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Evaluation of routine methods for determining SARS-CoV-2 serostatus

Dissertation

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Zusammenfassung

Impfungen gegen das Schwere Akute Atemwegs Syndrom Coronavirus Typ 2 (SARS-CoV-2) werden immer noch anhand von festen Impfschemata durchgeführt, anstatt individuelle (Re-)Immunisierungen entsprechend des persönlichen Immunschutzes und angepasst an die aktuelle Variante anzubieten.
Wir haben diagnostische Strategien zur individuellen (Re-)Immunisierung entwickelt, die auf Antikörpern gegen die SARS-CoV-2 Spike-Protein-Rezeptor-Bindungsdomäne (S1-AB) und deren Neutralisierungskapazität in Surrogat-Tests sowie in Virusneutralisationstests in Zellkultur basieren. Die Neutralisierung gegen Wildtyp B.1 und Omikron BA.5.1 sechs Monate nach der Impfung mit dem unveränderten mRNA-Impfstoff wurde bewertet und führte zu Vorschlägen für individualisierte Impfschemata. Zu diesem Zweck wurden 124 Probanden vor, während und sechs Monate nach der Impfung gegen SARS-CoV-2 mit dem mRNA-basierten Impfstoff Spikevax (Moderna, Cambridge, MA, USA) durch serologische Tests begleitet.
Die Immunreaktionen nach Impfung variierten erheblich zwischen den Probanden und ebenfalls in Bezug auf die untersuchte Variante. Ebenso konnte der langfristige Immunschutz nicht durch den unmittelbar nach der Impfung erreichten Antikörperspiegel vorhergesagt werden. Während 92% der Seren eine ausreichende Neutralisierungskapazität gegen Wildtyp B.1 aufwiesen, zeigten nur 20% eine Neutralisationskraft gegen Omikron BA.5.1 sechs Monate nach der zweiten Impfung. Zu diesem Zeitpunkt konnten die Teilnehmer, die noch positiv auf den Neutralisationstest in Zellkultur im B.1-Stamm reagierten, anhand von S1-AB-Spiegeln ≥1000 U/mL als diagnostisches Instrument zur Messung der *Ex-vivo*-Immunreaktion bestimmt werden, während Seren, die BA5.1 hemmten, anhand der S1-AB-Serumspiegel nicht von Seren ohne suffizienten Immunschutz unterschieden werden konnten.
Diese Studie unterstützt somit individualisierte Impfschemata auf der Grundlage serologischer Tests, die für die Routine der Gesundheitsversorgung geeignet sind und Impfprogramme zeit- und kosteneffizienter gestalten sowie die Zahl der Impfnebenwirkungen reduzieren können. Es zeigte sich aber auch, dass Impfstoffe gegen SARS-CoV-2 regelmäßig an die aktuelle Variante angepasst werden müssen und dass die diagnostischen Richtlinien für serologische Tests regelmäßig überarbeitet werden müssen, um ein *ex-vivo* Korrelat des Immunschutzes darzustellen. Da sich die Pandemie und die Virus-Varianten derzeit rasch weiterentwickeln, wäre eine solch regelmäßige Überarbeitung der SARS-CoV-2-Impfbegleitdiagnostik nicht sinnvoll und Auffrischungsimpfungen könnten in Zukunft eher auf einer individuellen Abwägung zwischen den Nebenwirkungen der Impfung und dem Risiko einer Infektion mit Covid-19 basieren.

Abstract

Vaccination against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is still carried out according to fixed immunization-schedules instead of individualized (re-)immunization in accordance with personal immune responsiveness and adapted to the current variant of concern.

We developed diagnostic strategies based on antibodies against SARS-CoV-2 spike protein receptor-binding domain (S1-AB) and their neutralizing capacity in surrogate assays as well as in full virus neutralization tests in cell culture. Neutralization against wildtype B.1 and Omikron BA.5.1 six months after vaccination with unmodified mRNA vaccine was evaluated and led to proposals for individualized (re-)vaccination schedules. For this purpose, 124 subjects were monitored by serological testing before, during and six months after vaccination against SARS-CoV-2 with the mRNA-based vaccine Spikevax (Moderna, Cambridge, MA, USA).

Vaccination responses varied substantially interindividual and regarding to the investigated variant of concern and long-term immune protection cannot be predicted by the achieved antibody level immediately after vaccination. While 92% of the sera exhibited sufficient neutralizing capacity against wildtype B.1, only 20% showed neutralization against Omikron BA.5.1 six months after the second vaccination. At this point participants still positive for the full virus NT in B.1 strain could be determined by S1-AB levels ≥1000 U/mL as a diagnostic tool for gauging ex-vivo immune-responsiveness, while sera inhibiting BA5.1 could not be distinguished from non-inhibiting sera by serum levels of S1-AB.

This study supports an individualized (re-)vaccination scheme based on serological tests suitable for health care routine shaping vaccination-programs more time- and cost efficient as well as reducing the amount of side-effects. Nevertheless, it also shows that vaccines against SARS-CoV-2 have to be regularly adapted to the current variant of concern as well as the diagnostic guidelines in serological testing have to be revised to depict an *ex-vivo* correlate of immune protection. As the pandemic situation and variants of concern currently develop rapidly a regular revision of SARS-CoV-2 vaccination companion diagnostic would be elaborate and re-vaccination schemes in the future might be rather based on an individual assessment of the side effects of the vaccination and the risk of an infection with Covid-19.

Abbreviations

ACE2	*Angiotensin-converting enzyme 2*
full-virus NT	*full virus endpoint dilution neutralization test*
N	*nucleocapsid protein*
N-AB	*antibodies against the SARS-CoV-2 nucleocapsid antigen*
PACS	*Post-acute Covid-19 syndrome*
PACVS	*Post-acute Covid-19 vaccination syndrome*
PRNT	*Plaque Reduction Neutralization Test*
RBD	*receptor-binding domain*
rt-PCR	*by reverse transcriptase polymerase chain reaction*
S1	*spike-protein*
S1-AB	*antibodies against the SARS-CoV-2 spike (S1) protein receptor-binding domain*
SAGE	*WHO´s Strategic Advisory Group on Immunization*
SARS-CoV-2	*severe acute respiratory syndrome coronavirus type 2*
STIKO	*Standing Commission on Vaccination*
TMPRSS2	*transmembrane protease serine subtype 2*
VOC	*variant of concern*
WHO	*World Health Organization*

Contents

1. Introduction

1.1 Covid-19

1.1.1 Etiology

Covid-19 is an acute pulmonary and systemic disease caused by an infection with severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) [1], which belongs to the group of Betacoronaviruses like the Middle East respiratory syndrome coronavirus (MERS-CoV) [2, 3]. SARS-CoV-2 is mainly transmitted by droplets or aerogenically by the release of respiratory particles [4-6]. Moreover, it can also be transmitted by indirect contact through surfaces and vertically diaplacental or perinatal [7]. Therefore, the main transmission happens from person-to-person contact. Main risk factors for an infection with SARS-CoV-2 are poverty, a low socio-economic status and local outbreaks for example in nursing homes [8-10]. Onset and duration of infectivity are very variable from five to over 20 days after symptom onset due to different variants of the virus and the heterogenous courses of Covid-19 disease [10-12].

SARS-CoV-2 is an RNA-virus with a diameter of 80-140 nm. It has a single stranded genome, which makes up the nucleocapsid together with the non-membrane-bound nucleocapsid protein (N). Furthermore, the virus contains membrane-bound structural proteins such as the spike-protein (S1) [13, 14]. This specific protein is responsible for binding to the host cell with the receptor-binding domain (RBD) on the S1-subunit of the spike-protein and for inducing neutralizing antibodies. The S-protein binds to the Angiotensin-converting enzyme 2 (ACE2) of human cells and penetrates it with its transmembrane protease serine subtype 2 (TMPRSS2) to enter the cell [15-18]. Moreover, the envelope-protein (E-protein), the matrix-protein (M-protein) and a huge number of other detected biomechanisms play a significant role in the pathophysiology and the lifecycle of SARS-CoV-2 [3, 14]. Each lifecycle is completed by the uncoating, translation, replication and transcription of the virus´ RNA followed by the assembly and exocytosis of newly created pathogens, which are about to infect other cells [14].

1.1.2 Epidemiology and variants of concern (VOCs)

SARS-CoV-2 led to a global pandemic after originating in Wuhan, China in December 2019 [19]. Two competing ideas about the origin of the virus were under public consideration. While the emergence as a zoonosis from bats is nowadays considered the more probable option there is no evidence for the idea of a laboratory escape of the virus [20]. On 11th of March 2020 the World Health Organization (WHO) declared Covid-19 a global pandemic situation, which has thus been the first pandemic caused by a coronavirus [21]. Until now (July 2024) more than 775 million cases of Covid-19 have been reported globally and more than seven million people lost their lives due to severe Covid-19 infections [22]. Case numbers show an exponential increase after exceeding a certain basis reproduction number leading to infectious waves with different variants of concern (VOCs) of Covid-19 [11, 23]. Each strain was named after a letter of the Greek alphabet by the WHO and evolved by new mutations of the viral genome leading to different characteristics in transmissibility and mortality. Different VOCs also show alternating epidemiological patterns. These altered characteristics arise from mutations encoding important sections of the structural proteins leading to different interactions with infected cells, the immune system of a patient and potential medication [24].

The first big infectious wave has been caused by the Alpha-variant, which originated in the United Kingdom in September 2020 and is characterized by higher virulence and transmissibility [25]. Over the time of the pandemic development new VOCs generally led to higher case numbers, a higher severity of the course of the infection and a different susceptibility of immunity achieved by vaccinations [26, 27]. Characteristics of the Covid-19 VOCs changed over time. Until now five strains called Alpha, Beta, Gamma, Delta and Omicron were of public concern, as VOCs and over one thousand strains were officially defined [28]. The nowadays predominant VOC Omicron emerged in spring 2022 is the most genetically diverse one but also shows a drastic decrease in hospitalizations and case fatality rates. This effect correlates with the average age of the patients, which is lower than in the high-mortality waves as mostly people in age groups under 65 years were infected by Omicron, and less tissue damage induced by mutations in the S-protein [24, 29-31]. An infection with a newer VOC therefore is

more probable due to mechanisms of immune escape after vaccination, but also less severe in most cases, which might have led to a higher rate of undocumented cases in the recent past [27, 32].

1.1.3 Clinical manifestation of Covid-19

Patients suffering from an infection with SARS-CoV-2 show a typical clinical presentation encompassing symptoms like fever, cough and dyspnea [19]. Moreover, due to a systemic hyperinflammation and dysregulation of the immune system, symptoms like changes in the gustatoric and olfactoric system called dysgeusia and anosmia appear. Pathogenesis behind these phenomena is still not understood completely [33]. Hyperinflammation leads to a massive production of proinflammatoric cytokines like interleukin 1 and 6 causing severe illness with acute respiratory distress syndrome and Covid-19 pneumonia with the need for ventilation as well as endothelial dysfunction causing thromboembolic events and acute kidney injury [34-36]. Other specific symptoms occurring in severe causes of Covid-19 are gastrointestinal manifestation, myocarditis mostly affecting young male patients and neurological disorders such as dysesthesia and strokes [37-39]. This diverse and complex cluster of clinical manifestation of Covid-19 can be explained by organotropism. As SARS-CoV-2 uses ACE2 and TMPRSS2 for entering cells, mostly tissues expressing this specific receptor get damaged during an infection with the virus [40]. Research even suggests that ACE2 gets upregulated by interferons during infection for enhancing SARS-CoV-2 pathogenicity [16].
According to the WHO, severe causes are classified by the need of hospitalization, ventilation, use of extracorporeal membrane oxygenation, kidney replacement therapy and other non-/invasive therapies [41]. Covid-19 tends to cause critical illness especially during infections with more aggressive VOCs, in old patients and in people already suffering from multimorbidity leading to significantly higher case fatality rates [30, 31, 42]. Nevertheless, asymptomatic and mild infections are very common especially in younger patients with good functional performance status. Since these patients are still able to transmit the virus, they constitute a challenge in infectious events and to their prevention [43]. However, even asymptomatic infections can reliably be detected and improve containment by different methods

of diagnostics when preventive testing in high-risk constellations is carried out [44].

1.1.4 Covid-19 diagnostics and therapeutic approaches

Two different methods are currently used in clinical routine for detecting an infection with SARS-CoV-2. Each is based on a nasopharyngeal swab test or other sampling of respiratory secretion. While the highly sensitive detection by reverse transcriptase polymerase chain reaction (rt-PCR) indicates the viral load of each specimen by measuring the cycle threshold and is the gold standard in medical institutions, rapid antigen tests detecting virus proteins are less sensitive but cheaper and available as self-test kits [45, 46]. Serological assays detecting antibodies against SARS-CoV-2 are of lower importance in primary care as their utility is limited due to a poor cost-benefit ratio and informative value concerning acute and undergone infections [47]. Seroconversion occurs 1-3 weeks after the onset of symptoms, making antibody tests obsolete as a tool for detecting SARS-CoV-2 infections in health care settings [48].

Therapeutic approaches to Covid-19 were of immediate urgency due to its massive impact on the health care system [49]. Besides supportive care in intensive care units such as ventilation, prophylaxis of thrombosis and management of fluids, electrolytes and nutrition, also specific drug therapy moved into focus [50, 51]. Steroids, antiviral therapeutics, plasma therapy and monoclonal antibodies were licenced and launched on the market to complete Covid-19 therapy [51, 52]. However, it soon became clear that the hazardous course of the pandemic cannot be contained by new therapeutics but has to be curbed by a dedicated concept of prevention [19, 53].

1.1.5 Prevention of Covid-19 and its relevance

As soon as the pandemic developed into a burden bearing down on the population, health care system and economy prevention concepts were set up to limit the damage the pandemic has already caused and to prevent public systems from collapsing [49, 54, 55]. Prevention control measures mainly encompass social distancing, hand hygiene, wearing face masks, preventive testing and the

isolation of cases [45, 56, 57]. These prevention techniques could flatten the curve and were partly able to control the spread of SARS-CoV-2. However, they were also associated with a massive impact on mental health due to shutdowns and major financial loss [58, 59]. Thus, a long-term solution to contain the pandemic had to be found and the development of a vaccination became the focus of attention [60].

In addition to the reasons for urgent prevention already mentioned another threat arose during the course of the pandemic. Long Covid or the Post-acute Covid-19 syndrome (PACS) is a new entity underestimated for a long time. Long Covid describes persistent symptoms after an infection with SARS-CoV-2 for more than four weeks after infection and occurs with an estimated incidence of 10-30% in un-hospitalized and 50-70% in hospitalized cases [61]. This new entity includes disorders of the nervous, respiratory and cardiovascular system as well as mental health generating symptoms like fatigue, myalgia, dyspnoea, depression and vertigo [62]. Symptoms are diverse and diagnostic tools are not yet standardized, which leads to serious consequences for patients and the health care system as a whole [63]. Pathomechanisms behind Long Covid remain unconfirmed until today but latest research suggests ongoing immune dysregulation, dysautonomia, virus persistence and endotheliopathy as potential pathogenesis behind the illness [64]. A severe cause and a low socio-economic status constitute main risk factors for suffering from PACS [61]. Research regarding Long Covid is still at a young age and therapeutic regimes have not been implemented so far. Beside therapeutic apharesis also anti-thrombotic therapy and antihistamines state possible therapeutic approaches [65, 66]. Since the complete impact of Long Covid cannot be estimated at the time, the importance of prevention of Covid-19 becomes even more concrete.

