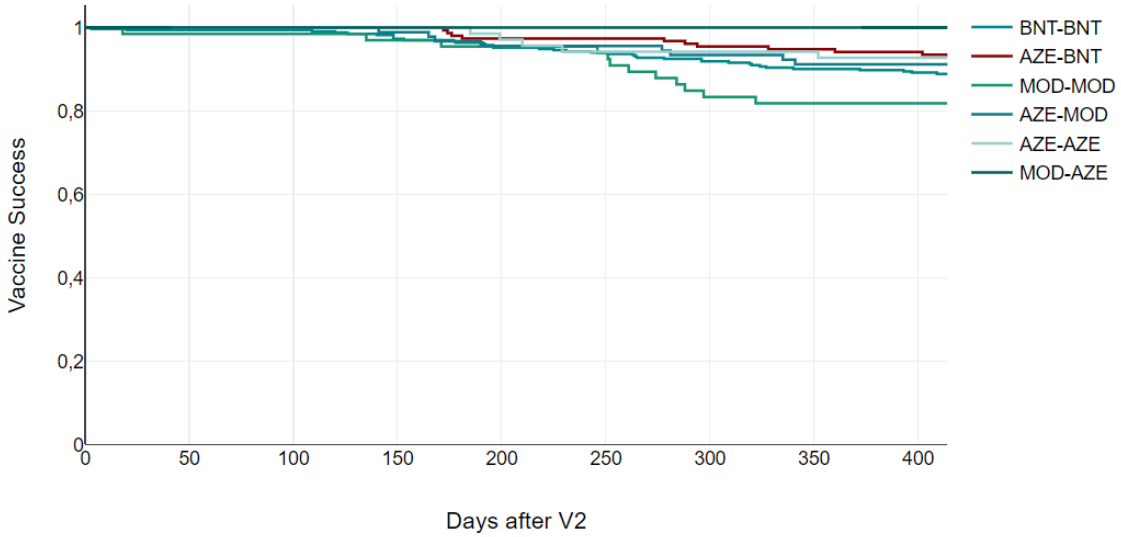

Figure 19: Kaplan-Meier survival function for the specific vaccine regimen considering V1 and V2. Vaccine breakthrough infection serves as measured event. For the present data, the log-rank test showed that there is no difference between the groups in terms of the distribution of time until the event occurs, p=.058.

It becomes apparent that the homologous regimen of Moderna declines the fastest among the observed. To test whether the difference is significant enough to cause a "shine through effect" in the first analysis by producing a type I error, the log-rank test of Figure 18 (*homologous vaccine regimen compared to the heterologous regimen*) was repeated, excluding MOD-MOD. When eliminating "MOD-MOD" from the analysis, the significance of the p-value of a log-rank test for Figure 18 increases to .036 and therefore remains significant. For Figure 19, obviously, *p* remains insignificant upon exclusion of MOD-MOD. Nevertheless, it is a relevant finding when examining the factors influencing the decline in vaccine upholding. A homologous V1-V2-regimen of Moderna seems to be the least favorable by far.

After the 3rd Vaccination

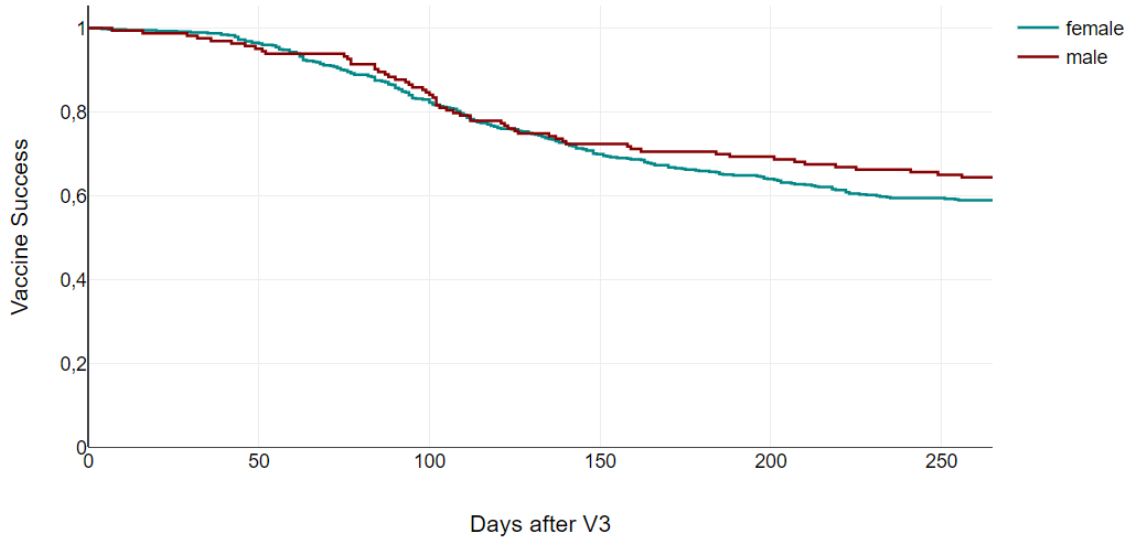

Figure 20: Kaplan-Meier survival function for gender after booster vaccination. Vaccine breakthrough infection after the third dose serves as measured event.

For the present data, the log-rank test showed that there is no difference between the genders in terms of the distribution of time until the event occurs, p=.153 (Fig. 20). The null hypothesis is thus not rejected.

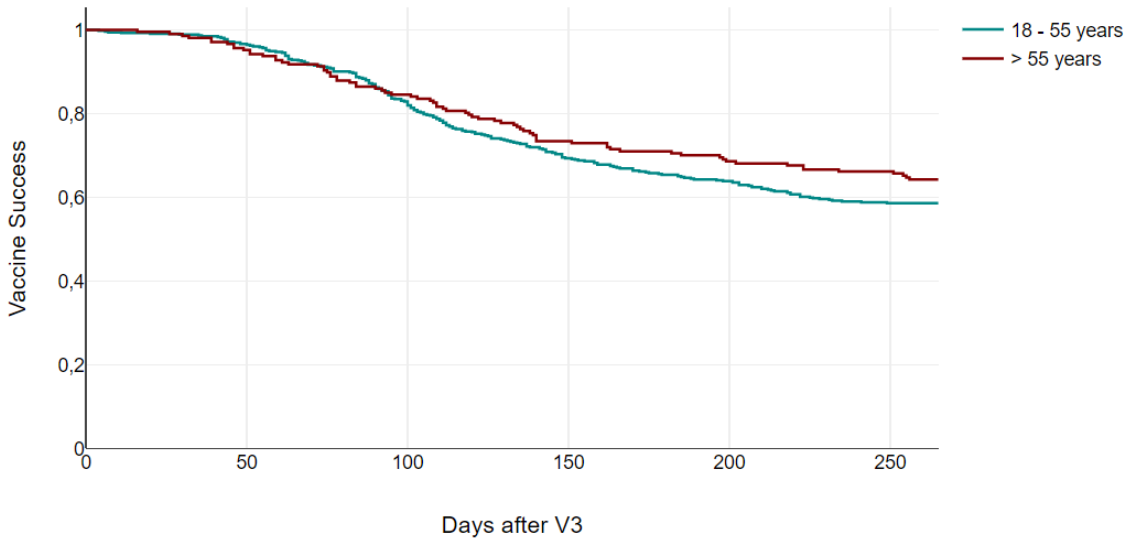

Figure 21: Kaplan-Meier survival function for the age groups 18-55 years and > 55 years after booster vaccination. Vaccine breakthrough infection serves as measured event.

For the present data, the log-rank test showed that there is no significant difference between the groups in terms of the distribution of time until the event occurs, p=.515. The null hypothesis is thus not rejected. Just like for vaccine breakthrough infections after the

"full vaccination" (Fig. 13) there is no significant difference between the age groups although in a merely visual approach younger participants' vaccine efficacy seems to decline faster than that of the older age group.

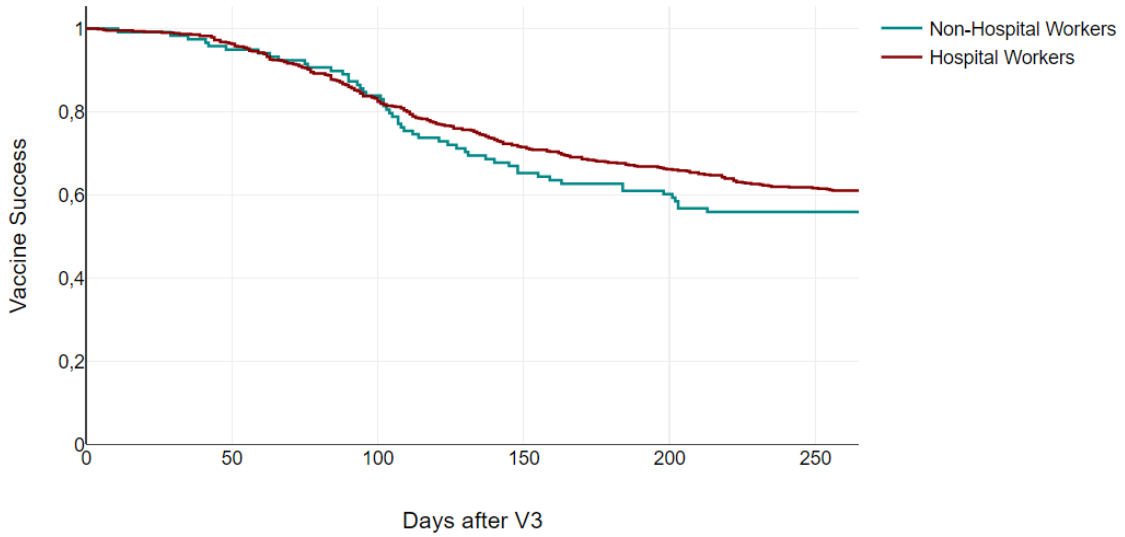

Figure 22: Kaplan-Meier survival function for the hospital workers group compared to the general population control group after the third dose vaccine breakthrough infection serves as measured event.

For the present data, the log-rank test showed that there is no difference between the occupational groups in terms of the distribution of time until the event occurs, p=.051 (Fig. 22), being just above the defined alpha level and the null hypothesis is thus still retained.

While the difference between the hospital workers in the general population control group was statistically significant after the so-called "full vaccination" (i.e., V2, Fig. 14), when looking at the vaccine breakthrough infections after the third, so called booster dose, the significance falls slightly below the defined alpha level regardless of the higher sample size. Yet we can observe that the effect of a faster decline in the general population control group, although not statistically significant, continues just like after V2 (Fig. 22). In a rather visual approach, it appears like there is increasing divergence between the compared populations.

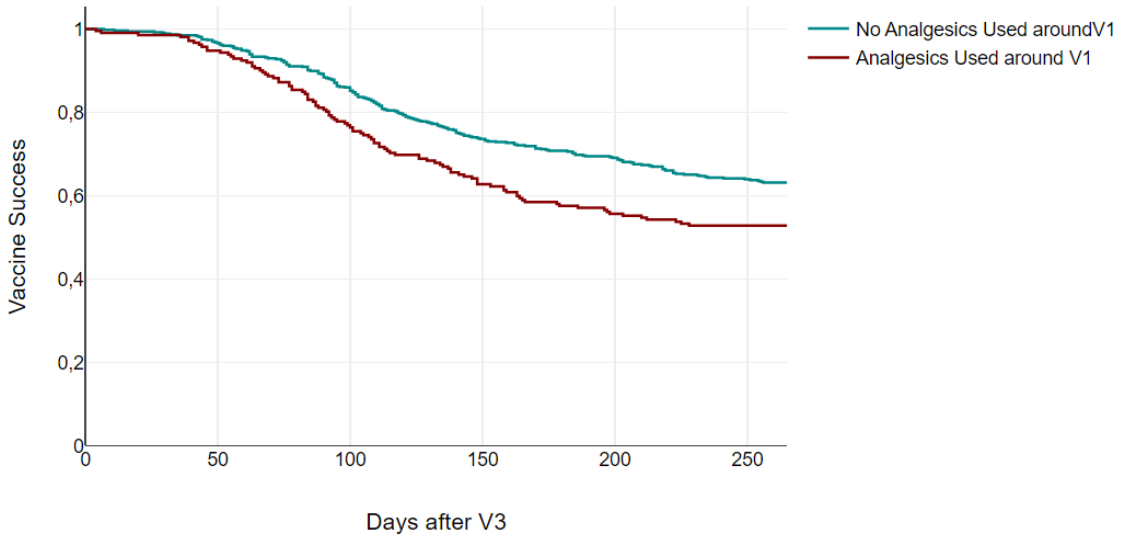

Figure 23: Kaplan-Meier survival function for the comparison of participants who used analgesics after the *first* vaccination and those who did not. Vaccine breakthrough infection after the *third* vaccination serves as measured event.

For the present data, the log-rank test showed that there is a difference between the analgesia groups in terms of the distribution of time until the event occurs, p=.006 (Fig. 23). The null hypothesis is thus rejected.

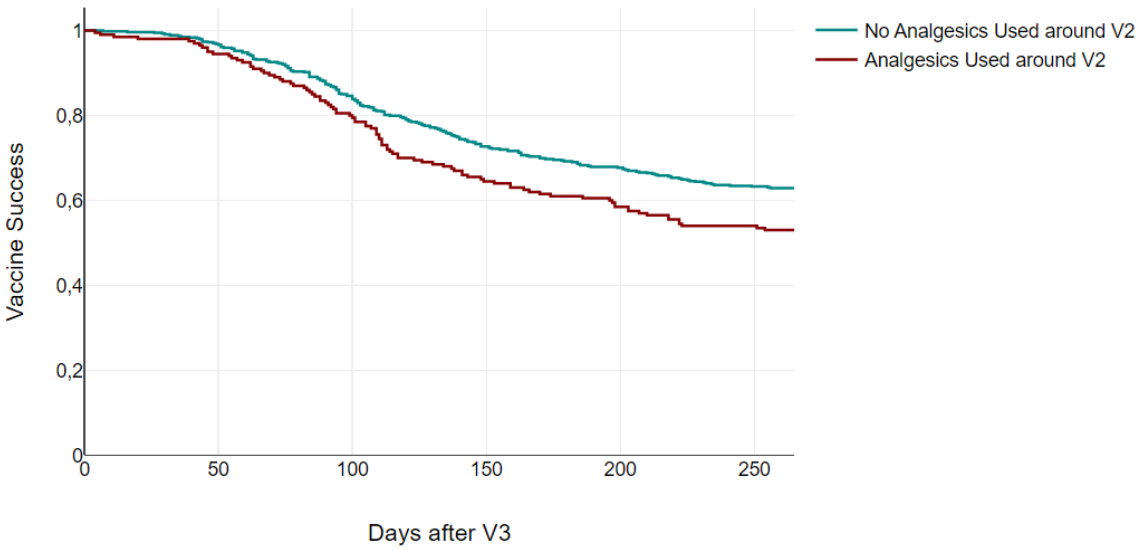

Figure 24: Kaplan-Meier survival function for the comparison of participants who used analgesics after the *second* vaccination and those who did not. Vaccine breakthrough infection after the *third* vaccination serves as measured event.

For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the event occurs, p=.041 (Fig. 24). The null hypothesis is thus rejected.

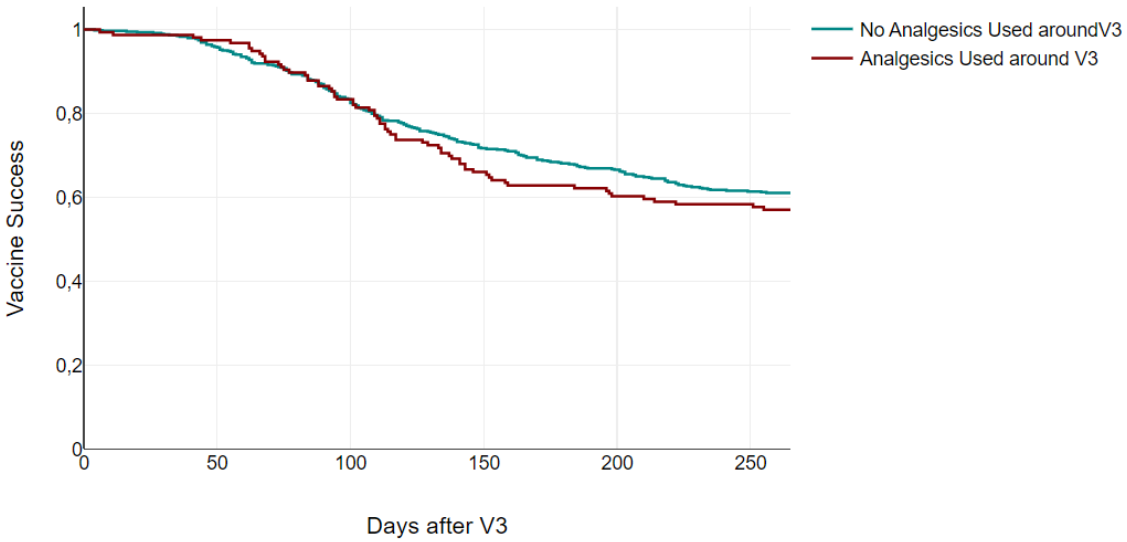

Figure 25: Kaplan-Meier survival function for the comparison of participants who used analgesics after the *third* vaccination and those who did not. Vaccine breakthrough infection after the *third* vaccination serves as measured event.

For the present data, the log-rank test showed that there is no significant difference between the groups in terms of the distribution of time until the event occurs, p=.404 (Fig. 25).

Interestingly enough when performing a log rank test, we yield a statistically significant difference in terms of time of breakthrough infection after a **3rd** dose depending on whether the participants used analgesics after the **2nd**, so called "full vaccination".

In contrast to the analysis of those who had a vaccine breakthrough infection before the third dose (Fig. 24) we now yield a significant difference depending on whether analgesics are used after the first dose as well.

Whether participants have used analgesics or not after the *third* dose does not result in a statistically significant p-value. All findings are summarized pictographically in the *Discussion* Section (Figure 60).

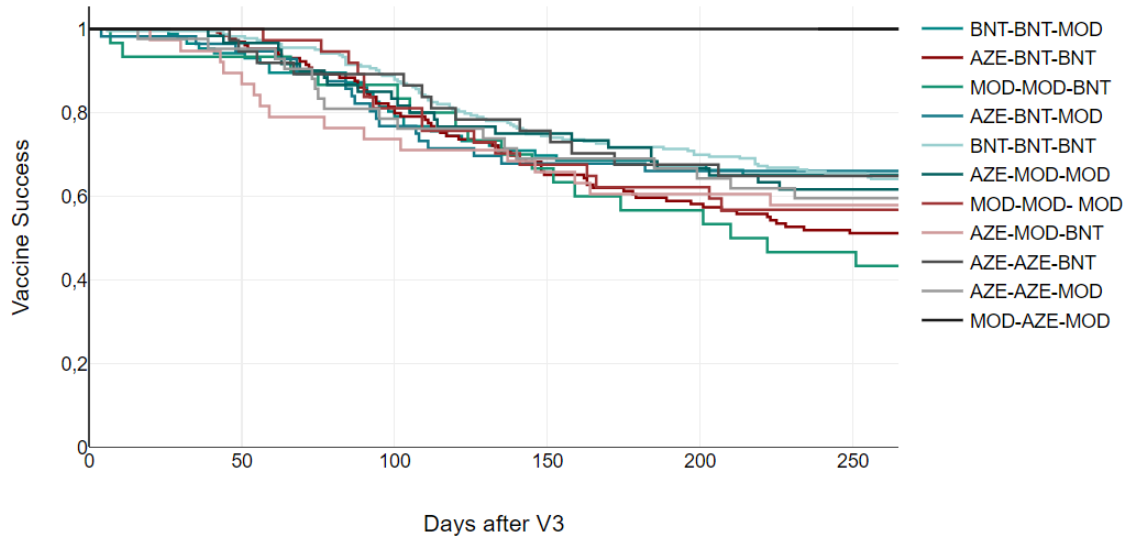

Figure 26: Kaplan-Meier survival function for the specific vaccine regimen considering V1, V2 and V3. Vaccine breakthrough infection after V3 serves as measured event. For the present data, the log-rank test showed that there is no difference between the groups in terms of the distribution of time until the event occurs, p=.347. The null hypothesis is thus not rejected.

