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Review Article

SARS-CoV-2 Vaccination Companion Diagnostics: A Novel Perspective in Optimizing COVID-19 (Re-) Immunization

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ABSTRACT

Until today, vaccination against COVID-19 is handled by fixed and blind immunization-schedules. This work overviews and discusses the opportunities and limitations of different Severe Acute Respiratory Syndrome Corona Virus type 2 (SARS-CoV-2) antibody tests and their suitability as SARS-CoV-2 vaccination companion diagnostics. These tests are not officially recommended for evaluating the necessity of (re-) immunization yet, but novel studies contribute to understanding their informative value, their complementary relationships and thus their potential for using them as a predictor for immune-protection against COVID-19.

We found that serological assays detecting antibodies against SARS-CoV-2 and their neutralizing capacity can form a diagnostic strategy for individualized vaccination schedules due to their specific informative values. Serological antibody tests as SARS-CoV-2 vaccination companion diagnostics thus constitute a novel perspective for optimized and individualized time points for (re-)immunization. Despite being time- and cost consuming for the health care system they represent a great benefit for closing gaps in immune-protection for people at risk. SARS-CoV-2 vaccination companion diagnostics may also reduce the amount of severe short term vaccination side effects and consequences like t-cell exhaustion or auto-immune diseases by over-vaccination in long term. Nonetheless, future research directions concerning SARS-CoV-2 vaccination companion diagnostics are manifold and official clinical guidelines still need to be established.

Keywords: Companion-diagnostic, COVID-19-serology, SARS-CoV-2-immunity, SARS-CoV-2-neutralization, SARS-CoV-2-vaccination

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ABBREVIATIONS

PRNT: Plaque Reduction Neutralization Test; SARS-CoV-2: Severe Acute Respiratory Syndrome Corona Virus Type 2; VOC: Variant of Concern

INTRODUCTION

The Severe Acute Respiratory Syndrome Corona Virus Type 2 (SARS-CoV-2) is a novel airborne-transmitted enveloped single-stranded RNA virus causing the respiratory disease COVID-19 (V'kovski P, *et al*., 2021; Kim D, *et al*., 2020; Zhang R, *et al*., 2020). The virus originates from Wuhan, China, where it had its zoonotic emergence in December 2019 (Nadeem MS *et al*., 2020; Platto S, *et al*., 2021; Hu B, *et al*., 2017). Since then, more than 400 million people suffered from an infection with SARS-CoV-2 comprising asymptomatic courses, mild to severe symptoms like dry cough, fever, pneumonia, Acute Respiratory Distress Syndrome (ARDS) and even nearly six million deaths globally (WHO, 2022; Tsang HF, *et al*., 2021; Tzotzos SJ, *et al*., 2020; Lane YM, *et al*., 2020).

In December 2020, one year after the outbreak of the COVID-19 pandemic, vaccinations against SARS-CoV-2 became available and were started being administered (Lamb YN, 2021). By February 2022, nine different mRNA-, vector-, and protein-based vaccines are in use and almost 11 billion doses have been administered globally (WHO, 2022; Paul-Ehrlich-Institute, 2022; Ndwandwe D and Wiysonge CS, 2021).

Soon, antibody tests entered the market as an instrument for monitoring the immune status of infected people. The commercially available antibody tests are quantitative serological assays detecting different kinds of antibodies in a patient´s blood sample (Baraniuk C, 2020). Most common are tests detecting antibodies against the SARS-CoV-2 spike protein receptor binding domain (spike-antibody) and against the nucleocapsid antigen (nucleocapsid-protein-antibodies). Currently, N-protein-AB can serve as a marker for infection only, while spike-antibodies are produced by the human body as a reaction both to infection and vaccination (Che XY, *et al*., 2004; Bao Y, *et al*., 2021). However, vaccines targeting both the SARS-CoV-2 spike protein receptor binding domain and the nucleocapsid antigen are currently under development and might enter the market soon. The clear discrimination of antibodies induced by vaccination or infection is then obsolete and has to be overhauled (Dutta NK, *et al*., 2020; Dangi T, *et al*., 2021). As these tests are only of quantitative value and do not allow any information on the functionality of the antibodies, surrogate assays measuring the neutralizing capacity of antibodies against SARS-CoV-2 and thus the extent of immune protection are carried out (Khoury DS, *et al*., 2021; Feng S, *et al*., 2021; Bergwerk M, *et al*., 2021). Nevertheless, the gold standard for testing the neutralizing capacity of antibodies against SARS-CoV-2 is the full virus endpoint dilution neutralization test or the Plaque Reduction Neutralization Test (PRNT), which need to be conducted in a biosafety level 3 facilities (Valcourt EJ, *et al*., 2021). In this method serum samples diluted to varying degrees get incubated with infectious SARS-CoV-2 in cell culture. The dilution of serum reducing or inhibiting the infection is given as the neutralizing antibody-titre (Valcourt EJ, *et al*., 2021; Bewley KR, *et al*., 2021). An approximation to full virus neutralization tests with infectious SARS-CoV-2 are Pseudovirus-Based Neutralization Assays (PBNAs) that make use of recombinant virus particles carrying a specific SARS-CoV-2 spike protein (Nie J, *et al*., 2020). The approach of implementing a reference neutralization test conductible in a biosafety level 1 facility has been pursued at the beginning of the COVID-19 pandemic but was not imposed until today (Zettl F, *et al*., 2020).