1.2 Vaccination against SARS-CoV-2

1.2.1 Different types of Covid-19 vaccines

On the 11th of December 2020, not even one year after the emergence of SARS-CoV-2, the first vaccine against Covid-19 was offered to the population by an emergency use authorization in the United States and the European Union [67]. The first vaccine type launched on the market was an mRNA-based vaccine. In

2023 Katalin Karikó and Drew Weissman were rewarded with the Nobel Prize in Physiology and Medicine for the discovery of foundational genomic mechanisms that enabled the development of mRNA-based vaccines against Covid-19 [68]. This type of vaccine has then soon been followed by DNA-based vaccines, viral vector-based ones and vaccines with inactivated or attenuated proteins. They induce cellular and humoral immune response [69]. mRNA-based vaccines contain synthetic mRNA encoding the S-protein protected by lipid nanoparticles. Human cells synthetize the S-protein after vaccination and transport it to the surface of the cell. It can then get recognized by the immune system and leads to the production of protective antibodies. This technology states an innovation in vaccinology since the S-protein itself is not pathogenic for the human body [67]. Until today more than 13 billion doses have been administered globally, which makes up a population share of 56% vaccinated with a complete primary series of a Covid-19 vaccine [70, 71].

Covid-19 vaccinations have positive effects on preventing symptomatic infections with SARS-CoV-2, reducing the number of severe cases, hospitalizations and mortality [72]. Moreover, Covid-19 vaccination is associated with a lower risk of suffering from Long Covid when vaccination has been administered prior infection [73].

Recommendations on Covid-19 vaccinations orientate to the patient´s age, health condition and vaccination history. According to the WHO´s Strategic Advisory Group on Immunization SAGE all adults as well as children and adolescents with comorbidities should receive one dose of a vaccine or two doses of an inactivated vaccine. Immunecompromised patients, health care workers and patients older than 75 years should receive 2-3 doses as they belong to high priority-use groups [74]. The Standing Commission on Vaccination STIKO at the Robert Koch Institute in Germany recommends at least three contacts with the SARS-CoV-2 antigen to achieve basic immunity. These contacts can be achieved either by infection or vaccination, while at least two of the three contacts should take place by vaccinations. Immunity achieved by the combination of undergone infection and vaccination was designated hybrid immunity [75].

1.2.2 Booster immunization and the adjustment to VOCs

Immune response after vaccination against SARS-CoV-2 is inter-individually heterogeneous depending not only on the use of different types of vaccines but also on e.g. the patient´s age and comorbidities [76-78]. Among groups of elderly and male patients even non-responders after basic immunization became apparent, which therefore state another group at risk [79]. In addition, vaccination against Covid-19 does not guarantee lifelong immune protection. Studies have shown that immunity already wanes one to four months after vaccination and half a year after administration there is already no protective effect of the vaccination left. Protection against severe illness remains up to nine months excluding male patients and people with comorbidities [80]. Due to bottlenecks in comprehensive provision of vaccination people were offered heterologous immunization schedules in which a patient primary vaccinated with an mRNA-based vaccination gets a second dose of another vaccination type or vice versa. Research found that heterologous vaccination leads to a longer immune protection against SARS-CoV-2 than homologous vaccination schedules [81]. Nevertheless, both strategies show weaning immunity after a certain time, which led to the instruction of administering booster immunization. Boosting the immunity with a third vaccination after six months leads to a reduction in infection rates and reduces the risk of a severe outcome. Immune protection thus is prolonged by booster immunization [82, 83]. In addition undergone infection do not provide life-long immunity against re-infection either and in cases of infections with Omicron protection against re-infection and severe causes was even lower than it was after infections with previous VOCs [84].

Because of waning immunity after infection and vaccination breakthrough infections have appeared more frequently. Due to the ability of immune escape this phenomenon became apparent especially since Omicron is the predominant VOC [85, 86]. As a reaction to that a bivalent Covid-19 vaccine producing antibodies both against ancestral VOCs and against Omicron has been released [87]. This bivalent vaccination was recommended as a booster dose after a monovalent primary series in September 2022 and vaccine effectiveness against severe causes was again determined [88]. Till the end of 2023 28% of total

population has been vaccinated with at least one monovalent or bivalent booster dose of a Covid-19 vaccine [70].

In addition to the high costs associated with Covid-19 vaccination governments had to bear also adverse effects have occurred during vaccination period [89]. Beside mild side effects like muscle pain, swelling and fever also severe side effects became apparent [90]. These rare but severe adverse effects include myocarditis primarily in young patients, vaccine-associated immune thrombosis and thrombocytopenia and other symptoms [91, 92]. Moreover, Covid-19 vaccination brought forth a new disease entity named Post-acute Covid-19 vaccination syndrome (PACVS). Patients suffering from PACVS report symptoms like chronic fatigue, cardiovascular dysautonomia, cognitive deficits and nausea. Symptom set on in close temporal context to vaccination and continue in waves associated with an extreme reduction in quality of life. Elevated levels of Interleukin 6 and altered levels of receptor autoantibodies are recently suggested as the somatic correlate of PACVS and thus could state probable therapeutical targets [93]. PACS and PACVS have the similarity that both entities have a long time to diagnosis and optional therapy as they are incompletely understood, and patients often are tainted with prejudices concerning these medical novelties. Due to the complexity of medical phenomena during the Covid-19 pandemic so far it becomes apparent that the long-term impacts of both the infection with SARS-CoV-2 and the vaccination against Covid-19 are not yet fully revealed [94].

1.2.3 Monitoring SARS-CoV-2 immune-status

Vaccination against SARS-CoV-2 results in humoral and cellular immune response. Main objects of interest when considering immunological parameters are antibody levels and T-cell response [95, 96]. Testing for cellular immunity is elaborate as methods like fluorescence activated cell sorting FACS are used [97]. Therefore, mainly serological evaluation of immune response after Covid-19 vaccination became the focus of attention.

Two different types of immune response regarding Anti-SARS-CoV-2 serology can be differentiated. Immunity after an infection with SARS-CoV-2 and immune response after Covid-19 vaccination constitute different antibody profiles. While

antibodies against the SARS-CoV-2 spike (S1) protein receptor-binding domain (S1-AB) serve as a marker both for infection and vaccination antibodies against the SARS-CoV-2 nucleocapsid antigen (N-AB) only occur after an infection with Covid-19 [98]. For the determination of antibody levels methods like Enzyme-linked Immunosorbent Assay (ELISA) and Electrochemiluminescence Immunoassay (ECLIA) are currently used [99, 100]. The WHO Expert Committee on Biological Standardization released the first WHO International Standard and International Reference Panel for anti-SARS-CoV-2 immunoglobulin in December 2020, which serves as a calibrator for serological tests and secondary methods [101]. Since the determination of antibody levels only deliver quantitative results regarding the levels of S1-AB or N-AB, they are not of qualitative value concerning the functionality of these circulating antibodies. Functionality of antibodies also known as neutralizing capacity can be measured by surrogate assays, which detect the inhibition of binding to ACE2 by formed antigen-antibody complexes with S1-protein and the patient´s serum [102]. However, the gold standard when assessing neutralizing capacity as a correlate to immune protection is the full virus endpoint dilution neutralization test (full-virus NT) or Plaque Reduction Neutralization Test (PRNT). This test has to be conducted in a containment level 3 facility in cell culture and thus is very time and cost consuming. It measures the varying dilution of serum inhibiting virus-plaque growth, from which the neutralizing antibody-titre is then derived [103].

1.3 Scope of the dissertation

This study assesses serological routine methods for establishing a diagnostic method for serostatus as a correlate of immune protection after vaccination against Covid-19.
Until today the WHO recommends booster immunization for elderly and immunocompromised patients, people with comorbidities and health care workers every 6-12 months and revaccination for women in every pregnancy [104]. Research also suggests annually revaccination against Covid-19 together with Influenza and vaccines for that purpose have already been experimentally developed [105]. The question as to whether revaccination following fixed

schedules is of a favourable cost-benefit ratio concerning economic, medical and social aspects or individual revaccination schemes are superior is core of this study.

The currently circulating Omicron VOC still leads to high infestation rates due to its potential of immune escape and the pandemic situation might already have turned into endemic conditions [24, 85, 86, 106]. In May 2023 the WHO announced that Covid-19 is no Public Health Emergency of International Concern (PHEIC) anymore [106]. Over 775 million cases have been reported globally since the beginning of the pandemic and vaccination rates are high in almost every country [70]. Many people achieved hybrid immunity as a consequence of the combination of both high infestation and immunization rates, which leads to a stronger immune protection [107, 108]. Considering the high rate of asymptomatic courses during the Omicron wave, this effect would be even stronger [43, 109]. However, immune protection became heterogenous regarding different vaccination schedules with numerous different types of vaccines and different severity of the cases during the course of the pandemic, which makes an individualized vaccination schedule necessary [109, 110]. Studies proved that this heterogeneity is even greater among elderly, frail and male patients [79]. Closing these gaps in immunization and thus preventing higher case rates and severe causes could be practicable by regular serological testing.

In contrast to that avoiding unnecessary booster vaccinations becomes even more relevant when considering severe side effects of Covid-19 vaccination like PACVS [93]. Serological antibody tests prior to revaccination could prevent patients from these adverse effects.

Since there has not been a method for determining immune protection against an infection with SARS-CoV-2 established in health care routine, we examined how levels of circulating immunoglobulins can serve as a correlate for immune protection [45]. We also aimed to adapt this algorithm to different VOCs using Omicron as a suitable and currently relevant example.

For evaluating routine methods determining SARS-CoV-2 serostatus and establishing a diagnostic algorithm for serostatus as a correlate of immune protection we examined sera drawn in the University hospital of Düsseldorf. Permission for the study from the ethics committee of the medical faculty at

Heinrich Heine University in Düsseldorf was granted (study numbers: 2021-1455 (23 April 2021) and 2020-1259 (22 January 2021)).

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A Diagnostic Strategy for Gauging Individual Humoral Ex Vivo Immune Responsiveness Following COVID-19 Vaccination

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Abstract: Purpose: We describe a diagnostic procedure suitable for scheduling (re-)vaccination against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) according to individual state of humoral immunization. Methods: To clarify the relation between quantitative antibody measurements and humoral ex vivo immune responsiveness, we monitored 124 individuals before, during and six months after vaccination with Spikevax (Moderna, Cambridge, MA, USA). Antibodies against SARS-CoV-2 spike (S1) protein receptor-binding domain (S1-AB) and against nucleocapsid antigens were measured by chemiluminescent immunoassay (Roche). Virus-neutralizing activities were determined by surrogate assays (NeutraLISA, Euroimmune; cPass, GenScript). Neutralization of SARS-CoV-2 in cell culture (full virus NT) served as an ex vivo correlate for humoral immune responsiveness. Results: Vaccination responses varied considerably. Six months after the second vaccination, participants still positive for the full virus NT were safely determined by S1-AB levels $\geq$1000 U/mL. The full virus NT-positive fraction of participants with S1-AB levels <1000 U/mL was identified by virus-neutralizing activities >70% as determined by surrogate assays (NeutraLISA or cPas). Participants that were full virus NT-negative and presumably insufficiently protected could thus be identified by a sensitivity of >83% and a specificity of >95%. Conclusion: The described diagnostic strategy possibly supports individualized (re-)vaccination schedules based on simple and rapid measurement of serum-based SARS-CoV-2 antibody levels. Our data apply only to WUHAN-type SARS-CoV-2 virus and the current version of the mRNA vaccine from Moderna (Cambridge, MA, USA). Adaptation to other vaccines and more recent SARS-CoV-2 strains will require modification of cut-offs and re-evaluation of sensitivity/specificity.

Keywords: COVID-19 serology; SARS-CoV-2 neutralization; SARS-CoV-2 vaccination; SARS-CoV-2 immunity; companion diagnostic

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1. Introduction

Since the emergence of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) in December 2019, more than 300,000,000 cases have been reported globally and nearly six million deaths have been confirmed [1,2]. The virus is transmitted human-to-human and can affect almost all human organs causing COVID-19, a potentially chronic disease comprising, inter alia, dry cough, fever, dyspnoea, anosmia, ageusia and pneumonia [2,3]. Apart from anti-inflammatory and virostatic approaches for treating manifest COVID-19, vaccination is considered the most crucial measure for stopping the SARS-CoV-2-pandemic [4].

SARS-CoV-2 constitutes a new scientific problem. Over the past two years, many questions have been addressed regarding disease management, and vaccination very quickly came into focus. The type of vaccine, which part of the virus should be targeted by vaccination and which immunization schemes should be employed had to be determined [5]. Several types of vaccines were developed rapidly, but initially limited supply of doses mandated decisions on whom to vaccinate first [6]. By January 2022, nearly ten billion doses had been administered globally [1], and the scientific focus shifted towards surveillance of individual vaccination responses and optimization of renewed immunization.

In the latter context, serological tests for monitoring humoral immune responses to infection and/or vaccination are of central importance. In December 2020, the World Health Organization established an international standard and reference panel allowing calibrated and standardized determinations of circulating levels of SARS-CoV-2 antibodies [7,8]. However, scientific guidance is lacking regarding which levels of circulating antibodies are sufficient for protecting vaccinated patients from severe causes of an infection and which serological tests are suited for surveillance of SARS-CoV-2 immune responsiveness following infections or vaccinations [7].

Here, we investigate how the state of humoral immune responsiveness against SARS-CoV-2 following vaccination (as specified by circulating serum levels of specific antibodies and functional ex vivo effects thereof) can be assessed under conditions of routine health care. We monitored the vaccination response of an adult cohort during two cycles of vaccination with the mRNA-based COVID-19 vaccine Spikevax (Moderna Biotech, Cambridge, MA, USA), using immunoassays for antibodies against the SARS-CoV-2 spike (S1) protein receptor-binding domain (S1-AB) and the SARS-CoV-2 nucleocapsid antigen (N-AB) and two surrogate assays for the virus-neutralizing activity of SARS-CoV-2 antibodies, for which measurements of suppression of cytopathic effects of the SARS-CoV-2 virus in cell culture (full virus neutralization test, full virus NT) served as a reference.

We thus evaluated a set of routine serological tests for circulating SARS-CoV-2 antibodies as COVID-19 companion diagnostics and aimed to establish criteria for judging their results quoad functional ex vivo responsiveness against the SARS-CoV-2 virus. Based on our results, we propose a staged diagnostic strategy that may allow laboratories to monitor the functional state of humoral immune responsiveness to SARS-CoV-2, without having access to a BSL-3 facility required for the full virus neutralization test, which is considered the gold standard.

2. Materials and Methods

2.1. Study Participants

A total of 124 study participants (83 female, 41 male, mean age 46 years, median age of 50 years) were recruited at the University Hospital of the Heinrich Heine University, Düsseldorf. All participants were employees of these institutions and underwent a program of two vaccinations with the COVID-19 vaccine Spikevax (Moderna Biotech, Cambridge, MA, USA) spaced exactly four weeks apart. Vaccinations were performed according to the instructions of the manufacturer and the recommendations of the German vaccination commission (STIKO). None of the participants tested positive for SARS-CoV-2 or exhibited symptoms of COVID-19, or exhibited debilitating symptoms of co-morbidities.

2.2. Sampling

Blood samples (18 mL) were collected by antecubital vein puncture 48 h before and four weeks following initial vaccination, and two weeks and six months after the booster dose (i.e., the second dose). Immune responses to mRNA-based vaccines are known to be reliably detectable 21 days after initial and seven days after a booster dose [9]. Following centrifugation (20 min, 1650× *g*), serum was separated and stored at −20 °C until testing. Aliquots (1 mL) for reflex testing were stored at −20 °C for up to 6 months.

Determination of Circulating Levels of Anti-SARS-CoV-2 Antibodies

Antibodies against the SARS-CoV-2 spike (S1) protein receptor-binding domain (S1-AB) encompassing all immunoglobulin classes (panIg) were determined using chemiluminescent immunoassay (ECLIA) (Elecsys Anti-SARS-CoV-2 S, Roche Diagnostics GmbH, Mannheim, Germany) on a COBAS 8000 analyzer (Roche, Basel, Switzerland) as prescribed by the manufacturer. Samples were measured at 10-fold dilution (Roche Cobas Universal Diluent) and re-measured at 400-fold dilution when exceeding the upper detection limit (250 U/mL). Results ≥0.80 U/mL are considered positive. PanIg antibodies against the SARS-CoV-2 nucleocapsid antigen (N-AB) were similarly determined with ECLIA (Elecsys Anti-SARS-CoV-2 assay, Roche, Basel, Switzerland) using a cut-off index based on positive and negative calibrators normalized to WHO standards [10]. Results presenting a ratio of signal/cut-off ≥1.0 are considered positive.