Because the analysis of Figure 26 includes ten groups that are tested simultaneously, and the null hypothesis is not rejected, it is important to examine whether a type II error has occurred.

Repeating the analysis in pairwise fashion, comparing the highest portion of vaccine success with the lowest, i.e., AZE-BNT-MOD and MOD-MOD-BNT results in a p-value of .11 in the log-rank test, hence confirming a correct result.

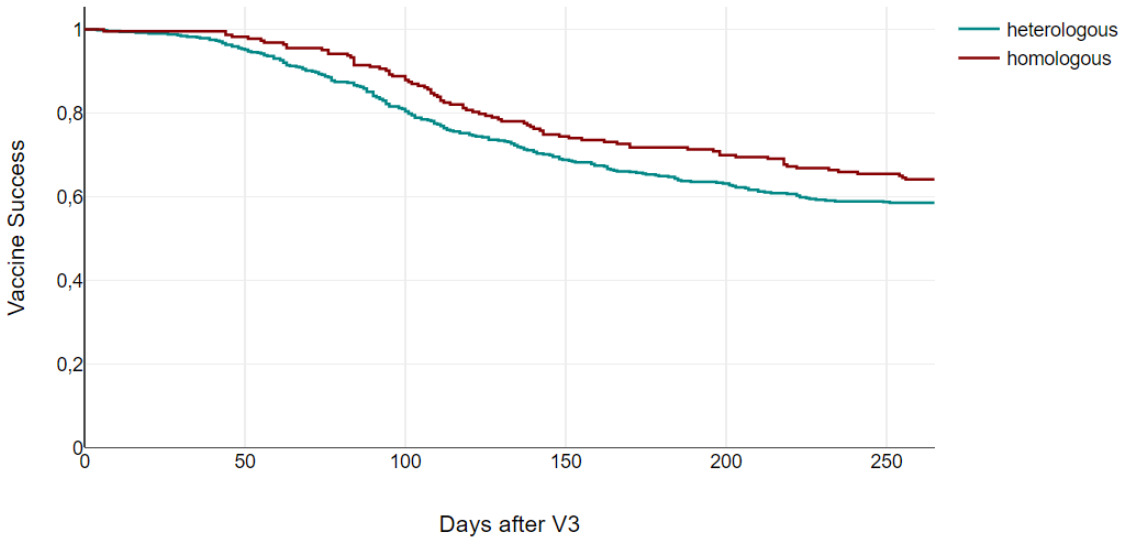

Figure 27: Kaplan-Meier survival function for the homologous vaccine regimen compared to the heterologous one considering V1, V2 and V3. Vaccine breakthrough infection serves as measured event.

For the present data, the log-rank test showed that there is a no significant difference between the groups in terms of the distribution of time until a breakthrough infection occurs, p=.224. The null hypothesis is thus retained. Although the p-value is below the defined significance threshold the infection dynamics are inverted in comparison to primary immunization in respect to homo- or heterology (Figure 27).

Other Factors

The same analysis has been performed between each and amnestic criterion (Table 3, section *Methods*) and vaccine breakthrough infections after the third vaccine as well as each side effect (i.e., each local and systemic side effect for each consecutive vaccination in a dichotomous format). The following figures show only the significant results.

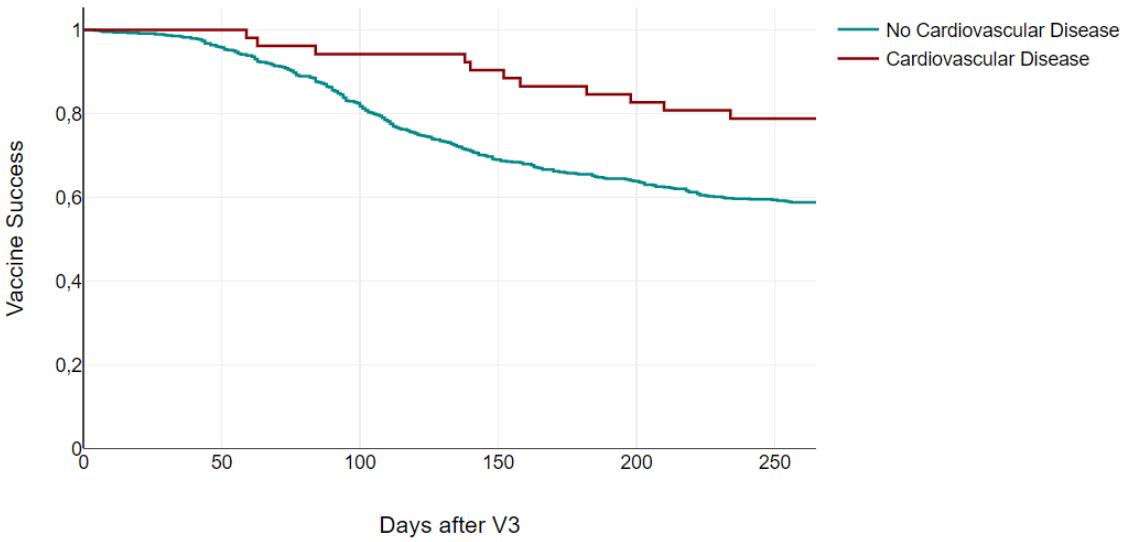

Figure 28: Kaplan-Meier survival function for the comparison of participants who reported suffering from a cardiovascular condition (n=58) and those who did not. Vaccine breakthrough infection after the *third* vaccination serves as measured event. For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the event occurs, p=.007. The null hypothesis is thus rejected.

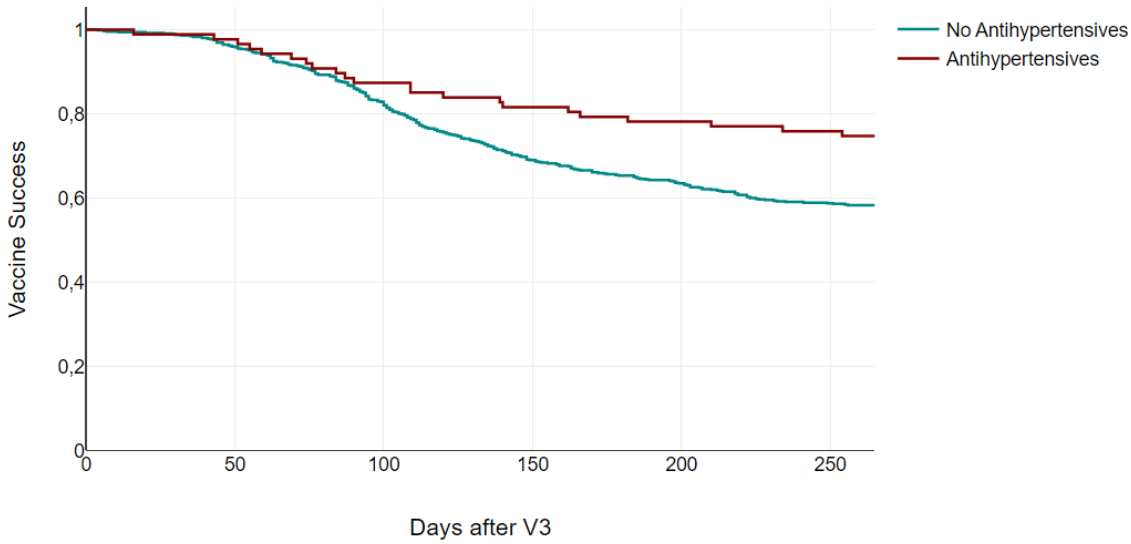

Figure 29: Kaplan-Meier survival function for the comparison of participants who reported using antihypertensive medication (n=95) and those who did not. Vaccine breakthrough infection after the

third vaccination serves as measured event. For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the event occurs, p=.005. The null hypothesis is thus rejected.

The finding of Figures 28 and 29 at first glance comes a bit as a surprise. Participants who report having a cardiovascular condition are significantly less likely to contract a SARS-CoV-2 infection or in other words have a significantly longer time to breakthrough infection than the control group. Somehow acting as a quality control of our data coherence, participants who report using antihypertensives demonstrate the same infection dynamic.

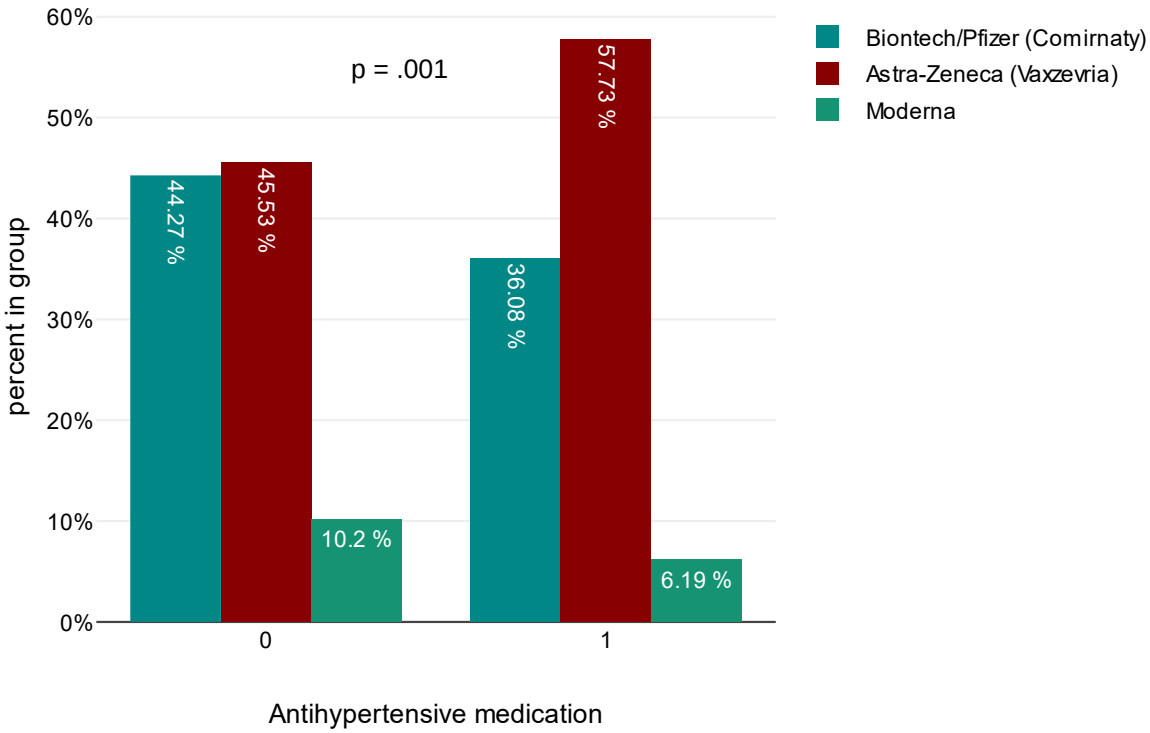

Figure 30: Kind of vaccine administered upon the first vaccination in comparison between those who report using antihypertensive medication and those who do not. The bar graphs show the percentage in the groups on the X axis. The difference is significant (p = .001, Chi-square test of independence).

A potential or at least partial explanation for this effect may be explained by Figure 16. A higher proportion of those on antihypertensives have received AZE as a first vaccine and a lower proportion has received MOD than the control group. Referring to Figure 29

the data has demonstrated that this constellation changes the infection breakthrough dynamics favorably.

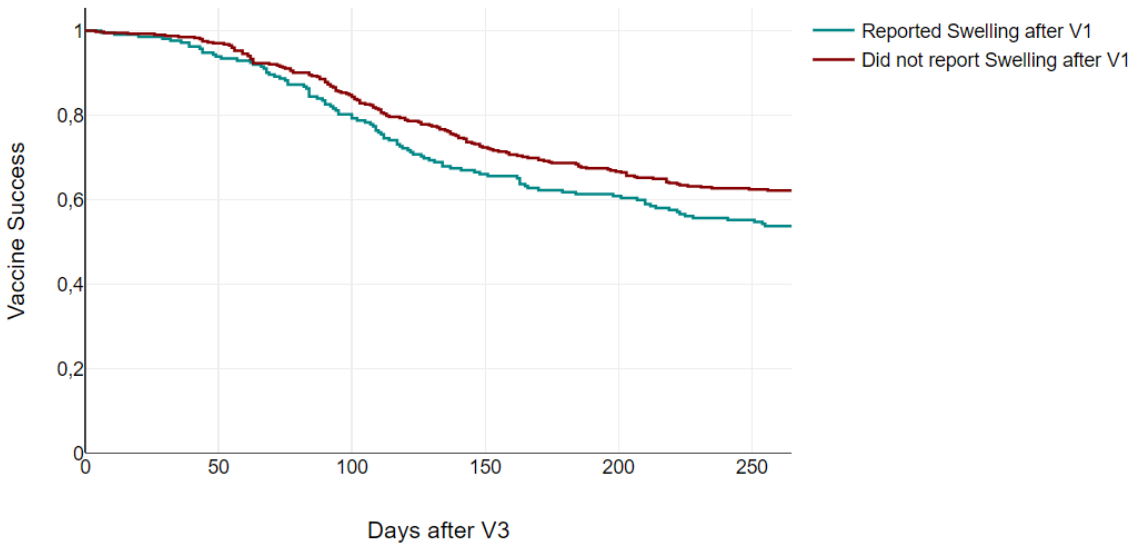

Figure 31: Kaplan-Meier survival function for the comparison of participants who reported local swelling and those who did not. Vaccine breakthrough infection after the *third* vaccination serves as measured event. For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the event occurs, p=.011. The difference is thus significant.

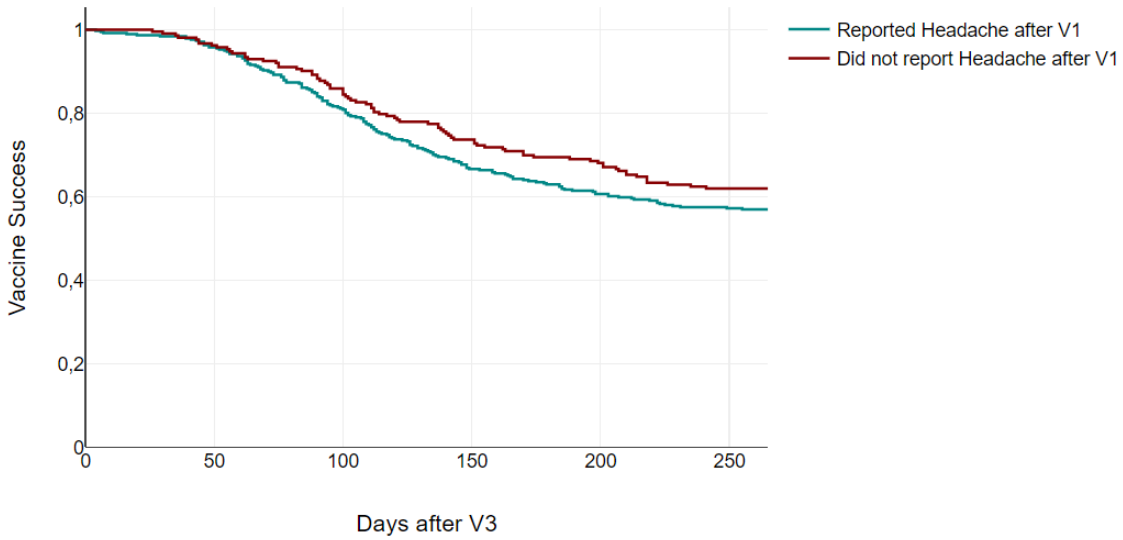

Figure 32: Kaplan-Meier survival function for the comparison of participants who reported headache and those who did not. Vaccine breakthrough infection after the *third* vaccination serves as measured event. For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the event occurs, p=.041. The null hypothesis is thus rejected.

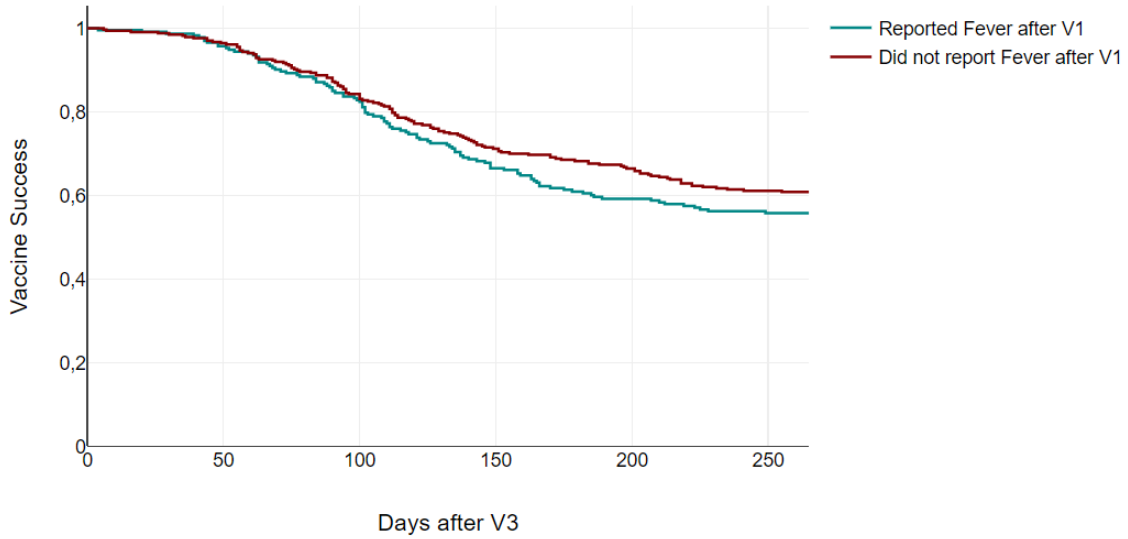

Figure 33: Kaplan-Meier survival function for the comparison of participants who reported Fever and those who did not. Vaccine breakthrough infection after the *third* vaccination serves as measured event. For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the event occurs, p=.049. The null hypothesis is thus rejected.