Until now, serological antibody tests are frequently used for the diagnostic of infections with SARS-CoV-2 and are not officially recommended for estimating the necessity for re-vaccination (CDC, 2022). Nevertheless, latest studies consider them as a reliable instrument for choosing optimized and individualized vaccination time points in the future (CDC, 2022; Kuechler AS, *et al*., 2022).

Having a variety of diagnostic tools for determining antibody levels and thus the immune protection of a person vaccinated against SARS-CoV-2, this work aims to overview and discuss the role of SARS-CoV-2 vaccination companion diagnostics and outlines their opportunities and limitations.

LITERATURE REVIEW

The issue with SARS-CoV-2 vaccination companion diagnostics

The question as to whether serological antibody testing as a SARS-CoV-2 vaccination companion diagnostic is useful and superior to fixed vaccination schedules without personalized monitoring of immune protection includes several different aspects-In the past months, the COVID-19 pandemic has been characterized by synchronous waves of infections, each dominated by a new Variant of Concern (VOC) and culminating in maximum cases reported in cold winter months (Salfi F, *et al*., 2021; Engelbrecht FA and Scholes RJ, 2021). These waves of infection could be encountered with periodic blind waves of vaccinations. Because of the enormously high number of reported cases during e.g. the omicron-wave, the infestation rate in each population is going to increase. This leads to synchronous pandemic infection waves converting into not less harmful asynchronous endemic conditions (Phillips N, 2021; Katzourakis A, 2022; Antia R and Halloran ME, 2021). The immune responses throughout a population will then be extremely heterogenous, wherefore re-vaccination will have to be handled individually.

The high rate of infestation is accompanied by a similarly increased number of asymptomatic and therefore partially even inapparent infections (Muller CP, 2021). People undergoing inapparent infections produce antibodies against SARS-CoV-2, too, even though they reach lower levels of antibodies than the ones found in mild to severe symptomatic patients. Previous studies have shown that asymptomatic patients are capable of producing neutralizing antibodies, which protect them from a new infection (Long QX, *et al*., 2020; Choe PG, *et al*., 2020). Those people then do not require a re-immunization by vaccination in the near future as they received a boost of their immune response by infection itself.

Avoiding booster vaccinations for patients having undergone inapparent infections even gains importance when considering studies reporting more frequent and severe side effects in vaccinated people with previous contemporary COVID-19 infection (Tissot N, *et al*., 2021; Krammer F, *et al*., 2021). Presenting a specific kind of antigen to the human body by multiple booster vaccinations repeatedly may lead to t-cell exhaustion. A weakened immune system by t-cell exhaustion as it also occurs in chronic viral infections like HIV might be the consequence (Wherry EJ, 2011; Blank CU, *et al*., 2019; Roth C, 2022). Moreover, mechanisms leading to the induction of humoral immune tolerance or autoimmune-diseases in the context of so called "over-vaccination" are currently discussed (Wraith DC, *et al*., 2003; Ungerer M, *et al*., 2018; Mariani G, *et al*., 2003). Serological antibody tests before a vaccination against SARS-CoV-2 would prevent those patients from profuse side effects and other long-term consequences based on the overstimulation of the immune-system and can estimate a suitable time point of re-vaccination for them.