2.3. Surrogate Assays for SARS-CoV-2-Neutralizing Activity

Virus neutralization activity of SARS-CoV-2 antibodies was measured with NeutraLISA (EUROIMMUN Medizinische Labodiagnostika AG, Lübeck, Germany) and cPass (GenScript Biotech, Piscataway, NJ, USA), which both measure binding of recombinant, biotin-labelled ACE2 receptor to recombinant SARS-CoV-2-S1/-receptor-binding domain immobilized on microtiter plates. Signals of ACE2 receptor bound in the presence of serum are inversely proportional to the neutralizing potency thereof. Following the manufacturers' instructions, duplicate samples were processed on a semiautomatic ELISA processor by EUROIMMUN or using ELISA washer and reader from Tecan (Männedorf, Switzerland). Inhibition values (%) were derived from raw luminescence at 450 nm referenced to 620–650 nm and normalized to background without antibody. Cut-off values as provided by the manufacturers were ≥35% (positive) and <20% (negative) for the NeutraLISA, and ≥30% (positive) for the cPass.

2.4. Full Virus Endpoint Dilution Neutralization Test

Neutralization of entire SARS-CoV-2 virus B.1 isolate (Wuhan Hu-1 wildtype, GISAID accession number EPI_ISL_425126) served as reference assay for the neutralization capacity of sera. Two-fold serial dilutions (1:10 to 1:5120) of heat-inactivated sera (56 °C, 30 min) were prepared with maintenance medium (Dulbecco's Modified Eagle Medium, Gibco (Waltham, MA, USA), Ref 11995-065, 100 U/mL Penicillin and 100 µg/mL Streptomycin, Gibco, Ref 11995-065, 2% Fetal Calf Serum, Pan Biotech, (Aidenbach, Germany) Cat P303031). A total of 50 µL of diluted serum samples was incubated (37 °C, 1 h) in 96-well cell culture TC plates (Sarstedt AG & Co. KG, Nümbrecht, Germany) with virus solution at an absolute TCID50 of 100. Subsequently, 100 µL of cell suspension containing 7 × 104 VERO cells/mL (ATCC-CCL-81, obtained from LGC Standards) was added to each sample, and incubation continued (37 °C, 5% CO_2, 96 h). Subsequently, cytopathic effects (CPEs) were determined by microscopic inspection. The effective neutralization titre was defined as the highest CPE-negative sample dilution. Titres of ≥ 1:10 were considered positive. Controls included in each test series encompassed neutralization-negative and -positive serum samples (previously determined and stored at −20 °C), the effect of virus in the absence of serum, and growth controls of cells exposed neither to virus nor to serum.

2.5. Statistical Methods

IBM SPSS Statistics 28 software (IBM Corp. released in 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY, USA: IBM Corp.) and Graph Pad Prism 9 (Graph Pad Software, Inc., San Diego, CA, USA: released in 2020. Graph Pad Prism 9 for Windows, San Diego, CA, USA: Graph Pad Inc.) were used for analysis. Normal distribution was tested according to Shapiro–Wilk and Q-Q graphs. Non-normally distributed data were descriptively analyzed by mean/median values, interquartile range and boxplots. Correlations were analyzed by Spearman correlation. Friedman's test was used to detect differences between paired samples within a given dataset over time. Differences between infected and non-infected participants were analyzed by the Mann–Whitney-U test. Linear regression analysis was used to analyze relationships between vaccination responses at various timepoints. Correlation and effect size was assumed to be good at $r \geq 0.5$ and moderate at $r \geq 0.3$. For all tests, statistical significance was assumed at $p < 0.05$. Missing data (about 12%) were handled by listwise deletion.

3. Results

Samples obtained at each timepoint were tested for serum levels of antibodies against SARS-CoV-2 spike (S1) protein receptor-binding domain (RBD) (S1-AB) and antibodies against the SARS-CoV-2 nucleocapsid antigen (N) (N-AB). S1-AB served as a marker of infection as well as vaccination, whereas N-ABs served as a marker of infection only [11].

N-ABs are not expected to increase upon mRNA vaccination against spike (S1) protein receptor-binding domain (RBD) in the absence of infection. Consequently, N-AB-positive participants were assumed to have undergone asymptomatic infection with SARS-CoV-2 virus before vaccination or during the post-vaccination period monitored in the study. A total of 1.8% (n = 2) of the participants (n = 113, details: Supplementary Table S1) were positive for N-ABs and S1-AB in the sample obtained before the first vaccination, indicating that they had already undergone inapparent or unregistered infection(s) with the SARS-CoV-2 virus. Another participant tested positive for a SARS-CoV-2 infection according to a PCR test. According to the vaccination regimen of the institution, all participants were vaccinated irrespective of their serological state. However, in our study, N-AB- and PCR-positive participants were kept separate in statistical analyses. There were no N-AB-positive samples obtained six months after vaccinations, ruling out intercurrent inapparent SARS-CoV-2 infections.

S1-AB levels before and at various timepoints after vaccination were as follows: 99.1% (n = 109) of the N-negative participants (n = 110) and 100% (n = 116, details: Supplementary Table S1) tested positive after first and second vaccination, respectively. All N-negative participants monitored six months after the second vaccination (n = 95, details: Supplementary Table S1) were still S1-AB-positive. These values are in good agreement with vaccination responses observed elsewhere [12]. Mean values of S1-AB were 169 U/mL (0.4–1.004 U/mL) after the first vaccination and increased to 5704 U/mL (213–17.764 U/mL) after the second vaccination and dropped again to 1.019 U/mL (69–5.220 U/mL) six months after the second vaccination. A synoptic representation of S1-AB values obtained at the various timepoints of observation is given in Figure 1A.

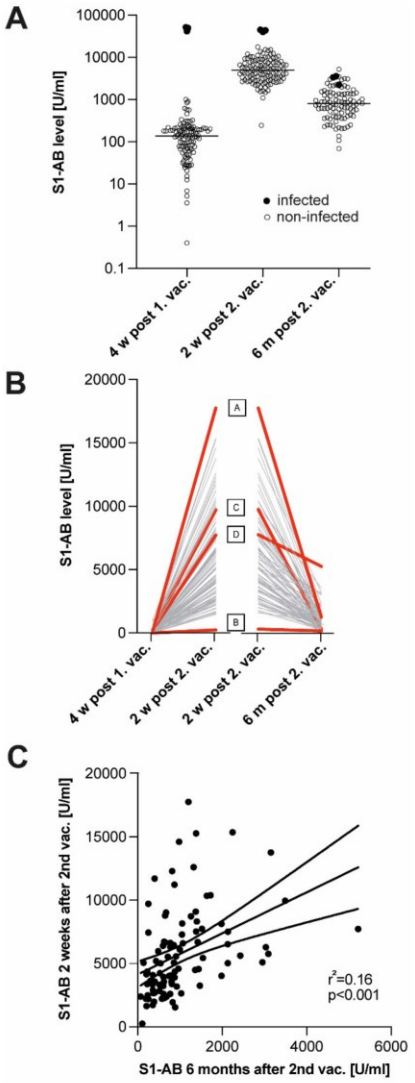

Figure 1. Serum levels of S1-AB after vaccination with COVID-19 vaccine Spikevax. (**A**) S1-AB levels measured four weeks after first (**left**), two weeks after second (**middle**) and six months after second vaccination (**right**). Median values indicated by horizontal bars. Brackets indicate significant ($p < 0.001$) differences of the means. Closed symbols: SARS-CoV-2 infection prior to vaccination. (**B**) Developments of S1-AB serum levels between first and second vaccination (**left**) and 6 months after second vaccination (**right**). Exemplary time courses are highlighted (red) and indexed (capitals). (**C**) Correlation of S1-AB serum levels at two weeks and six months after second vaccination. Linear regression of the data and 95% confidence interval indicated by solid and dashed lines, respectively ($r^2 = 0.16$, $p < 0.001$). For numbers of included participants, see Supplementary Table S1.

Alterations in antibody levels over time were highly significant ($p < 0.001$), giving rise to highly inhomogeneous time courses of sero-responses (Figure 1B). Interindividual divergence started with immediate vaccination responses: certain participants showed a huge increase in antibody levels from an above-median level after the first vaccination to an even higher level above the median after the second vaccination (Index Pat. A), whereas other participants responded with sub-median rises in S1-AB to the first vaccination and exhibited no significant further increase following the second vaccination (Index Pat. B); see Figure 1B. Time courses of S1-AB levels during the six months after the second vaccination were even more heterogeneous, encompassing a drop to as low as 2.6% (250 U/mL of 9.724 U/mL, Index Pat. C) as well as maintenance of as much as 67.5% (5.220 of 7.725 U/mL, Index Pat. D) of the initial S1-AB level reached after the second vaccination. Differences in immediate and long-term humoral vaccination responses exhibited no significant correlation with age, gender, or any known co-pathologies of the study participants. Most notably, S1-AB levels immediately after the second vaccination exhibited only a very moderate correlation ($r = 0.54$, $p < 0.001$) with corresponding residual S1-AB levels observed six months later. The rather poor linear regression of those data ($r^2 = 0.16$, $p < 0.001$) suggests that immediate humoral vaccination response and long-term maintenance of humoral immunity are not stringently linked in quantitative terms. (Figure 1C).

Samples of participants having undergone SARS-CoV-2 infection before vaccination (n = 3, details: Supplementary Table S1) were identified by increased serum levels of N-ABs and/or a positive PCR result. These samples exhibited many-fold higher levels of S1-AB. After the first vaccination, the mean value of S1-AB in post-infection samples was 47,738 ± 3.002 U/mL as opposed to 169 ± 16.6 U/mL in non-infected samples. After the second vaccination, the mean value of S1-AB in post-infection samples was 43,001 ± 1.532 U/mL as opposed to 5.704 ± 322.9 U/mL in non-infected samples. These differences were highly significant ($p < 0.001$). In the long run, the augmenting effect of SARS-CoV-2 infection on vaccination response started to diminish. At six months after the second vaccination, the mean value of S1-AB in post-infection samples was 3.070 ± 417 U/mL as opposed to 1.019 ± 88.5 U/mL in non-infected samples. This difference was still significant ($p = 0.001$) but quantitatively less pronounced than at the timepoints directly after vaccination (see Figure 1A and Supplementary Figure S1).

In the next step, we compared S1-AB serum levels with corresponding virus-neutralizing activity of the sera. For that purpose, all samples were probed for their potency to inhibit the binding of biotin-labelled ACE2 receptor to immobilize recombinant SARS-CoV-2-S1/-RBD (NeutraLISA, EUROIMMUN, Lübeck, Germany), which is considered a practical diagnostic surrogate for the neutralization of cytopathic effects of the full viable virus as determined in cell culture. After the first vaccination and six months after the second vaccination (see Figure S2), levels of S1-AB correlated strongly with the corresponding virus neutralization capacity of the sera (r2 = 0.774 to 0.845). Immediately after the second vaccination, a similar analysis was determined not to be meaningful since the upper measuring limit of the NeutraLISA at 100% was already attained by sub-median levels of S1-AB. Thus, the limited dynamic range rendered the NeutraLISA uninformative in the situation of recent re-immunization. Similar results were obtained by cPass (not shown). The two surrogate assays exhibited excellent linear correlations across all timepoints ($r^2 = 0.774$ to 0.932, $p < 0.001$) (Supplementary Figure S1). It should be noted that the cPass assay appeared slightly more sensitive in the low range (after the first vaccination) but yielded similar values (around 98%) after the second vaccination.

In summary, the two surrogate assays for virus neutralization capacity failed to provide meaningful additional information regarding immediate vaccination responses. However, they may be useful in long-term monitoring of humoral vaccination responses. To follow up on the latter notion, NeutraLISA data obtained at six months after the second vaccination was scrutinized for relevance. Based on comparisons with WHO standards and a full virus endpoint dilution neutralization test (full virus NT), inhibition values of $\geq$35% obtained by the NeutraLISA in post-infection sera are proposed to indicate effective virus

neutralization potency [13]. However, according to our own unpublished observations, the neutralization potency of antibodies induced by S1-spike protein-directed vaccination may be overestimated by these surrogate assays as compared to the results obtained with the full virus NT, which is currently considered the reference assay. To follow up on this notion, samples collected six months after the second vaccination were re-tested with a full virus NT. For 95 samples, interpretable results were obtained. Within these samples, the surrogate assays showed strong correlations with the full virus NT ($r^2 = 0.79$, $p < 0.001$ for NeutraLISA, $r^2 = 0.77$, $p < 0.001$ for cPass) (Figure 2A,B), which confirms the results of previous studies [13]. However, in the low range, positive–negative discrimination by the surrogate assays did not sufficiently match the results of the full virus NT. Most notably, the surrogate assays yielded a significant number of false-positive results (5/89 in both tests) (Figure 2A,B, inserts), suggesting that they may not be a safe companion diagnostic for long-term monitoring of vaccination with mRNA-based vaccines such as Spikevax (Moderna, Cambridge, MA, USA).

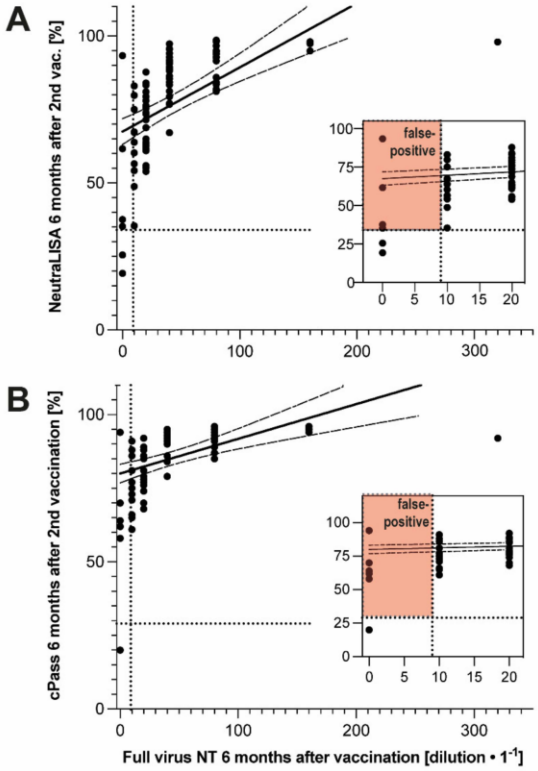

Figure 2. Virus neutralization capacity six months after second vaccination, as determined by surrogate assays and full virus NT. (**A**) Correlation of NeutraLISA (Lübeck, Germany) with full virus NT six months after second vaccination; linear regression of the data and 95% confidence interval indicated by solid and dashed lines, respectively ($r^2 = 0.79$, $p < 0.001$); insert: blow ups of low-level range. (**B**) Correlation of cPass with full virus NT six months after second vaccination; linear regression of the data and 95% confidence interval indicated by solid and dashed lines, respectively ($r^2 = 0.77$, $p < 0.001$); insert: blow up of low-level range. For numbers of included participants, see Supplementary Table S1.

Consequently, we addressed the question of which other diagnostic tools or staged strategies could possibly improve the safety of serologic monitoring of long-term vaccination responses. First, we investigated whether a full virus NT titre ≥10 at six months after vaccination could possibly be predicted from the quantitative levels of S1-AB measured either directly (see Figure S3) or six months after second vaccination. S1-AB levels measured directly after the second vaccination were poorly correlated with the full virus NT obtained six months later ($r^2 = 0.54$, $p < 0.001$), which was expected given the equally poor correlation with quantitative S1-AB determined six months later (Figure 1C). However, S1-AB levels measured six months after the second vaccination exhibited a reasonably strong correlation with neutralizing capacity as determined by the full virus NT at the same time ($r^2 = 0.79$, $p < 0.001$) (Figure 3A), allowing for determining a cut-off at 1000 U/mL to discriminate a major portion (35/89 of the full virus NT-positive samples from all full virus NT-negative samples (Figure 3A, insert). Incidentally, the fraction above that cut-off encompassed all samples having undergone infection in addition to double vaccination (Figure 3B, black circles).