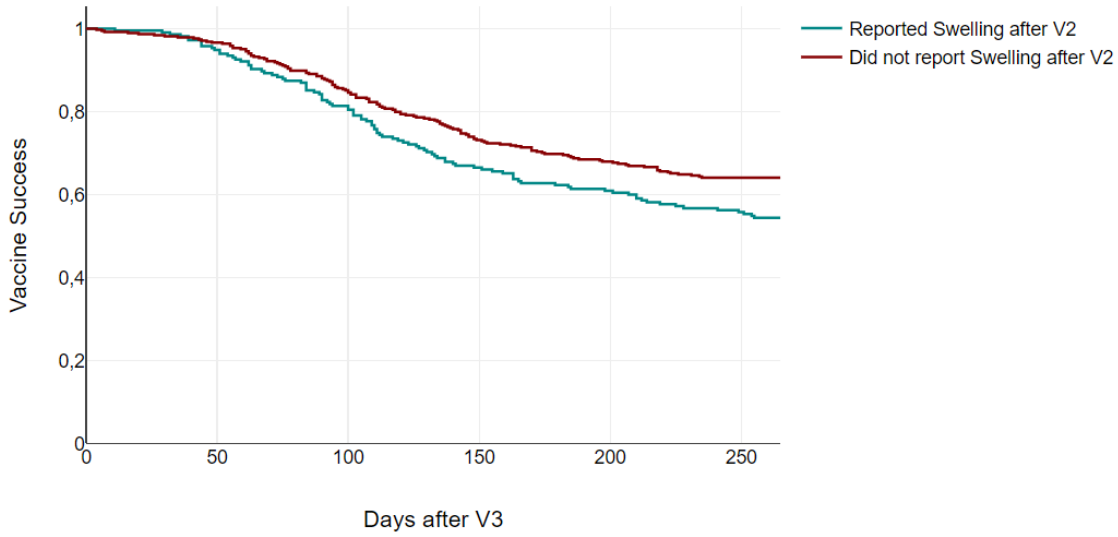

Figure 34: Kaplan-Meier survival function for the comparison of participants who reported local swelling and those who did not. Vaccine breakthrough infection after the *third* vaccination serves as measured event. For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the event occurs, p=.022. The null hypothesis is thus rejected.

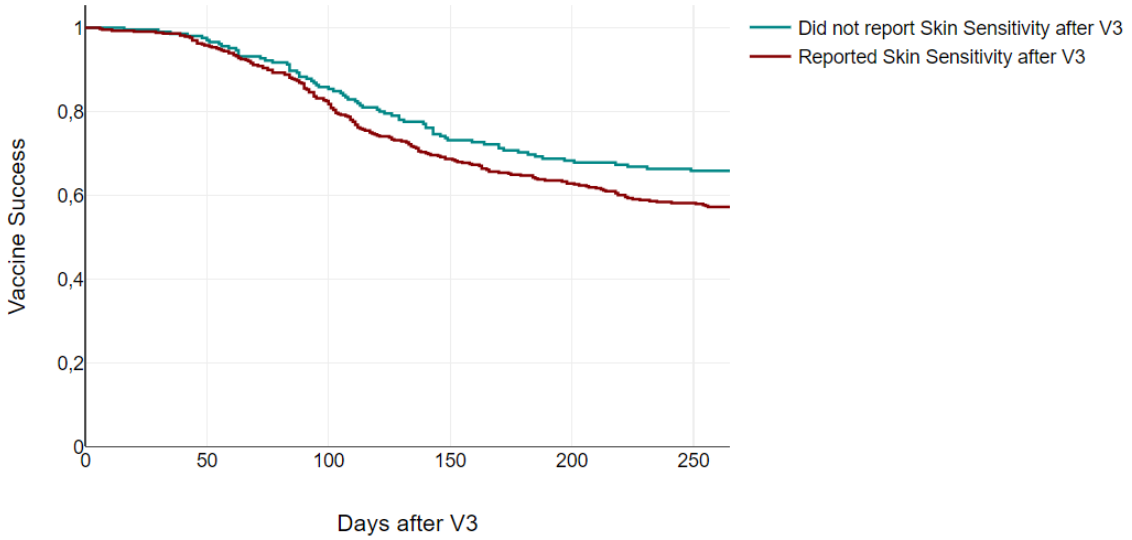

Figure 35: Kaplan-Meier survival function for the comparison of participants who reported local skin sensitivity and those who did not. Vaccine breakthrough infection after the *third* vaccination serves as measured event. For the present data, the log-rank test showed that there is a difference between the groups in terms of the distribution of time until the event occurs, p=.031. The null hypothesis is thus rejected.

In summary, when interpreting Figure 31 through Figure 35, it is fair to say that the occurrence of local swelling (Fig. 31), headache (Fig. 32) and fever (Fig. 33) for the first vaccination, local swelling on the second vaccination (Fig. 34) and local skin sensitivity on the 3rd (Fig. 35) are predictive of vaccine upholding. An increasing divergence between these groups appears around day 100 after the third dose. In other words, for these just mentioned local and systemic side effects, higher reactogenicity seems to be negatively related to specific SARS-CoV-2 immunocompetence. Other than the somewhat obvious explanation of the effect of antihypertensive medication, no such effect becomes immediately evident in this case. Inevitably an attempted correlation with serum antibody levels must be performed (see Figure 44).

Antibody Levels

Although our rather large sample size is able to make up for a couple of individual differences, most of the following analyses rely on a certain chain of events. Three separate vaccine applications (V), up to three blood samples (A), of which two maybe the analysis of the cellular immune response (T) and up to two infections (I) in the overall sample size of 1060 participants lead to 124 different combinations of these events. The most common are shown in Table 18.

Sequence of Events	Frequency
V1 V2 A1 V3 A2 A3	255
V1 V2 A1	127
V1 V2 A1 V3 A2 I1 A3	108
V1 V2 A1 V3 A2	95
V1 V2 A1 V3 A2 I1 A3 T1 A4	36
V1 V2 A1 A2	35
V1 V2 A1 A2 A3	25
V1 V2 A1 V3	23
V1 V2 A1 V3 A2 I1	20
V1 V2 A1 V3 A2 A3 I1	19
V1 V2 A1 V3 A2 I1 A3 T1	18
V1 V2 V3 A1	16
V1 V2 A1 V3 I1 A2	15
V1 V2 V3 A1 A2	15
V1 V2 A1 A2 I1 A3	14
V1 V2 A1 V3 I1 A2 A3	14
V1 V2 V3 A1 I1 A2	13
V1 V2 V3 A1 A2 A3	13
V1 V2 A1 A2 I1	10
I1 V1 V2 A1 V3 A2 A3	10
V1 V2 A1 I1 A2 A3	9
V1 V2 A1 A2 T1 V3 A3 T2 I1 A4	8
I1 V1 V2 A1	7
V1 V2 V3 A1 A2 I1 A3	6
V1 V2 A1 A2 T1 V3 A3 T2 A4	5
V1 V2 A1 I1 A2	5

Table 18: Sequence of events and their frequency in our sample. Only those of a frequency of five and above are shown.

In order to compare the antibody levels and efficiency effectively, the following sequence of events is used: V1 V2 A1 V3 A2 A3 for the following analysis. Summarized in the clear words we are now focusing on participants who received a “full vaccination” consisting of two consecutive doses first, got their antibody levels measured, then

received a third dose followed by two blood samples, that are not interrupted by another immunizing event (i.e. another vaccine or an infection).

524 participants matched these criteria. The arithmetic mean of days between the first and the second vaccination in this subsample was 58 days (± 29). The arithmetic mean between the second and the third dose was 202 days (± 35). On average, 166 (±47) days passed between the full vaccination and the first blood sample, 47 (±42) days between the booster dose and the second blood sample and 204 days (±31) between the booster dose and the third sampling. Obviously, the sample is thinning out the more consecutive criteria are applied. 523 participants matching the sequence as mentioned above of events had a first blood sample, 358 the second one and only 50 a third one.

Antibody Dynamics over Time

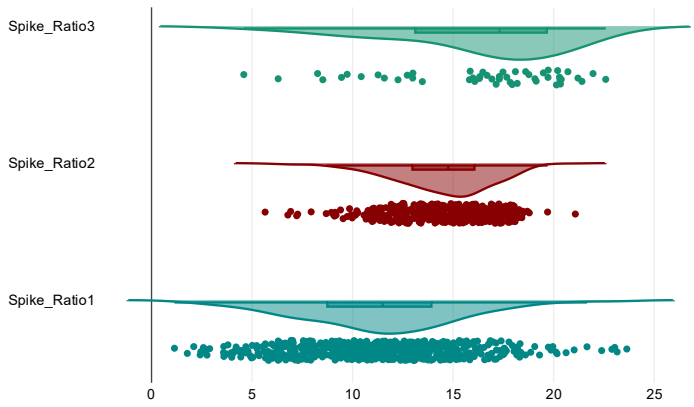

Figure 36: Raincloud plot of the spike ratio measured on first, second and third sample.

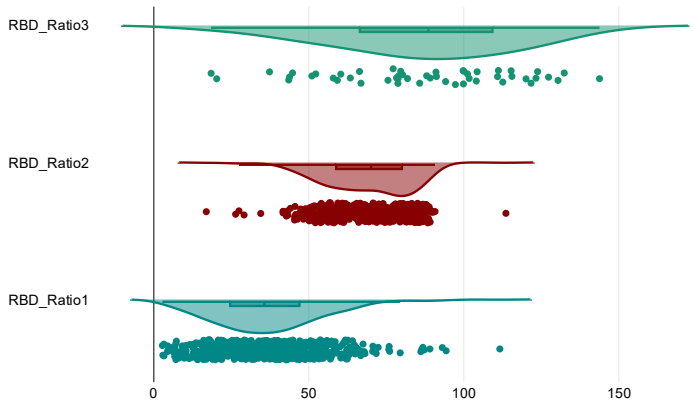

Figure 37: Raincloud plot of the RBD ratio measured on first, second and third sample.

What is true when examining Figures 36 and 37 is also true for the other quantitative measurements of humoral immune response when comparing the first, second and third blood sample. The central tendency of each measure increases over time. A booster dose can explain this change from the first sample to the second, that has been administered in between. However, without a recorded immunizing event between the second and third blood sample, the central tendency further increases. Looking at the measurement of anti-nucleocapsid antibodies can help to serve as an internal quality control to double check if an infection has occurred "silently" without the participant noticing or reporting it as such. Only three participants tested positive for nucleocapsid (anti-N ratio ³ 1) in this particular subsample newly at the third blood sample. Hence this alone does not explain the dynamics.

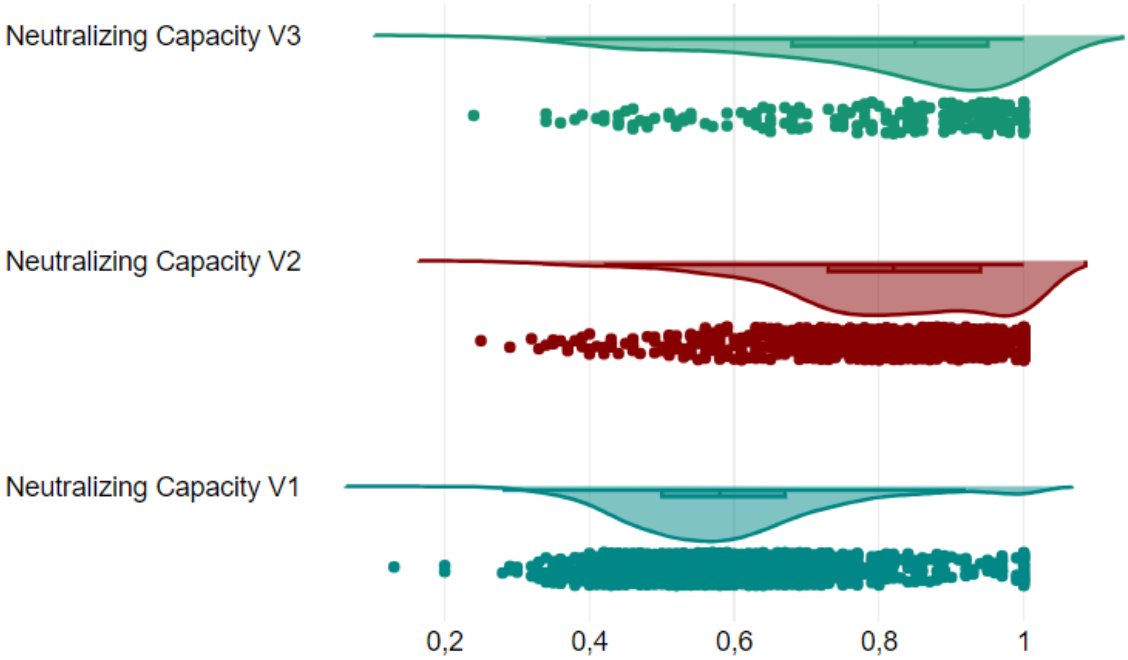

Figure 38: Raincloud plot of the NBP RBD ratio measured on first, second and third sample.

Figure 38 demonstrates a more comprehensive dynamic. In opposite to the quantitative analysis of the other parameters, NeutrobodyPlex analysis being a competitive assay for the receptor binding domain, clearly shows that the capability of the present antibodies in the participants' serum shows some degree of efficiency after a “full vaccination” but an even higher efficiency after the booster dose (represented by the second blood sample shown in red). Without another immunizing event, the central tendency moves to the left on the scale, decreasing neutralizing capacity (as indicated by the green data points, sample three). An ANOVA analysis confirms this merely visual finding for dependent samples with a Bonferroni post-hoc correction demonstrating a pairwise, intergroup-p value of <.001.

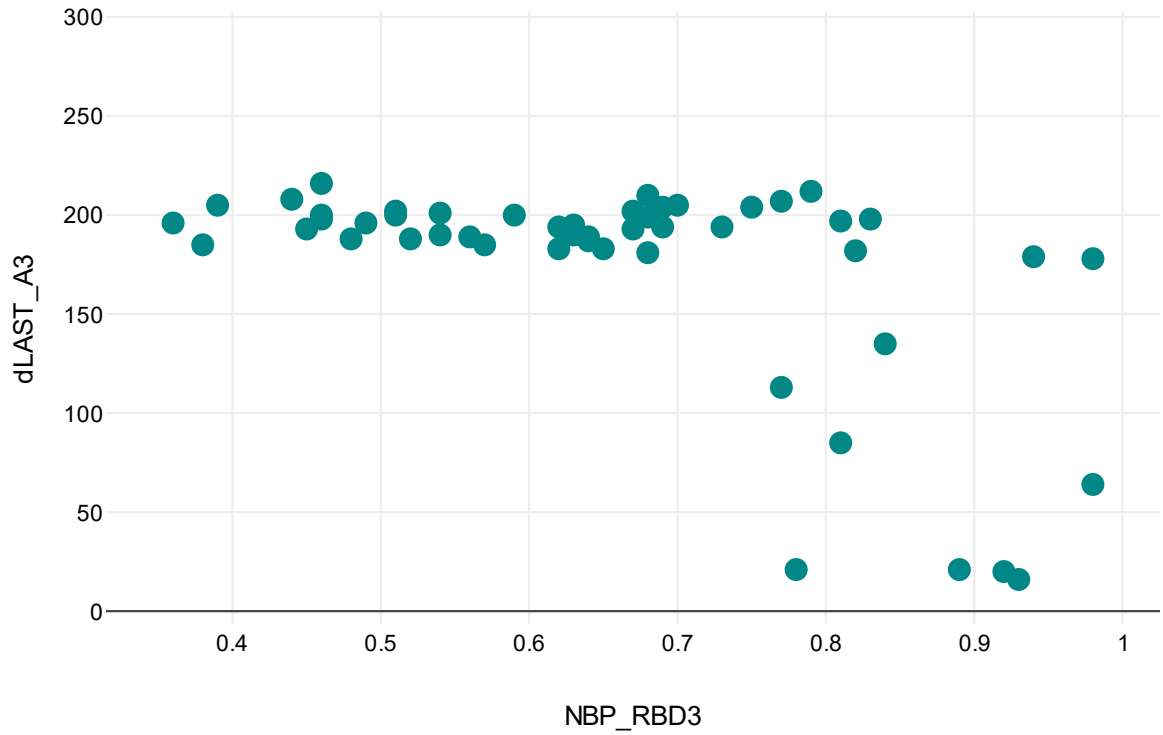

Figure 39**:** Scatter diagram of NeutrobodyPlex on the third sample on the x-axis and the last immunizing event before the sample, i.e., third vaccination in this subsample.

What is demonstrated here is a statistically significant correlation between the time distance of the booster vaccine to blood sampling and the neutralizing capacity of the participants antibodies. Due to our study design most participants got that a third blood sample roughly after 200 days after booster vaccine. Their neutralizing capacity was between 35% and 83%. Those who received the booster vaccine more recently demonstrated a significantly higher neutralizing capacity as a Pearson correlation test confirmed ($p<.001$). The correlation coefficient r was calculated as -0.57, the correlation is therefore strong.

		Anti-Spike ratio	Anti-RBD ratio	Anti-S1 ratio	Anti-S2 ratio	RBD ratio Neutrobodyplex	Days after last immunizing event
Anti-Spike ratio	Correlation	1	0.9	0.9	0.38	0.39	-0.38
	p (2-tailed)		<.001	<.001	<.001	<.001	<.001
Anti-RBD ratio	Correlation	0.9	1	0.93	0.37	0.46	-0.29
	p (2-tailed)	<.001		<.001	<.001	<.001	<.001
Anti-S1 ratio	Correlation	0.9	0.93	1	0.43	0.51	-0.32
	p (2-tailed)	<.001	<.001		<.001	<.001	<.001
Anti-S2 ratio	Correlation	0.38	0.37	0.43	1	0.26	-0.15
	p (2-tailed)	<.001	<.001	<.001		<.001	0.001
RBD ratio Neutrobodyplex	Correlation	0.39	0.46	0.51	0.26	1	-0.13
	p (2-tailed)	<.001	<.001	<.001	<.001		0.006
Days after last immunizing event	Correlation	-0.38	-0.29	-0.32	-0.15	-0.13	1
	p (2-tailed)	<.001	<.001	<.001	0.001	0.006	

Table 19: Spearman correlation between the days after the last immunizing event (i.e., second dose) and the antibody measurements on the first blood sample.

A significant correlation between all antibody parameters and the time that has passed since the last immunizing event, the second dose in this analysis, is demonstrated. Furthermore, all coefficients are negative, meaning that the more time passes the lower the measured humoral immunity. A scatterplot for visualization is portrayed in Figure 40.

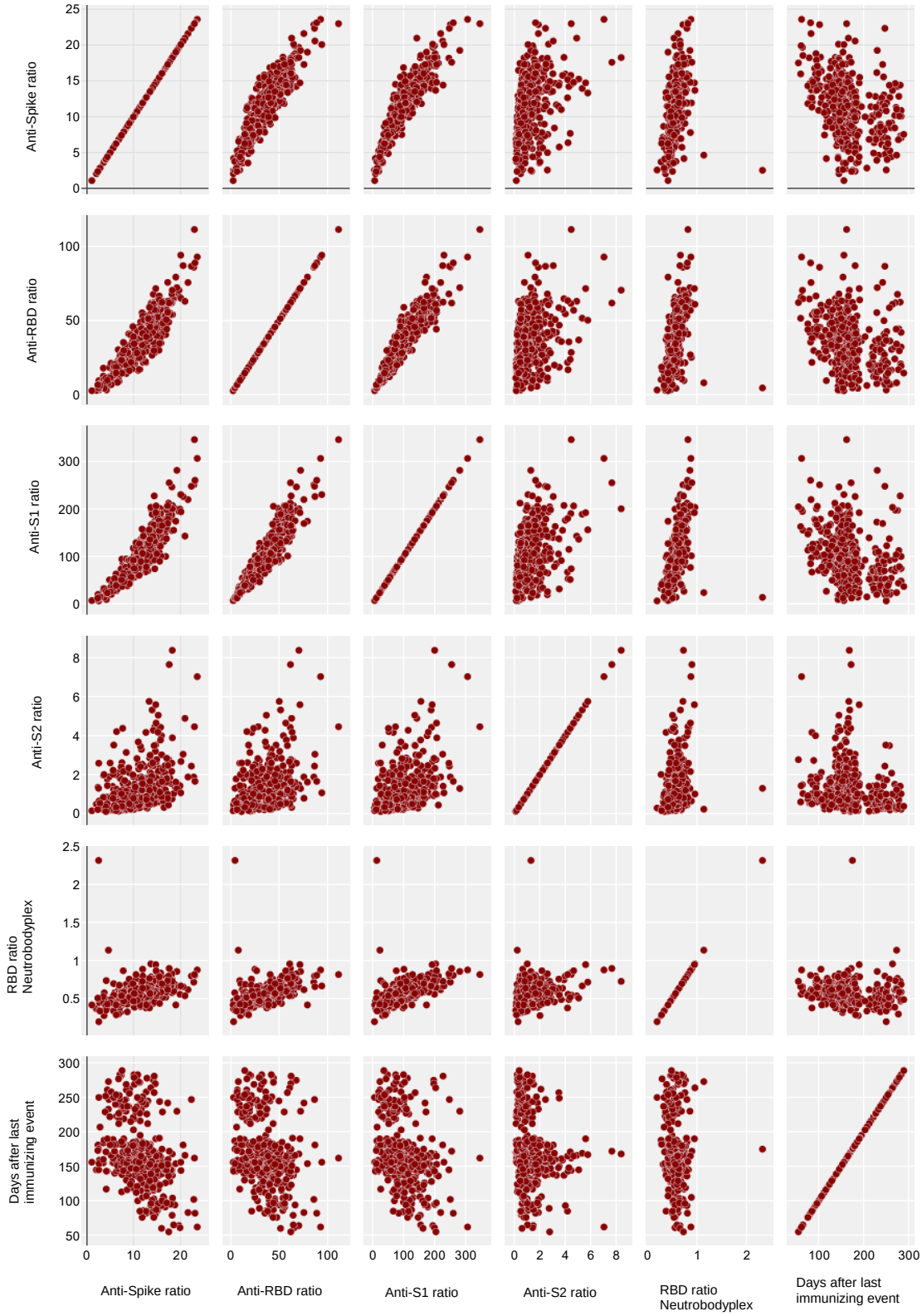

Figure 40: Scatterplot corresponding to Table 18.