Another aspect which should be taken into account when considering more frequent side effects of patients receiving a vaccination on top of already high antibody levels is the diversity of disposable vaccines. Previous studies have shown that the different types of vaccines show great disparities in the period of time in which they can attain safe humoral immune protection. According to the findings of Nordström, *et al.* vector-based vaccines lead to a significantly faster waning of immune response than mRNA-based vaccines (Nordström P, *et al*., 2022). The situation becomes even more complex as heterologous prime and boost vaccination schedules show higher antibody levels and a slower waning of immune protection. The mix of an mRNA-based vaccine with a vector-based one in the first two and the boost vaccination therefore leads to prolonged immune protection and provides the opportunity to postpone another re-immunization (Ho TC, *et al*., 2021; He Q, *et al*., 2021; Atmar RL, *et al*., 2022). Patients vaccinated with a combination of different vaccines therefore need to be treated differently than patients receiving only one kind of vaccine throughout the prime and boost succession. Moreover, patients that have undergone vaccination and infection, also in reversed order, need to be taken into special consideration as well since they show prolonged immune responses in comparison to patients without hybrid immunity (Goldberg Y, *et al*., 2021). This even extends the operational area of SARS-CoV-2 companion diagnostics.

The final and maybe most important reason to incorporate SARS-CoV-2 vaccination companion diagnostics in health care routine is the urgent need of a permanent and sufficient immune protection against COVID-19 of elderly, comorbid and immunosuppressed patients. This large patient collective subjects to the risk of significantly more severe outcomes of infections and a higher mortality (Villalobos NVF, *et al*., 2021; Pedreañez A, *et al*., 2021). Comorbidities like respiratory-, renal- and cardiovascular diseases, diabetes, cancer, and immune deficiencies are only a small selection of diseases affecting the course of COVID-19 patients negatively (Yang J, *et al*., 2020; Ng WH, *et al*., 2021). Additionally, these people at risk also show lower levels of spike-antibodies, weaker neutralizing capacities and a higher rate of non-responders after vaccinations (Naaber P, *et al*., 2021; Collier DA, *et al*., 2021; Müller L, *et al*., 2021). They additionally show a faster waning of immunity, which makes a gapless immune protection more challenging and the benefit of SARS-CoV-2 vaccination companion diagnostic apparent in this special group of patients (Nordström P, *et al*., 2022; Bosetti P, *et al*., 2021).

DISCUSSION

What can SARS-CoV-2 companion diagnostics tell us?

SARS-CoV-2 companion diagnostics are based on a selection of serological tests with different informative values.

Spike-antibodies as well as nucleocapsid-protein-antibodies are produced by the human body during a SARS-CoV-2 infection while vaccines target the production of spike-antibodies only (Bao Y, *et al*., 2021). These kinds of antibodies are protective to some extent against a SARS-CoV-2 infection and can mitigate the course of COVID-19 (Feng S, *et al*., 2021; Harvey RA, *et al*., 2021; Kuno T, *et al*., 2021). The value of spike-antibodies is generally expressed in International Units (IU) or the more standardized Binding Antibody Units per millilitre (BAU/ml) as recommended by WHO. This measuring unit constitutes the values of the laboratory test results multiplied by the factor 2.6. It measures the quantity of antibodies in a sample binding to a fixed antigen in a test container. Since the introduction of a calibration reference, the WHO International Standard and Reference Panel for anti-SARS-CoV-2 immunoglobulin, measurements of spike-antibodies (Immunoglobulin G) are comparable with each other and can be calibrated against different tests (Kristiansen PA, *et al*., 2021; Knezevic I, *et al*., 2021). Soon the question arose which level of spike-antibodies conveys a sufficient immune protection, and the approach of SARS-CoV-2 vaccination companion diagnostic arose.

Latest studies suggest values of spike-antibodies around 1000 U/ml as being reliably protective when correlating them with surrogate assays and plaque reduction neutralization test. Testing a patient for spike-antibodies

several weeks after vaccination therefore provides a suitable method for monitoring the immune response obtained by vaccination. Nevertheless, these studies are of limited validity as the results cannot directly be applied to different variants of concern and combinations of vaccines (Kuechler AS, *et al*., 2022; Dimeglio C, *et al*., 2022). During the omicron-wave many people who suffered from COVID-19 and had already received their booster vaccination showed at least frequently mild courses, a lower hospitalisation rate and lower morbidity (Yang J, *et al*., 2020; Gruell H, *et al*., 2022; Kuhlmann C, *et al*., 2021). However, the suggested cut-off of 1000 U/ml spike-antibodies adjusted to the predominant variant of concern provides a significant basis for companion diagnostics and constitutes the least elaborate and expensive antibody test (Kuechler AS, *et al*., 2022).