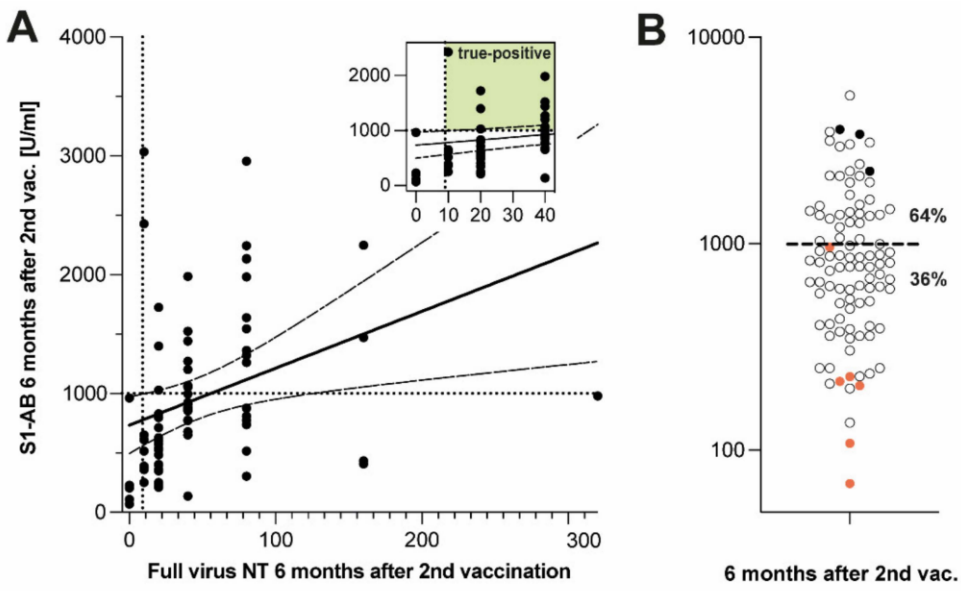

Figure 3. Levels of S1-AB and titres of full virus NT six months after second vaccination. (**A**) Comparison of S1-AB and full virus NT six months after second vaccination; linear regression of the data and 95% confidence interval indicated by solid and dashed lines, respectively ($r^2 = 0.79$, $p < 0.001$); insert: blow up of low-level range. (**B**) S1-AB levels six months after second vaccination; horizontal dashed bar: cut-off for full virus NT negatives. Percentages: fractions of samples located above and below cut-off. Closed symbols (black): SARS-CoV-2 infection prior to vaccination, closed symbols (red): full virus NT negatives. For numbers of included participants, see Supplementary Table S1.

The remaining 63/89 samples below the cut-off (i.e., exhibiting S1-AB levels <1000 U/mL six months after second vaccination) (Figure 3B, symbols below dashed line) encompassed all 6 full virus NT-negative samples (Figure 3B, closed red circles) but also 57 full virus NT-positive samples (Figure 3B, open circles below dashed line). In search of a practical diagnostic tool allowing us to discriminate within this group between NT-negative and

-positive samples, we reassessed the corresponding results of the surrogate assays for virus neutralization. Upon re-adjusting the cut-off level of NeutraLISA and cPass to 64 and 72%, respectively, it was thereby possible to discriminate between 5/6 true full virus NT-negative samples within the samples having S1-AB levels <1000 U/mL (Figure 4).

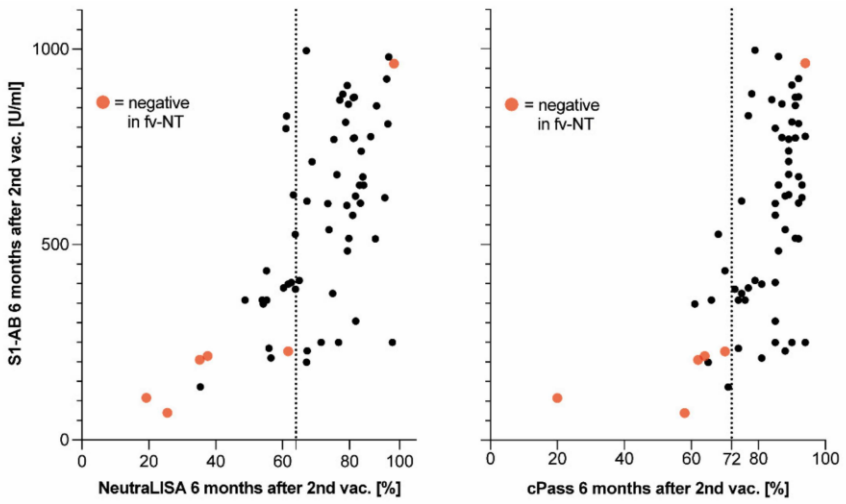

Figure 4. Adjustment of surrogate assays' cut-offs for the discrimination of full virus NT-negative samples with S1-AB levels <1000 U/mL at six months after second vaccination compared with corresponding values of NeutraLISA (**left**) and cPass (**right**). Dashed lines: optimized cut-offs of surrogate assays for discrimination of full virus NT-positive from full virus NT-negative samples. For numbers of included participants, see Supplementary Table S1.

In summary, the staged diagnostic strategy applied six months after the second vaccination detected five out of six full virus NT-negative samples, i.e., it had a sensitivity for a presumably insufficient virus neutralization capacity of 83.3%. As few as 14/89 (using NeutraLISA) or 6/89 (using cPass) were thereby falsely classified as virus NT-negative, i.e., corresponding specificity values were 84.2 and 96.2% for NeutraLISA and cPass, respectively.

4. Discussion

4.1. Rationale and Aim

Currently, SARS-CoV-2 vaccinations follow fixed temporal schedules prescribed by the manufacturers of the vaccines that are corroborated by guidelines and recommendations of national and international health agencies [14–17]. The ongoing appearance of new virus mutants and the increasing incidence of SARS-CoV-2-infection and COVID-19 disease in vaccinated people [18–20] demonstrate that vaccinations often fail to convey permanent immunity, and regular re-vaccinations are to remain a necessity in routine health care [21].

The rigidly scheduled regimen of re-vaccination currently employed to break the pandemic waves appears to be safe in terms of undesired side effects [22]. However, a more flexible vaccination strategy may have to be adopted eventually for the following reasons: (i) the duration of protection conveyed by current mRNA- and vector-based vaccines differs considerably [23]; (ii) the heterogeneity of duration of vaccination protection will further increase as protein- and whole-virus-based types of vaccines (such as Nuvaxovid (Novavax, Gaithersburg, MD, USA)) are introduced [24]; (iii) individual SARS-CoV-2 immunity and COVID-19 protection of vaccinated people is bound to vary even more due to unknown re-immunization by asymptomatic SARS-CoV-2-infections [25,26]; and (iv) ultimately, synchronous pandemic infection waves will

lead to continuous asynchronous endemic re-infection, making it even more difficult to select optimal timepoints for re-vaccination [27,28].

In summary, the above arguments suggest that a rigidly scheduled regimen for SARS-CoV-2 vaccination may soon become obsolete. Instead, it may become necessary to adapt re-vaccination to the individual immune status. This expectation implies the need to gauge the individual state of SARS-CoV-2 immunity using a diagnostic test [29]. Currently, there is no analytical correlate of protection against SARS-Cov-2 infection or against COVID-19 disease. Nevertheless, the question of which companion diagnostics may possibly be suitable to support an individualized vaccination strategy is raised.

To address this question, here we have here evaluated several tests for humoral SARS-CoV-2 immune responses, which are currently commercially available and practical in the setting of routine health care diagnostics [30–32]. We have investigated which of these tests could be used to monitor the waning of vaccination. In addition, we have compared our results to the full virus NT, which is not practical in routine health care but considered the serological test most closely reflecting humoral immunity [33,34].

4.2. Salient Findings

1. Humoral vaccination responses exhibited a huge interindividual heterogeneity in the study collective in terms of (i) maximal serum levels of S1-AB induced by vaccination, (ii) time courses of S1-AB levels over six months and (iii) residual S1-AB levels after six months. These observations are in line with other studies [35].
2. Immediate response and long-term maintenance of vaccination-induced antibodies were not stringently linked in quantitative terms, precluding judgement of durability of vaccination response from antibody levels measured shortly thereafter.
3. Four types of time courses of vaccination response could be identified: (i) high initial response followed by rapid decline, (ii) middling initial response followed by slow decline, (iii) middling initial response followed by fast decline and (iv) low initial and overall response. Types (iii) and (iv) tended to result in sub-average S1-AB levels after six months and were found in about half of the participants.
4. Surrogate assays for gauging the vaccination-induced serological potential of virus neutralization failed to provide meaningful information shortly after vaccinations due to limitations of measuring range and upper measuring limits.
5. At six months after vaccination, the serological potential of virus neutralization tended to be overestimated by surrogate assays as compared to the full virus NT, supporting previous notions that indiscriminate use of these assays would not provide adequate warning of crucial waning of immunity [36].
6. Lack of functional virus protection (as defined by full virus NT negativity) can possibly be detected during prolonged waning periods by a staged strategy employing surrogate assays to detect S1-AB levels below a cut-off of <1000 U/mL, which were judged by an elevated cut-off of around 70%. At six months after the second vaccination, virus NT-negative samples could thus be detected with a sensitivity of >80% and a specificity of between 80 and 90%, depending on which surrogate assay was used.

4.3. Limitations

1. All participants were vaccinated with Spikevax (Moderna Biotech), which rendered the study collective homogenous and produced significant results for this specific kind of vaccine. Our findings cannot be readily applied to other vaccines, especially protein- or vector-based ones.
2. Our serological tests were not adapted to virus mutants. Thus, our results apply only to the initial SARS-CoV-2 Wuhan virus isolate. Existing SARS-CoV-2 vaccines have been found to be less efficient against Delta, Omicron and other SARS-CoV-2 variants of concern (VOCs) [18]. Consequently, application of the proposed strategy to assess vaccinations against such VOCs will require adaption of the immunogenic assay target and re-evaluation of sensitivities, specificities and cut-offs.

3. Having included only three infected people in our study, we cannot add significantly to previous studies comparing antibody levels in persons with or without SARS-CoV-2 infection before and during vaccination on a larger scale [31,37]. However, we clearly show that the proposed diagnostic strategy worked similarly for vaccinated persons with and without infection.
4. The full virus NT is currently considered as the ex vivo test that most closely reflects functional humoral immune response [34]. However, it is not a direct measure of immunity itself, and it remains unknown how these data are related to real-life immunity. The same limitation applies to our data, which have been calibrated to the full virus NT. Yet, it is not fully understood which serological or cellular parameter is a direct correlate to sufficient immune protection. Therefore, measuring neutralizing antibodies can indicate the necessity for re-vaccination but cannot be understood as a strict recommendation.

4.4. Concluding Remarks

Current epidemiologic studies predict that regular re-vaccination against the SARS-CoV-2 virus will become a future necessity in routine health care [21], which poses a number of challenges: even a single type of COVID-19 vaccine exhibits considerable interindividual variability regarding levels and persistence of humoral immune responses thereby induced (shown here and in previous studies [38,39]). Variance of immune responses further increases when several types of vaccines are in play [23]. Moreover, intercurrent (possibly asymptomatic) infections have to be taken into account, since undesired vaccination effects tend to be more severe following recent infection [40]. In conclusion, it is to be expected that re-vaccination will soon have to be tailored to individual immune status. Serological surveillance of individual SARS-CoV-2 immunity will become even more important when synchronous pandemic infection waves lead to continuous asynchronous endemic re-infection. The diagnostic strategy proposed here may be useful in facing the above challenge, as it provides a reliable way of gauging levels and functionality of circulating SARS-CoV-2 antibodies, which so far requires ex vivo tests of virus neutralization in cell culture impracticable in routine health care [41]. Strictly speaking, the data presented here are already superseded, because they are only valid for the determination of antibody activity against the original Wuhan type of SARS-CoV-2. However, following re-calibration of the immunological tests and their cut-offs, the procedure can readily be applied to any virus mutant dominating the COVID-19 endemic in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/vaccines10071044/s1, Figure S1: Impact of prior SARS-CoV-2 infection on vaccination-induced Antibodies against the SARS-CoV-2 spike (S1) protein receptor binding domain (RBD) (S1-AB) serum levels, Figure S2:Correlation of NeutraLISA and cPass six months after second vaccination, Figure S3: Levels of Antibodies against the SARS-CoV-2 spike (S1) protein receptor binding domain (RBD) (S1-AB) and full virus neutralization test (full virus NT) measured two weeks after second vaccination, Table S1: Numbers of participants included in each analysis of the study. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions: Conceptualization, M.U., S.W., F.B. (Fritz Boege), F.B. (Florian Babor) and S.B.B.; Data curation, A.S.K., K.S.-B., M.U., S.W., F.B. (Fritz Boege), O.A., L.M., F.B. (Florian Babor), S.B.B., T.-X.U.P., M.H. and S.B.R.; Formal analysis, A.S.K., K.S.-B., F.B. (Fritz Boege), S.B.B. and D.H.; Investigation, A.S.K., K.S.-B., M.U., S.W., F.B. (Fritz Boege), O.A., L.M. and F.B. (Florian Babor); Methodology, M.U., S.W., F.B. (Fritz Boege), O.A., L.M., F.B. (Florian Babor), S.B.B., M.H. and S.B.R.; Project administration, K.S.-B., M.U., S.W., F.B. (Fritz Boege) and F.B. (Florian Babor); Resources, K.S.-B., M.U., S.W., F.B. (Fritz Boege), O.A., L.M. and S.B.B.; Software, A.S.K. and F.B. (Fritz Boege); Supervision, K.S.-B., M.U., S.W., F.B. (Fritz Boege) and D.H.; Validation, A.S.K., K.S.-B., F.B. (Fritz Boege) and S.B.B.; Visualization, A.S.K. and F.B. (Fritz Boege); Writing—original draft, A.S.K. and F.B. (Fritz Boege); Writing—review and editing, A.S.K., K.S.-B., M.U., S.W., F.B. (Fritz Boege), O.A., L.M., F.B. (Florian Babor), S.B.B., T.-X.U.P., M.H., S.B.R. and D.H. All authors have read and agreed to the published version of the manuscript.