		Anti-Spike ratio	Anti-RBD ratio	Anti-S1 ratio	Anti-S2 ratio	RBD ratio Neutrobodyplex	Days after last immunizing event
Anti-Spike ratio	Correlation	1	0.72	0.05	-0.22	-0.31	0.01
	p (2-tailed)		<.001	.334	<.001	<.001	.903
Anti-RBD ratio	Correlation	0.72	1	0.41	0.07	0.1	0.05
	p (2-tailed)	<.001		<.001	.22	.061	.313
Anti-S1 ratio	Correlation	0.05	0.41	1	0.42	0.56	-0.19
	p (2-tailed)	.334	<.001		<.001	<.001	<.001
Anti-S2 ratio	Correlation	-0.22	0.07	0.42	1	0.47	-0.04
	p (2-tailed)	<.001	.22	<.001		<.001	.481
RBD ratio Neutrobodyplex	Correlation	-0.31	0.1	0.56	0.47	1	-0.25
	p (2-tailed)	<.001	.061	<.001	<.001		<.001
Days after last immunizing event	Correlation	0.01	0.05	-0.19	-0.04	-0.25	1
	p (2-tailed)	.903	.313	<.001	.481	<.001	

Table 20: Spearman correlation between the days after the last immunizing event (i.e., third dose) and the antibody measurements on the second blood sample.

A significant correlation between antibody levels against the S1 subunit as well as neutralizing capacity and the time that has passed since the last immunizing event, the third dose in this analysis, is demonstrated (Table 20). Furthermore, both coefficients are negative, meaning that the more time passes the lower the measured humoral immunity. A scatterplot for visualization is portrayed in Figure 41.

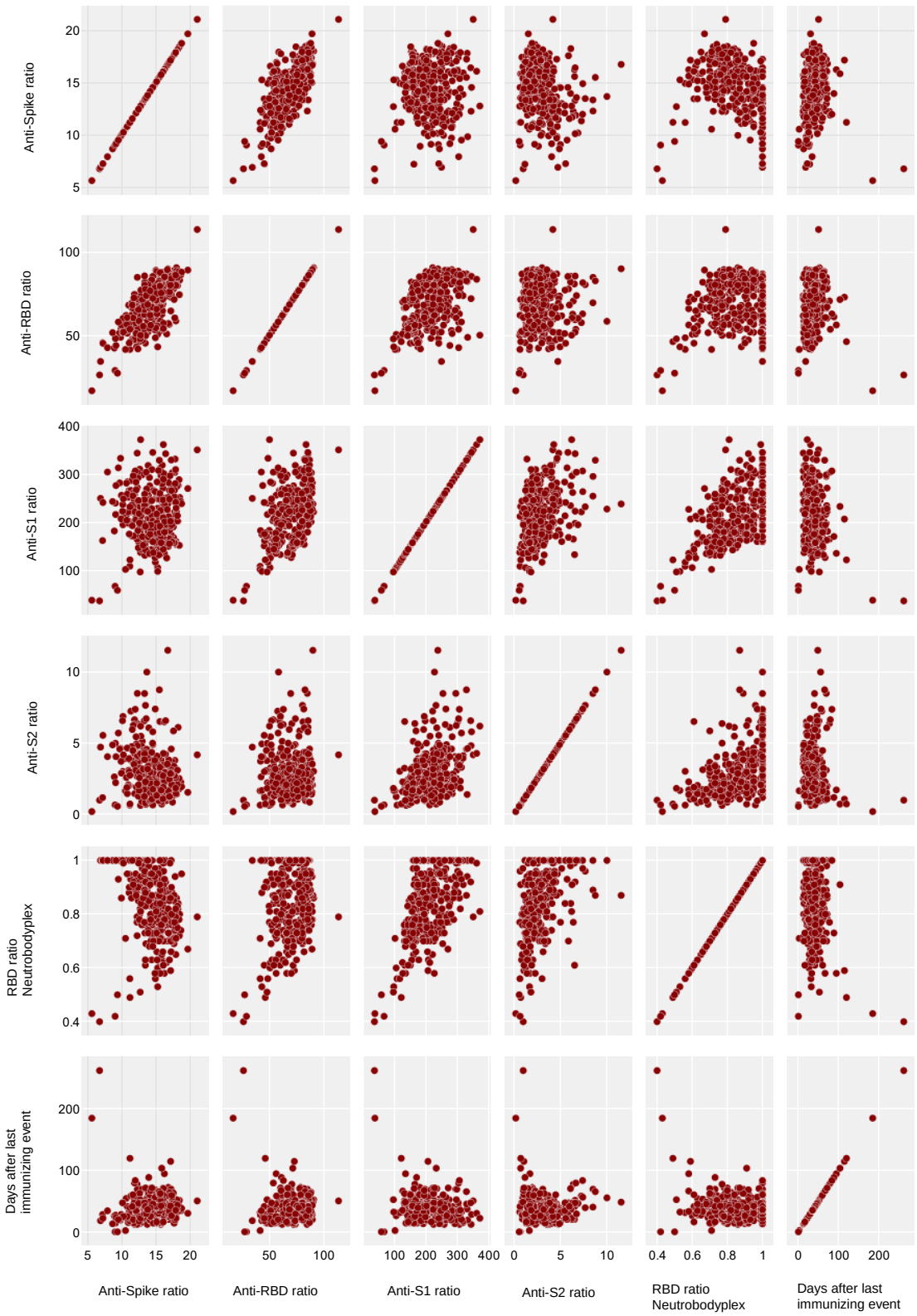

Figure 41: Scatterplot corresponding to Table 19.

		Anti-Spike ratio	Anti-RBD ratio	Anti-S1 ratio	Anti-S2 ratio	RBD ratio Neutrobodyplex	Days after last immunizing event
Anti-Spike ratio	Correlation	1	0.95	0.9	0.48	0.6	0.08
	p (2-tailed)		<.001	<.001	<.001	<.001	.595
Anti-RBD ratio	Correlation	0.95	1	0.93	0.54	0.68	0.1
	p (2-tailed)	<.001		<.001	<.001	<.001	.478
Anti-S1 ratio	Correlation	0.9	0.93	1	0.61	0.82	-0.18
	p (2-tailed)	<.001	<.001		<.001	<.001	.22
Anti-S2 ratio	Correlation	0.48	0.54	0.61	1	0.53	-0.09
	p (2-tailed)	<.001	<.001	<.001		<.001	.54
RBD ratio Neutrobodyplex	Correlation	0.6	0.68	0.82	0.53	1	-0.53
	p (2-tailed)	<.001	<.001	<.001	<.001		<.001
Days after last immunizing event	Correlation	0.08	0.1	-0.18	-0.09	-0.53	1
	p (2-tailed)	.595	.478	.22	.54	<.001	

Table 21: Spearman correlation between the days after the last immunizing event (i.e., third dose) and the antibody measurements on the third blood sample.

A significant correlation only between the neutralizing capacity and the time that has passed since the last immunizing event, the third dose in this analysis, is demonstrated in Table 21. Furthermore, the coefficient is negative, meaning that the more time passes the lower the measured humoral immunity. A scatterplot for visualization is portrayed in Figure 42.

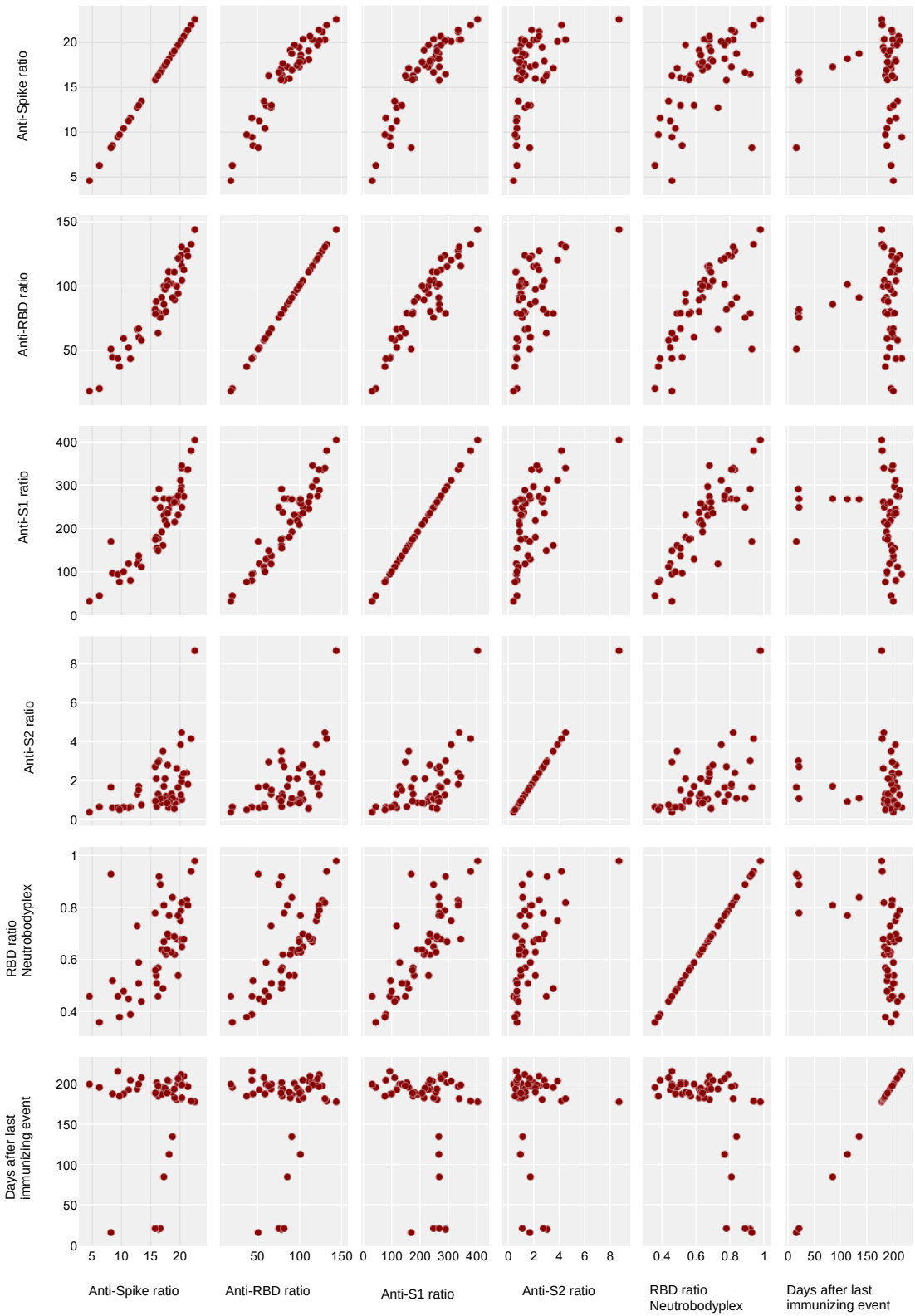

Figure 42: Scatterplot corresponding to Table 20.

Anamnestic Factors and Humoral Immune Response after Full Vaccination

	spike ratio		RBD ratio		S1 ratio		S2 ratio		NBP_RBD	
	p	r_{pb}	p	r_{pb}	p	r_{pb}	p	r_{pb}	p	r_{pb}
Anamnesis										
Cardiovascular	.002	-.1	.006	-.09	.04	-.1	.19		.282	
Neurologic	.453		.386		.236		.662		.118	
Skin	.339		.233		.286		.997		.605	
Hematogenic	.515		.757		.264		.547		.857	
Pulmonologic	.976		.849		.921		.234		.709	
Hepatic / Nephrologic	.791		.96		.87		.249		.671	
Gastrointestinal	.456		.554		.401		.067		.087	
Any Chronic Condition	.951		.732		.957		.716		.9	
Tumor	.352		.68		.36		.453		.903	
Antihypertensives	.026	-.07	.132		.092		.202		.879	
Antilipidemics	<.001	-.12	<.001	-.12	<.001	-.11	.203		.604	
Immunosuppressants	.59		.607		.954		.692		.247	
Anticoagulants	.244		.602		.76		.22		.985	
Antidiabetics	.727		.79		.701		.31		.193	
Regular Analgesia	.507		.492		.983		.415		.971	
Antidepressants	.068		.294		.187		.711		.248	
Thyroid Hormones	.559		.68		.888		.052		.142	
Chemotherapy	.091		.255		.166		.287		.648	

Table 22: A point-biserial test has been performed between each anamnestic condition and the level of each parameter of humoral immune response. The correlation coefficient r is only displayed if $p \leq .05$. Antibody levels were measured *after two consecutive doses* with no reported vaccine breakthrough before the blood sample.

Table 22 shows a low yet significant correlation between several quantitative antibody parameters and the medical history of the vaccinees. However, the neutralization capacity (NBP_RBD) does not appear to be influenced (causality assumed) by pre-existing conditions. Exemplary data is visualized in Figures 43 through 46 (below). Note that the sample size of participants on antilipidemics is, although large enough to produce significance, rather small. All significant factors have a negative influence on antibody levels.

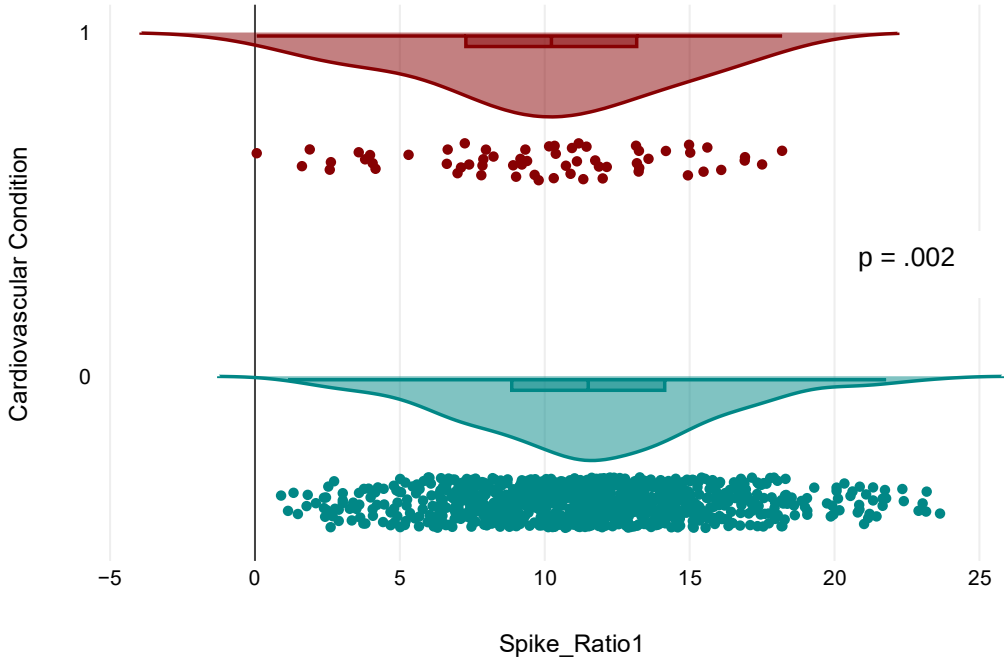

Figure 43: Raincloud plot of anti-spike antibody levels in comparison between participants who reported suffering from a cardiovascular condition and those who did not.

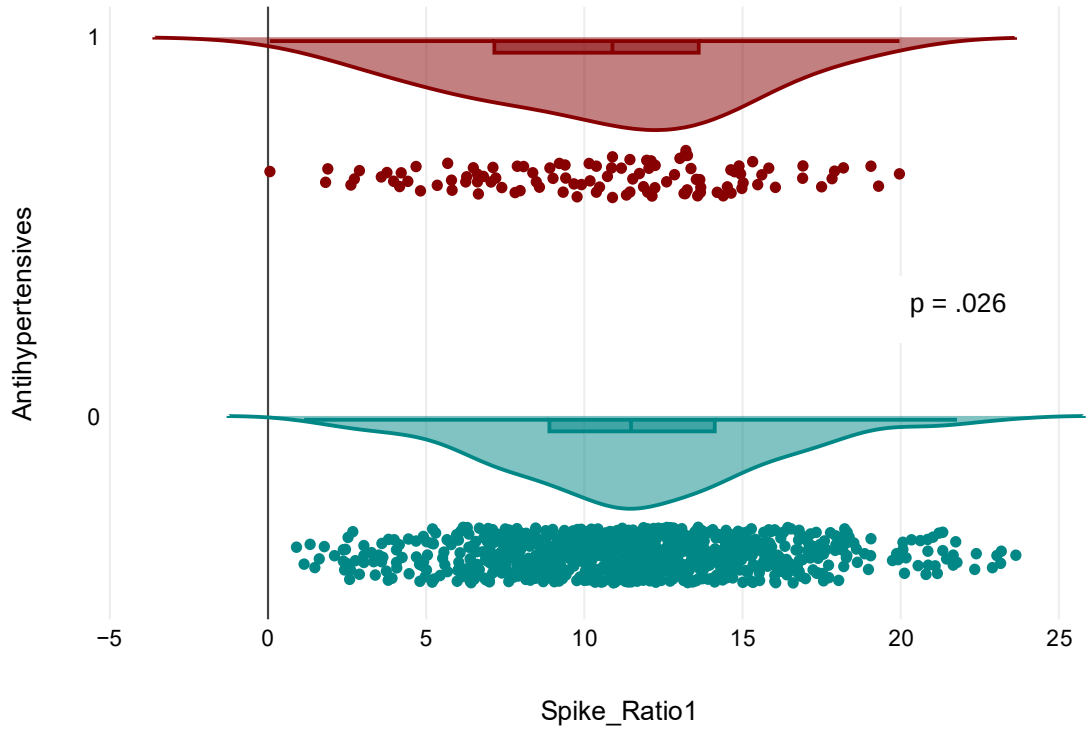

Figure 44: Raincloud plot of anti-spike antibody levels in comparison between participants who reported taking antihypertensives and those who did not.