Surrogate assays measure the neutralizing capacity of spike-antibodies in a sample expressed in percent. Thus, the value gives information about the functionality of antibodies and their capability to prevent a SARS-CoV-2 infection and a severe outcome (Khoury DS, *et al*., 2021; Legros V, *et al*., 2021). Surrogate assays have shown strong correlation with levels of spike-antibodies. However, plaque reduction neutralization test as the time and cost consuming gold standard of neutralization tests demonstrated that values of surrogate assays do not faithfully describe the potency of a given serum to protect a model cell from the cytotoxic effect of the virus and that corrected cut-offs of their values needed to be developed (Kuechler AS, *et al*., 2022; Meyer B, *et al*., 2020). These new cut-offs adapted to the correlation with plaque reduction neutralization test are located at 70% and therefore higher than specified by the manufacturer´s instructions. They can now provide reliable information about the immune protection of a patient by the implementation of surrogate assays in health care routine (Kuechler AS, *et al*., 2022; Kitagawa Y, *et al*., 2022). However, it is of note that, similarly to spike-antibodies tests, the measured values of surrogate assays have to be individually interpreted and thus their thresholds adjusted to the predominant variant of concern (Kuechler AS, *et al*., 2022).

It also has to be kept in mind that antibodies as the humoral part of the immune system are only one column immune protection is based on. Cellular components of the immune system like T-and B-lymphocytes or natural killer cells are not detected by antibody tests. However, they are also produced after a vaccination against SARS-CoV-2 and are an important mechanism of the protection against a SARS-CoV-2 infection. Antibody tests are therefore no complete correlate of immune protection (Painter MM, *et al*., 2021; Barouch DH, *et al*., 2021). Nevertheless analyzing the cellular immune response after vaccination is far more time and cost consuming than a monitoring confined to humoral aspects only and not suitable for health care routine and commercial purposes. It is still not clear how such cellular assays work, which role they play in SARS-CoV-2 immune protection and whether they have to be implemented in health care routine (Karlsson AC, *et al*., 2020).

Recommendation for future use of SARS-CoV-2 vaccination companion diagnostics

SARS-CoV-2 vaccination companion diagnostics compete with standardized blind vaccination schedules (CDC, 2022). As this work assesses opportunities as well as limitations of SARS-CoV-2 vaccination companion diagnostics, not only scientific but also economical and organizational perspectives need to be considered.

A fixed vaccination schedule, as it is implemented still today, constitutes the less time and cost consuming handling of re-/immunization. Determining the individual immune status of each patient prior vaccination against SARS-CoV-2 would lead to a high workload for medical personnel, rising costs for health insurances and medical infrastructure and also social inequalities if antibody tests need to be paid for by the patient itself (Garcia MS and Szech N, 2020). Nonetheless, routine SARS-CoV-2 vaccination companion diagnostics could detect gaps of immune-protection and fill them by personalized vaccination schedules. Consequently, companion diagnostics could reduce the appearance of vaccination side effects, optimize the frequency of individual re-vaccination and protect people from severe courses of COVID-19 due to a closed gap of immune-protection (Kuechler AS, *et al*., 2022; Krammer F, *et al*., 2021; Villalobos NVF, *et al*., 2021). In return, medical budgets and personnel could be disburdened and would therefore benefit from SARS-CoV-2 vaccination companion diagnostics as well.

During the COVID-19 pandemic pharmacies play a special role concerning the comprehensive offer of vaccinations and managing the workload of administering billions of vaccine doses. Legal issues concerning the special right of administering vaccinations by pharmacists and dealing with preventable side effects caused by non-medical personnel remain obscure. Liability insurances compensate for the incurred damages but reducing the amount of side effects by SARS-CoV-2 vaccination companion diagnostics preceding vaccinations constitutes a great benefit for the medical safety of patients and the legal hedge of pharmacies (Poudel A, *et al*., 2019; Wickware C, 2022; PDA, 2022).