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References

1. World Health Organization. WHO Coronavirus (COVID-19) Dashboard. Available online: https://covid19.who.int/table (accessed on 30 October 2021).
2. Tsang, H.F.; Chan, L.W.C.; Cho, W.C.S.; Yu, A.C.S.; Yim, A.K.Y.; Chan, A.K.C.; Ng, L.P.W.; Wong, Y.K.E.; Pei, X.M.; Li, M.J.W.; et al. An update on COVID-19 pandemic: The epidemiology, pathogenesis, prevention and treatment strategies. *Expert Rev. Anti-Infect. Ther.* **2021**, *19*, 877–888. [CrossRef] [PubMed]
3. The Lancet. Facing up to long COVID. *Lancet* **2020**, *396*, 1861. [CrossRef]
4. Pollard, C.A.; Morran, M.P.; Nestor-Kalinoski, A.L. The COVID-19 pandemic: A global health crisis. *Physiol Genom.* **2020**, *52*, 549–557. [CrossRef] [PubMed]
5. Silveira, M.M.; Moreira, G.; Mendonça, M. DNA vaccines against COVID-19: Perspectives and challenges. *Life Sci.* **2021**, *267*, 118919. [CrossRef]
6. Bubar, K.M.; Reinholt, K.; Kissler, S.M.; Lipsitch, M.; Cobey, S.; Grad, Y.H.; Larremore, D.B. Model-informed COVID-19 vaccine prioritization strategies by age and serostatus. *medRxiv* **2021**. [CrossRef]
7. Knezevic, I.; Mattiuzzo, G.; Page, M.; Minor, P.; Griffiths, E.; Nuebling, M.; Moorthy, V. WHO International Standard for evaluation of the antibody response to COVID-19 vaccines: Call for urgent action by the scientific community. *Lancet Microbe* **2021**, *3*, e235–e240. [CrossRef]
8. World Health Organization. *Establishment of the WHO International Standard and Reference Panel for Anti-SARS-CoV-2 Antibody*; Expert Committee on Biological Standardization: Geneva, Switzerland, 2020; pp. 9–10.
9. Sahin, U.; Muik, A.; Derhovanessian, E.; Vogler, I.; Kranz, L.M.; Vormehr, M.; Baum, A.; Pascal, K.; Quandt, J.; Maurus, D.; et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature* **2020**, *586*, 594–599. [CrossRef]
10. Roche. Elecsys®Anti-SARS-CoV2 S Quantitativer Assay—Abgeglichen am WHO Standard 12. May. 2021. Available online: https://a.storyblok.com/f/94122/x/f2860942d8/who-standard.pdf (accessed on 6 June 2022).
11. Narayanan, K.; Chen, C.J.; Maeda, J.; Makino, S. Nucleocapsid-independent specific viral RNA packaging via viral envelope protein and viral RNA signal. *J. Virol.* **2003**, *77*, 2922–2927. [CrossRef]
12. Bradley, T.; Grundberg, E.; Selvarangan, R.; LeMaster, C.; Fraley, E.; Banerjee, D.; Belden, B.; Louiselle, D.; Nolte, N.; Biswell, R.; et al. Antibody Responses after a Single Dose of SARS-CoV-2 mRNA Vaccine. *N. Engl. J. Med.* **2021**, *384*, 1959–1961. [CrossRef]
13. Hofmann, N.; Grossegesse, M.; Neumann, M.; Schaade, L.; Nitsche, A. Detection of neutralizing antibodies against SARS-CoV-2 by using a commercial surrogate virus neutralization ELISA: Can it substitute the classical neutralization test? *medRxiv* **2021**. [CrossRef]
14. CDC; CfDCaP. 2021. Available online: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines.html (accessed on 6 June 2022).
15. Paul-Ehrlich-Institute. 2021. Available online: https://www.pei.de/EN/newsroom/dossier/coronavirus/coronavirus-content.html?cms_pos=2 (accessed on 6 June 2022).
16. Koch-Institut Ständige Impfkommission (STIKO) beim Robert Koch-InstitutSISbR. Available online: https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2022/Ausgaben/02_22.pdf?__blob=publicationFile (accessed on 13 January 2022).
17. Germany BfG. 2021. Available online: https://www.bundesgesundheitsministerium.de/fileadmin/Dateien/3_Downloads/C/Coronavirus/Impfstoff/National_COVID-19_Vaccination_Strategy_June_2021.pdf (accessed on 6 June 2022).
18. Chia, P.Y.; Ong, S.W.X.; Chiew, C.J.; Ang, L.W.; Chavatte, J.-M.; Mak, T.-M.; Cui, L.; Kalimuddin, S.; Chia, W.N.; Tan, C.W.; et al. Virological and serological kinetics of SARS-CoV-2 Delta variant vaccine-breakthrough infections: A multi-center cohort study. *Clin. Microbiol. Infect.* **2021**, *28*, 612.e1–612.e7. [PubMed]
19. Hacisuleyman, E.; Hale, C.; Saito, Y.; Blachere, N.E.; Bergh, M.; Conlon, E.G.; Schaefer-Babajew, D.J.; DaSilva, J.; Muecksch, F.; Gaebler, C.; et al. Vaccine Breakthrough Infections with SARS-CoV-2 Variants. *N. Engl. J. Med.* **2021**, *384*, 2212–2218. [CrossRef] [PubMed]

20. Wang, R.; Chen, J.; Hozumi, Y.; Yin, C.; Wei, G.-W. Emerging vaccine-breakthrough SARS-CoV-2 variants. *arXiv* **2021**, arXiv:2204.0947. [CrossRef] [PubMed]
21. Monto, A.S. The Future of SARS-CoV-2 Vaccination—Lessons from Influenza. *N. Engl. J. Med.* **2021**, *385*, 1825–1827. [CrossRef]
22. Bar-On, Y.M.; Goldberg, Y.; Mandel, M.; Bodenheimer, O.; Freedman, L.; Kalkstein, N.; Mizrahi, B.; Alroy-Preis, S.; Ash, N.; Milo, R.; et al. Protection of BNT162b2 Vaccine Booster against COVID-19 in Israel. *N. Engl. J. Med.* **2021**, *385*, 1393–1400. [CrossRef]
23. Nordström, P.; Ballin, M.; Nordström, A. Effectiveness of COVID-19 vaccination against risk of symptomatic infection, hospitalization, and death up to 9 months: A Swedish total-population cohort study. *SSRN Pap.* **2021**, *9*. [CrossRef]
24. Mabrouk, M.T.; Huang, W.C.; Martinez-Sobrido Lu Lovell, J.F. Advanced Materials for SARS-CoV-2 Vaccines. *Adv. Mater.* **2022**, *34*, 2107781. [CrossRef]
25. Kosiorek, P.; Kazberuk, D.; Hryniewicz, A.; Milewski, R.; Stróż, S.; Stasiak-Barmuta, A. Systemic COVID-19 vaccination also enhances the humoral immune response after SARS CoV-2 infection. An approach to criteria for COVID-19 re-immunization is needed. Do we need a third dose? *Vaccines* **2022**, *10*, 334. [CrossRef]
26. Ivanova, E.; Devlin, J.; Buus, T.; Koide, A.; Cornelius, A.; Samanovic, M.; Herrera, A.; Zhang, C.; Desvignes, L.; Odum, N.; et al. Discrete immune response signature to SARS-CoV-2 mRNA vaccination versus infection. *medRxiv* **2021**. [CrossRef]
27. Kissler, S.M.; Tedijanto, C.; Goldstein, E.; Grad, Y.H.; Lipsitch, M. Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period. *Science* **2020**, *368*, 860–868. [CrossRef]
28. Phillips, N. The coronavirus is here to stay—Here's what that means. *Nature* **2021**, *590*, 382–384. [CrossRef] [PubMed]
29. Criscuolo, E.; Diotti, R.A.; Strollo, M.; Rolla, S.; Ambrosi, A.; Locatelli, M.; Burioni, R.; Mancini, N.; Clementi, M.; Clementi, N. Weak correlation between antibody titers and neutralizing activity in sera from SARS-CoV-2 infected subjects. *J. Med. Virol.* **2021**, *93*, 2160–2167. [CrossRef] [PubMed]
30. Lumley, S.F.; O'Donnell, D.; Stoesser, N.E.; Matthews, P.C.; Howarth, A.; Hatch, S.B.; Marsden, B.D.; Cox, S.; James, T.; Warren, F.; et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. *N. Engl. J. Med.* **2021**, *384*, 533–540. [CrossRef] [PubMed]
31. Buonfrate, D.; Piubelli, C.; Gobbi, F.; Martini, D.; Bertoli, G.; Ursini, T.; Moro, L.; Ronzoni, N.; Angheben, A.; Rodari, P.; et al. Antibody response induced by the BNT162b2 mRNA COVID-19 vaccine in a cohort of health-care workers, with or without prior SARS-CoV-2 infection: A prospective study. *Clin. Microbiol. Infect.* **2021**, *27*, 1845–1850. [CrossRef]
32. Lustig, Y.; Sapir, E.; Regev-Yochay, G.; Cohen, C.; Fluss, R.; Olmer, L.; Indenbaum, V.; Mandelboim, M.; Doolman, R.; Amit, S.; et al. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: A prospective, single-centre, longitudinal cohort study in health-care workers. *Lancet Respir. Med.* **2021**, *9*, 999–1009. [CrossRef]
33. Khoury, D.S.; Cromer, D.; Reynaldi, A.; Schlub, T.E.; Wheatley, A.K.; Juno, J.A.; Subbarao, K.; Kent, S.J.; Triccas, S.J.; Davenport, M.P. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* **2021**, *27*, 1205–1211. [CrossRef]
34. Matusali, G.; Colavita, F.; Lapa, D.; Meschi, S.; Bordi, L.; Piselli, P.; Gagliardini, R.; Corpolongo, A.; Nicastri, E.; Antinori, A.; et al. SARS-CoV-2 Serum Neutralization Assay: A Traditional Tool for a Brand-New Virus. *Viruses* **2021**, *13*, 655. [CrossRef]
35. Ibarrondo, F.J.; Hofmann, C.; Fulcher, J.A.; Goodman-Meza, D.; Mu, W.; Hausner, M.A.; Ali, A.; Balamurugan, A.; Taus, E.; Elliott, J.; et al. Primary, Recall, and Decay Kinetics of SARS-CoV-2 Vaccine Antibody Responses. *ACS Nano* **2021**, *15*, 11180–11191. [CrossRef]
36. Goldberg, Y.; Mandel, M.; Bar-On, Y.M.; Bodenheimer, O.; Freedman, L.; Haas, E.J.; Milo, R.; Alroy-Preis, S.; Ash, N.; Huppert, A. Waning immunity of the BNT162b2 vaccine: A nationwide study from Israel. *medRxiv* **2021**. [CrossRef]
37. Dittadi, R.; Seguso, M.; Bertoli, I.; Afshar, H.; Carraro, P. Antibodies against SARS-CoV-2 Time Course in Patients and Vaccinated Subjects: An Evaluation of the Harmonization of Two Different Methods. *Diagnostics* **2021**, *11*, 1709. [CrossRef]
38. De la Monte, S.M.; Long, C.; Szczepanski, N.; Griffin, C.; Fitzgerald, A.; Chapin, K. Heterogeneous Longitudinal Antibody Responses to COVID-19 mRNA Vaccination. *Clin. Pathol.* **2021**, *14*, 2632010X211049255. [CrossRef] [PubMed]
39. Collier, D.A.; Ferreira, I.A.T.M.; Kotagiri, P.; Datir, R.P.; Lim, E.Y.; Touizer, E.; Meng, B.; Abdullahi, A.; Elmer, A.; Kingston, N.; et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature* **2021**, *596*, 417–422. [CrossRef] [PubMed]
40. Efrati, S.; Catalogna, M.; Abu Hamad, R.; Hadanny, A.; Bar-Chaim, A.; Benveniste-Levkovitz, P.; Levtzion-Korach, O. Safety and humoral responses to BNT162b2 mRNA vaccination of SARS-CoV-2 previously infected and naive populations. *Sci. Rep.* **2021**, *11*, 16543. [CrossRef] [PubMed]
41. Valcourt, E.J.; Manguiat, K.; Robinson, A.; Chen, J.C.-Y.; Dimitrova, K.; Philipson, C.; Lamoureux, L.; McLachlan, E.; Schiffman, Z.; Drebot, M.A.; et al. Evaluation of a commercially-available surrogate virus neutralization test for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *Diagn. Microbiol. Infect. Dis.* **2021**, *99*, 115294. [CrossRef]

3. Published Original Article 2

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Communication

Ex Vivo Immune Responsiveness to SARS-CoV-2 Omicron BA.5.1 Following Vaccination with Unmodified mRNA-Vaccine

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Abstract: (1) Background: The high incidence of SARS-CoV-2 infection in vaccinated persons underscores the importance of individualized re-vaccination. PanIg antibodies that act against the S1/-receptor binding domain quantified in serum by a routine diagnostic test (ECLIA, Roche) can be used to gauge the individual ex vivo capacity of SARS-CoV-2 neutralization. However, that test is not adapted to mutations in the S1/-receptor binding domain, having accumulated in SARS-CoV-2 variants. Therefore, it might be unsuited to determine immune-reactivity against SARS-CoV-2 BA.5.1. (2) Method: To address this concern, we re-investigated sera obtained six months after second vaccinations with un-adapted mRNA vaccine Spikevax (Moderna). We related serum levels of panIg against the S1/-receptor binding domain quantified by the un-adapted ECLIA with full virus neutralization capacity against SARS-CoV-2 B.1 or SARS-CoV-2 BA5.1. (3) Results: 92% of the sera exhibited sufficient neutralization capacity against the B.1 strain. Only 20% of the sera sufficiently inhibited the BA5.1 strain. Sera inhibiting BA5.1 could not be distinguished from non-inhibiting sera by serum levels of panIg against the S1/-receptor binding domain quantified by the un-adapted ECLIA. (4) Conclusion: Quantitative serological tests for an antibody against the S1/-receptor binding domain are unsuited as vaccination companion diagnostics, unless they are regularly adapted to mutations that have accumulated in that domain.

Keywords: COVID 19-serology; SARS-CoV-2-neutralization; SARS-CoV-2-vaccination; SARS-CoV-2-immunity; companion-diagnostic; SARS-CoV-2 BA.5.1

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1. Introduction

The continued emergence of new SARS-CoV-2 mutants and the high incidence of re-infection and COVID-19 disease in vaccinated populations [1–3] clearly corroborate the need for regular re-vaccination, similar to vaccinations for influenza [4]. Initially, SARS-CoV-2 vaccinations followed fixed temporal schedules that were designed to break pandemic waves [5–8]. By now, health care deals with the outcome of a heterogeneous vaccination regimen and continuous asynchronous endemic re-infection by various SARS-CoV-2 mutants. As a consequence, immune responsiveness to SARS-CoV-2 exhibits considerable variability within the population [9], and individualization of vaccination has become an issue in several countries [10–13].

We [14] and others [10,11,15] have argued that diagnostic tests for humoral SARS-CoV-2 immune responses that are commercially available and practical in the setting of routine health care diagnostics [16–18] can be used to gauge humoral ex vivo immune responsiveness to SARS-CoV-2 and possibly provide a companion diagnostic for individualized re-vaccination. We observed that simple and rapid measurements of circulating SARS-CoV-2

antibody levels in serum were reasonably well correlated with virus-neutralizing activities determined by ex vivo surrogate assays or by the neutralization of the full SARS-CoV-2 virus in cell culture. We derived from these investigations a cut-off value for panIg antibodies against Spike S1-protein in serum. Above this cut-off value, immune responsiveness to SARS-CoV-2 could be assumed to be sufficient, as deduced from effective virus neutralization ex vivo [14].

However, these data were only valid for the original SARS-CoV-2 B.1 isolate and for vaccination and immune assays based on the unmodified spike S1-protein domain derived from the original virus strain. By now, the available serological assays are still the same and most persons still rely on protection by unmodified vaccines, but infectious challenges originate from mutant virus strains, in which the protein domain targeted by vaccines and putative companion diagnostics has accumulated many mutations and, thereby, is considerably altered [19]. Therefore, our published data [14] are probably superseded. To address that concern, we re-tested the post-vaccination sera of the above study with respect to their potency to neutralize the virus strain Omicron BA.5.1, which currently dominates the SARS-CoV-2-endemy in many countries [12,13].

2. Materials and Methods

Study features and most assays were previously described [14]. Initially, a total of 124 study participants (83 female, 41 male, mean age 46 years, median age 50 years) were recruited at the University Hospital of the Heinrich Heine University, Düsseldorf. All participants were employees of that institution and underwent a program of two vaccinations with the COVID-19 vaccine Spikevax (Moderna Biotech, Cambridge, MA, USA), spaced exactly four weeks apart. Vaccinations were performed according to the instructions of the manufacturer and the recommendations of the German vaccination commission (STIKO). None of the participants tested positive for SARS-CoV-2 or exhibited symptoms of COVID-19, nor did they exhibit debilitating symptoms of co-morbidities. We also retested 90 serum samples obtained six months after the second vaccinations (70 female, 20 male, mean age of 47 years, median age of 49 years) for their neutralization capacity against the SARS-CoV-2 Omicron variant BA5.1 (GISAID accession number EPI_ISL_16100719).

Antibodies against the SARS-CoV-2 spike (S1) protein receptor-binding domain (S1-AB) were determined using chemiluminescent immunoassay (ECLIA) (Elecsys Anti-SARS-CoV-2 S, Roche Diagnostics GmbH, Mannheim, Germany) on a COBAS 8000 analyzer (Roche, Basel, Switzerland), as prescribed by the manufacturer. Samples were measured at tenfold dilution (Roche Cobas Universal Diluent) and remeasured at 400-fold dilution when exceeding the upper detection limit (250 U/mL) [14].

The virus neutralization activity of the SARS-CoV-2 antibodies was measured with the surrogate assays NeutraLISA (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) and cPass(GenScript Biotech, Piscataway, NJ, USA), both of which measure the binding of the recombinant, biotin-labelled ACE2 receptor to the recombinant SARS-CoV-2-S1/-receptor-binding domain immobilized on microtiter plates [14].