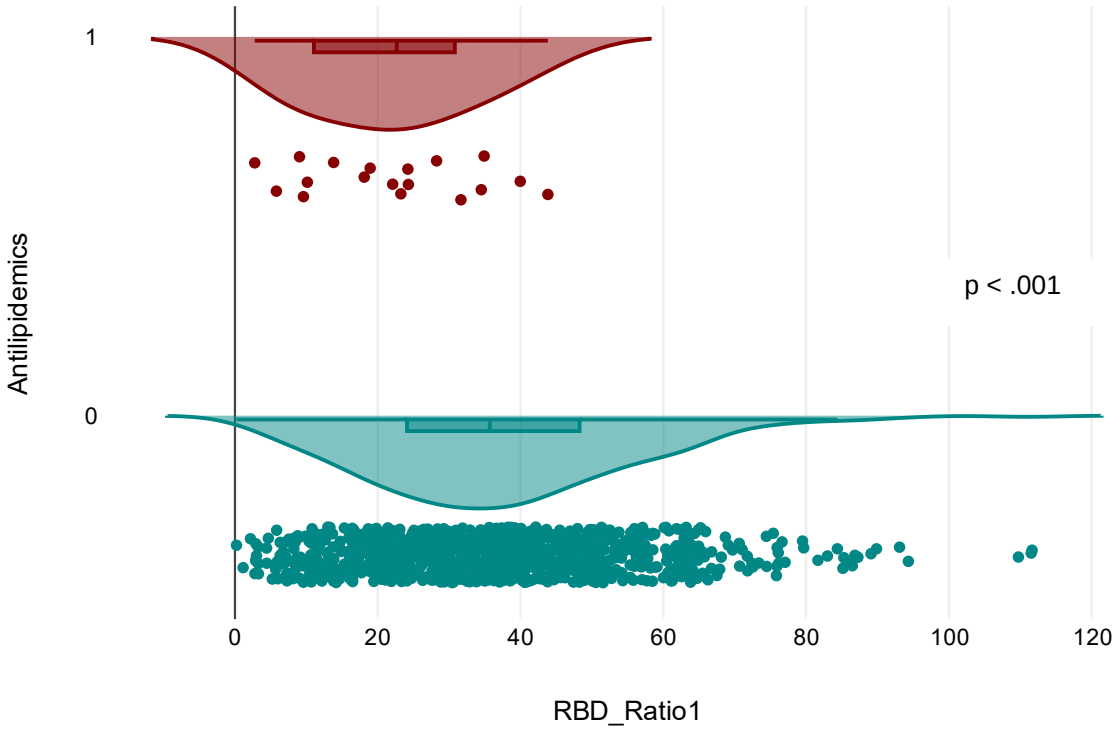

Figure 45: Raincloud plot of anti-RBD antibody levels in comparison between participants who reported using antilipidemics and those who did not.

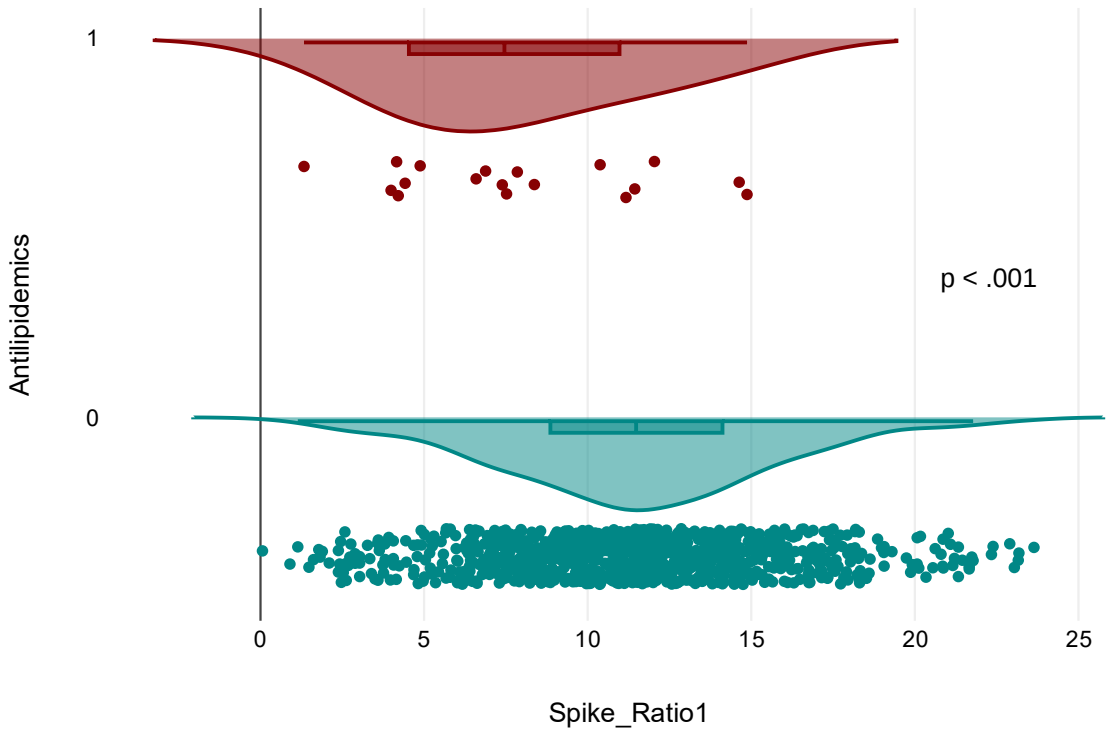

Figure 46: Raincloud plot of anti-Spike antibody levels in comparison between participants who reported using antilipidemics and those who did not.

A2 V3	spike ratio		RBD ratio		S1 ratio		S2 ratio		NBP_RBD	
	p	d	p	d	p	d	p	d	p	d
Anamnesis										
Cardiovascular	.941		.891		.516		.932		.756	
Neurologic	.036	.71	.022	.77	.639		.858		.585	
Skin	.739		.992		.075		.759		.128	
Hematogenic	.285		.256		.613		.525		.491	
Pulmonologic	.094		.082		.058		.972		.355	
Hepatic / Nephrologic	.838		.569		.863		.896		.866	
Gastrointestinal	.114		.354		.859		.971		.242	
Any Chronic Condition	.456		.408		.505		.305		.838	
Tumor	.686		.601		.636		.753		.336	
Antihypertensives	.148		.101		.467		.03	.25	.413	
Antilipidemics	.9		.944		.34		.538		.369	
Immunosuppressants	.269		.458		.221		.58		.327	
Anticoagulants	.065		.224		.765		.827		.469	
Antidiabetics	.617		.573		.932		.598		.715	
Regular Analgesia	.327		.895		.214		.403		.214	
Antidepressants	.046		.694		.046	.49	.316		.056	
Thyroid Hormones	.387		.469		.837		.269		.449	
Chemotherapy	n/a		n/a		n/a		n/a		n/a	

Table 23: As a pendant to Table 22 the correlations between each anamnestic condition and AB levels have been examined for the second blood sample, i.e. after *three consecutive doses* of a SARS-CoV-2 vaccine without a vaccine breakthrough reported until that point. Because of the smaller sample size (n = 560, n of each condition considerably lower) the medical conditions recorded were more scattered and less frequent. Hence, first a Lavene test of variance equality followed by a t-test of independent samples was performed. The effect size d is only displayed if $p \leq .05$. Both, significance level and effect size are according to variance (in-)equality.

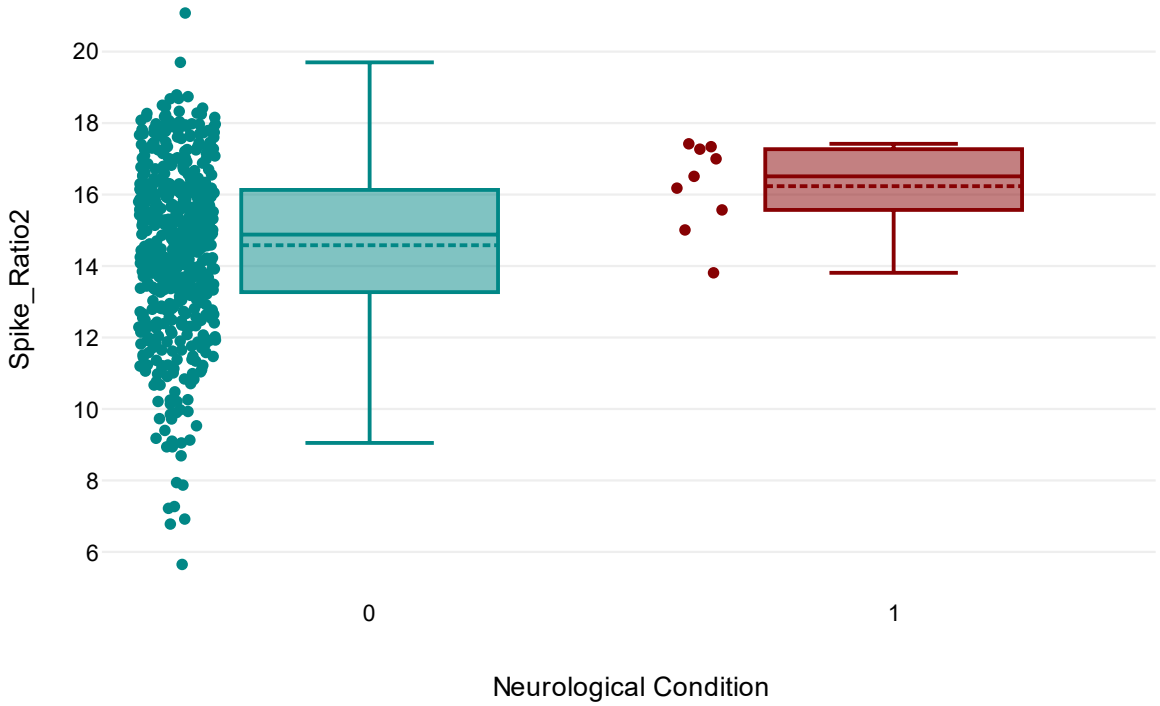

Figure 47: Scatter-Box-Plot of the Spike Ratio of participants with (1) and without (0) a neurological condition after *three consecutive doses* of a SARS-CoV-2 vaccine without a vaccine breakthrough reported until that point.

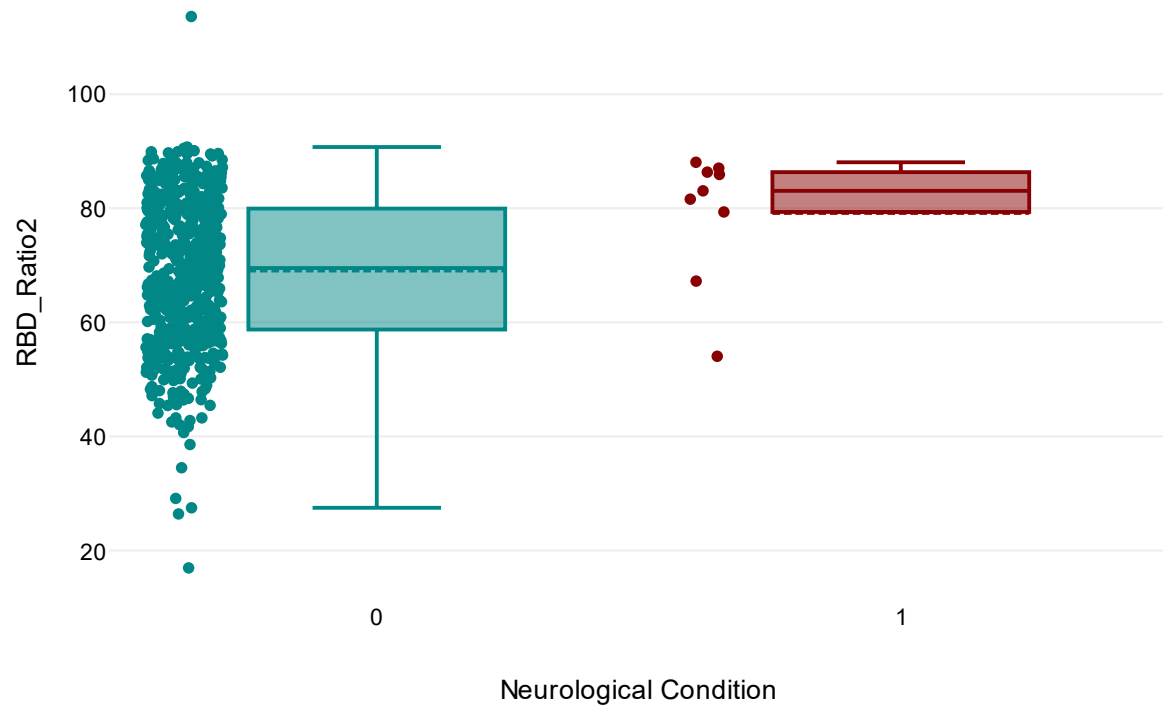

Figure 48 Scatter-Box-Plot of the RBD Ratio of participants with (1) and without (0) a neurological condition after *three consecutive doses* of a SARS-CoV-2 vaccine without a vaccine breakthrough reported until that point.

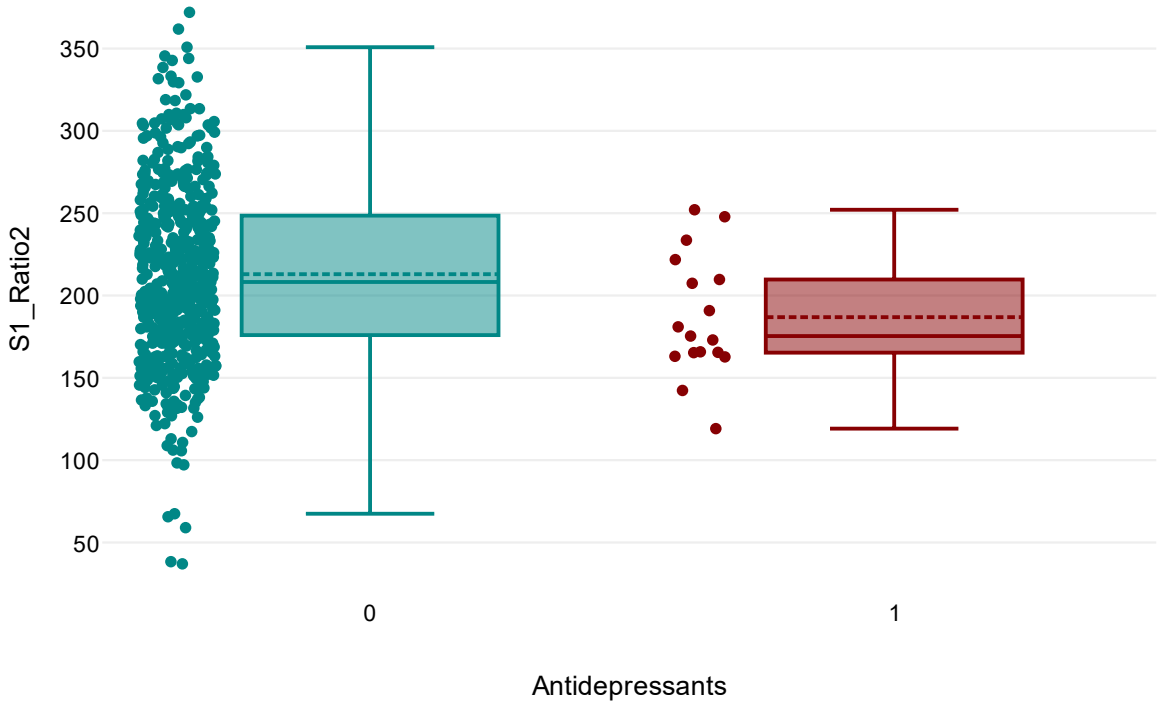

Figure 49 Scatter-Box-Plot of the S1-Ratio of participants with (1) and without (0) antidepressantiv medication after *three consecutive doses* of a SARS-CoV-2 vaccine without a vaccine breakthrough reported until that point.

When running a correlation analysis for the antibody levels after participants have received a booster dose (Table 23, Figures 47-49), the correlations found after the second dose (Table 22, Figures 43-46) no longer show. However, new significant correlations with an effect size in the medium range do emerge. Now a neurological anamnesis or taking antidepressive medication shows significance to some degree. Interestingly enough, antihypertensive medication and anti-S2 antibody ratio do correlate after a booster dose, but anti-S2 was the only parameter that did not show this correlation in the previous analysis. Note the relatively small sample size of the subgroups in Figures 47-49. Although the statistical difference turns out significant, the conclusion to be drawn has limited power of prediction.

	spike ratio		RBD ratio		S1 ratio		S2 ratio		NBP_RBD	
	p	$r_{Spearman}$	p	$r_{Spearman}$	p	$r_{Spearman}$	p	$r_{Spearman}$	p	$r_{Spearman}$
ordinal SE										
pain	.033	.07	.015	.08	.023	.07	.155	.08	.198	
skin sensitivity	.083		.105		.079		.137		.08	
swelling	.079		.094		.072		.141		.037	.07
erythema	.47		.152		.081		.08		<.001	.12
headache	.1		.281		.062		<.001	.18	<,001	.11
fever	.114		.184		.076		<.001	.19	.014	.08
shivers	.304		.185		.056		<.001	.19	<.001	.11
muscle pain	.031	.07	.043	.07	.019	.08	<.001	.15	.1	.9
joint pain	.779		.941		.545		<.001	.17	.045	.07
fatigue	.965		.95		.695		<.001	.13	.321	
nausea	.09		.289		.042	.07	.078		.052	
diarrhea	.07		.136		.153		.166		.257	
	p	r_{pb}	p	r_{pb}	p	r_{pb}	p	r_{pb}	p	r_{pb}
side effects dichotomous										
any local SE	.225		.136		.225		.859		.805	
pain	.07		.042	.07	.07	.06	.515		.572	
skin sensitivity	.27		.151		.27		.284		.497	
swelling	.131		.106		.131		.257		.225	
erythema	.174		.188		.174		.107		.006	.09
any systemic SE	.645		.914		.645		.006	.09	.419	
headache	.2		.453		.2		<.001	.11	.032	.07
fever	.469		.387		.469		<.001	.18	.135	
shivers	.166		.33		.166		<.001	.15	.007	.09
muscle pain	.125		.14		.125		<.001	.11	.081	
joint pain	.827		.628		.875		<.001	.15	.308	
fatigue	.613		.495		.613		.001	.08	.554	
nausea	.297		.585		.294		.351		.323	
diarrhea	.026	.07	.024	.07	.026	.07	.165		.336	

Table 24: A spearman correlation has been performed for each side effect (SE) in its *ordinal* form (none, mild, moderate, severe) to examine the data for a correlation between the strength of each side effect upon fist vaccination and the levels of antibodies (AB). A point-biserial test has been performed between each SE in *dichotomous* format and the level of each parameter of humoral immune response to check if the frequency of each reported SE has an effect on AB levels. The correlation coefficient r is only displayed if $p \leq .05$. Antibody levels were measured after two consecutive doses with no reported vaccine breakthrough before the blood sample.

Only a very limited number of side effects after the first dose does turn out statistically significant in examination of the influence on antibody levels (Table 24). The same is true for the strength of side effects as well as their frequency. Furthermore, those that are statistically significant return a positive but low to very low correlation efficient (assuming $r_{Spearman}$ and $r_{pb(Ponit\ Bisearial)} < .3$ as low[109]).