A novel study has suggested the design of a reliable and efficient diagnostic strategy combining different serological assays: As a first step, the spike-antibody level of a patient should be determined. Values of >1000 AU/ml are a positive predictor for sufficient immune-protection due to correlations with surrogate assays and plaque reduction neutralization test. Patients' not exceeding values of 1000 AU/ml need to be tested in a surrogate assay with an adjusted cut-off at 70%. A promptly re-vaccination needs to be implemented only for the group of patients with spike-antibodies <1000 U/ml and surrogate assay <70%. All other patients are sufficiently protected and can postpone re-immunization (Kuechler AS, *et al*., 2022).

Fixed and blind vaccination schedules superseded by the establishment of this diagnostic strategy could renew the current situation of anti-SARS-CoV-2 vaccination. This strategy would lead to an individualized vaccination regime with personalized timepoints of revaccination and a possible decrease of vaccination side effects and severe courses of COVID-19 consequently (Kuechler AS, *et al*., 2022).

Appropriate time points for the use of SARS-CoV-2 companion diagnostics are when expecting weak immune protection of a patient. There are two time points predilected for insufficient immune protection: The first one is located at two weeks after the second vaccination. Patients responding adequately to the vaccination develop stable levels of antibodies several weeks after vaccination and companion diagnostics could reliably detect whether a patient is able to produce antibodies after the first two vaccinations (Sahin U, *et al*., 2020). If SARS-CoV-2 vaccination companion diagnostics detect insufficient immune protection, a patient should receive the booster vaccination earlier to reach higher levels of antibodies and thus build a proper extent of protection. Patients concerned by this scenario are most commonly old, comorbid, and immunosuppressed (Naaber P, *et al*., 2021; Collier DA, *et al*., 2021; Müller L , *et al*., 2021; Goldberg Y, *et al*., 2021). The second time point is the one with the bigger target group as it constitutes the waning immunity after several months after vaccination. Three to four months after the second vaccination humoral immune protection starts to subside and SARS-CoV-2 vaccination companion diagnostics can detect whether a patient should already receive re-immunization or if the patient is still sufficiently protected (Nordström P, *et al*., 2022; Bosetti P, *et al*., 2022, Goldberg Y, *et al*., 2021, Krause PR, *et al*., 2021; Bubar KM, *et al*., 2021; Buonfrate D, *et al*., 2021).

CONCLUSION

Serological antibody tests as SARS-CoV-2 vaccination companion diagnostics constitute a novel perspective in optimizing COVID-19 vaccination schedules. The urgent need of a permanent sufficient immune protection of old and comorbid patients as well as heterologous immune

protection among people due to the conceivable endemic situation and the great variety in different vaccines are only a few reasons for implementing SARS-CoV-2 vaccination companion diagnostics in health care routine. Although serological tests after basic immunization and prior booster vaccinations are additional strains for the already overburdened health care system during the COVID-19 pandemic, companion diagnostics constitute a great benefit for a huge group of patients. Not only the gap of immunity between basic and booster immunization could get revealed and filled, thus reliably protecting patients at risk by vaccination, but also severe side effects by vaccinating on top of already high antibody levels could be avoided.

The staged setup by starting SARS-CoV-2 vaccination companion diagnostics with determining spike-antibody level and following values of spike-antibodies <1000 U/ml with adjusted surrogate assays constitutes the novel and optimized diagnostic strategy.

Nonetheless, research concerning SARS-CoV-2 antibody tests is as new as the virus itself and therefore not advanced. This review is thus based on a limited amount of data and scientific opinions. The whole field of SARS-CoV-2 vaccination companion diagnostics brings forth future research potential like adjusting clinical tests to the respective variant of concern, the better understanding of immune protection and their serological correlates, as well as the official establishment of a diagnostic strategy concerning monitoring and implementation of anti-COVID-19 vaccination.

At this state of research SARS-CoV-2 vaccination companion diagnostics entail huge advantages comprising a permanent reliable immune protection by its monitoring with a certain test combination and thus medical secureness against COVID-19. Nonetheless, huge costs and a massive workload for the healthcare system as well as a higher access threshold for vaccinations associated with SARS-CoV-2 companion diagnostics stand against their comprehensive implementation.

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