Full-virus endpoint dilution neutralization (BA.5NT) was measured in duplicate in five-fold serial dilutions (1:10 to 1:1250) of heat-inactivated sera (56 °C, 30 min). A total of 10 µL of serum samples was incubated (37 °C, 1 h) with a SARS-CoV-2 Omicron BA5.1 virus solution at an absolute TCID50 of 100. Subsequently, 50 µL of cell suspension containing 25×10^4 VeroE6 cells/mL (ATCC-CRL-1586) was added to each sample, and incubation continued (37 °C, 5% CO_2, 96 h). Subsequently, cytopathic effects (CPEs) were determined by microscopic inspection. The effective neutralization titer was defined as the highest CPE-negative sample dilution. Titers of ≥1:10 were considered positive. Controls included in each test series encompassed neutralization-negative and -positive serum samples (previously determined and stored at −20 °C), the effect of virus in the absence of serum, and the growth controls of cells exposed to neither the virus nor the serum.

Graph Pad Prism 9 (Graph Pad Software Inc., San Diego, CA, USA) was used for analysis. Normal distribution was tested according to Shapiro–Wilk. Correlations were

analyzed by Spearman correlation. Correlations were assumed to be good at $r \geq 0.7$ and moderate at $r \geq 0.5$. For all tests, statistical significance was assumed at $p < 0.05$. Missing data (about 12%) was handled by listwise deletion.

3. Results

The ex vivo immune responsiveness of the tested serum samples differed markedly between the B.1 and BA.5.1 strains. Of the previous samples, 92% (85/92) exhibited sufficient full-virus neutralization capacity against the B.1 strain [14]. In contrast, in this study, only 20% (18/90) of the samples exhibited sufficient full-virus neutralization capacity against the BA.5.1 strain (BA.5-NT). These differences were also apparent in quantitative comparisons: B1-NT titers exhibited reasonable correlations with two surrogate assays of virus neutralization that measure the inhibition of the binding of the recombinant, biotin-labelled ACE2-receptor to the recombinant SARS-CoV-2-S1/-receptor binding domain immobilized on microtiter plates (NeutraLISA, EUROIMMUN AG, Lübeck, Germany, and cPass, GenScript Biotech, Piscataway, NJ, USA). In contrast, BA.5-NT titers showed no quantitative correlations with these surrogate assays and were only moderately correlated with B.1-NT titers (Figure 1).

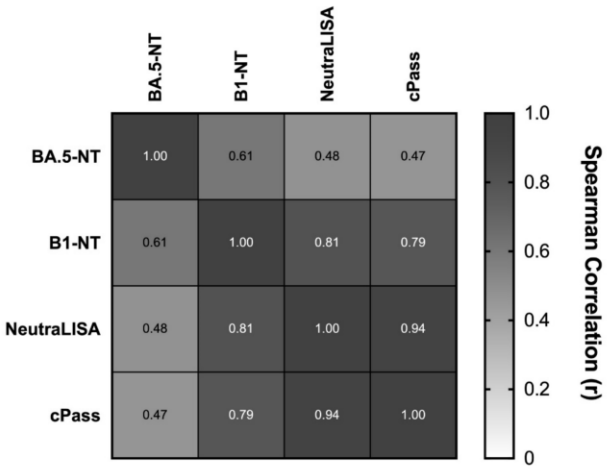

Figure 1. Comparison of virus neutralization capacity of 92 sera obtained six months after vaccination with the original mRNA vaccine (Spikevax, Moderna). Serological neutralization potency was determined by inhibition of the binding of the recombinant, biotin-labelled ACE2-receptor to the recombinant SARS-CoV-2-S1/-receptor binding domain (NeutraLISA, Euroimmun and cPass, Genscript Biotech) or by the endpoints of full-virus dilution neutralization using either the B.1-strain (B.1-NT) or the BA.5.1-strain (BA.5-NT) as targets. R-values of Spearman's correlation test are shown.

In keeping with the data demonstrated in Figure 1, it was not possible to define cut-off values for the two surrogate assays that would allow a discrimination of BA.5-NT-positive samples. In fact, BA.5-NT-negative and -positive samples were completely intermingled relative to their capacity to inhibit the binding between the ACE2-receptor to the recombinant SARS-CoV-2-S1/-receptor binding domain (Figure 2a). Similarly, serum levels of panIg antibodies against the SARS-CoV-2 spike (S1) protein receptor binding domain (S1-AB) determined by chemiluminescent immunoassay (Roche Diagnostics, Mannheim, Germany) allowed, at best, a moderate discrimination of BA.5-NT-positive samples: 70% (12/17) of the samples above an S1-AB cut-off value of 1700 U/mL (Figure 2b, dotted line) were BA.5-NT-positive, while below that S1-AB value, 91% (67/73) of the samples were BA.5-

NT-negative. We assume that this discriminatory power is not sufficient for diagnostic purposes.

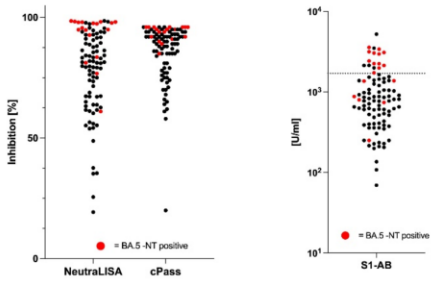

Figure 2. Discrimination of BA.5-NT-positive and -negative sera by quantitative serology. (**a**) Serological neutralization potency determined by inhibition of the binding of the recombinant, biotin-labelled ACE2-receptor to the recombinant SARS-CoV-2-S1/-receptor binding domain (cPass, Genscrip Biotech and NeutraLISA, Euroimmun); (**b**) serum levels of panIg against the SARS-CoV-2 spike (S1) protein receptor binding domain (S1-AB) determined by chemiluminescent immunoassay (ECLIA, Roche Diagnostics). Black dots: BA.5-NT-negative samples; red dots: BA.5-NT-positive samples; dotted line: cut-off at 1700 U/mL.

4. Discussion

The salient findings of this study are:

- Vaccination with mRNA corresponding to the original sequence of the S1/-receptor binding domain (derived from SARS CoV-2 B.1) confers a much lower humoral ex vivo neutralizing potency against SARS CoV-2 Omicron B.A5.1 than against SARS CoV-2 B.1.
- Commercial serological tests based on the original S1/-receptor binding domain (derived from SARS CoV-2 B.1) have only limited predictive power for ex vivo neutralizing potency against SARS CoV-2 Omicron BA.5.1.

In summary, these observations are in line with published findings regarding the diminishing power of un-adapted SARS-CoV-2-vaccinations to protect against immune escape variants of the virus that have accumulated mutations in the S1/-receptor binding domain [12,13,19].

Our previous investigation addressed the immune responsiveness of post-vaccination sera against the SARS CoV-2 B.1 strain. We could derive from that investigation a diagnostic strategy mainly based on serum levels of panIg against the SARS-CoV-2 spike (S1) protein receptor binding domain, which provided a reliable way of gauging levels and functionality of circulating SARS-CoV-2 antibodies [14]. However, reanalysis of these samples with respect to the currently most abundant and clinically relevant mutant SARS CoV-2 BA.5.1 reveals that the above strategy is severely compromised by the immunological drift of the virus and can no longer be safely applied.

A limitation of our study is that our results refer only to the B.1 and B.5.1 variants studied here and not to other variants that have evolved in the meantime. Furthermore, since our collective was exclusively vaccinated with Spikevax (Moderna Biotech), our findings cannot be easily applied to other vaccines and additional investigations on later-developed protein- or vector-based vaccines would be necessary.

5. Conclusions

It is not astonishing that serological assays investigating antibody interactions with the S1/-receptor binding domain are compromised by mutations that accumulate in that

domain and gradually lose their predictive power for humoral immune responsiveness to virus mutants such as SARS CoV-2 Omicron BA.5.1. We conclude that currently un-adapted serological assays are of low value as vaccination companion diagnostics, and that they must be adapted to the mutations of the clinically relevant virus strains, in a similar fashion to that of the vaccines themselves.

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Informed Consent Statement: Informed written consent was obtained from all subjects involved in the study. Informed written consent was obtained from all individual participants to publication of their anonymized data.

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References

1. Chia, P.Y.; Ong, S.W.X.; Chiew, C.J.; Ang, L.W.; Chavatte, J.-M.; Mak, T.-M.; Cui, L.; Kalimuddin, S.; Chia, W.N.; Tan, C.W. Virological and serological kinetics of SARS-CoV-2 Delta variant vaccine-breakthrough infections: A multi-center cohort study. *Clin. Microbiol. Infect.* **2021**, *28*, 612.e1–612.e7.
2. Hacisuleyman, E.; Hale, C.; Saito, Y.; Blachere, N.E.; Bergh, M.; Conlon, E.G.; Schaefer-Babajew, D.J.; DaSilva, J.; Muecksch, F.; Gaebler, C.; et al. Vaccine Breakthrough Infections with SARS-CoV-2 Variants. *N. Engl. J. Med.* **2021**, *384*, 2212–2218. [CrossRef] [PubMed]
3. Wang, R.; Chen, J.; Hozumi, Y.; Yin, C.; Wei, G.-W. Emerging vaccine-breakthrough SARS-CoV-2 variants. *ACS Infect. Dis. 2022 8*, 546–556. [CrossRef]
4. Monto, A.S. The Future of SARS-CoV-2 Vaccination—Lessons from Influenza. *N. Engl. J. Med.* **2021**, *385*, 1825–1827. [CrossRef] [PubMed]
5. CDC; C.f.D.C.a.P. Stay Up to Date with COVID-19 Vaccines Including Boosters. Available online: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines.html (accessed on 13 January 2023).
6. Paul-Ehrlich-Institute. Coronavirus and COVID-19. Available online: https://www.pei.de/EN/newsroom/dossier/coronavirus/coronavirus-content.html?cms_pos=2 (accessed on 13 January 2023).
7. Koch-Institut, S.I.S.b.R. Epidemiologisches Bulletin. Available online: https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2022/Ausgaben/02_22.pdf?__blob=publicationFile (accessed on 13 January 2023).
8. Germany, B.f.G. Strategy for the Continued Delivery and Evaluation of Vaccination against SARS-CoV-2 in Germany (Updated). Available online: https://www.bundesgesundheitsministerium.de/fileadmin/Dateien/3_Downloads/C/Coronavirus/Impfstoff/National_COVID-19_Vaccination_Strategy_June_2021.pdf (accessed on 13 January 2023).
9. Efrati, S.; Catalogna, M.; Abu Hamad, R.; Hadanny, A.; Bar-Chaim, A.; Benveniste-Levkovitz, P.; Levtzion-korach, O. Safety and humoral responses to BNT162b2 mRNA vaccination of SARS-CoV-2 previously infected and naive populations. *Sci. Rep.* **2021**, *11*, 16543. [CrossRef] [PubMed]
10. Bonanni, P.; Cantón, R.; Gill, D.; Halfon, P.; Liebert, U.G.; Crespo, K.A.N.; Martín, J.J.P.; Trombetta, C.M. The Role of Serology Testing to Strengthen Vaccination Initiatives and Policies for COVID-19 in Europe. *COVID* **2021**, *1*, 20–38. [CrossRef]

11. dos Santos Ferreira, C.E.; Gómez-Dantés, H.; Junqueira Bellei, N.C.; López, E.; Nogales Crespo, K.A.; O'Ryan, M.; Villegas, J. The Role of Serology Testing in the Context of Immunization Policies for COVID-19 in Latin American Countries. *Viruses* **2021**, *13*, 2391. [CrossRef] [PubMed]
12. Noruzi, A.; Gholampour, B.; Gholampour, S.; Jafari, S.; Farshid, R.; Stanek, A.; Saboury, A.A. Current and future perspectives on the COVID-19 vaccine: A scientometric review. *J. Clin. Med.* **2022**, *11*, 750. [CrossRef] [PubMed]
13. Gan, P.; Pan, X.; Huang, S.; Xia, H.; Zhou, X.; Tang, X. Current status of coronavirus disease 2019 vaccine research based on bibliometric analysis. *Hum. Vaccines Immunother.* **2022**, *18*, 2119766. [CrossRef] [PubMed]
14. Kuechler, A.S.; Weinhold, S.; Boege, F.; Adams, O.; Muller, L.; Babor, F.; Bennstein, S.B.; Pham, T.U.; Hejazi, M.; Reusing, S.B.; et al. A Diagnostic Strategy for Gauging Individual Humoral Ex Vivo Immune Responsiveness Following COVID-19 Vaccination. *Vaccines* **2022**, *10*, 1044. [CrossRef] [PubMed]
15. Criscuolo, E.; Diotti, R.A.; Strollo, M.; Rolla, S.; Ambrosi, A.; Locatelli, M.; Burioni, R.; Mancini, N.; Clementi, M.; Clementi, N. Weak correlation between antibody titers and neutralizing activity in sera from SARS-CoV-2 infected subjects. *J. Med. Virol.* **2021**, *93*, 2160–2167. [CrossRef] [PubMed]
16. Lumley, S.F.; O'Donnell, D.; Stoesser, N.E.; Matthews, P.C.; Howarth, A.; Hatch, S.B.; Marsden, B.D.; Cox, S.; James, T.; Warren, F. Antibody status and incidence of SARS-CoV-2 infection in health care workers. *N. Engl. J. Med.* **2021**, *384*, 533–540. [CrossRef] [PubMed]
17. Buonfrate, D.; Piubelli, C.; Gobbi, F.; Martini, D.; Bertoli, G.; Ursini, T.; Moro, L.; Ronzoni, N.; Angheben, A.; Rodari, P.; et al. Antibody response induced by the BNT162b2 mRNA COVID-19 vaccine in a cohort of health-care workers, with or without prior SARS-CoV-2 infection: A prospective study. *Clin. Microbiol. Infect.* **2021**, *27*, 1845–1850. [CrossRef] [PubMed]
18. Lustig, Y.; Sapir, E.; Regev-Yochay, G.; Cohen, C.; Fluss, R.; Olmer, L.; Indenbaum, V.; Mandelboim, M.; Doolman, R.; Amit, S. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: A prospective, single-centre, longitudinal cohort study in health-care workers. *Lancet Respir. Med.* **2021**, *9*, 999–1009. [CrossRef] [PubMed]
19. Tao, K.; Tzou, P.L.; Nouhin, J.; Gupta, R.K.; de Oliveira, T.; Kosakovsky Pond, S.L.; Fera, D.; Shafer, R.W. The biological and clinical significance of emerging SARS-CoV-2 variants. *Nat. Rev. Genet.* **2021**, *22*, 757–773. [CrossRef] [PubMed]

4. Discussion

4.1 Salient findings

99% of the participants were positive for S1-antibodies after first vaccination and 100% showed positive antibody levels for S1-protein after second vaccination indicating no poor- and non-responders among the participants. Six months after second vaccination average S1-antibody-levels reduced to 18% of the initial value after second vaccination (from 5.704 U/ml to 1.019 U/ml after six months). These results underline the fast waning of immune protection after vaccination and correspond to immune response observed in different research [80, 111]. Time course of immune response was highly heterogenous throughout the study participants and correlation between antibody-levels immediately after second vaccination and six months later was poor. Waning of immunity thus cannot be predicted by the initial antibody level achieved immediately after vaccination and might depend according to current research on the patients age, number of prior infections and the interval between first and second dose of vaccine [112]. Exhibiting higher antibody-levels after vaccination after undergoing prior infection was a central result of this study, too. Nevertheless, this augmentation in antibody-levels when prior infected decreased after six months and did not lead to a preserved immune protection in the long-term. Research confirms this effect of higher antibody levels the first months after immunization when considering hybrid immunity and simultaneously affirms the fast waning of this special type of immune protection [113].