A1 V2	spike ratio		RBD ratio		S1 ratio		S2 ratio		NBP_RBD	
	p	$r_{Spearman}$	p	$r_{Spearman}$	p	$r_{Spearman}$	p	$r_{Spearman}$	p	$r_{Spearman}$
ordinal SE										
pain	<.001	.15	<.001	.14	.023	.07	.177		.001	.11
skin sensitivity	.001	.11	.001	.11	.079		.0667		.014	.08
swelling	.003	.1	.11	.08	.072		.032	.07	.001	.11
erythema	.038	.07	.007	.09	.081		.015	.08	<.001	.13
headache	<.001	.15	<.001	.15	.062		<.001	.12	<.001	.16
fever	<.001	.18	<.001	.18	.076		<.001	.13	<.001	.14
shivers	<.001	.19	<.001	.18	.056		.063		<.001	.18
muscle pain	<.001	.21	<.001	.19	.019	.08	.001	.11	<.001	.14
joint pain	<.001	.15	<.001	.14	.545		.001	.11	<.001	.13
fatigue	<.001	.14	<.001	.13	.695		.014	.08	.009	.09
nausea	.422		.587		.042	.07	.92		.524	
diarrhea	.455		.5		.153		.496		.321	
	p	r_{pb}	p	r_{pb}	p	r_{pb}	p	r_{pb}	p	r_{pb}
side effects dichotomous										
any local SE	<.001	.13	.134		.007	.09	.942		.068	
pain	<.001	.17	.001	.14	.001	.12	.939		.055	
skin sensitivity	.003	.1	.01	.09	.051		.191		.3	
swelling	.002	.1	.01	.09	.008	.09	.269		.025	.07
erythema	.042	.07	.014	.08	.01	.09	.033	.07	.01	.08
any systemic SE	<.001	.14	.001	.12	.001	.12	.091		.05	.06
headache	<.001	.13	.001	.12	<.001	.13	.006	.09	.002	.1
fever	<.001	.19	<.001	.18	<.001	.18	<.001	.13	.001	.11
shivers	<.001	.19	<.002	.17	.001	.2	.208		<.001	.13
muscle pain	<.001	.21	<.003	.18	<.001	.19	.021	.08	.001	.11
joint pain	<.001	.14	.001	.11	<.001	.14	.008	.09	.005	.09
fatigue	.001	.12	.002	.1	.003	.1	.148		.34	
nausea	.151		.24		.125		.936		.783	
diarrhea	.336		.358		.233		.348		.221	

Table 25: Significance and correlation coefficients between side effects that have been reported after the second vaccination and antibody levels measured after two consecutive doses without breakthrough infection having been reported by the participants before that. For side effects in *ordinal* format a two tailed Spearman correlation analysis was used. For the side effects in *dichotomous* format a point biserial correlation has been performed. The correlation coefficient r is only displayed if $p \leq .05$.

In comparison, when correlating the side effects that were reported after the second vaccination against antibody levels (Table 25), a vast majority of side effects (ordinal and dichotomous) return with a statistical significance. Nevertheless, again the correlation coefficients are low to very low. The effect is, therefore, to be considered present yet limited. The fact that more side effects than portrayed in Table 24 show significance may be explained through a shorter time span between the point of antibody level measurement and experienced side effects.

	spike ratio		RBD ratio		S1 ratio		S2 ratio		NBP_RBD	
	p	$r_{Spearman}$	p	$r_{Spearman}$	p	$r_{Spearman}$	p	$r_{Spearman}$	p	$r_{Spearman}$
ordinal SE										
pain	.703		.772		.29		.007	.12	.056	
skin sensitivity	.237		.894		.811		.598		.894	
swelling	.462		.954		.063		.145		.001	.14
erythema	.297		.228		.922		.805		.021	.1
headache	.278		.994		.121		<.001	.15	.016	.1
fever	.026	-.1	.164		.126		.01	.11	<.001	.17
shivers	.004	-.13	.328		.184		.011	.11	<.001	.16
muscle pain	.201		.869		.049	.09	.004	.13	.004	.12
joint pain	.041	-.09	.467		.081		.002	.13	.006	.12
fatigue	.128		.439		.093		.005	.12	<.001	.15
nausea	.843		.963		.06		.307		.627	
diarrhea	.484		.613		n/a		.163		.123	
	p	**d**	**p**	**d**	**p**	**d**	**p**	**d**	**p**	**d**
side effects dichotomous										
any local SE	.681		.815		.677		.183		.042	.24
pain	.704		.915		.587		.281		.119	
skin sensitivity	.16		.721		.925		.611		.99	
swelling	.54		.946		.056		.648		.001	.3
erythema	.321		.226		.952		.483		.042	.22
any systemic SE	.79		.455		.022	.22	.11		.002	.3
headache	.493		.862		.191		.047	.17	.078	
fever	.015		.102		.217		.064		<,001	.38
shivers	.001	.32	.148		.304		.037	.21	.002	.31
muscle pain	.101		.85		.02	.2	.036	.18	.002	.28
joint pain	.041	.18	.437		.188		.022	.2	.005	.25
fatigue	.183		.414		.242		.094		.002	.28
nausea	.614		.703		.067		.489		.751	
diarrhea	.426		.647		.017	.55	.525		.11	

Table 26: Significance and correlation coefficients between side effects that have been reported after the third vaccination and antibody levels measured after three consecutive doses without breakthrough infection having been reported by the participants before that. For side effects in ordinal format a two tailed Spearman correlation analysis was used. For the side effects in dichotomous format first a Lavene test of variance equality followed by a t-test of independent samples was performed. The correlation coefficient r and the effect size d respectively are only displayed if $p \leq .05$.

In this third analysis (Table 26), again roughly a third of parameters return with statistically significant influence of side effect strength and frequency on antibody levels. Perceived intensity of fever, shivers and joint pain even return a low yet negative correlation coefficient. This result is an exception in the current data, and although the analysis has been repeated to double check might be the result of a minor database error. There is no clear other explanation at hand.

In conclusion, when studying the severity and frequency of side effects experienced after a first (Table 24), second (Table 25) and third (Table 26) vaccination dose, there is a notable effect, especially in the most critical parameter, the NeutobodyPlex level, hence demonstrating a low but measurable positive correlation between experienced side effects and antibody functionality.

Age, Analgesics and Vaccination Scheme and Humoral Immune Response

other	spike ratio		RBD ratio		S1 ratio		S2 ratio		NBP_RBD	
	p	r	p	r	p	r	p	r	p	r
Age	<.001	-.25	<.001	-.25	<.001	-.25	.638		<.001	-.14
Analgesics after V1	.677		.71		.677		.001	.15	.984	
homologous vs heterologous 1/2	.001	.07	.025	.07	.042	.07	.001	.2	.003	.1

Table 27: To test for a possible correlations Spearman correlation has been performed between age and AB levels, for whether participants reported having used analgesia after the first vaccination or not and AB levels and whether their vaccination scheme was homologous or heterologous and AB levels. The correlation coefficient r is only displayed if $p \leq .05$.

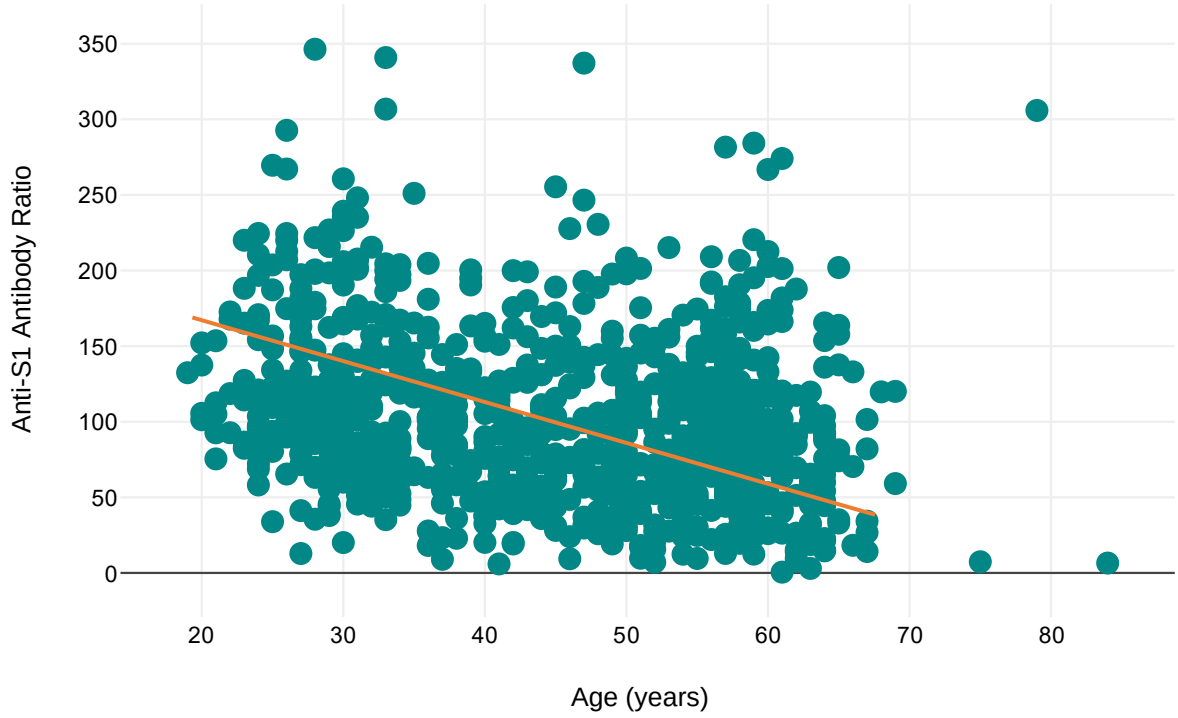

Figure 50: Scatter plot of anti-S1 antibody levels and participant age.

As Table 27 indicates, there is a statistically significant correlation between age and antibody levels for all parameters except for anti-S2. With increasing age, the immunogenicity decreases. Figure 50 shows the effect visually.

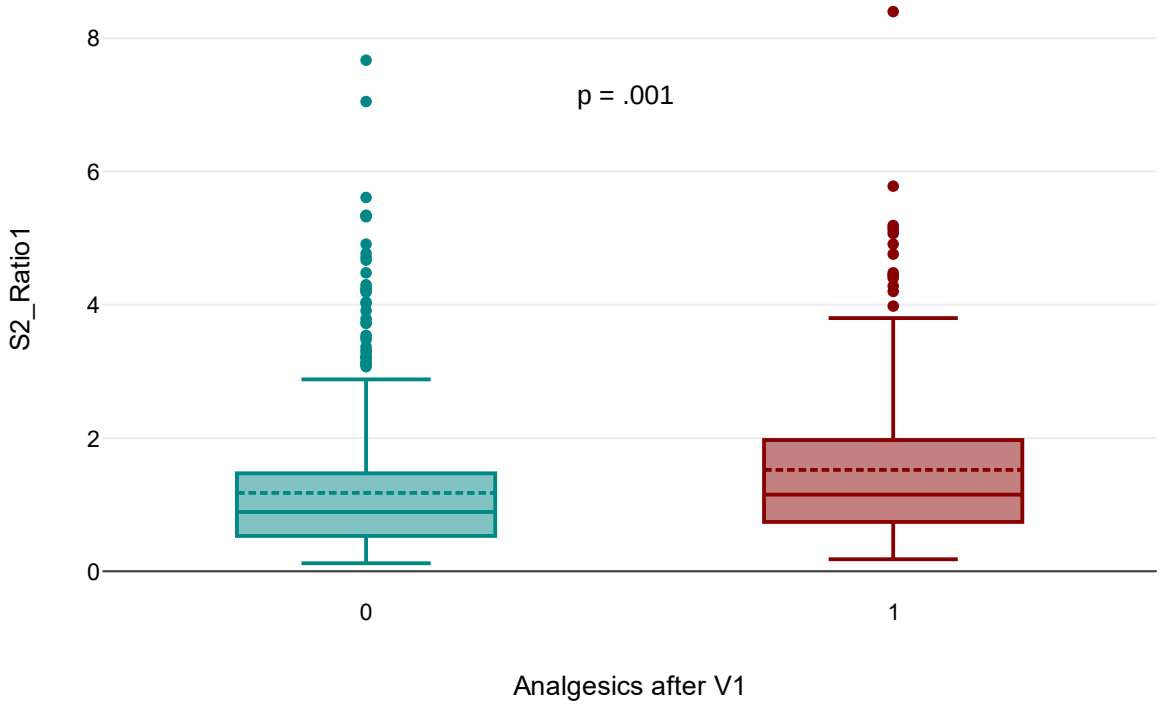

Figure 51: Box plots comparing the S2-ratio of participants that reported using analgesia after the first vaccination (1) and those who did not (0).

Figure 51 demonstrates that the S2 ratio is higher in participants that reported using analgesics around the first vaccination then those who did not. Table 27 shows that the difference is statistically significant, and the correlation coefficient is small.

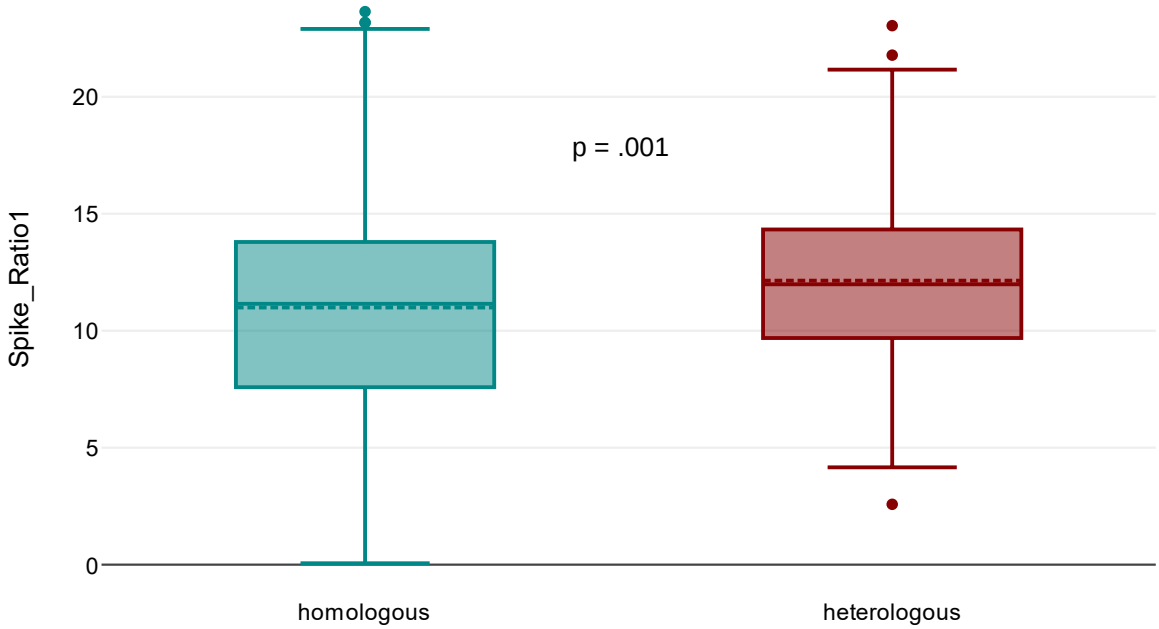

Figure 52: Box plots comparing the spike ratio of those who received a homologous vaccination scheme versus those who received a heterologous scheme for the first two vaccinations. Antibody levels were measured after the second vaccination.

Figure 52 and the values listed in Table 27 depict the statistically significant positive relationship on the spike ratio of receiving a heterologous vaccination pattern.

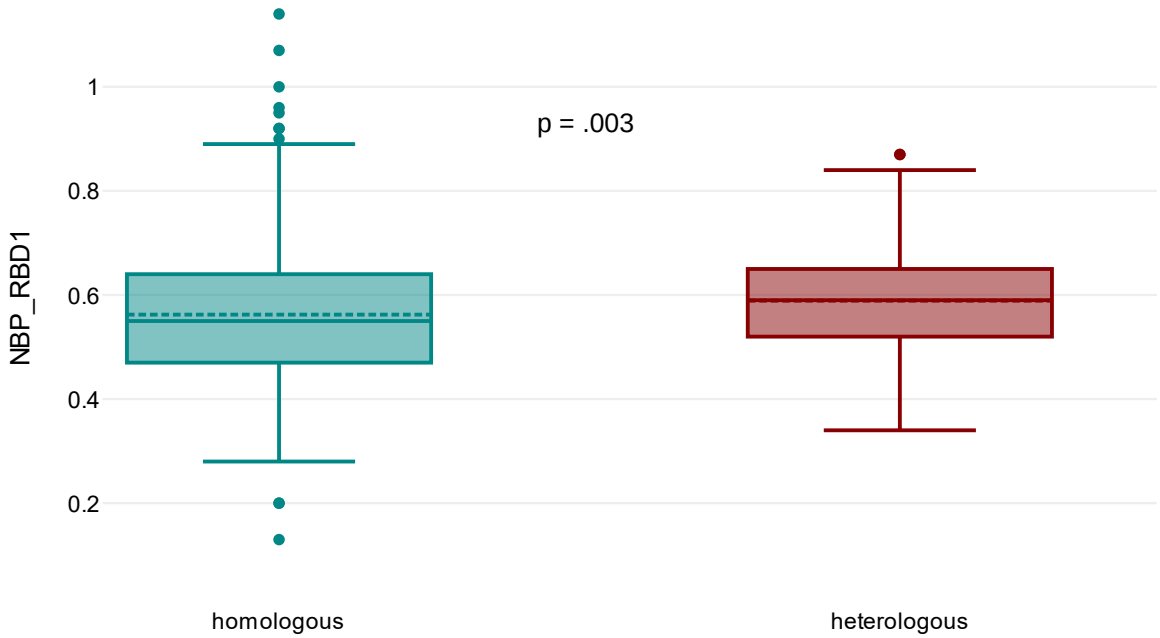

Figure 53: Box plots comparing the NBP ratio of those who received a homologous vaccination scheme versus those who received a heterologous scheme for the first two vaccinations. Antibody levels were measured after the second vaccination.

Just as demonstrated for the spike ratio, a heterologous pattern of vaccination does have a small, yet significantly positive effect on antibody functionality.

	spike ratio		RBD ratio		S1 ratio		S2 ratio		NBP_RBD	
other	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**
Analgesics after V2	.006	.09	.002	.1	.001	.1	.002	.1	.072	

Table 28: A Spearman correlation between weather an algetic has been used after the second vaccine dose and the AB levels measured after two consecutive doses has been performed. The correlation coefficient r is only displayed if $p \leq .05$.

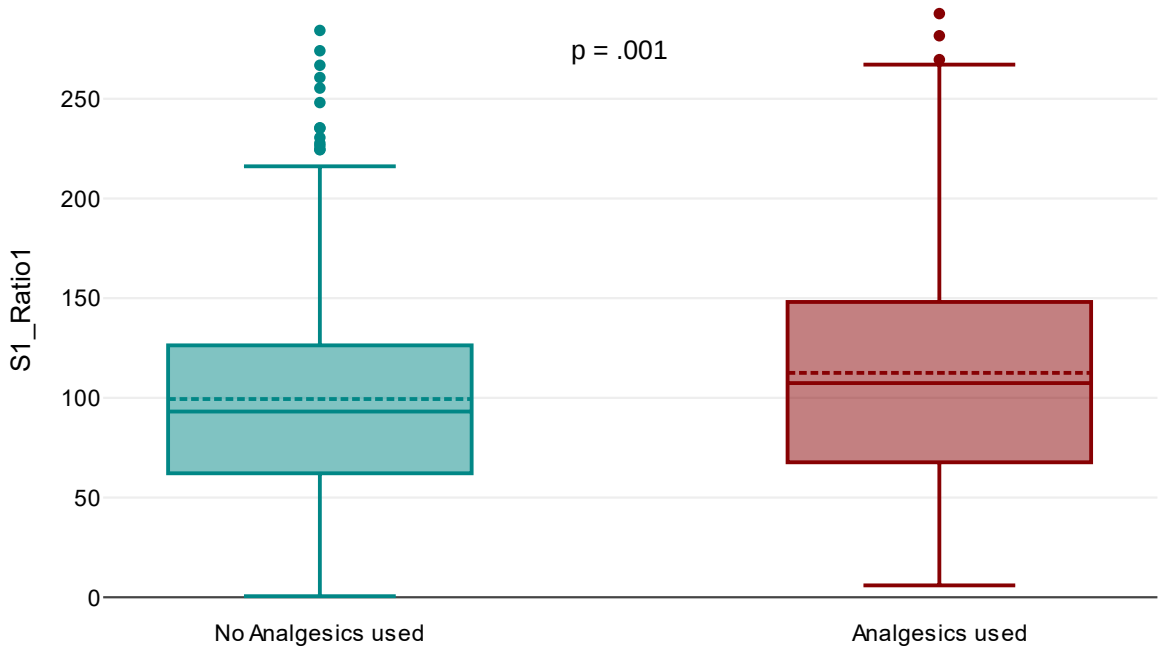

Figure 54: As an exemplary graph the box plots show the higher level of S1 ratio after a full vaccination (to vaccination doses) when participants reported using analgesics around the second application.

All measured antibody parameters except NBP show the significant positive relationship in the way depicted in Figure 54. Therefore, there is to conclude that some effect exists but is not to be overinterpreted as in this analysis it is not to be seen in the functionality of the antibodies, only in the level of the subunits.

	spike ratio		RBD ratio		S1 ratio		S2 ratio		NBP_RBD	
other	**p**	**d**	**p**	**d**	**p**	**d**	**p**	**d**	**p**	**d**
Analgesics after V3	<,001	.36			0,141		.007	.27	.003	.3
homologous vs heterologous 1_2	.345		.961		0,242		.507		.134	
homologous vs heterologous 1_2_3	.012	.29	.268		0,27		<,001	.05	.025	.23

Table 29: A Lavene test of variance equality followed by a t-test of independent samples was performed between AB levels and weather participants reported using analgesics after the third dose,

whether their vaccine regimen was homologous or heterologous in V1 and V2 as well as in V1, V2 and V3. The effect size d is only displayed if $p \leq .05$.

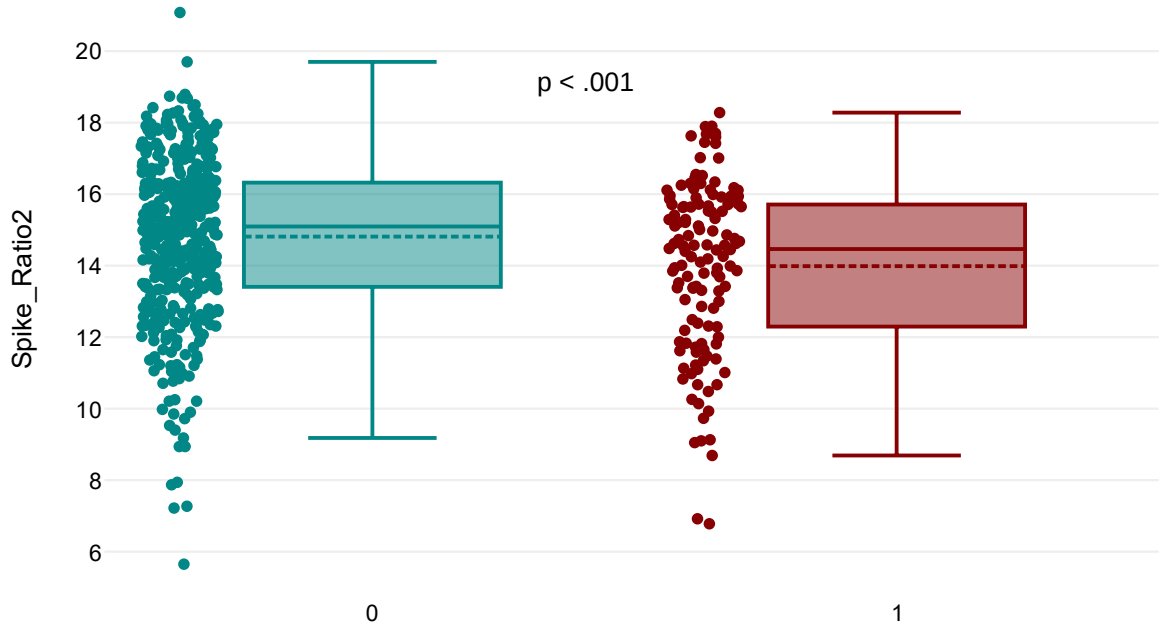

Figure 55: Box plots comparing the Spike ratio of participants that reported using analgesia after the *third* vaccination (1) and those who did not (0). Antibody levels were measured after the third dose.

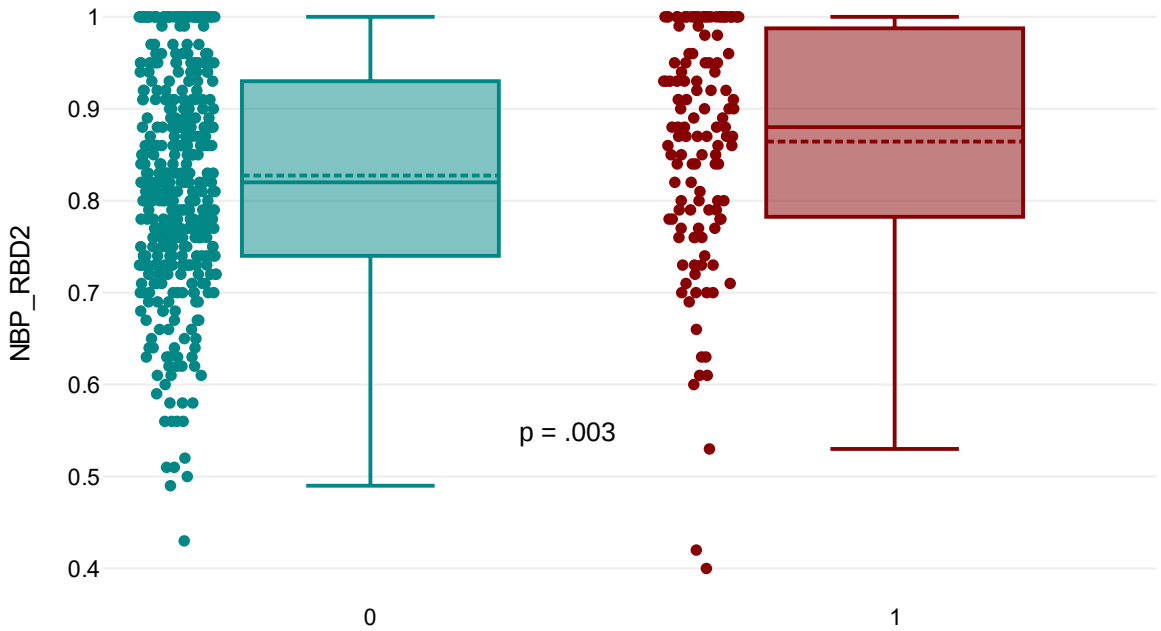

Figure 56: Box plots comparing the NBP levels of participants that reported using analgesia around the *third* vaccination (1) and those who did not (0). Antibody levels were measured after the third dose.

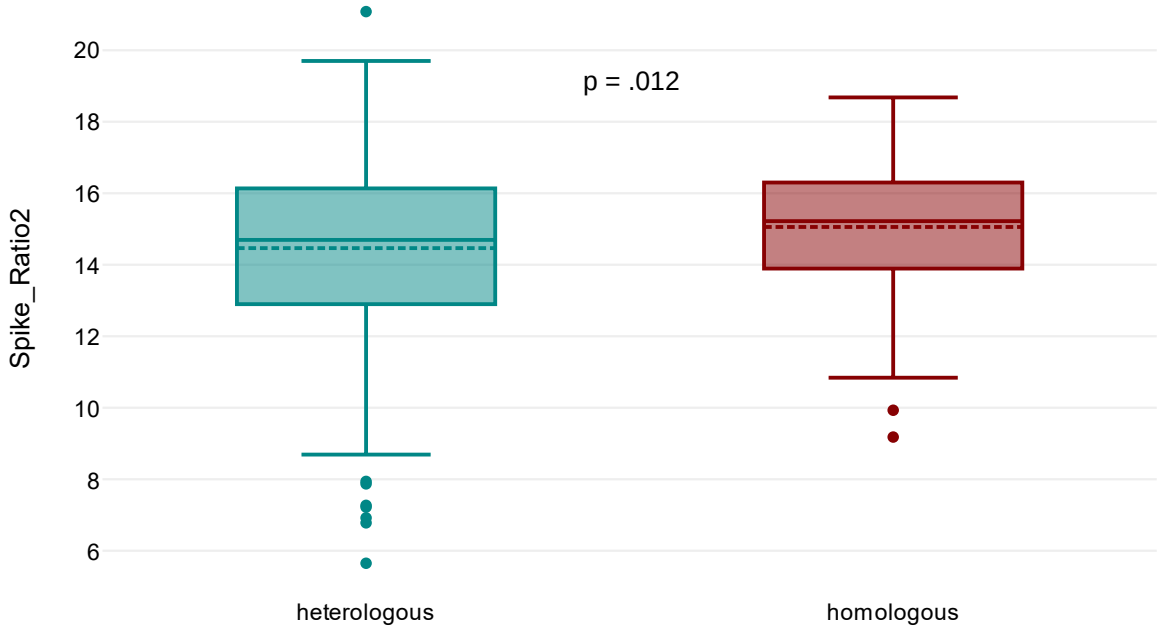

Figure 57: Box plots comparing the Spike ratio of those who received a homologous vaccination scheme versus those who received a heterologous scheme for *all three* vaccinations. Antibody levels were measured after the third vaccination.

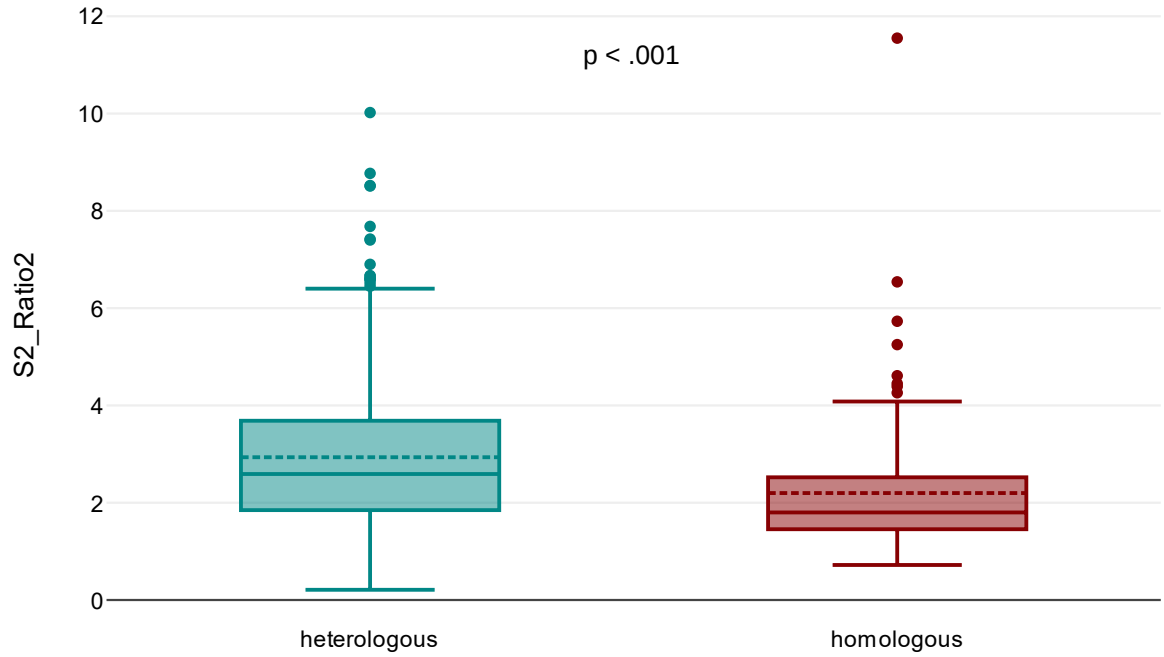

Figure 58: Box plots comparing the S2 ratio of those who received a homologous vaccination scheme versus those who received a heterologous scheme for *all three* vaccinations. Antibody levels were measured after the third vaccination.

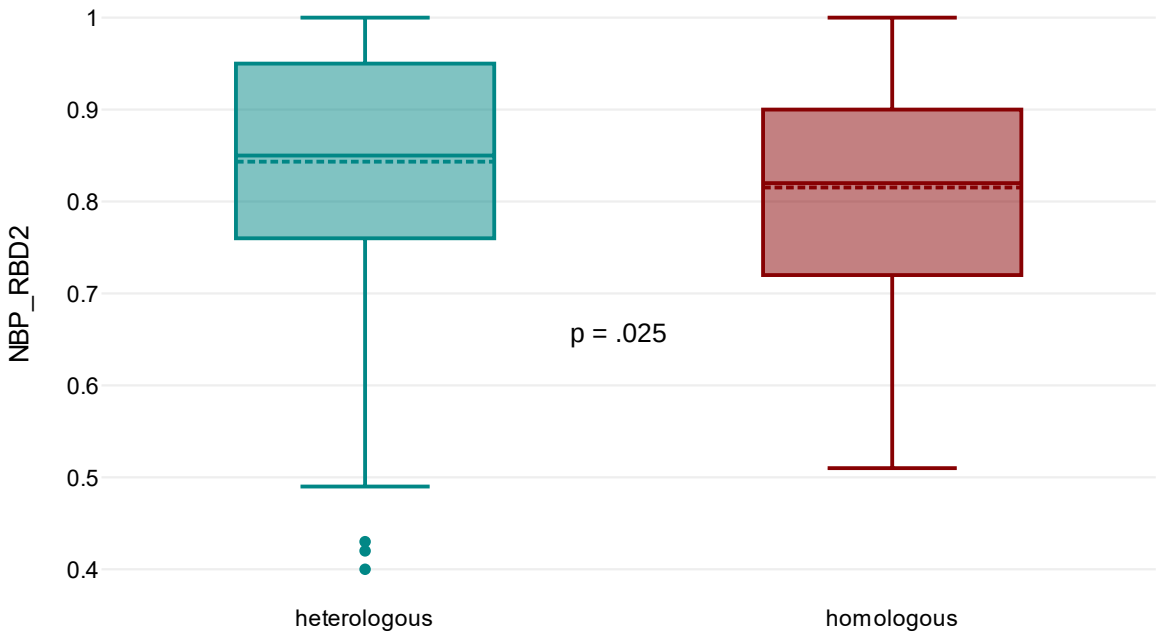

Figure 59 Box plots comparing the NBP levels of those who received a homologous vaccination scheme versus those who received a heterologous scheme for *all three* vaccinations. Antibody levels were measured after the third vaccination.

Table 29 and Figures 55 and 56 demonstrate the statistically significant difference between the subgroup that reported using analgesics after the third dose and those who did not. What appears to be noteworthy is that the data appears somewhat contradictory when analyzing the influence after the third dose. While spike levels are lower in the group that used analgesics, their NBP level and, therefore, their antibody functionality is increased.

The data also suggests a mixed signal when being examined for the influence of a heterologous versus a homologous vaccination scheme across all three applications. While the overall spike level is increased in the homologous regimen (Table 29, Figure 57), the level of the measured S2 subunit as well as the NBP level, decreased (Table 29, Figures 58 and 59).

Discussion

Side Effects

Upon the first vaccination MOD showed the highest frequency for local side effects (SE) and the least frequency for systemic SE. AZE demonstrated the opposite and outranked MOD and BNT by far. The SE after AZE were also significantly more severe. Probands who received AZE were slightly yet statistically significantly older than the BNT or MOD population.

In the second dose, the proportion of participants who received AZE decreased drastically, and the age went up and is now clearly above the age of the mRNA population. This dynamic is backed up and explained by a change in regulations by the standing vaccination committee, recommending AZE only for vaccinees of 60 years and above.[113] The proportion of participants who received MOD doubled. MOD dominated the frequency of participants who reported having at least one both local and systemic SE. Just like after the first dose, pain on the injection site was the most common local SE and headache followed by fatigue was the most common systemic SE. Some SE were slightly, yet statistically significantly higher in severity for heterologous vaccinated participants. Some publications back this data up[114], others contradict[115]. One might postulate that the findings about variations in side effects depending on whether vaccines were mixed or not are more of an academic discussion rather than a practical approach when trying to tackle a pandemic. A large consensus and the most important part, however, is that mixing vaccines is safe and effective.[114–116] The latter might even be enhanced.[116] On a larger scale, the “mix-and-match” vaccination strategy has the ability to overcome a shortage of a particular manufacturer, leading to a faster supply of the population and slowing the pandemic progress.

All booster doses registered were mRNA-based vaccines in accordance with German regulations and recommendations.[117] MOD showed to cause SE more often and more severely, although the difference to BNT is often very little and statistically significant in less than half of the considered SE. BNT recipients were slightly younger than MOD recipients.

Use of Analgesics

Matching the findings from the previous section for the first vaccination, AZE showed to be a significant positive factor with respect to whether participants decided to take analgesics. Correspondingly MOD caused an unproportionate high frequency of analgesia after the second dose. No difference has been found for the third vaccine, reflecting the findings about side effects in which there was very little difference for the third dose. For the effect on vaccine efficacy, please refer to the next section.

Age turns out to be of limited influence on the decision to use analgesics after vaccination. Participants who reported using “painkillers” after the first and second doses were statistically significantly younger. The arithmetic mean for the third dose demonstrates a similar dynamic, yet the difference is not significant. What makes this interesting is that the overall distribution of over-the-counter pain relievers strongly tends to the elderly.[118] One might, therefore, assume that younger participants did not take analgesics more frequently due to some sort of default mode. Far more likely that the choice of whether analgesics are used is based on the experienced symptoms, which our data, as well as literature suggests are more frequent and intense in the younger population.[119,120] Well consistent with intuitive understanding, systemic side effects were of greater influence on the decision of analgesia than local side effects. Primarily the appearance of headache and fever determined whether participants took a pill.

Female participants were significantly more likely to ease the side effects pharmaceutically after every dose. They were also more likely to experience side effects, which was to expect.[119,121,122]

To draw a practical conclusion for future studies, the study design should consider investigating the timing of analgesic use relative to vaccination, as well as the duration, frequency and kind of analgesic use. This will provide significant insight into what impact on vaccination outcomes prophylactic, early or prolonged analgesic use has. Plus, the potential influence of comorbidities and information on participants' pain tolerance to better understand the individual factors that influence analgesic use needs further attention. There are well validated methods for evaluation of this.

Vaccine Breakthrough

A comparison between the time and proportion of vaccine breakthroughs in the age groups 18-55 and 56 and above has been performed. Although a higher proportion of the younger participants had a vaccine breakthrough infection at the end of the observation period, the difference in the age groups was insignificant. It is discussed that more social and less cautious interaction, more contact with by regulation unvaccinated minors and increased professional contacts might be at the source of this.[123]

Non-Healthcare workers were more prone to get a breakthrough infection than healthcare workers. The difference is statistically significant when observing breakthroughs after the 2nd dose and ends up just slightly above the significance threshold for observation after the 3rd dose. One might speculate that the latter might become significant when increasing the observation time frame as now, naturally, it is shorter than the one after the 2nd dose. Although some studies show that working in healthcare environment increases the risk of contracting SARS-CoV-2 our finding is matched by a retrospective Belgic large scale study does come to the same conclusion.[123] Greater use and adherence to personal safety equipment and less exposure time might be a driving force of this effect.