Surrogate assays as a link between antibody-levels and PRNT as the gold standard did not provide useful information immediately after vaccination as immune protection was overestimated by surrogate assays in comparison to results supplied by PRNT. For estimating long-term immune protection surrogate assays showed excellent correlation with PRNT six months after vaccination when considering medium to high antibody-levels and a 6% rate of false-positive results when considering low-range antibody-levels. These findings are in line with previous studies [114]. Addressing the strong correlation between humoral quantitative immune response and results in PRNT six months after vaccination

we defined a cut-off at 1.000 U/ml of S1-AB as a predictor for immune protection. This value could be transferred to the surrogate assay and corresponds to results of 64% and 72% considering different manufacturers of surrogate assays. Moreover, our study showed that surrogate assays also enable to discriminate between strong and poor immune protection when falling below the recommended cut-off of 1000 U/ml of S1-AB with a sensitivity of 83,3% and a specificity of 84,2% to 96,2%.

In the second publication of this study, we examined whether this diagnostic algorithm can be transferred to Omicron BA5.1. Central results were only 20% of the specimen exhibiting neutralizing capacity in BA5.1 PRNT and a poor correlation between PRNT and surrogate assays. Moreover, BA5.1-titres and B1-titres did not correlate in PRNT either. Deriving a diagnostic algorithm for humoral immune response as a correlate to immune protection against Omicron BA5.1 was not sensible in the latter context. Consequently, these results support the necessity for booster immunization and adapting the vaccination to the circulating VOC as soon as immune escape mechanisms become apparent [86, 87, 115].

4.2 Significance for Covid-19 vaccination companion diagnostics

This study proves that qualitative immune protection can be derived from quantitative humoral immune response, which enables implementing Covid-19 vaccination companion diagnostics. This could lead away from fixed reimmunization schedules to individual immunization schemes when referring to the B1-strain. In the context of heterogenous effects on immunity by different vaccination schedules, numerous different types of vaccines and different amounts of undergone infection with again different types of VOCs fixed reimmunization schedules appear to be outdated. On account of the pandemic situation turning into endemic conditions fixed timepoints for revaccination to encounter infectious waves became obsolete and individualized revaccination gains importance [116, 117]. Moreover, Covid-19 vaccination companion diagnostics can provide patients from severe side effects of the vaccination by avoiding unnecessary reimmunization without prior serological assessment of the immune state. PACVS

and other adverse effects of Covid-19 vaccination state enormous reduction in life quality and lead to economic loss [93, 118]. Covid-19 vaccination companion diagnostics could prevent these negative impacts on patients and society [119].

The diminishing power of monovalent Covid-19 vaccination against new VOCs able to escape immune response with mutations in S1-RBD makes our diagnostic strategy for gauging individual humoral immune responsiveness after Covid-19 vaccination inapplicable in the current state. Therefore, unadapted serological assays need to be adapted to new VOCs serving as correlate to immune protection furthermore. A new diagnostic algorithm could be then applied to adapted laboratory methods and vaccines.

After initially high demands of Covid-19 vaccines hesitancy against the vaccination has been an issue during the whole pandemic. Doubts about vaccine effectiveness, its fast development, fear of severe side effects and individual believes made herd immunity almost unattainable [120]. Declaring Covid-19 vaccination as mandatory was highly controversial but let immunization coverage rise in many countries [121, 122]. Since Covid-19 vaccination is not compulsory anymore new discourse about the vaccination´s benefit arouse. It has been unambiguous ever since that the vaccination prevents critical illness and hospitalization. This effect persists during Omicron as the predominant VOC despites high infestation rates and breakthrough infections maintaining the benefits of Covid-19 vaccination [72, 88]. Since basic immunity is achieved by 1-3 vaccinations depending on the risk profile of each patient according to the WHO and alternatively by three antigen contacts either by vaccination or infection according to the STIKO a huge share of population possesses basic immunity [74, 75]. Booster immunization is currently recommended for elderly and immunocompromised patients, people with comorbidities and health care workers every 6-12 months and for women in every pregnancy. There is no recommendation for revaccination for healthy children, adolescents and adults which make up a huge proportion of society [104].
Taking the basic immunization coverage, the high number of mild courses in cases with current VOCs and a missing recommendation for booster immunization for huge parts of the population into account the relevance of determining individual vaccination titres has to be scrutinized.

4.3 Limitations of the study

All participants of the study received basic immunization with the mRNA-based vaccine Spikevax by Moderna Biotech. This standardized the conditions under which the study has been performed and excluded different vaccine types as a confounder. Results thus are of a huge comparability and highly representative for the vaccine used in this study on the one hand. On the other hand, results cannot be applied on the entirety of the vaccinated population as different kind of vaccines are under current use and they might lead to different results. Differing efficacy and effectiveness depending on the vaccine type as well as varying longevity of immune protection after vaccination is described in current research [123, 124].
Moreover, serological tests used for this study are only adapted to the SARS-CoV-2 B1 Wuhan virus isolate but not to any kind of VOC that has arose during the pandemic course. Since surrogate assays showed only poor correlation when comparing results obtained by testing against the B1 strain and Omicron BA5.1 it becomes comprehensible that every VOC has its own characteristics and performance in serological tests might differ greatly.
In addition, the plaque reduction neutralization test which serves as gold standard for estimating immune protection has been used as correlate of immune protection in this study. However, it can only be understood as approximation to immune protection as immunity is based on complex interaction of humoral and cellular mechanisms including t-cell response and other parameters, which were left out in these investigations [97, 125].
Another limitation of the study is that we cannot suggest optimized timepoints for serological testing after vaccination since the tempo of immunity waning is not correlated with the initially achieved level of antibodies after vaccination. We can recommend the level of antibodies that indicates the necessity for reimmunization if it has been undercut but we cannot estimate the interval for retesting the patient if the cut-off has still been exceeded. This leads to the dilemma of determining serostatus too often which is associated with high costs on the one hand and performing serological testing in bigger intervals which might lead to missing the optimal timepoint for revaccination out leading to gaps in immunization on the other hand.

4.4 Conclusion and future implications

According to leading health - and vaccination authorities it is indisputable that Covid-19 and vaccinations against SARS-CoV-2 will belong to health care routine at least for mid-term future and will be a part of the daily life in our society for an indefinite time. Regular revaccination at least for people at risk and health care personnel thus is currently recommended [75, 104].
Summarizing the findings of this study and their future implications it becomes distinct that revaccination against SARS-CoV-2 is a subject of high complexity. Several phenomena have to be taken into account when evaluating revaccination schemes against Covid-19 in the future: Humoral and cellular immune response exhibit huge interindividual variability and long-term immune protection cannot be derived by the initially achieved antibody level immediately after vaccination [80, 126, 127]. Variance in immune response and prediction of long-term immunity becomes even more complex when considering combinations of different types of vaccines and effects of hybrid immunity [110, 113, 128, 129]. Both an infection with SARS-CoV-2 and the vaccination against it are associated with the risk of severe side effects encompassing PACS and PACVS, which both state new disease entities. As their epidemiology and impact on the patient´s physical and mental health, the economy and society cannot be comprehensively estimated by now PACS and PACVS state new challenges when considering opportunities and disadvantages of regular reimmunization against Covid-19 [93, 94].
Disregarding the fast development of the pandemic and the accumulation of genomic changes of each VOC this study suggests a practicable diagnostic algorithm for individualized reimmunization adapted to SARS-CoV-2 companion diagnostics and the patient´s serostatus. The diagnostic algorithm allows the estimation of immune protection by determining serostatus with methods practicable in health care routine and thus helps facing the above challenges. When considering SARS-CoV-2 B.1 Wuhan virus isolate this study enables optimized revaccination schemes and the assessment of a patient´s immunity against SARS-CoV-2 with serological tests as a correlate to immune state without time- and cost consuming methods like PRNT.
However, the rapid development of VOCs and the strongly deviating characteristics of each VOC concerning transmissibility, pathogenicity and their

capability of immune escape makes the algorithm for SARS-CoV-2 vaccination companion diagnostics lose its predictive value [24, 26, 27, 130]. Each serological assay would have to be adapted to new VOCs and new vaccines to obtain predictive power. These circumstances make establishing SARS-CoV-2 companion diagnostics in health care routine elaborate and unattractive when taking Covid-19 becoming an endemic situation into account [106, 117].
In conclusion, risks and long-term consequences of an infection with SARS-CoV-2 including severe causes and the possibility of suffering from Long-Covid have to be weighed up individually against the side effects of (re-)vaccination against Covid-19 in the future. Covid-19 will state a part of the daily life as well as questions regarding opportunities and disadvantages of vaccination against SARS-CoV-2 will.

References

1. Ramos-Casals, M., P. Brito-Zerón, and X. Mariette, Systemic and organ-specific immune-related manifestations of COVID-19. Nat Rev Rheumatol, 2021. 17(6): p. 315-332.
2. Tang, D., P. Comish, and R. Kang, The hallmarks of COVID-19 disease. PLoS Pathog, 2020. 16(5): p. e1008536.
3. Li, X., et al., Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal, 2020. 10(2): p. 102-108.
4. Chu, D.K., et al., Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis. Lancet, 2020. 395(10242): p. 1973-1987.
5. Tsang, H.F., et al., An update on COVID-19 pandemic: the epidemiology, pathogenesis, prevention and treatment strategies. Expert Rev Anti Infect Ther, 2021. 19(7): p. 877-888.
6. Zhang, R., et al., Identifying airborne transmission as the dominant route for the spread of COVID-19. Proceedings of the National Academy of Sciences, 2020. 117(26): p. 14857-14863.
7. Musa, S.S., et al., Vertical Transmission of SARS-CoV-2: A Systematic Review of Systematic Reviews. Viruses, 2021. 13(9).
8. Lone, N.I., et al., Influence of socioeconomic deprivation on interventions and outcomes for patients admitted with COVID-19 to critical care units in Scotland: A national cohort study. Lancet Reg Health Eur, 2021. 1: p. 100005.
9. Hodge, E., S. Oversby, and J. Chor, Why are some outbreaks worse than others? COVID-19 outbreak management strategies from a PHU perspective. BMC Public Health, 2023. 23(1): p. 597.
10. Seok, H., et al., Infectivity of Coronavirus Disease 2019: A Prospective Cohort Study in the Korean Metropolitan Area. J Korean Med Sci, 2022. 37(14): p. e106.
11. Du, Z., et al., Serial Interval of COVID-19 among Publicly Reported Confirmed Cases. Emerg Infect Dis, 2020. 26(6): p. 1341-1343.
12. Drain, P.K., et al., Duration of viral infectiousness and correlation with symptoms and diagnostic testing in non-hospitalized adults during acute SARS-CoV-2 infection: A longitudinal cohort study. J Clin Virol, 2023. 161: p. 105420.
13. Bao, Y., et al., Dynamic anti-spike protein antibody profiles in COVID-19 patients. International Journal of Infectious Diseases, 2021. 103: p. 540-548.
14. Yang, H. and Z. Rao, Structural biology of SARS-CoV-2 and implications for therapeutic development. Nat Rev Microbiol, 2021. 19(11): p. 685-700.
15. Jackson, C.B., et al., Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol, 2022. 23(1): p. 3-20.
16. Ziegler, C.G.K., et al., SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. Cell, 2020. 181(5): p. 1016-1035.e19.
17. Muus, C., et al., Single-cell meta-analysis of SARS-CoV-2 entry genes across tissues and demographics. Nat Med, 2021. 27(3): p. 546-559.

18. Pollard, C.A., M.P. Morran, and A.L. Nestor-Kalinoski, The COVID-19 pandemic: a global health crisis. Physiol Genomics, 2020. 52(11): p. 549-557.
19. Ochani, R., et al., COVID-19 pandemic: from origins to outcomes. A comprehensive review of viral pathogenesis, clinical manifestations, diagnostic evaluation, and management. Infez Med, 2021. 29(1): p. 20-36.
20. Holmes, E.C., et al., The origins of SARS-CoV-2: A critical review. Cell, 2021. 184(19): p. 4848-4856.
21. Organization, W.H., WHO Director-General's opening remarks at the media briefing on COVID-19. 2020.
22. Organization, W.H. WHO COVID-19 dashboard. 2024 [cited 2024; Available from: https://data.who.int/dashboards/covid19/cases?n=c.
23. Pasetto, D., et al., Range of reproduction number estimates for COVID-19 spread. Biochem Biophys Res Commun, 2021. 538: p. 253-258.
24. Carabelli, A.M., et al., SARS-CoV-2 variant biology: immune escape, transmission and fitness. Nat Rev Microbiol, 2023. 21(3): p. 162-177.
25. Dutta, A., COVID-19 waves: variant dynamics and control. Sci Rep, 2022. 12(1): p. 9332.
26. Choi, J.Y. and D.M. Smith, SARS-CoV-2 Variants of Concern. Yonsei Med J, 2021. 62(11): p. 961-968.
27. Andre, M., et al., From Alpha to Omicron: How Different Variants of Concern of the SARS-Coronavirus-2 Impacted the World. Biology (Basel), 2023. 12(9).
28. Gorbalenya, A.E., et al., The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nature Microbiology, 2020. 5(4): p. 536-544.
29. de Boer, P.T., et al., Age-specific severity of severe acute respiratory syndrome coronavirus 2 in February 2020 to June 2021 in the Netherlands. Influenza and Other Respiratory Viruses, 2023. 17(8): p. e13174.
30. Hasan, M.N., et al., The Global Case-Fatality Rate of COVID-19 Has Been Declining Since May 2020. Am J Trop Med Hyg, 2021. 104(6): p. 2176-2184.
31. Green, M.S., et al., The confounded crude case-fatality rates (CFR) for COVID-19 hide more than they reveal-a comparison of age-specific and age-adjusted CFRs between seven countries. PLoS One, 2020. 15(10): p. e0241031.
32. Li, R., et al., Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). Science, 2020. 368(6490): p. 489-493.
33. Krishnakumar, H.N., et al., Pathogenesis and progression of anosmia and dysgeusia during the COVID-19 pandemic. Eur Arch Otorhinolaryngol, 2023. 280(2): p. 505-509.
34. Tufan, A., A. Avanoğlu Güler, and M. Matucci-Cerinic, COVID-19, immune system response, hyperinflammation and repurposing antirheumatic drugs. Turk J Med Sci, 2020. 50(Si-1): p. 620-632.
35. Nie, X., et al., Multi-organ proteomic landscape of COVID-19 autopsies. Cell, 2021. 184(3): p. 775-791.e14.
36. Silva, B.M., et al., Acute kidney injury outcomes in covid-19 patients: systematic review and meta-analysis. J Bras Nefrol, 2022. 44(4): p. 543-556.

37. Fairweather, D., et al., COVID-19, Myocarditis and Pericarditis. Circ Res, 2023. 132(10): p. 1302-1319.
38. Jin, B., et al., Pathophysiological mechanisms underlying gastrointestinal symptoms in patients with COVID-19. World J Gastroenterol, 2021. 27(19): p. 2341-2352.
39. Rajendran, B., S. N, and G. Ramkumar, COVID-19-associated Neurological Manifestations and Complications: An Observational Study. J Assoc Physicians India, 2022. 70(12): p. 11-12.
40. Yao, X.H., et al., A cohort autopsy study defines COVID-19 systemic pathogenesis. Cell Res, 2021. 31(8): p. 836-846.
41. Marshall, J.C., et al., A minimal common outcome measure set for COVID-19 clinical research. The Lancet Infectious Diseases, 2020. 20(8): p. e192-e197.
42. Zhang, J.J., et al., Risk and Protective Factors for COVID-19 Morbidity, Severity, and Mortality. Clin Rev Allergy Immunol, 2023. 64(1): p. 90-107.
43. Gao, Z., et al., A systematic review of asymptomatic infections with COVID-19. J Microbiol Immunol Infect, 2021. 54(1): p. 12-16.
44. Zhang, J., S. Wu, and L. Xu, Asymptomatic carriers of COVID-19 as a concern for disease prevention and control: more testing, more follow-up. Biosci Trends, 2020. 14(3): p. 206-208.
45. Peeling, R.W., et al., Diagnostics for COVID-19: moving from pandemic response to control. Lancet, 2022. 399(10326): p. 757-768.
46. Rai, P., et al., Detection technologies and recent developments in the diagnosis of COVID-19 infection. Appl Microbiol Biotechnol, 2021. 105(2): p. 441-455.
47. Zarif, A., et al., The role of antibody tests for COVID-19 in primary care. Br J Gen Pract, 2021. 71(704): p. 131-134.
48. Liu, W., et al., Predictors of Nonseroconversion after SARS-CoV-2 Infection. Emerg Infect Dis, 2021. 27(9): p. 2454-2458.
49. Haileamlak, A., The impact of COVID-19 on health and health systems. Ethiop J Health Sci, 2021. 31(6): p. 1073-1074.
50. Ağababaoğlu, İ. and K. İnci, Management of COVID-19 in the Intensive Care Unit. Adv Exp Med Biol, 2021. 1353: p. 81-89.
51. Rello, J., et al., Update in COVID-19 in the intensive care unit from the 2020 HELLENIC Athens International symposium. Anaesth Crit Care Pain Med, 2020. 39(6): p. 723-730.
52. Majumder, J. and T. Minko, Recent Developments on Therapeutic and Diagnostic Approaches for COVID-19. Aaps j, 2021. 23(1): p. 14.
53. Zhou, Z., Y. Zhu, and M. Chu, Role of COVID-19 Vaccines in SARS-CoV-2 Variants. Front Immunol, 2022. 13: p. 898192.
54. Umakanthan, S., et al., Origin, transmission, diagnosis and management of coronavirus disease 2019 (COVID-19). Postgrad Med J, 2020. 96(1142): p. 753-758.
55. Chen, J., et al., Epidemiological and economic impact of COVID-19 in the US. Scientific Reports, 2021. 11(1): p. 20451.
56. Li, Y., et al., Face masks to prevent transmission of COVID-19: A systematic review and meta-analysis. Am J Infect Control, 2021. 49(7): p. 900-906.
57. Daher-Nashif, S., et al., COVID-19 exit strategy during vaccine implementation: a balance between social distancing and herd immunity. Arch Virol, 2022. 167(9): p. 1773-1783.

58. Liu, J., et al., Social Distancing in the COVID-19 Pandemic: Associated Factors, Health Outcomes, and Implications. Fam Community Health, 2024. 47(1): p. 80-94.
59. Rothwell, J.T., et al., Global evidence on the economic effects of disease suppression during COVID-19. Humanities and Social Sciences Communications, 2024. 11(1): p. 78.
60. Rehman, S.U., S.U. Rehman, and H.H. Yoo, COVID-19 challenges and its therapeutics. Biomed Pharmacother, 2021. 142: p. 112015.
61. Davis, H.E., et al., Long COVID: major findings, mechanisms and recommendations. Nature Reviews Microbiology, 2023. 21(3): p. 133-146.
62. Al-Aly, Z., Y. Xie, and B. Bowe, High-dimensional characterization of post-acute sequelae of COVID-19. Nature, 2021. 594(7862): p. 259-264.
63. Chen, C., et al., Global Prevalence of Post-Coronavirus Disease 2019 (COVID-19) Condition or Long COVID: A Meta-Analysis and Systematic Review. J Infect Dis, 2022. 226(9): p. 1593-1607.
64. Kenny, G., et al., Long COVID: Clinical characteristics, proposed pathogenesis and potential therapeutic targets. Front Mol Biosci, 2023. 10: p. 1157651.
65. Achleitner, M., et al., Clinical improvement of Long-COVID is associated with reduction in autoantibodies, lipids, and inflammation following therapeutic apheresis. Molecular Psychiatry, 2023. 28(7): p. 2872-2877.
66. Kenny, G., et al., Long COVID: Clinical characteristics, proposed pathogenesis and potential therapeutic targets. Frontiers in Molecular Biosciences, 2023. 10.
67. Verbeke, R., et al., The dawn of mRNA vaccines: The COVID-19 case. J Control Release, 2021. 333: p. 511-520.
68. Sterner, E., Analyses of the 2023 Nobel Prize in Physiology or Medicine: Nucleoside Base Modifications and Effective mRNA Vaccines. Science & Technology Libraries, 2024. 43(1): p. 1-17.
69. Li, M., et al., COVID-19 vaccine development: milestones, lessons and prospects. Signal Transduct Target Ther, 2022. 7(1): p. 146.
70. World Health Organization. WHO Coronavirus (COVID-19) Dashboard. 30.10.2021 13.08.2024]; Available from: https://covid19.who.int/table.
71. Statista. Impfungen gegen das Coronavirus (COVID-19) nach ausgewählten Ländern weltweit seit Beginn der Impfkampagne im Dezember 2020. 2024 13.08.2024]; Available from: https://de.statista.com/statistik/daten/studie/1195157/umfrage/impfungen-gegen-das-coronavirus-nach-laendern-weltweit/.
72. Fiolet, T., et al., Comparing COVID-19 vaccines for their characteristics, efficacy and effectiveness against SARS-CoV-2 and variants of concern: a narrative review. Clin Microbiol Infect, 2022. 28(2): p. 202-221.
73. Watanabe, A., et al., Protective effect of COVID-19 vaccination against long COVID syndrome: A systematic review and meta-analysis. Vaccine, 2023. 41(11): p. 1783-1790.
74. Organization, w.H. COVID-19 advice for the public: Getting vaccinated. 2023; Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines/advice.
75. Impfkommission, S., Decision on the implementation of the COVID-19 vaccination into the general recommendations of the STIKO 2023. 2023.
76. Collier, D.A., et al., Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. Nature, 2021. 596(7872): p. 417-422.

77. Zhang, Z., et al., Humoral and cellular immune memory to four COVID-19 vaccines. Cell, 2022. 185(14): p. 2434-2451.e17.
78. El Karoui, K. and A.S. De Vriese, COVID-19 in dialysis: clinical impact, immune response, prevention, and treatment. Kidney Int, 2022. 101(5): p. 883-894.
79. Zeng, Q., et al., Immunological Findings in a Group of Individuals Who Were Poor or Non-Responders to Standard Two-Dose SARS-CoV-2 Vaccines. Vaccines, 2023. 11(2): p. 461.
80. Nordström, P., M. Ballin, and A. Nordström, Effectiveness of Covid-19 vaccination against risk of symptomatic infection, hospitalization, and death up to 9 months: a Swedish total-population cohort study. Hospitalization, and Death Up to, 2021. 9.
81. Orlandi, C., et al., Comparing Heterologous and Homologous COVID-19 Vaccination: A Longitudinal Study of Antibody Decay. Viruses, 2023. 15(5).
82. Zhu, Y., S. Liu, and D. Zhang, Effectiveness of COVID-19 Vaccine Booster Shot Compared with Non-Booster: A Meta-Analysis. Vaccines, 2022. 10(9): p. 1396.
83. Agrawal, U., et al., Severe COVID-19 outcomes after full vaccination of primary schedule and initial boosters: pooled analysis of national prospective cohort studies of 30 million individuals in England, Northern Ireland, Scotland, and Wales. The Lancet, 2022. 400(10360): p. 1305-1320.
84. Past SARS-CoV-2 infection protection against re-infection: a systematic review and meta-analysis. Lancet, 2023. 401(10379): p. 833-842.
85. Woldemeskel, B.A., et al., SARS-CoV-2-specific immune responses in boosted vaccine recipients with breakthrough infections during the Omicron variant surge. JCI Insight, 2022. 7(10).
86. Wang, Q., et al., Alarming antibody evasion properties of rising SARS-CoV-2 BQ and XBB subvariants. Cell, 2023. 186(2): p. 279-286.e8.
87. Chalkias, S., et al., A Bivalent Omicron-Containing Booster Vaccine against Covid-19. N Engl J Med, 2022. 387(14): p. 1279-1291.
88. Link-Gelles, R., et al., Estimates of Bivalent mRNA Vaccine Durability in Preventing COVID-19-Associated Hospitalization and Critical Illness Among Adults with and Without Immunocompromising Conditions - VISION Network, September 2022-April 2023. MMWR Morb Mortal Wkly Rep, 2023. 72(21): p. 579-588.
89. Reddy, K.P., et al., Clinical outcomes and cost-effectiveness of COVID-19 vaccination in South Africa. Nature Communications, 2021. 12(1): p. 6238.
90. Meo, S.A., et al., COVID-19 vaccines: comparison of biological, pharmacological characteristics and adverse effects of Pfizer/BioNTech and Moderna Vaccines. Eur Rev Med Pharmacol Sci, 2021. 25(3): p. 1663-1669.
91. Franchini, M., G.M. Liumbruno, and M. Pezzo, COVID-19 vaccine-associated immune thrombosis and thrombocytopenia (VITT): Diagnostic and therapeutic recommendations for a new syndrome. Eur J Haematol, 2021. 107(2): p. 173-180.
92. Altman, N.L., et al., Vaccination-Associated Myocarditis and Myocardial Injury. Circ Res, 2023. 132(10): p. 1338-1357.
93. Semmler, A., et al., Chronic Fatigue and Dysautonomia following COVID-19 Vaccination Is Distinguished from Normal Vaccination Response by Altered Blood Markers. Vaccines (Basel), 2023. 11(11).

94. Scholkmann, F. and C.A. May, COVID-19, post-acute COVID-19 syndrome (PACS, "long COVID") and post-COVID-19 vaccination syndrome (PCVS, "post-COVIDvac-syndrome"): Similarities and differences. Pathol Res Pract, 2023. 246: p. 154497.
95. Seekircher, L., et al., Immune response after two doses of the BNT162b2 COVID-19 vaccine and risk of SARS-CoV-2 breakthrough infection in Tyrol, Austria: an open-label, observational phase 4 trial. The Lancet Microbe, 2023. 4(8): p. e612-e621.
96. Dalla Gasperina, D., et al., Humoral and Cellular Immune Response Elicited by the BNT162b2 COVID-19 Vaccine Booster in Elderly. Int J Mol Sci, 2023. 24(18).
97. Sureshchandra, S., et al., Single-cell profiling of T and B cell repertoires following SARS-CoV-2 mRNA vaccine. JCI Insight, 2021. 6(24).
98. Loesche, M., et al., Longitudinal SARS-CoV-2 Nucleocapsid Antibody Kinetics, Seroreversion, and Implications for Seroepidemiologic Studies. Emerg Infect Dis, 2022. 28(9): p. 1859-1862.
99. Uysal, E.B., et al., Evaluation of antibody response after COVID-19 vaccination of healthcare workers. J Med Virol, 2022. 94(3): p. 1060-1066.
100. Fox, T., et al., Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev, 2022. 11(11): p. Cd013652.
101. Knezevic, I., et al., WHO International Standard for evaluation of the antibody response to COVID-19 vaccines: call for urgent action by the scientific community. The Lancet Microbe.
102. McDade, T.W., et al., Durability of antibody response to vaccination and surrogate neutralization of emerging variants based on SARS-CoV-2 exposure history. Scientific Reports, 2021. 11(1): p. 17325.
103. Valcourt, E.J., et al., Evaluating humoral immunity against SARS-CoV-2: validation of a plaque-reduction neutralization test and a multilaboratory comparison of conventional and surrogate neutralization assays. Microbiology spectrum, 2021. 9(3): p. e00886-21.
104. Organization, W.H. COVID-19 advice for the public: Getting vaccinated. 21.01.2022; Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines/advice.
105. Li, Y., et al., Rational design of an influenza-COVID-19 chimeric protective vaccine with HA-stalk and S-RBD. Emerg Microbes Infect, 2023. 12(2): p. 2231573.
106. Contreras, S., E.N. Iftekhar, and V. Priesemann, From emergency response to long-term management: the many faces of the endemic state of COVID-19. The Lancet Regional Health – Europe, 2023. 30.
107. Bobrovitz, N., et al., Protective effectiveness of previous SARS-CoV-2 infection and hybrid immunity against the omicron variant and severe disease: a systematic review and meta-regression. The Lancet Infectious Diseases, 2023. 23(5): p. 556-567.
108. Virk, A., et al., Hybrid Immunity Provides Protective Advantage Over Vaccination or Prior Remote Coronavirus Disease 2019 Alone. Open Forum Infect Dis, 2023. 10(5): p. ofad161.
109. Boyton, R.J. and D.M. Altmann, The immunology of asymptomatic SARS-CoV-2 infection: what are the key questions? Nature Reviews Immunology, 2021. 21(12): p. 762-768.

110. Zhao, T., X. Huang, and Y. Shu, Comparing the immune response and protective effect of COVID-19 vaccine under different vaccination strategies. Hum Vaccin Immunother, 2023. 19(3): p. 2273155.
111. Kolarić, B., et al., Waning immunity six months after BioNTech/Pfizer COVID-19 vaccination among nursing home residents in Zagreb, Croatia. Croat Med J, 2021. 62(6): p. 630-633.
112. Pérez-Alós, L., et al., Modeling of waning immunity after SARS-CoV-2 vaccination and influencing factors. Nat Commun, 2022. 13(1): p. 1614.
113. de Gier, B., et al., Effects of COVID-19 vaccination and previous infection on Omicron SARS-CoV-2 infection and relation with serology. Nature Communications, 2023. 14(1): p. 4793.
114. Hofmann, N., et al., Detection of neutralizing antibodies against SARS-CoV-2 by using a commercial surrogate virus neutralization ELISA: can it substitute the classical neutralization test? medRxiv, 2021: p. 2021.10.12.21264881.
115. Shete, A.M., et al., Waning natural and vaccine-induced immunity leading to reinfection with SARS-CoV-2 Omicron variant. Hum Vaccin Immunother, 2022. 18(6): p. 2127289.
116. Phillips, N., The coronavirus is here to stay - here's what that means. Nature, 2021. 590(7846): p. 382-384.
117. Antia, R. and M.E. Halloran, Transition to endemicity: Understanding COVID-19. Immunity, 2021. 54(10): p. 2172-2176.
118. Amer, S.A., et al., Exploring the reported adverse effects of COVID-19 vaccines among vaccinated Arab populations: a multi-national survey study. Scientific Reports, 2024. 14(1): p. 4785.
119. Kuechler, A.S., K. Schulze-Bosse, and L. Müller, SARS-CoV-2 Vaccination Companion Diagnostics: A Novel Perspective in Optimizing COVID-19 (Re-) Immunization. Systematic Reviews in Pharmacy, 2022. 13(6).
120. Pourrazavi, S., et al., COVID-19 vaccine hesitancy: A Systematic review of cognitive determinants. Health Promot Perspect, 2023. 13(1): p. 21-35.
121. Politis, M., et al., Healthcare Workers' Attitudes towards Mandatory COVID-19 Vaccination: A Systematic Review and Meta-Analysis. Vaccines (Basel), 2023. 11(4).
122. Mills, M.C. and T. Rüttenauer, The effect of mandatory COVID-19 certificates on vaccine uptake: synthetic-control modelling of six countries. The Lancet Public Health, 2022. 7(1): p. e15-e22.
123. Tregoning, J.S., et al., Progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. Nat Rev Immunol, 2021. 21(10): p. 626-636.
124. Jamshidi, E., et al., Longevity of immunity following COVID-19 vaccination: a comprehensive review of the currently approved vaccines. Hum Vaccin Immunother, 2022. 18(5): p. 2037384.
125. Kumar, R., et al., COVID-19 vaccination: Immune response in healthcare workers-A study with review of literature. Indian J Pathol Microbiol, 2023. 66(4): p. 758-763.
126. Collier, D.A., et al., Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. Nature, 2021. 596(7872): p. 417-422.
127. de la Monte, S.M., et al., Heterogeneous Longitudinal Antibody Responses to Covid-19 mRNA Vaccination. Clin Pathol, 2021. 14: p. 2632010x211049255.

128. Goldberg, Y., et al., Protection and waning of natural and hybrid COVID-19 immunity. MedRxiv, 2021.
129. Bobrovitz, N., et al., Protective effectiveness of previous SARS-CoV-2 infection and hybrid immunity against the omicron variant and severe disease: a systematic review and meta-regression. Lancet Infect Dis, 2023. 23(5): p. 556-567.
130. Flores-Vega, V.R., et al., SARS-CoV-2: Evolution and Emergence of New Viral Variants. Viruses, 2022. 14(4).

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