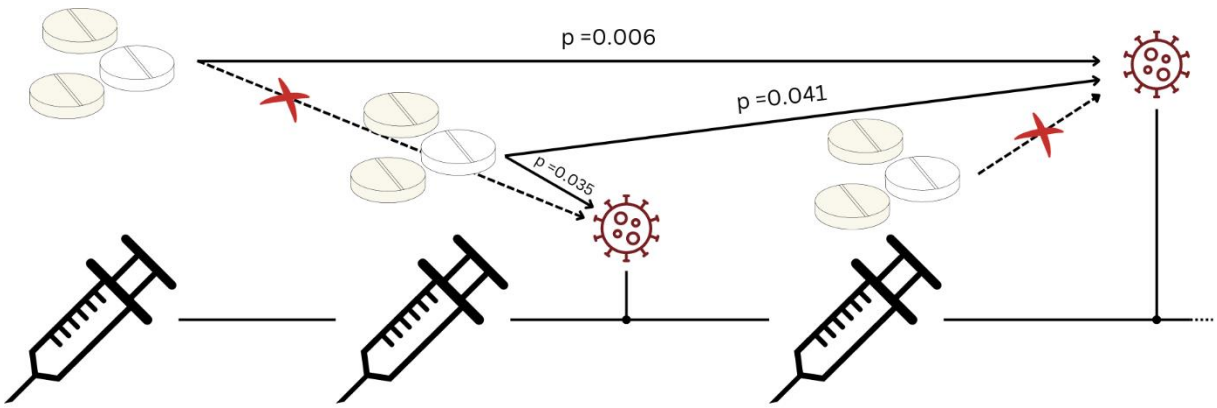

Figure 60: Summarizing overview of the influence of analgesics (pill pictograph) on breakthrough infections (pictographed as virions). Syringes indicate the respective consecutive vaccine dose.

The data suggests that using analgesia after the first and second dose negatively influences vaccine efficacy after a later 3rd dose. The same applies to analgesia after the second vaccine dose and a breakthrough after that. Participants who did use analgesics suffered an earlier vaccine breakthrough infection. A similar effect was recorded for

analgesia after V3, yet the result is not statistically significant. As of today, it remains of speculation whether the analysis timeframe is the reason for that, considering that obviously less time has passed since the third vaccination than the time since V1 and V2. The use of non-steroidal anti-inflammatory drugs has been demonstrated to downregulate cytokines in the inflammatory response to SARS-CoV-2 in mice.[124] A similar effect might be at play here.

There is a significant difference in vaccine efficacy *after two doses* over time, depending on the specific vaccine administered *first*. Participants having received MOD as a first dose showed the worst efficacy. Contrary to most literature[123,125] those who received AZE as a first dose showed the longest resistance against a breakthrough before the booster dose. However, a systematic review found a higher cellular immune response in AZE-primed individuals postulation a potential benefit of heterogenous vaccination.[126] No other such effect was recorded, neither for each specific vaccine or specific vaccination scheme for the 2nd and 3rd dose.

An interesting picture emerges when grouping the schemes according to their homo- or heterology. Those who received a homologous primary vaccination scheme (i.e., V1 & V2) had significantly worse vaccine efficacy than the heterologous group (Figure 18). Although the effect is not statistically significant, the opposite is true for breakthroughs after the booster dose and the respective homo- or heterology for three consecutive doses. Here in a merely visual approach (Figure 27), the homologous scheme does perform better. What seems to be the more practical approach in the light of a broadly vaccinated population, a weaning pandemic, and an ever-increasing rate of breakthrough infections is that the highest protective value lies in the combination of *any* approved vaccine and infection, that hopefully is experienced mildly and left behind quickly, due to vaccine-induced immune priming.[121,125–127]

Our findings suggest that there might be a link between cardiovascular conditions, antihypertensive medication use, and enhanced vaccine efficacy. This finding should be taken with care as those who reported using antihypertensives had a higher proportion of AZE as a first dose, which in and of itself demonstrated a better efficacy. Causality can,

therefore, only be assumed with further investigation. Although considered a risk factor for severe COID-19 manifestations if contracted, this finding does match into current peer-reviewed literature, postulating hypertension as a potential protective factor against contraction.[128]

Experiencing local swelling, headache and fever after the 1st dose, local swelling and skin sensitivity after the 2nd dose, and skin sensitivity after the 3rd dose demonstrate a negative correlation with vaccine efficacy over time. Causality should be assumed with caution, as headache and fever were the main cause of the use of analgesics. So some, yet different for each vaccine administration, side effects have shown to be predictive of vaccine efficacy. A potential explanation of these primarily local side effects is that if the vaccine reaction occurs primarily locally, to the disadvantage of the systemic and therefore intended immune reaction. Vaccine administration errors or imperfections might play a role.[129]

Overall, in a merely visual approach, we notice an increasing slope of the Kaplan-Maier curves after roughly 120 days after the second vaccination and around 45-50 days after the 3rd vaccination reflecting an increased infection dynamic from this time point on.
As a noteworthy limitation of the Kaplan-Meier survival function in this context should be mentioned that the exposure to and the virulence of SARS-CoV-2 underlies a highly dynamical fluctuation with the pandemic still going on. Yet it is fair to assume that our rather vast sample size makes up for these differences between individuals and the groups compared each underly the same dynamics in average.

As the topic of vaccine breakthroughs was one of the central discussions in the COVID pandemic, future research should place an emphasis on it. Our study had the disadvantage, that during data acquisition the infectious dynamic was highly volatile. Examining the above-mentioned factors in a more controlled, predictable and long-term setting could benefit the entire field of vaccination as significant part of the field of preventive medicine especially when expanded to other vaccination technologies and kinds.

Antibodies

In our analysis, the quantitative signal-to-cutoff ratios (i.e., Spike, S1, S2, RBD) had increased its central tendency from sample 1 to sample 2, at least partially explained by the third dose of covid vaccine in between. However, we also measured an increase from sample 2 to sample 3, although only considering those probands who did not report an immunizing event between those. At first glance, this finding contradicts common literature about waning antibody titers over time. It is important, though, to acknowledge several practical points before dismissing the finding. One is that fluorescence-based immunoassays have inherent limitations and are rarely entirely accurate. Varying antigen density between the beads, unspecific binding tendency of other antibodies, and inter-lot differences in bead reactions are some of them.[130] The next consideration is that the Luminex assay is an immunofluorescence assay based on the direct detection of antigens.[131] An increase in antigen levels over time in assays of direct measurement has been described and is potentially explained by increased antibody avidity.[132] Antibody avidity does increase after infection but also does after repeated vaccination.[133] One could also proceed to assume that long lived plasma cells are contributing to this finding.[134]
In coherence to literature the proportion of neutralizing antibodies increased after vaccination and decreased in a follow-up without an immunizing event in between.[135–137]

Anti-Spike, anti-RBD, and anti-S1 antibody levels are marginally yet statistically significantly reduced after two consecutive doses without a previous infection for participants with a cardiovascular condition and on antilipidemics. Those who use antihypertensives demonstrate a slightly lower anti-spike ratio reflecting literature.[138] The effect may in part be explained through a downregulation of angiotensin converting enzyme 2 (ACE2) through freely circulating spike proteins after vaccination, which in turn leads to an inflammatory response.[139] As pro-inflammatory cytokines are always accompanied by downregulatory set of immune mediators the intended, specific buildup of anti-spike antibodies may be compromised.[140]

A different set of anamnestic conditions significantly influences certain antibody measurements after the third dose. Participants who reported having an unspecified

neurological condition had higher anti-spike and anti-RBD levels. Participants on antidepressants demonstrated lower levels of anti-S1. However, the sample size for these findings is relatively small, possibly resulting in an incidental finding by random chance.

Across three blood samples and across the evaluation of reactogenicity of all three vaccination doses, several side effects have demonstrated a positive effect of varying antibody measurements and of varying degrees. The presence of fever and shivers after vaccination increases the binding capacity of anti-RBD-antibodies the strongest after all vaccinations. Although the influence is relatively small, this finding conditionally demonstrates the link between reactogenicity and immunogenicity.

Age is a well-known negative factor for antibody levels and their dynamic over time.[135,141–145] A negative influence has been demonstrated across all measured antibodies and binding capacity from our data as well (Table 27, Figure 50).

The use of analgesics around the first vaccine correlated positively with the S2-ratio measured after the second dose. The use around the second dose correlated positively with the quantitative levels but not with the neutralization capacity—the use of analgesics around the third dose did influence the antibody ratio at anti-spike and anti-S1. Interestingly when participants used analgesics after the third dose, in opposition to the previous vaccines, they demonstrated an increase in neutralization capacity. Although surprising at first glance, others have found the same dynamics yet concluded no negative effects on immunity based on seroconversion.[146] What possibly constitutes this effect is that higher reactogenicity might promote the decision to use analgesics (assuming that analgesics were used to treat symptoms of vaccination – see limitations for discussion) and is, therefore, at best indirectly correlated. However, the finding does contradict that of an earlier breakthrough infection when analgesics have been used.

The neutralizing capacity of probands antibodies who received a heterologous regimen was slightly higher than that of those who received a homologous regimen after two vaccines. The effect increased after receiving a homologous booster vaccine increasing not just the neutralizing capacity but the anti-S2-ratio as well.

Limitations

A vast advantage of the study is the large sample size, including a population of healthcare workers and a control group large enough to draw reliable conclusions. The prospective nature and availability of laboratory utilities for measuring humoral and cellular immune response (not part of this dissertation) has little matches in the literature.
An unforeseen limitation resulted from a change in vaccine regulation after the start of the study, which was initially conceptualized as a long-term longitudinal acquisition of humoral and cellular response after the full vaccination. The broad introduction of a booster dose has caused a reset in baseline after data gathering started. Variants that have newly emerged during the study and drastically changing the infection dynamics pose a difficulty in study organization and data interpretation.
Methodically the study relied on active and prolonged participation, requiring quite a bit of knowledge around the use of online platforms and QR codes, potentially reducing adherence and data consistency. The algorithm allowed participants to view their serology results only after filling out the respective side effect questionnaire. This was intended to increase the willingness to provide information. It may although as well lead to frustration and entering wrong data “to get over with” the bureaucratic part.

A linguistic limitation might lie in the term “joint pain”. The German term “Gliederschmerzen” of the online questionnaire does in fact in common sense refer to all three myalgia, arthralgia, and ostealgia.

While the questionnaire was designed to be interrelatable with existing literature and perhaps even extend the currently existing scope of medical conditions concerning vaccination, a compromise was made. Questions about the medical history and medication were kept superficial, so non-specialists would be able and more likely to answer them. On a practical scale and from a physician's viewpoint, this led to imprecisions that partially made it difficult to reach practical conclusions. One example would be that hepatic and renal pathologies were generalized into one category. At best, this could have led to the conclusion that an unspecified effect might exist in this area and

that further research is needed. Another imprecision in this context is the questioning around analgesia. Neither had the participant to specify whether the medication was used prophylactically or upon noticing symptoms, nor was a distinction made between nonsteroidal anti-inflammatory drug (NSAID) and acetaminophen or other substances. Both of this information is vital to draw practical conclusions as the literature is still debating this issue.

Lookout and Perspectives

Although limitations exist, we were able to leverage the existing infrastructure of the IKET (human resources, experience, laboratory equipment, recruiting) to conduct a longitudinal study with a vast amount of both subjective and quantitative data to shed more light on one of the most critical aspects of the still ongoing COVID pandemic. The quality and depth of our results allow for a plea that institutions with the respective infrastructure in place should engage in large-scale research.

Like most scientific research, while some questions were answered, others came up. Especially of value would be a follow-up study of a similar scale that investigates the effect of analgesics on vaccine efficacy, taking the time-point and exact kind of medication into consideration as the topic is broadly relevant.

Non-Commercial and therefore independent post-market vaccine studies like ours are of tremendous importance to public health, especially with the emerging establishment of mRNA vaccines to monitor safety and efficacy but also increase the population's trust.

Conclusion

The use of all three spotlighted vaccines, Vaxzevria by AstraZeneca, mRNA-1273 by Moderna and BNT162b2 by BioNTech/Pfizer are generally safe and efficacious upon each individual administration and used in interchangeable vaccine regimens. Moderna causes slightly more and more severe side effects upon the booster dose. Receiving Moderna as a primary, first vaccine may influence vaccine efficacy negatively. Receiving the vector-based Vaxzevria first may increase vaccine performance.

Higher reactogenicity, especially for systemic side effects, younger age and female gender were associated with more frequent use of analgesics. The data suggests that analgesia has a limited, statistically significant negative influence on vaccine efficacy. In contrast to that, the use of analgesics caused a slight increase in some antibody ratios. Having used analgesics after the booster dose even influenced the binding capacity of anti-RBD-antibodies positively. The topic remains therefore controversial and requires further scientific attention, tailored to this specific question at hand.

Heterologous primary vaccination with a booster of a vaccine that has already been administered should be preferred and showed to improve both the binding capacity of anti-RBD antibodies and vaccine efficacy over time.

Hypertension may be a protective factor against contracting SARS-CoV-2 but is correlated with slightly lower immunogenicity of COVID-vaccines.

Although several side effects in frequency and severity across several dose administrations correlate positively with humoral immunogenicity, some primarily local side effects correlate with worse vaccine efficacy. Extensive education of staff that handles and administers vaccines should be aimed for.

The frequency of vaccine breakthrough infections picks up speed at roughly 4 months after the second and 1.5 months after the booster dose. A recommendation for an earlier booster and second booster dose should be considered, especially for the elderly as already implemented in Germany.

From a global perspective, the COVID-19 pandemic has revealed many weaknesses in the healthcare system and preparedness for pandemics. While progress has been made and learnings were implemented in developing and distributing COVID-19 vaccines, there is still much work to be done to ensure the world is better prepared for the next pandemic. The COVID-19 pandemic showed that early detection and rapid response are critical to controlling the spread of a new virus. Countries that were better prepared and had more robust public health systems were generally able to respond more effectively to the pandemic.[147,148] The value of high-paced international cooperation and coordination in response to a global health crisis became highly evident. Sharing of information, resources, and expertise across countries has shown to be essential for an effective response to the pandemic.

While there have been tremendous efforts to address these issues, much more must be done to ensure the world is better prepared for the next pandemic. This includes investing in public health systems, strengthening international cooperation and coordination and supporting scientific research and innovation.

To end this section and manuscript on a notion that closes the loop and puts our current point in time into perspective I want to quote Philip Mackowiak, professor and vice chairman of the Department of Medicine of the University of Maryland School of Medicine:

> *"COVID-19 is not the world's first pandemic, not its worst, or likely to be its last."*
>
> Philip Mackowiak [149]

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Zusammenfassung

Die bis zum heutigen Tage fortwährende SARS-CoV-2 Pandemie konnte durch eine Reihe an Maßnahmen eingedämmt werden. Die Impfung spielt bei der Bewältigungsstrategie eine zentrale Rolle. Die Impfnebenwirkungen sind häufig mild bis mäßig, selten jedoch auch bedrohlich. Die Diskussion und Sorge um diesen Umstand ist häufig ein treibender Faktor für eine zögerliche Impfbereitschaft der Bevölkerung und sollte daher kontinuierlich überwacht und nach Möglichkeit optimiert werden. Die heterologe Verabreichung in der Primärimmunisierung scheint der homologen in Bezug auf die humorale Immunantwort und die Impfwirksamkeit im zeitlichen Vergleich überlegen zu sein.

Die Einnahme von Schmerzmitteln perivakzinär scheint trotz marginal verbesserter Immunantwort einen negativen Einfluss auf die Impfwirksamkeit je nach Betrachtungszeitraum zu haben. Eine für diese Fragestellung dezidierte Untersuchung außerhalb eines hochdynamischen Pandemiegeschehens sollte erfolgen.

Arterieller Bluthochdruck ist möglicherweise ein protektiver Faktor im Infektionsgeschehen, wenn auch assoziiert mit einem marginal geringeren Antikörperspiegeln. Der Stellenwert der messbaren Antikörpermenge sollte somit kritisch betrachtet werden und misst nur bedingt den Impferfolg. Die Antikörperqualität sollte ebenfalls berücksichtigt werden.

Unsere Daten zeigen, dass einige Impfnebenwirkungen einen signifikant positiven Einfluss auf die humorale Immunantwort haben und somit eingeschränkt den Zusammenhang zwischen Reaktogenität und Immunogenität demonstrieren. Zeitgleich jedoch stellt die Ausprägung vereinzelter Impfnebenwirkungen möglicherweise einen negativen prädiktiven Faktor bezüglich der Impfwirksamkeit dar.

Schlussendlich bleibt der Stellenwert eines höheren Antikörperspiegels bei der Frage nach langfristigem Impferfolg fraglich.

Summary

The SARS-CoV-2 pandemic, which continues to this day, has been contained through a number of measures. Vaccination plays a central role in the coping strategy. Vaccination side effects are often mild to moderate, but rarely life threatening. Discussion and concern about this is often a driving factor for reluctance to vaccinate in the population and should therefore be continuously monitored and optimized when possible. Heterologous administration in primary immunization appears to be superior to homologous in terms of humoral immune response and vaccine efficacy over time.

The use of analgesics perivaccinarily seems to have a negative impact on vaccine efficacy depending on the time period under consideration, despite marginally improved immune response. A dedicated study for this issue should be performed outside a highly dynamic pandemic event.

Arterial hypertension may be a protective factor in the infection event, albeit associated with marginally lower antibody levels. Thus, the importance of measurable antibody levels should be considered critically and only partially measures vaccination success. Antibody quality should also be considered.

Our data show that some dsitinct vaccine side effects have a significant positive impact on the humoral immune response, thus demonstrating in a limited way the relationship between reactogenicity and immunogenicity. At the same time, however, the expression of certain vaccine side effects may represent a negative predictive factor with respect to vaccine efficacy.

Ultimately, the role of higher antibody levels in long-term vaccination success remains questionable.

Erklärung zum Eigenanteil der Dissertationsschrift

Die Arbeit wurde im Zentrum für klinische und experimentelle Transfusionsmedizin, Teil des Universitätsklinikums Tübingen unter Betreuung von Prof. Dr. Tamam Bakchoul durchgeführt.

Die initiale Konzeption der Studie erfolgte durch Dr. med. Günalp Uzun, Prof. Bakchoul und Dr. med. Karina Althaus. An unterjährigen Verfahrensanpassungen war ich zentral durch aktive Teilnahme am Lenkungskreis beteiligt.

Sämtliche Recherche, Datenbereinigung und konsekutive Auswahl von Probenden und probandenbezogenen Daten wurden von mir eigenständig durchgeführt.

Die Messungen der Antikörperspiegel wurden von Marco Mikus, MTA HLA/PLT am ZKT durchgeführt.

Die statistische Auswertung erfolgte vollumfänglich eigenständig. Alle Grafiken, Diagramme und Tabellen wurden eigenständig durch mich erstellt.

Ich versichere das Manuskript selbständig verfasst zu haben und keine weiteren, als die von mir angegebenen Quellen verwendet zu haben.

Nehren, den 10.05.2023

Alan Bareiß

Veröffentlichung

Folgende Publikation ging aus der Arbeit hervor:

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Die entsprechenden Passagen sind mit einem entsprechenden Verweis markiert.

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